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Review Article

B-Cell-Directed Therapies: A New Era in Multiple Sclerosis Treatment

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ABSTRACT: Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS) that often progresses to severe disability. Previous studies have highlighted the role of T cells in disease pathophysiology; however, the success of B-cell-targeted therapies has led to an increased interest in how B cells contribute to disease immunopathology. In this review, we summarize evidence of B-cell involvement in MS disease mechanisms, starting with pathology and moving on to review aspects of B cell immunobiology potentially relevant to MS. We describe current theories of critical B cell contributions to the inflammatory CNS milieu in MS, namely (i) production of autoantibodies, (ii) antigen presentation, (iii) production of proinflammatory cytokines (bystander activation), and (iv) EBV involvement. In the second part of the review, we summarize medications that have targeted B cells in patients with MS and their current position in the therapeutic armamentarium based on clinical trials and real-world data. Covered therapeutic strategies include the targeting of surface molecules such as CD20 (rituximab, ocrelizumab, ofatumumab, ublituximab) and CD19 (inebilizumab), and molecules necessary for B-cell activation such as B cell activating factor (BAFF) (belimumab) and Bruton's Tyrosine Kinase (BTK) (evobrutinib). We finally discuss the use of B-cell-targeted therapeutics in pregnancy.

RÉSUMÉ : Les traitements ciblant les lymphocytes B dans la sclérose en plaques : nouvelle ère thérapeutique en vue. La sclérose en plaques (SP) est une maladie auto-immune chronique démyélinisante du système nerveux central (SNC) qui aboutit souvent à une grande incapacité. Le rôle des lymphocytes T dans la physiopathologie de la maladie a déjà été mis en évidence dans des études antérieures, mais les bons résultats des traitements ciblant les lymphocytes B ont suscité de l'intérêt pour le rôle de ces derniers dans l'immunopathologie de la maladie. Aussi présenterons-nous dans l'article de synthèse des données probantes qui font ressortir l'action des lymphocytes B dans les mécanismes d'évolution de la SP, depuis la maladie elle-même jusqu'aux éléments immunobiologiques des lymphocytes B potentiellement associés à la SP. Dans la première partie, il sera question des théories existantes sur le rôle fondamental que jouent les lymphocytes B dans le milieu inflammatoire du SNC, dans la SP, à savoir i) la production d'autoanticorps; ii) la présentation d'antigènes; iii) la production de cytokines pro-inflammatoires (activation de voisinage); iv) le rôle du virus d'Epstein-Barr. Dans la seconde partie, nous présenterons un résumé des médicaments qui ciblent les lymphocytes B chez les patients atteints de la SP, et discuterons de leur place dans l'arsenal thérapeutique de la maladie d'après les résultats d'essais cliniques et des données réelles. Les stratégies thérapeutiques traitées dans l'article porteront notamment sur la prise pour cible des molécules présentes à la surface des lymphocytes telles que la CD20 (par le rituximab, l'ocrélizumab, l'ofatumumab ou l'ublituximab) et la CD19 (par l'inébilizumab), ainsi que sur la prise pour cible des molécules nécessaires à l'activation des lymphocytes B tel que le facteur d'activation des lymphocytes B (BAFF, en anglais) (par le bélimumab), et à l'inhibition de la tyrosine-kinase de Bruton (par l'évobrutinib). Enfin, il sera question de l'emploi des traitements ciblant les lymphocytes B chez les femmes enceintes.

Keywords: Multiple sclerosis; B cells; Monoclonal antibodies; CD20; CD19

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune demyelinating inflammatory disease of the central nervous system (CNS), in the majority of cases gradually leading to progressive, severe disability if left untreated. MS is the leading cause of non-traumatic disability among young adults in the developed world. It is most often diagnosed between 20 and 40 years of age and affects women and men at a ratio of approximately 2:1.[1–3] The clinical course of MS can be characterized as (i) clinically isolated syndrome (CIS), (ii) relapsing-remitting (RRMS), (iii) primary progressive (PPMS), or (iv) secondary progressive (SPMS). Each of the above MS categories can be further subcategorized as either active or inactive, based on both the clinical relapse rate and MRI findings (new T2 lesions and/or active, gadolinium-enhancing lesions-GdELs). Further, progressive forms can be subcategorized as actively progressive or stable.[4]

Significant progress in understanding MS pathophysiology has been accomplished in the past decades. Two hundred and thirty-

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three genetic variants have been identified as risk factors for MS, 32 of which refer to the major histocompatibility complex family (MHC).[5] Prominent among the many risk variants, MHC Class II DR15 molecule entails mechanistically relevant susceptibility to the disease rather than just being a genetic marker.[6] Additional genetic variants associated with the disease refer to other genes of the immune system, such as genes involved in T-cell activation and proliferation (IL-2, IL-7R), tumor necrosis factor-alpha (TNF-α)-related pathways, and vitamin D metabolic pathways (GC, CYP24A1).[3,7–11]

In MS, myelin is phagocytosed by CD68-positive macrophages, while immune cells including B and T cells seem to be activated in the periphery and to express adherence molecules that enable them to cross blood-brain barrier (BBB) in order to participate in the formation of MS lesions. Accumulating evidence suggests an important contribution of $CD4^+$ T cells to disease pathophysiology.[12] Often being present from the beginning and increasing in quantity as disease progresses, axonal degeneration is regarded as a correlate of disability progression.[13]

MS was long considered mainly T-cell-mediated; however, intrathecal IgG synthesis,[14] a hallmark of MS, supports B-cell involvement.[15,16] The T-cell-dominated view of MS pathogenesis was further challenged by the remarkable efficiency of $CD20^+$ B-cell depletion in eliminating inflammatory activity in patients with MS. In this review, we aim to shed light on the key role B cells play in the pathogenesis of MS and present current advances in MS treatment strategies based on promising and effective B-cell-targeted therapeutic regimens.

B Cells in MS Pathology

Histological studies of active MS lesions have demonstrated that B cells can reside in the perivascular space and the CSF but also within the parenchyma.[17] Moreover, ectopic lymphoid follicles are found primarily in the intrameningeal spaces.[18] and are associated with subpial cortical demyelination in patients with SPMS.[19,20] In addition, four histopathological patterns have been proposed for the classification of acute MS plaques. Type I lesions (15% of MS patients) are characterized by a T-cell and activated microglia inflammatory environment without immunoglobulin deposition and complement activation. On the contrary, type II lesions (58% of MS patients) develop in an inflammatory milieu with immunoglobulin production and complement activation. Demyelination in type III lesions (26% of MS patients) is accompanied by oligodendrocyte apoptosis without immunoglobulin deposition or complement activation. Finally, in type IV lesions (rare; 1% of MS patients) inflammatory modulators result in non-apoptotic death of oligodendrocytes in the white matter surrounding the plaque due to metabolic disorganization processes.[21] However, it is important to note that IgG deposits in MS histopathological specimens are not specific for MS[22] and that no disease-characterizing autoantibodies have been defined to date. Nevertheless, pattern II has been linked to better response to plasma exchange.[23]

Potential Roles of B Cells in MS Pathophysiology

Several studies have explored potential roles of B cells in the development of MS: antibody production, antigen presentation, and secretion of pro- and anti-inflammatory mediators are three prominent research directions that have been explored.[24,25] In addition, clear epidemiological associations of B-lymphotropic Epstein-Barr virus (EBV) infection to MS have led to the explorations of its pathophysiological relevance.[26]

Autoantibodies

Autoreactive B cells that escape peripheral tolerance checkpoint selection could target antigens of the CNS and cause autoimmune inflammation; however, no consistent B-cell antigen that is specific for MS and that causes demyelination has been identified to date despite numerous attempts. Intrathecal oligoclonal bands, a hallmark of MS diagnosis found in up to 95% of patients,[27,28] are not specific for MS (found also in e.g. meningitis and subacute sclerosing panencephalitis) and have been found to target intracellular antigens in patients with MS.[29,30] In addition, detection of intrathecal IgM synthesis has been associated with onset of relapses and a more aggressive disease course.[31] Similarly to antibodies of oligoclonal bands, B cells of MS lesions have been found to target intracellular antigens.[30] Antibodies previously thought to be present in MS such as antibodies against myelin oligodendrocyte glycoprotein (MOG) rather characterize a distinct disease entity (MOG-antibody disease) that encompasses pediatric acquired demyelinating syndrome, recurrent optic neuritis, acute disseminated encephalomyelitis, and neuromyelitis optica without anti-aquaporin four autoantibodies. A minority of MS patients harbor antibodies against a variety of antigens (some of them cell surface proteins) such as contactin-2,[32] OMGP,[33] and other peptide and lipid antigens.[34,35]

It must be noted that autoantibodies can also be responsible for the activation and chemotaxis of CD4+ T cells. The opsonization of myelin antigens, even at low concentrations, enhances the presentative competence of resident antigen-presenting cells (APCs), such as macrophages and dendritic cells, leading to increased recruitment of effector T cells and, consequently, aggravation of the disease severity, as explained in more detail below.[36–38]

B Cells as Antigen-Presenting Cells

B cells are efficient APCs and express MHC class II and costimulatory molecules, such as CD40, CD80, and CD86.[25,39] They can capture soluble and membrane-tethered antigens via their B-cell receptor (BCR) and present them to T cells in an up-to-a 10.000 times more efficient way compared to myeloid APCs.[40] Evidence from experimental autoimmune encephalitis, a rodent model simulating "efferent" MS pathophysiology, opposes the hypothesis that the antigen-presenting function of B cells is central to the pathophysiology of MS. Specifically, MOG-specific B cells may initiate CNS inflammation and, consequently, the symptomatic onset of the disease, but do not affect either the proliferation or the molecular profile (i.e. secreted cytokines, activation markers) of MOG-specific T cells in the spleen and the draining lymph nodes.[37]

On the other hand, a recently published report focusing on human leukocyte antigen (HLA)-DR15, which is the major genetic risk factor for MS, addresses how the immunopeptidomes presented by both DR15 allomorphs, DR2a and DR2b, on different APCs in the thymus, peripheral blood, and brain – including B cells – could affect autoimmune T cells. The results showed that DR2a and DR2b immunopeptidomes on B cells are significantly skewed toward HLA-DR-self peptides (HLA-DR-SPs) – compared to monocytes – which are consequently presented to autoreactive $CD4^+$ T cells. These T cells responded robustly to individual and pooled HLA-DR-SPs in MS patients, compared to healthy

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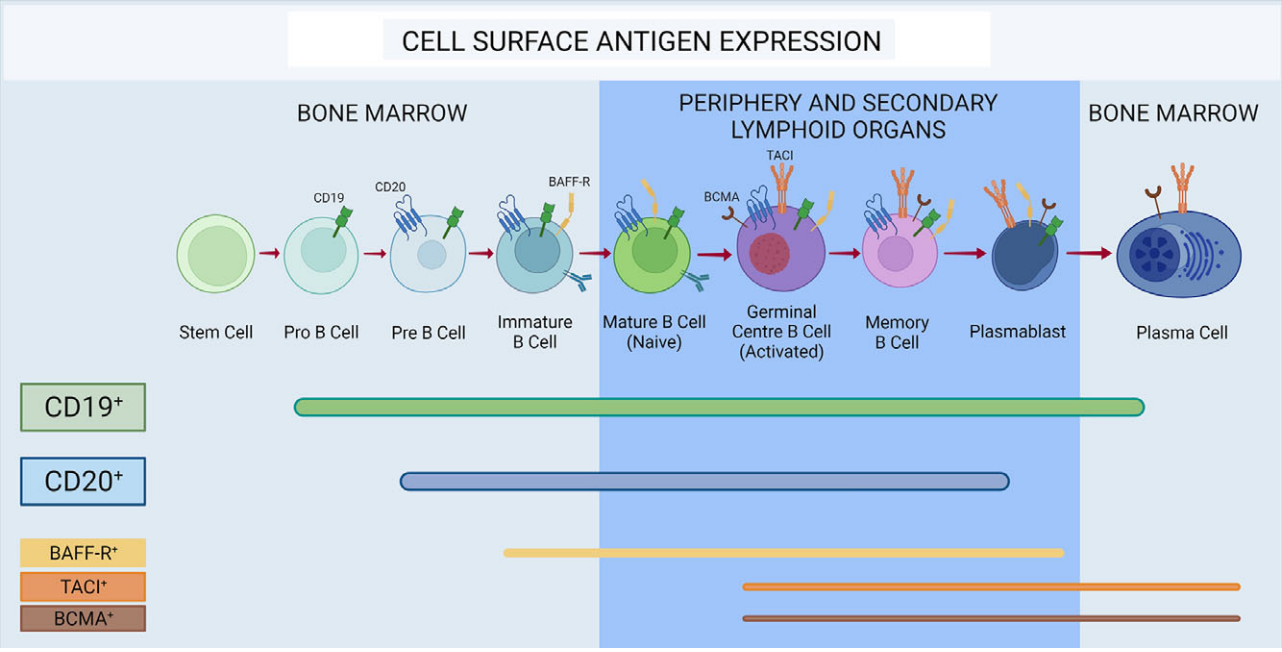

Figure 1: *Expression of cell surface antigens throughout B-cell maturation. CD19 is expressed in all stages of B-cell development, with the exception of stem cells and the majority of plasma cells. CD20 is not present on plasma cells, most plasmablasts, pro-B cells, and stem cells. BAFF-receptor (BAFF-R) is expressed on both immature and mature B cells in the germinal center, as well as memory B cells and late plasmablasts. Transmembrane activator and CAML interactor (TACI) and B-cell maturation antigen (BCMA) are expressed on germinal center B cells, memory cells, and antibody-secreting cells.*

donors, suggesting that DR2a and DR2b could jointly shape an autoreactive T-cell repertoire in MS.[41]

B Cells as a Source of Cytokines

Physiologically, B cells can be a source of both proinflammatory and anti-inflammatory (regulatory) cytokines. B cells of RRMS patients however feature a profile that is skewed towards an abnormally hyperactive proinflammatory response. In mice, high levels of B-cell-secreted IL-6 can foster the differentiation of Th17 cells, while preventing the generation of T regulatory cells.[42–44] In MS patients, B-cell production of lymphotoxin alpha (LT-α), TNF-α, and granulocyte macrophage-colony stimulating factor (GM-CSF) appears elevated, forging a chronically inflammatory milieu within the CNS.[19,25,45] At the same time, anti-inflammatory, regulatory cytokines that are produced by B cells, such as IL-35,[46] but also TGF-β1 and IL-10, are instrumental in controlling inflammation in experimental models of MS.[47]

EBV in MS

Among the infectious factors examined, the B-lymphotropic EBV has been shown to confer increased risk of developing MS, via, as yet, unclear mechanisms.[48] Ninety-six percent of the general population is positive for IgG antibodies against EBV (indicating past infection), while in MS patients this percentage is almost 100%.[26] Moreover, a prospective cohort study of 955 incident MS patients showed a 97% of EBV (but not other viruses) seroconversion before development of the disease, significantly higher compared to controls.[49] Studies and experiments have shaped four major theories about EBV's role in the pathogenesis of MS: the cross-reactivity hypothesis,[50] the bystander damage hypothesis,[51] the αβ-crystallin hypothesis,[52] and the EBV-infected autoreactive B-cell hypothesis.[53] Cellular and CSF findings however only partially match the pathophysiology of MS as far as the first three hypotheses are concerned. One important finding is the recent demonstration of molecular mimicry between EBV transcription factor EBNA1 and CNS protein glial cell adhesion molecule (GlialCAM), leading to the production of cross-reactive antibodies with higher affinity towards an intracellular GlialCAM epitope.[54] The fourth hypothesis postulates that EBV-infected autoreactive B cells accumulate in the target organ and orchestrate the disease by producing antibodies and stimulating T cells due to a defect in their elimination by the antiviral $CD8^+$ T cells. Moreover, the EBV anti-apoptotic protein BHRF1, produced by both latently and lytically infected cells, inhibits B-cell apoptosis,[55] resulting in immortalization of the autoreactive B cells it infects. In support of this hypothesis, substantial EBV persistence in B and plasma cells as well as meningeal B-cell lymphoid follicles of all MS cases examined was reported,[56] but could not be reproduced in multiple independent replication studies.[57–60] Overall, the epidemiological associations remain; however, no underlying biological mechanism has been consequently supported by experimental data.

Anti-B-cell Agents as a Therapeutic Strategy

Most anti-B-cell agents are monoclonal antibodies (mAbs); however, small molecules have also emerged as promising agents and have better CNS penetrance (Table 1). B-cell-depleting antibodies can be categorized in 1st-, 2nd-, or 3rd-generation. 1st-generation monoclonal antibodies (mAbs) can be either fully murine (suffix: -omab) or chimeric (65% human, suffix: -ximab), while 2nd-generation ones can be humanized (>90% human, suffix: -zumab) or even fully human (suffix: -mumab). 3rd-generation mAbs consist of a modified Fc region, chimeric, or humanized. Immunogenicity in theory ranges from higher in 1st-generation mAbs to lower in 2nd- and 3rd-generation ones.[61]

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Table 1: A summary of medicines targeting B cells that have been used in MS

Drug	Type	Target molecule	Clinical trials	Pregnancy	Dosing
Rituximab	Chimeric IgG1	Large extracellular loop of CD20 molecule	Phase 1; Phase 2 (Hermes); Phase 3 (Olympus). *Not FDA-approved*	Last infusion 3.5 months prior to conception	Intravenous dose of 2 × 1000 mg in a 2-week period
Ocrelizumab	Humanized glycosylated IgG1	Large extracellular loop of CD20 molecule	Phase 2; Phase 3 (OPERA I & II, ORATORIO). *FDA-approved*	Last infusion 6 months prior to conception	Intravenous doses of 600 mg twice a year
Ofatumumab	Fully human IgG1	Large & small extracellular loop of CD20 molecule	Phase 2b (MIRROR); Phase 3 (ASCLEPIOS I & II). *FDA-approved*	N/A	20 mg/0.4 mL once per week for the first 3 weeks, once a month thereafter
Ublituximab	Glycoengineered chimeric IgG1	Large extracellular loop of CD20 molecule	Phase 2 (NEDA); Phase 3 (ULTIMATE I & II). *Not yet FDA-approved*	N/A	N/A
MEDI-551	Glycoengineered, humanized, fucozylated IgG1κ	CD19	Phase 1; Phase 2/3 (N-MOmmentum). *FDA-approved*	N/A	Intravenous infusions of 300 mg every 6 months.
Atacicept	Human recombinant fusion protein (Fc IgG + TACI)	BAFF-APRIL	Phase 2 (ATAMS & ANOS). *Failed*	N/A	–
Evobrutinib	Small molecule drug	BTK	Phase 2; Phase 3. Not FDA-approved	N/A	75 mg dose daily

Anti-CD20 mAbs

CD20 is a 33-37kDa transmembrane protein, which spans the membrane four times, thus consisting of two extracellular loops and intracellular C- and N-termini. Although some T cells with CD20 surface expression can be found in all lymphatic organs, are often CD8-positive, can be myelin-specific,[62–64] and may correlate positively with disease severity,[65] CD20 serves a more important role on B cells. The molecule is not expressed throughout the entirety of the B-cell line of differentiation, but only in pre-B cells and mature B cells, with stem cells and the majority of antibody-secreting cells being CD20-negative (Figure 1).[66–68] Physiologically, CD20 plays a key role as regulator of calcium influx in the signaling pathways that lead to B-cell differentiation into antibody-secreting plasma cells[69] and its presence on the surface of most, but not all, B cells makes it an attractive target for monoclonal antibody-based therapy. B cells targeted by anti-CD20 monoclonal antibodies are eliminated via three main mechanisms: programmed cell death / apoptosis, complement-dependent cytotoxicity (CDC), or antibody-dependent cellular cytotoxicity (ADCC) processes.[70] Evidence from animal studies shows that anti-CD20 antibody-mediated B-cell depletion may be incomplete in lymph node germinal centers.[71] Moreover, cerebrospinal fluid B cells seem to be less affected than peripheral B cells by intravenous rituximab (the first anti-CD20 monoclonal antibody) administration, although the drug itself can be detected in a very low concentration (up to 1000 times lower than in the periphery) behind the BBB.[72–75] Of note, the limited access of anti-CD20 mAbs to the CNS due to their relatively high molecular weight could, at least to some extent, be overcome with intrathecal (IT) administration of anti-CD20 mAbs. The four main antibodies evaluated for anti-CD20 MS therapy are analyzed below.

Rituximab

Rituximab is a 1st-generation chimeric monoclonal antibody (IgG1), engineered by fusing a murine Fab with a human Fc domain.[61] Its elimination half-time is estimated at around 20 days;[76] it may, however, vary according to sex, body weight, and renal function.[77] Rituximab depletes B cells via ADCC and CDC and has been found to be extremely effective in patients with RRMS. A landmark 48-week, phase 2, double-blind, placebo-controlled study convincingly highlighted the efficacy of rituximab monotherapy in reducing gadolinium-enhanced lesions in patients with RRMS (n = 104).[78] In addition, a retrospective observational study of 808 patients with RRMS revealed absence of rebound disease activity upon rituximab cessation,[79] whereas rebound activity has been reported with natalizumab[80] and fingolimod cessation.[81,82]

In regard to progressive forms, a phase 3 (n = 439 PPMS patients), double-blind and placebo-controlled trial concluded in 2009 that CD20+ B-cell depletion can slow disease progression in a subgroup of younger patients with PPMS, particularly those with inflammatory lesions (GdELs), as rituximab-treated patients had less increase in T2 lesions and confirmed disease progression was delayed in the subgroup with GdELs.[83] Overall, however, the study was negative. Moreover, a large observational, retrospective study from the Swedish MS registry included 822 patients (557 RRMS, 198 SPMS, and 67 PPMS) and confirmed both rituximab's safety as well as its efficacy in reducing GdELs; GdELs went from 26.2% (pretreatment) to 4.6% in the pooled post-treatment cohort.[84] Interestingly, disability remained constant in RRMS patients but increased in SPMS and more so in PPMS patients. The question of whether disability progression differs in treated and untreated patients was tackled by a retrospective cohort study of 88 SPMS patients. This study resulted in significantly lower Expanded Disability Status Scale scores ($p < 0.001$) and delayed disease progression ($p = 0.02$) in the rituximab-treated group in comparison to the matched control group.[85] It should be noted however that the rituximab-treated group included more patients with radiologic activity, which may have driven the difference between the two groups.

In clinical practice, rituximab is widely used as an off-label treatment for the management of RRMS, as well as active SPMS, as its safety profile is acceptable, well-characterized,[86,87] and the efficacy evident, despite the lack of phase III trials.[88] The drug is

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generally well-tolerated by patients all throughout the MS type spectrum, and the main adverse effects are mild to moderate infusion-related reactions (IRRs), typically with the first dose, as well as mild to moderate infections. No cases of progressive multifocal leukoencephalopathy (PML) due to John Cunningham virus, which is mostly seen with natalizumab treatment,[89,90] have been recorded in MS patients treated with rituximab, and the frequency of PML in non-neurologic patients treated with rituximab seems to range around 1:4000; however, usually these patients have received multiple immunosuppressants.[91,92] Finally, an added advantage of rituximab is its relatively low cost (biosimilars are also available); however, its off-label prescription is complex and time-consuming for physicians. While open questions remain about optimal dosing and frequency strategies, a common tactic is 2 × 500 or 1000 mg, in a 14-day period, and repeat dosing of 500–1000 mg every 6 months or yearly.[61]

Ocrelizumab

Ocrelizumab, an IgG1 immunoglobulin, is a 2nd-generation recombinant humanized anti-CD20 mAb.[93] The drug has a terminal elimination half-time of around 26 days, which is not affected by mild renal or hepatic impairment.[94] Compared to rituximab, ocrelizumab mobilizes *in vitro* lower CDC, but higher ADCC action[95] and as a humanized molecule is expected to be less immunogenic than rituximab with lower titles of neutralizing anti-drug antibodies.[96,97]

In OPERA I (n = 821 patients) and OPERA II (n = 835 patients), two phase 3, double-blind trials published in 2017, ocrelizumab was associated with a lower annualized relapse rate (by 46–47%) and an impressive reduction of the mean number of GdELs (by 94%) over a 96-week time period compared to interferon beta-1a ($p < 0.001$). The drug effectively depleted CD19 B cells (CD19 B cells serve as index of B-cell count in anti-CD20 treatment) within 2 weeks (which is when CD19 cells were measured).[98] ORATORIO, a phase 3, double-blind, placebo-controlled trial, examined ocrelizumab's efficacy in managing PPMS progression. Results from 732 patients revealed that ocrelizumab was associated with lower rates of clinical and MRI progression than placebo. Because in this study the effect was driven by a fraction of PPMS that had evident MRI inflammation, EMA has approved the drug only in inflammatory PPMS, whereas other agencies such as the FDA and Swissmedic have not applied this restriction.[99]

The most common adverse effects of ocrelizumab include mild and manageable IRRs, like pruritus, rashes, throat irritations, and flushing, but their severity and frequency decrease with the number of infusions. Generally, mild to moderate infections occur in 30% of patients, but severe ones are relatively rare. Other adverse events such as extremity pain, diarrhea, and peripheral edema may also occur in rare cases.[100,101]

Ocrelizumab is administered intravenously according to a fixed dosing schedule, as approved based on the phase 3 studies. An initial dose of 600 mg is divided in 2 × 300 mg with a 2-week time interval. Subsequent doses of 600 mg are given in a single infusion once every 6 months.[94] Interestingly, a post hoc analysis from ORATORIO, where patients with lower body weight (and respectively higher ocrelizumab dose per kg) suffered less progression of deficits, prompted a currently ongoing clinical trial that examines the safety and efficacy of higher than standard ocrelizumab doses (1200 mg for body weights <75 kg, or 1800 mg for body weights >75 kg) in PPMS.[102,103]

Ofatumumab

Ofatumumab is a 2nd-generation, fully human IgG1 mAb[104] that depletes circulating CD20 B cells via ADCC[105] and CDC.[106] Two identically designed, double-blind, phase 3 clinical trials, ASCLEPIOS I and II, compared the efficacy of subcutaneously administered ofatumumab to that of oral teriflunomide, the oral pyrimidine synthesis inhibitor. The trials enrolled 1882 patients in total in 1.6 years, and their results indicated a statistically significant advantage of ofatumumab over teriflunomide in suppressing both new relapses and GdEL activity (the latter by 94–97%). Side effects were reported to be mild to moderate and included injection-related reactions, headache, and infections (in 51.6% of patients treated with ofatumumab) such as nasopharyngitis, upper respiratory, and urinary tract infection.[107] Consequently, the FDA approved ofatumumab as a therapy for RRMS, CIS, and active SPMS in the form of an auto-injector pen, while the EMA for relapsing, active MS.[106] Ofatumumab was approved for subcutaneous use at a dose of 20 mg/0.4 mL once per week for the first 3 weeks of treatment and once monthly thereafter.[108]

Ublituximab

Ublituximab is a 3rd-generation anti-CD20 glycoengineered chimeric IgG1 mAb that exerts its action primarily via ADCC, which is facilitated by defucosylation of its Fc region and thereby increased affinity for FcγRIIIa.[61] A 48-week, placebo-controlled, phase 2 trial of ublituximab in 45 RRMS patients established that 150 mg iv on day 1 and 450–600 mg on day 15 and week 24 were able to efficiently deplete B cells within 4 weeks (which is when B cells were measured); moreover, 74% of patients achieved no evidence of disease activity status (NEDA), that is had no relapses, no radiological disease activity, and no progression of disability. Similarly to the other CD20 agents, adverse effects comprised mild to moderate IRRs and upper respiratory infections, influenza, nasopharyngitis, sinusitis, and fungal infections.[109] In follow-up, two double-blind, phase 3 trials [ULTIMATE I (NCT03277261) and II (NCT03277248)] will assess ublituximab's efficacy and safety compared to teriflunomide in 880 patients with RRMS.[110,111]

Anti-CD19 mAbs

CD19 belongs to the Ig superfamily and along with CD21, CD82, and CD225 contributes to the formation of a multimolecular signal-transduction complex that ultimately leads to the activation of PI-3 kinase.[112] Compared to CD20, CD19 is expressed on B cells of earlier developmental stages as well as in more antibody-secreting cells and is thus an appealing therapeutic target (Figure 1).[113] In addition to having a broader expression during B-cell stages of development and differentiation, CD19, unlike CD20, is selectively expressed on B cells and not T cells.[62] A phase 1 study assessing the pharmacokinetic (intravenous and subcutaneous) profile of inebilizumab, a humanized afucosylated IgG1κ anti-CD19 mAb,[114] has been conducted in patients with relapsing MS with positive results,[115] but no phase III trials for MS are currently known to be underway.

Atacicept

Atacicept is a human recombinant fusion protein, consisting of a human IgG Fc portion and the extracellular domain of TACI receptor that binds both BAFF and a proliferation-inducing ligand (APRIL).[116] The drug therefore competes for BAFF and APRIL binding with native TACI, which is both membrane-bound and soluble,[117] as well as, to a lesser extent, with the other receptors

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of the BAFF-APRIL system (BAFF-R and BCMA).[118,119] After improving rheumatoid arthritis and systemic lupus erythematosus (SLE),[120] atacicept was tried in MS.

Subcutaneous atacicept was evaluated in a 36-week, phase 2, double-blind, and placebo-controlled trial in 255 patients with relapsing MS. The trial was prematurely terminated when an increase in inflammatory disease activity was noticed despite immunoglobulin and naïve B-cell decrease, which led to the suspension of every atacicept trial in MS.[119,121,122] Another 36-week, phase 2, double-blind, and placebo-controlled atacicept trial in 34 patients with unilateral optic neuritis as clinical isolated syndrome also showed disease exacerbation, with a significantly higher proportion of patients converting to clinically definite MS compared with placebo.[123] As atacicept effectively depletes naive B cells and induces a transient but marked increase in memory B cells (especially class-switched ones),[117,124,125] possible reasons why atacicept aggravated MS include elimination of regulatory naïve B cells and enhancement of pathogenic memory B-cell function.[126,127]

Belimumab

Belimumab is a human IgG1λ recombinant monoclonal antibody directed against BAFF that prevents BAFF from interacting with its three receptors on the surface of B cells, thereby reducing B-cell survival, differentiation, and antibody production.[128,129] Interestingly, belimumab administration does not result in overt immunosuppression.[130] While being moderately effective and FDA-approved for the treatment of SLE since 2011, it failed in myasthenia gravis,[131] a disease mediated by pathogenic autoantibodies.[132] A phase 2, open-label trial of subcutaneous belimumab in addition to ocrelizumab (standard dose) in 40 patients with RRMS was scheduled to start within 2021.[130]

Evobrutinib

Evobrutinib is a small molecule drug that binds permanently to and deactivates Bruton's Tyrosine Kinase (BTK). BTK is an integral part of the BCR signaling cascade that affects B-cell activation and is essential for B-cell maturation and their ultimate, terminal differentiation into memory or plasma cells. Of interest, BTK is involved in the entry of B cells into follicular structures. Knockout or absence of BTK results in lack of B-cell activation, moreover almost complete lack of peripheral B and plasma cells and low circulating immunoglobulin.[133–136] Importantly, about 75% of the CNS cells that express BTK are microglial, while BTK expression levels in the brain increase after demyelination.[137] As evobrutinib can bypass the BBB and enter the CNS, it can affect microglial cells and B cells within the CNS.

Evobrutinib was the first BTK inhibitor (BTKI) to be tested as a monotherapy in relapsing MS.[138] In a double-blind, phase II trial ($n = 267$), evobrutinib was tested against placebo and dimethyl fumarate. The results showed that patients who received 75 mg of daily evobrutinib had significantly fewer GdELs during weeks 12 through 24 than those who received placebo (1.69 ± 4.69 against 3.85 ± 5.44, $p = 0.005$), while adverse effects were minimal (e.g. nasopharyngitis, alanine aminotransferase, and aspartate aminotransferase level elevation).[139,140] Evobrutinib is now being advanced to phase III evaluation, along with several other BTKI (some of them with reversible BTK binding); fenebrutinib, ibrutinib, and tolebrutinib.[141]

While CD19/20 B-cell depletion has shown tremendous efficacy in reducing clinical and radiological MS activity, it raises several safety concerns on humoral deficiency with long-term usage in addition to a reduced response to vaccination.[86,142,143] These disadvantages could possibly be avoided with inhibition of B-cell activation and maturation with small molecules such as BTKIs.[136,144] In contrast with antibody-based B-cell depletion, BTKIs do not destroy or lastingly minimize the frequency of peripheral B cells, but seem to prevent the development of pathogenic B cells.[145] Their effect on disease activity does not seem to be as impressive as that of anti-B-cell antibodies, and they cannot control the pathogenic properties of B cells as rapidly; however, they are smaller in size, can penetrate the CNS, target microglia, and might therefore have a better effect on disability progression.[146]

B-Cell-Targeted Therapies and Pregnancy

As MS largely affects female patients with childbearing potential, the utilization of B-cell-targeted therapies in women of childbearing age deserves special mention.[147,148] Rituximab-associated B-cell depletion persists long after the drug's elimination, which occurs approximately 3 months after the last infusion. Thus, conception can be considered safe 3 months after the last infusion without significant risk of fetal exposure. But even if a woman conceives before rituximab's effective elimination, IgG1 subclass mAbs cannot cross the placenta barrier during the first trimester, resulting in low chance of fetal exposure.[149] Importantly, rituximab administration and concurrent B-cell depletion have not been linked to increased risk of adverse pregnancy outcomes compared with the expected incidence in population.[150] Also, infants breastfed under anti-CD20 treatment had normal B-cell counts, and no negative impact on health and development was attributed to breastfeeding in the 1-year follow-up period.[151] Although data regarding ocrelizumab administration in this population group are limited, it is reasonable to apply the same principles as with rituximab. One additional advantage of CD20 depletion in terms of family planning is that discontinuation of therapy is not associated with a rebound phenomenon, as has been observed with natalizumab. In that regard, a cohort study regarding the safety of anti-C20 mAbs rituximab and ocrelizumab during the last 12 months before or during pregnancy concluded that the drugs are effective in controlling disease in women with RRMS, during and partly after pregnancy. However, B-cell monitoring is essential both for the newborn and for the mother after delivery, and larger studies are required to assess their safety profile and to establish the best time to restart the therapy after delivery.[152] Recent recommendations suggest prioritization of MS management and conception postponement in cases of highly active MS and contraception for up to 4 months after ocrelizumab administration.[153]

Conclusion

The therapeutic criterion underlines that B cells not only participate in the pathogenesis of MS but can act as the orchestrators of the inflammatory processes. As shown by clinical trials and real-world data, B-cell-targeting agents (in particular CD20-depleting agents) have established a new era in MS therapeutics and immunotherapy in general, considering their remarkable efficacy and safety profile. Long-term safety, especially increased risk of infection with slowly but gradually decreasing total serum immunoglobulin levels, remains a significant concern that has a limiting effect on anti-CD20 usage in clinical practice. Regular monitoring of immunoglobulin levels (e.g. before each follow-up infusion) can help timely detection of a decrease and lowers the risk of infection due to associated immunodeficiency.[154–156]

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Future studies will further inform on long-term effects of CD20-targeting medications, on the use of oral BTKI agents and determine the new therapeutic algorithm that will likely move more towards induction rather than escalation.

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References

1. Yeshokumar AK, Narula S, Banwell B. Pediatric multiple sclerosis. Curr Opin Neurol. 2017;30:216–21. DOI 10.1097/WCO.0000000000000452.
2. Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. Lancet. 2018;391:1622–36. DOI 10.1016/S0140-6736(18)30481-1.
3. Filippi M, Bar-Or A, Piehl F, et al. Multiple sclerosis. Nat Rev Dis Primers. 2018;4:43. DOI 10.1038/s41572-018-0041-4.
4. Lublin FD. New multiple sclerosis phenotypic classification. Eur Neurol. 2014;72 Suppl 1:1–5. DOI 10.1159/000367614.
5. International Multiple Sclerosis Genetics Consortium. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. Science (1979). 2019;365:eaav7188. DOI 10.1126/science.aav7188.
6. O'Connor KC, Bar-Or A, Hafler DA. The neuroimmunology of multiple sclerosis: possible roles of T and B lymphocytes in immunopathogenesis. J Clin Immunol. 2001;21:81–92. DOI 10.1023/a:1011064007686.
7. De Jager PL, Jia X, Wang J, et al. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet. 2009;41:776–82. DOI 10.1038/ng.401.
8. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat Rev Neurol. 2017;13:25–36. DOI 10.1038/nrneurol.2016.187.
9. Cotsapas C, Mitrovic M. Genome-wide association studies of multiple sclerosis. Clin Transl Immunol. 2018;7:e1018. DOI 10.1002/cti2.1018.
10. Beecham AH, Patsopoulos NA, Xifara DK, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet. 2013;45:1353–60. DOI 10.1038/ng.2770.
11. Mokry LE, Ross S, Ahmad OS, et al. Vitamin D and risk of multiple sclerosis: a mendelian randomization study. PLoS Med. 2015;12:e1001866. DOI 10.1371/journal.pmed.1001866.
12. Ota K, Matsui M, Milford EL, Mackin GA, Weiner HL, Hafler DA. T-Cell recognition of an immuno-dominant myelin basic protein epitope in multiple sclerosis. Nature. 1990;346:183–7. DOI 10.1038/346183a0.
13. Bjartmar C, Yin X, Trapp BD. Axonal pathology in myelin disorders. J Neurocytol. 1999;28:383–95.
14. Bonnan M. Intrathecal IgG synthesis: a resistant and valuable target for future multiple sclerosis treatments. Mult Scler Int. 2015;2015:296184. DOI 10.1155/2015/296184.
15. Cepok S, Rosche B, Grummel V, et al. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. Brain. 2005;128:1667–76. DOI 10.1093/brain/awh486.
16. Kowarik MC, Cepok S, Sellner J, et al. CXCL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation. J Neuroinflammation. 2012;9:93.
17. Krumbholz M, Derfuss T, Hohlfeld R, Meinl E. B cells and antibodies in multiple sclerosis pathogenesis and therapy. Nat Rev Neurol. 2012;8:613–23. DOI 10.1038/nrneurol.2012.203.
18. Kivisäkk P, Imitola J, Rasmussen S, et al. Localizing central nervous system immune surveillance: meningeal antigen-presenting cells activate T cells during experimental autoimmune encephalomyelitis. Ann Neurol. 2009;65:457–69. DOI 10.1002/ana.21379.
19. Moreno Torres I, García-Merino A. Anti-CD20 monoclonal antibodies in multiple sclerosis. Expert Rev Neurother. 2017;17:359–71. DOI 10.1080/14737175.2017.1245616.
20. Lassmann H. Pathogenic mechanisms associated with different clinical courses of multiple sclerosis. Front Immunol. 2018;9:3116. DOI 10.3389/fimmu.2018.03116.
21. Gh Popescu BF, Pirko I, Lucchinetti CF. Pathology of multiple sclerosis: where do we stand? Continuum (Minneap Minn). 2013;19:901–21.
22. Barnett MH, Parratt JDE, Cho ES, Prineas JW. Immunoglobulins and complement in postmortem multiple sclerosis tissue. Ann Neurol. 2009;65:32–46. DOI 10.1002/ana.21524.
23. Keegan M, König F, McClelland R, et al. Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. Lancet. 2005;366:579–82. DOI 10.1016/S0140-6736(05)67102-4.
24. Gasperi C, Stüve O, Hemmer B. B cell-directed therapies in multiple sclerosis. Neurodegener Dis Manag. 2016;6:37–47. DOI 10.2217/nmt.15.67.
25. Häusser-Kinzel S, Weber MS. The role of B cells and antibodies in multiple sclerosis, neuromyelitis optica, and related disorders. Front Immunol. 2019;10:201. DOI 10.3389/fimmu.2019.00201.
26. Guan Y, Jakimovski D, Ramanathan M, Weinstock-Guttman B, Zivadinov R. The role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to in vivo imaging. Neural Regen Res. 2019;14:373–86.
27. Abdelhak A, Hottenrott T, Mayer C, et al. CSF profile in primary progressive multiple sclerosis: re-exploring the basics. PLOS ONE. 2017;12:e0182647.
28. Thouvenot E. Multiple sclerosis biomarkers: helping the diagnosis? Rev Neurol (Paris). 2018;174:364–71. DOI 10.1016/j.neurol.2018.04.002.
29. Brändle SM, Obermeier B, Senel M, et al. Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins. Proc Natl Acad Sci U S A. 2016;113:7864–9. DOI 10.1073/pnas.1522730113.
30. Willis SN, Stathopoulos P, Chastre A, Compton SD, Hafler DA, O'Connor KC. Investigating the antigen specificity of multiple sclerosis central nervous system-derived immunoglobulins. Front Immunol. 2015;6:600. DOI 10.3389/fimmu.2015.00600.
31. Villar LM, Masjuan J, González-Porqué P, et al. Intrathecal IgM synthesis predicts the onset of new relapses and a worse disease course in MS. Neurology. 2002;59:555–9. DOI 10.1212/wnl.59.4.555.
32. Boronat A, Sepúlveda M, Llufriu S, et al. Analysis of antibodies to surface epitopes of contactin-2 in multiple sclerosis. J Neuroimmunol. 2012;244:103–6. DOI 10.1016/j.jneuroim.2011.12.023.
33. Gerhards R, Pfeffer LK, Lorenz J, et al. Oligodendrocyte myelin glycoprotein as a novel target for pathogenic autoimmunity in the CNS. Acta Neuropathol Commun. 2020;8:207. DOI 10.1186/s40478-020-01086-2.
34. Yeste A, Quintana FJ. Antigen microarrays for the study of autoimmune diseases. Clin Chem. 2013;59:1036–44. DOI 10.1373/clinchem.2012.194423.
35. Kanter JL, Narayana S, Ho PP, et al. Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat Med. 2006;12:138–43. DOI 10.1038/nm1344.
36. Trotter J, DeJong LJ, Smith ME. Opsonization with antimyelin antibody increases the uptake and intracellular metabolism of myelin in inflammatory macrophages. J Neurochem. 1986;47:779–89. DOI 10.1111/j.1471-4159.1986.tb00679.x.
37. Flach AC, Litke T, Strauss J, et al. Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease. Proc Natl Acad Sci U S A. 2016;113:3323–8. DOI 10.1073/pnas.1519608113.
38. Getahun A, Dahlström J, Wernersson S, Heyman B. IgG2a-mediated enhancement of antibody and T cell responses and its relation to inhibitory and activating Fc gamma receptors. J Immunol. 2004;172:5269–76. DOI 10.4049/jimmunol.172.9.5269.

39. Kinzel S, Weber MS. B cell-directed therapeutics in multiple sclerosis: rationale and clinical evidence. CNS Drugs. 2016;30:1137–48. DOI 10.1007/s40263-016-0396-6.
40. Ancau M, Berthele A, Hemmer B. CD20 monoclonal antibodies for the treatment of multiple sclerosis: up-to-date. Expert Opin Biol Ther. 2019;19:829–43. DOI 10.1080/14712598.2019.1611778.
41. Wang J, Jelcic I, Mühlenbruch L, et al. HLA-DR15 molecules jointly shape an autoreactive T cell repertoire in multiple sclerosis. Cell. 2020;183:1264–1281.e20. DOI 10.1016/j.cell.2020.09.054.
42. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441:235–8. DOI 10.1038/nature04753.
43. Molnarfi N, Schulze-Topphoff U, Weber MS, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. J Exp Med. 2013;210:2921–37. DOI 10.1084/jem.20130699.
44. Korn T, Mitsdoerffer M, Croxford AL, et al. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. Proc Natl Acad Sci U S A. 2008;105:18460–5. DOI 10.1073/pnas.0809850105.
45. Li R, Rezk A, Miyazaki Y, et al. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Sci Transl Med. 2015;7:310ra166.
46. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature. 2014;507:366–70. DOI 10.1038/nature12979.
47. Arneth BM. Impact of B cells to the pathophysiology of multiple sclerosis. J Neuroinflammation. 2019;16:128. DOI 10.1186/s12974-019-1517-1.
48. Morandi E, Jagessar SA, 't Hart BA, Gran B. EBV infection empowers human B cells for autoimmunity: role of autophagy and relevance to multiple sclerosis. J Immunol. 2017;199:435–48. DOI 10.4049/jimmunol.1700178.
49. Kjetil B, Marianna C, H.B. C, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science (1979). 2022;375:296–301. DOI 10.1126/science.abj8222.
50. Lang HLE, Jacobsen H, Ikemizu S, et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol. 2002;3:940–3. DOI 10.1038/ni835.
51. Angelini DF, Serafini B, Piras E, et al. Increased CD8+ T cell response to Epstein-Barr virus lytic antigens in the active phase of multiple sclerosis. PLoS Pathog. 2013;9:e1003220.
52. Pender MP, Burrows SR. Epstein-Barr virus and multiple sclerosis: potential opportunities for immunotherapy. Clin Transl Immunol. 2014;3:e27.
53. Pender MP. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. Trends Immunol. 2003;24:584–8. DOI 10.1016/j.it.2003.09.005.
54. Lanz Tv, Brewer RC, Ho PP, et al. Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. Nature. 2022;603:321–7. DOI 10.1038/s41586-022-04432-7.
55. McCarthy NJ, Hazlewood SA, Huen DS, Rickinson AB, Williams GT. The Epstein-Barr virus gene BHRF1, a homologue of the cellular oncogene Bcl-2, inhibits apoptosis induced by gamma radiation and chemotherapeutic drugs. Adv Exp Med Biol. 1996;406:83–97. DOI 10.1007/978-1-4899-0274-0_9.
56. Serafini B, Rosicarelli B, Franciotta D, et al. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med. 2007;204:2899–912. DOI 10.1084/jem.20071030.
57. Sargsyan SA, Shearer AJ, Ritchie AM, et al. Absence of Epstein-Barr virus in the brain and CSF of patients with multiple sclerosis. Neurology. 2010;74:1127–35. DOI 10.1212/WNL.0b013e3181d865a1.
58. Peferoen LAN, Lamers F, Lodder LNR, et al. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. Brain. 2010;133:e137.
59. Willis SN, Stadelmann C, Rodig SJ, et al. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain. 2009;132:3318–28. DOI 10.1093/brain/awp200.
60. Torkildsen Ø., Stansberg C, Angelskår SM, et al. Upregulation of immunoglobulin-related genes in cortical sections from multiple sclerosis patients. Brain Pathol. 2010;20:720–9. DOI 10.1111/j.1750-3639.2009.00343.x.
61. Whittam DH, Tallantyre EC, Jolles S, et al. Rituximab in neurological disease: principles, evidence and practice. Pract Neurol. 2019;19:5. DOI 10.1136/practneurol-2018-001899.
62. Schuh E, Berer K, Mulazzani M, et al. Features of human CD3+CD20+ T cells. J Immunol. 2016;197:1111–7. DOI 10.4049/jimmunol.1600089.
63. Palanichamy A, Jahn S, Nickles D, et al. Rituximab efficiently depletes increased CD20-expressing T cells in multiple sclerosis patients. J Immunol. 2014;193:580–6. DOI 10.4049/jimmunol.1400118.
64. Sabatino JJ Jr, Wilson MR, Calabresi PA, Hauser SL, Schneck JP, Zamvil SS. Anti-CD20 therapy depletes activated myelin-specific CD8(+) T cells in multiple sclerosis. Proc Natl Acad Sci U S A. 2019;116:25800–7. DOI 10.1073/pnas.1915309116.
65. von Essen MR, Ammitzbøll C, Hansen RH, et al. Proinflammatory CD20+ T cells in the pathogenesis of multiple sclerosis. Brain. 2019;142:120–32. DOI 10.1093/brain/awy301.
66. Payandeh Z, Bahrami AA, Hoseinpoor R, et al. The applications of anti-CD20 antibodies to treat various B cells disorders. Biomed Pharmacother. 2019;109:2415–26. DOI 10.1016/j.biopha.2018.11.121.
67. Dalakas MC. B cells in the pathophysiology of autoimmune neurological disorders: a credible therapeutic target. Pharmacol Ther. 2006;112:57–70. DOI 10.1016/j.pharmthera.2006.03.005.
68. Tedder TF, Streuli M, Schlossman SF, Saito H. Isolation and structure of a CDNA encoding the B1 (CD20) cell-surface antigen of human B lymphocytes. Proc Natl Acad Sci U S A. 1988;85:208–12. DOI 10.1073/pnas.85.1.208.
69. Santos MAO, Lima MM. CD20 role in pathophysiology of Hodgkin's disease. Rev Assoc Med Bras (1992). 2017;63:810–3. DOI 10.1590/1806-9282.63.09.810.
70. Maloney DG. Anti-CD20 antibody therapy for B-cell lymphomas. N Engl J Med. 2012;366:2008–16. DOI 10.1056/NEJMct1114348.
71. Schroder C, Azimzadeh AM, Wu G, Price JO, Atkinson JB, Pierson RN. Anti-CD20 treatment depletes B-cells in blood and lymphatic tissue of cynomolgus monkeys. Transpl Immunol. 2003;12:19–28.
72. Piccio L, Naismith RT, Trinkaus K, et al. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. Arch Neurol. 2010;67:707–14. DOI 10.1001/archneurol.2010.99.
73. Ramwadhdoebe TH, van Baarsen LGM, Boumans MJH, et al. Effect of rituximab treatment on T and B cell subsets in lymph node biopsies of patients with rheumatoid arthritis. Rheumatology (Oxford), 58:1075–85. DOI 10.1093/rheumatology/key428.
74. Petereit HF, Rubbert-Roth A. Rituximab levels in cerebrospinal fluid of patients with neurological autoimmune disorders. Mult Scler J. 2008;15:189–92. DOI 10.1177/1352458508098268.
75. Monson NL, Cravens PD, Frohman EM, Hawker K, Racke MK. Effect of rituximab on the peripheral blood and cerebrospinal fluid B cells in patients with primary progressive multiple sclerosis. Arch Neurol. 2005;62:258–64. DOI 10.1001/archneur.62.2.258.
76. Bar-Or A, Calabresi PAJ, Arnold D, et al. Rituximab in relapsing-remitting multiple sclerosis: a 72-week, open-label, phase I trial. Ann Neurol. 2008;63:395–400. DOI 10.1002/ana.21363.
77. Ng CM, Bruno R, Combs D, Davies B. Population pharmacokinetics of rituximab (anti-CD20 monoclonal antibody) in rheumatoid arthritis patients during a phase II clinical trial. J Clin Pharmacol. 2005;45:792–801. DOI 10.1177/0091270005277075.
78. Hauser SL, Arnold DL, Vollmer T, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med. 2008;358:676–88.
79. Juto A, Fink K, al Nimer F, Piehl F. Interrupting rituximab treatment in relapsing-remitting multiple sclerosis; no evidence of rebound disease activity. Mult Scler Relat Dis. 2020;37:101468. DOI 10.1016/j.msard.2019.101468.
80. lo Re M, Capobianco M, Ragonese P, et al. Natalizumab discontinuation and treatment strategies in patients with multiple sclerosis (MS): a retrospective study from two Italian MS centers. Neurol Ther. 2015;4:147–57. DOI 10.1007/s40120-015-0038-9.

https://doi.org/10.1017/cjn.2022.60 Published online by Cambridge University Press

81. Hatcher SE, Waubant E, Nourbakhsh B, Crabtree-Hartman E, Graves JS. Rebound syndrome in patients with multiple sclerosis after cessation of fingolimod treatment. JAMA Neurol. 2016;73:790–4. DOI 10.1001/jamaneurol.2016.0826.
82. Sacco R, Emming S, Gobbi C, Zecca C, Monticelli S. Rebound of disease activity after fingolimod withdrawal: immunological and gene expression profiling. Mult Scler Relat Dis. 2020;40:101927. DOI 10.1016/j.msard.2020.101927.
83. Hawker K, O'Connor P, Freedman MS, et al. Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol. 2009;66:460–71. DOI 10.1002/ana.21867.
84. Salzer J, Svenningsson R, Alping P, et al. Rituximab in multiple sclerosis. Neurology. 2016;87:2074–81. DOI 10.1212/WNL.0000000000003331.
85. Naegelin Y, Naegelin P, von Felten S, et al. Association of rituximab treatment with disability progression among patients with secondary progressive multiple sclerosis. JAMA Neurol. 2019;76:274–81. DOI 10.1001/jamaneurol.2018.4239.
86. Luna G, Alping P, Burman J, et al. Infection risks among patients with multiple sclerosis treated with fingolimod, natalizumab, rituximab, and injectable therapies. JAMA Neurol. 2020;77:184–91. DOI 10.1001/jamaneurol.2019.3365.
87. Alping P, Askling J, Burman J, et al. Cancer risk for fingolimod, natalizumab, and rituximab in multiple sclerosis patients. Ann Neurol. 2020;87:688–99. DOI 10.1002/ana.25701.
88. Yamout BI, El-Ayoubi NK, Nicolas J, Kouzi Yel, Khoury SJ, Zeineddine MM. Safety and efficacy of rituximab in multiple sclerosis: a retrospective observational study. J Immunol Res. 2018;2018:1–9. DOI 10.1155/2018/9084759.
89. Ghajarzadeh M, Azimi A, Valizadeh Z, Sahraian MA, Mohammadifar M. Efficacy and safety of rituximab in treating patients with multiple sclerosis (MS): a systematic review and meta-analysis. Autoimmun Rev. 2020;19:102585. DOI 10.1016/j.autrev.2020.102585.
90. Erickson KD, Garcea RL. Viral replication centers and the DNA damage response in JC virus-infected cells. Virology. 2019;528:198–206. DOI 10.1016/j.virol.2018.12.014.
91. Clifford DB, Ances B, Costello C, et al. Rituximab-associated progressive multifocal leukoencephalopathy in rheumatoid arthritis. Arch Neurol. 2011;68:1156–64. DOI 10.1001/archneurol.2011.103.
92. Kapoor T, Mahadeshwar P, Hui-Yuen J, et al. Prevalence of progressive multifocal leukoencephalopathy (PML) in adults and children with systemic lupus erythematosus. Lupus Sci Med. 2020;7:e000388. DOI 10.1136/lupus-2020-000388.
93. Syed YY. Ocrelizumab: a review in multiple sclerosis. CNS Drugs. 2018;32:883–90.
94. Ocrevus | European Medicines Agency.Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/ocrevus; accessed December 1, 2021.
95. Klein C, Lammens A, Schäfer W, et al. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. MAbs. 2013;5:22–33. DOI 10.4161/mabs.22771.
96. Vugmeyster Y, Beyer J, Howell K, et al. Depletion of B cells by a humanized anti-CD20 antibody PRO70769 in macaca fascicularis. J Immunother. 2005;28:212–9. DOI 10.1097/01.cji.0000155050.03916.04.
97. Sorensen PS, Blinkenberg M. The potential role for ocrelizumab in the treatment of multiple sclerosis: current evidence and future prospects. Ther Adv Neurol Disord. 2016;9:44–52. DOI 10.1177/1756285615601933.
98. Hauser SL, Bar-Or A, Comi G, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. N Engl J Med. 2017;376:221–34. DOI 10.1056/NEJMoa1601277.
99. Montalban X, Hauser SL, Kappos L, et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. N Engl J Med. 2017;376:209–20. DOI 10.1056/NEJMoa1606468.
100. Rommer PS, Zettl UK. Managing the side effects of multiple sclerosis therapy: pharmacotherapy options for patients. Expert Opin Pharmacother. 2018;19:483–98.
101. Rommer PS, Dudesek A, Stüve O, Zettl UK. Monoclonal antibodies in treatment of multiple sclerosis. Clin Exp Immunol. 2014;175:373–84. DOI 10.1111/cei.12197.
102. Gibiansky E, Petry C, Mercier F, et al. Ocrelizumab in relapsing and primary progressive multiple sclerosis: pharmacokinetic and pharmacodynamic analyses of OPERA I, OPERA II and ORATORIO. Br J Clin Pharmacol. 2021;87:2511–20. DOI 10.1111/bcp.14658.
103. A study to evaluate the efficacy, safety and pharmacokinetics of a higher dose of ocrelizumab in adults with primary progressive multiple sclerosis (PPMS). ClinicalTrials. Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT04548999; accessed May 18, 2021.
104. Florou D, Katsara M, Feehan J, Dardiotis E, Apostolopoulos V. Anti-CD20 agents for multiple sclerosis: spotlight on ocrelizumab and ofatumumab. Brain Sci. 2020;10:758. DOI 10.3390/brainsci10100758.
105. Masoud S, McAdoo SP, Bedi R, Cairns TD, Lightstone L. Ofatumumab for B cell depletion in patients with systemic lupus erythematosus who are allergic to rituximab. Rheumatology (Oxford). 2018;57:1156–61. DOI 10.1093/rheumatology/key042.
106. Ofatumumab. Am J Health Syst Pharm. 2020;77:2025–8. DOI 10.1093/ajhp/zxaa322.
107. Hauser SL, Bar-Or A, Cohen JA, et al. Ofatumumab versus teriflunomide in multiple sclerosis. N Engl J Med. 2020;383:546–57. DOI 10.1056/NEJMoa1917246.
108. Kesimpta (Ofatumumab SC) dosing, indications, interactions, adverse effects, and more. Available at: https://reference.medscape.com/drug/kesimpta-ofatumumab-sc-4000083#0; accessed January 23, 2017.
109. Fox E, Lovett-Racke AE, Gormley M, et al. A phase 2 multicenter study of ublituximab, a novel glycoengineered anti-CD20 monoclonal antibody, in patients with relapsing forms of multiple sclerosis. Mult Scler. 2021;27:420–9. DOI 10.1177/1352458520918375.
110. Mealy MA, Levy M. A pilot safety study of ublituximab, a monoclonal antibody against CD20, in acute relapses of neuromyelitis optica spectrum disorder. Medicine. 2019;98:e15944. DOI 10.1097/MD.0000000000015944.
111. Study to Assess the Efficacy and Safety of Ublituximab in Participants With Relapsing Forms of Multiple Sclerosis (RMS) (ULTIMATE II) (NCT03277248). Available online: https://clinicaltrials.gov/ct2/show/NCT03277248
112. Tedder TF. CD19: a promising B cell target for rheumatoid arthritis. Nat Rev Rheumatol. 2009;5:572–7.
113. Chen D, Gallagher S, Monson NL, Herbst R, Wang Y. Inebilizumab, a B cell-depleting anti-CD19 antibody for the treatment of autoimmune neurological diseases: insights from preclinical studies. J Clin Med. 2016;5:107. DOI 10.3390/jcm5120107.
114. Herbst R, Wang Y, Gallagher S, et al. B-cell depletion in vitro and in vivo with an afucosylated anti-CD19 antibody. J Pharmacol Exp Ther. 2010;335:213–22. DOI 10.1124/jpet.110.168062.
115. Safety and tolerability study of MEDI-551, a B-cell depleting agent, to treat relapsing forms of multiple sclerosis.Available at: https://clinicaltrials.gov/ct2/show/NCT01585766
116. Magliozzi R, Marastoni D, Calabrese M. The BAFF/APRIL system as therapeutic target in multiple sclerosis. Expert Opin Ther Targets. 2020;24:1135–45. DOI 10.1080/14728222.2020.1821647.
117. Hoffmann FS, Kuhn P-H, Laurent SA, et al. The immunoregulator soluble TACI is released by ADAM10 and reflects B cell activation in autoimmunity. J Immunol. 2015;194:542–52. DOI 10.4049/jimmunol.1402070.
118. Benson MJ, Dillon SR, Castigli E, et al. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. J Immunol. 2008;180:3655–9. DOI 10.4049/jimmunol.180.6.3655.
119. Hartung H-P, Kieseier BC. Atacicept: targeting B cells in multiple sclerosis. Ther Adv Neurol Disord. 2010;3:205–16. DOI 10.1177/1756285610371146.
120. van Vollenhoven RF, Kinnman N, Vincent E, Wax S, Bathon J. Atacicept in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase II, randomized, placebo-controlled trial. Arthritis Rheum. 2011;63:1782–92. DOI 10.1002/art.30372.
121. Kappos L, Hartung H-P, Freedman MS, et al. Atacicept in Multiple Sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial. Lancet Neurol. 2014;13:353–63. DOI 10.1016/S1474-4422(14)70028-6.

https://doi.org/10.1017/cjn.2022.60 Published online by Cambridge University Press

122. A phase 2 study of atacicept in subjects with relapsing multiple sclerosis (ATAMS). Available at: https://clinicaltrials.gov/ct2/show/NCT00642902
123. Sergott RC, Bennett JL, Rieckmann P, et al. Results from a phase II randomized trial of the B-cell-targeting agent atacicept in patients with optic neuritis. J Neurol Sci. 2015;351:174–8. DOI 10.1016/j.jns.2015.02.019.
124. Lühder F, Gold R. Trial and error in clinical studies: lessons from ATAMS. Lancet Neurol. 2014;13:340–1. DOI 10.1016/S1474-4422(14)70050-X.
125. Jelcic I, al Nimer F, Wang J, et al. Autoreactive CD4+ T cells in multiple sclerosis. Cell. 2018;175:85–100.e23. DOI 10.1016/j.cell.2018.08.011.
126. Baker D, Pryce G, James LK, Schmierer K, Giovannoni G. Failed B cell survival factor trials support the importance of memory B cells in multiple sclerosis. Eur J Neurol. 2020;27:221–8.
127. Baker D, Marta M, Pryce G, Giovannoni G, Schmierer K. Memory B cells are major targets for effective immunotherapy in relapsing multiple sclerosis. EBioMedicine. 2017;16:41–50. DOI 10.1016/j.ebiom.2017.01.042.
128. Halpern WG, Lappin P, Zanardi T, et al. Chronic administration of belimumab, a BLyS antagonist, decreases tissue and peripheral blood B-lymphocyte populations in cynomolgus monkeys: pharmacokinetic, pharmacodynamic, and toxicologic effects. Toxicol Sci. 2006;91:586–99. DOI 10.1093/toxsci/kfj148.
129. Dubey AK, Handu SS, Dubey S, Sharma P, Sharma KK, Ahmed QM. Belimumab: first targeted biological treatment for systemic lupus erythematosus. J Pharmacol Pharmacother. 2011;2:317–9. DOI 10.4103/0976-500X.85930.
130. Addition of belimumab to B-cell depletion in relapsing-remitting multiple sclerosis. ClinicalTrials. Gov. Available at: https://clinicaltrials.gov/ct2/show/study/NCT04767698; accessed May 18, 2021.
131. Hewett K., Sanders D.B., Grove R.A., et al. Randomized study of adjunctive belimumab in participants with generalized myasthenia gravis. Neurology. 2018;90:e1425–e1434. DOI 10.1212/WNL.0000000000005323.
132. Stohl W, Hilbert DM. The discovery and development of belimumab: the anti-BLyS-lupus connection. Nat Biotechnol. 2012;30:69–77. DOI 10.1038/nbt.2076.
133. Dingjan GM, Middendorp S, Dahlenborg K, Maas A, Grosveld F, Hendriks RW. Bruton's tyrosine kinase regulates the activation of gene rearrangements at the lambda light chain locus in precursor B cells in the mouse. J Exp Med. 2001;193:1169–78. DOI 10.1084/jem.193.10.1169.
134. Middendorp S, Dingjan GM, Hendriks RW. Impaired precursor B cell differentiation in Bruton's tyrosine kinase-deficient mice. J Immunol. 2002;168:2695–703. DOI 10.4049/jimmunol.168.6.2695.
135. Nomura K, Kanegane H, Karasuyama H, et al. Genetic defect in human X-linked agammaglobulinemia impedes a maturational evolution of pro-B cells into a later stage of pre-B cells in the B-cell differentiation pathway. Blood. 2000;96:610–7.
136. Torke S, Weber MS. Inhibition of bruton's tyrosine kinase as a novel therapeutic approach in multiple sclerosis. Expert Opin Investig Drugs. 2020;29:1143–50. DOI 10.1080/13543784.2020.1807934.
137. Martin E, Aigrot M-S, Grenningloh R, et al. Bruton's tyrosine kinase inhibition promotes myelin repair. Adv Exp Med Biol. 2020;5:123–33. DOI 10.3233/bpl-200100.
138. Becker A, Martin EC, Mitchell DY, et al. Safety, Tolerability, Pharmacokinetics, Target Occupancy, and Concentration-QT Analysis of the Novel BTK Inhibitor Evobrutinib in Healthy Volunteers. Clin Transl Sci. 2020 Mar;13(2):325-336. DOI 10.1111/cts.12713
139. A study of efficacy and safety of M2951 in participants with relapsing multiple sclerosis. Available at: https://www.clinicaltrials.gov/ct2/show/NCT02975349?term=evobrutinib&cond=Multiple+Sclerosis&draw=2&rank=5
140. Montalban X, Arnold DL, Weber MS, et al. Placebo-controlled trial of an oral BTK inhibitor in multiple sclerosis. N Engl J Med. 2019;380:2406–17. DOI 10.1056/NEJMoa1901981.
141. Study of evobrutinib in participants with relapsing multiple sclerosis (RMS) (EvolutionRMS 2). Available at: https://www.clinicaltrials.gov/ct2/show/NCT04338061?term=evobrutinib&cond=Multiple+Sclerosis&draw=2&rank=1
142. Nazi I, Kelton JG, Larché M, et al. The effect of rituximab on vaccine responses in patients with immune thrombocytopenia. Blood. 2013;122:1946–53. DOI 10.1182/blood-2013-04-494096.
143. Bar-Or A, Calkwood JC, Chognot C, et al. Effect of ocrelizumab on vaccine responses in patients with multiple sclerosis. Neurology. 2020;95:e1999–e2008. DOI 10.1212/WNL.0000000000010380.
144. Dolgin E. BTK blockers make headway in multiple sclerosis. Nat Biotechnol. 2021;39:3–5. DOI 10.1038/s41587-020-00790-7.
145. Torke S, Pretzsch R, Häusler D, et al. Inhibition of Bruton's tyrosine kinase interferes with pathogenic B-cell development in inflammatory CNS demyelinating disease. Acta Neuropathol. 2020;140:535–48. DOI 10.1007/s00401-020-02204-z.
146. Boschert U, Crandall T, Pereira A, et al. T cell mediated experimental CNS autoimmunity induced by PLP in SJL mice is modulated by evobrutinib (M2951) a novel Bruton's tyrosine kinase inhibitor. Mult Scler J. 2017;23:327.
147. Tisovic K, Amezcua L. Women's health: contemporary management of MS in pregnancy and post-partum. Biomedicines. 2019;7:32. DOI 10.3390/biomedicines7020032.
148. Wallin MT, Culpepper WJ, Campbell JD, et al. The prevalence of MS in the United States: a population-based estimate using health claims data. Neurology. 2019;92:e1029–e1040. DOI 10.1212/WNL.0000000000007035.
149. Das G, Damotte V, Gelfand JM, et al. Rituximab before and during pregnancy: a systematic review, and a case series in MS and NMOSD. Neurol Neuroimmunol Neuroinflamm. 2018;5:e453. DOI 10.1212/NXI.0000000000000453.
150. Smith JB, Hellwig K, Fink K, Lyell DJ, Piehl F, Langer-Gould A. Rituximab, MS, and pregnancy. Neurol Neuroimmunol Neuroinflamm. 2020;7, 10.1212/NXI.0000000000000734.
151. Ciplea AI, Langer-Gould A, de Vries A, et al. Monoclonal antibody treatment during pregnancy and/or lactation in women with MS or neuromyelitis optica spectrum disorder. Neurol Neuroimmunol Neuroinflamm. 2020;7:e723. DOI 10.1212/NXI.0000000000000723.
152. Kümpfel T, Thiel S, Meinl I, et al. Anti-CD20 therapies and pregnancy in neuroimmunologic disorders: a cohort study from Germany. Neurol Neuroimmunol Neuroinflamm. 2021;8:e913. DOI 10.1212/NXI.0000000000000913.
153. Wiendl H, Gold R, Berger T, et al. Multiple Sclerosis Therapy Consensus Group (MSTCG): position statement on disease-modifying therapies for multiple sclerosis (white paper). Ther Adv Neurol Disord. 2021;14:175628642110396. DOI 10.1177/17562864211039648.
154. Keystone E, Fleischmann R, Emery P, et al. Safety and efficacy of additional courses of rituximab in patients with active rheumatoid arthritis: an open-label extension analysis. Arthritis Rheum. 2007;56:3896–908. DOI 10.1002/art.23059.
155. Stathopoulos P, Dalakas MC. Evolution of anti-B cell therapeutics in autoimmune neurological diseases. Neurotherapeutics. 2022;112:57. DOI 10.1007/s13311-022-01196-w.
156. van Vollenhoven RF, Emery P, Bingham CO 3rd, et al. Longterm safety of patients receiving rituximab in rheumatoid arthritis clinical trials. J Rheumatol. 2010;37:558–67. DOI 10.3899/jrheum.090856.

https://doi.org/10.1017/cjn.2022.60 Published online by Cambridge University Press