B LYMPHOCYTE-TARGETING THERAPIES IN MULTIPLE SCLEROSIS: A NARRATIVE REVIEW

Eleftheria Koropouli, MD[1], John Tzartos, MD[1], Dimitrios Tzanetakos, MD[1], Dimitrios Kitsos, MD[1], Maria Chondrogianni, MD[1], Anna Keramida, MD[1], Vasileios Giannopapas, MD[1,2], Maria Maili, MD[1], Konstantinos Melanis, MD[1], Maria-Ioanna Stefanou, MD[1], Sotirios Giannopoulos, MD[1], Georgios Tsivgoulis MD[1]

[1] Second Department of Neurology, National and Kapodistrian University of Athens, School of Medicine, "Attikon" University Hospital, Athens, Greece
[2]Department of Physical Therapy, University of West Attica, Athens, Greece

Abstract
Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system mediated by aberrant activation of the immune system. This leads to inflammatory and degenerative processes that cause both discrete relapses and chronic progression and are mediated by T and B lymphocytes and microglial cells. B lymhocytes have emerged as critical regulators of both relapsing and progressive forms of MS and their targeting has proven to be highly effective for active disease (relapsing MS and primary active MS). Therapies that target B lymphocytes include compounds directed against the B cell surface protein cluster of differentiation 20 (CD20), which have been approved for the treatment of MS, and compounds directed against the intracellular signaling effector Bruton's tyrosine kinase (BTK) that are currently under evaluation in randomized-controlled clinical trials. These agents are deemed to target both relapse-associated and progression-associated inflammatory pathways, albeit their efficacy in halting disease progression needs to be further evaluated. The current narrative review provides a brief outline of the roles that B cells exert in MS and highlights the safety and efficacy of B cell-targeting therapies in MS. We also provide practical recommendations regarding the use of B cell-targeting therapies in different MS subtypes.

Key Words: *Multiple sclerosis, lymphocytes, B cells, monoclonal antibodies, Bruton's tyrosine kinase inhibitors*

ΘΕΡΑΠΕΙΕΣ ΠΟΥ ΣΤΟΧΕΥΟΥΝ ΤΑ Β ΛΕΜΦΟΚΥΤΤΑΡΑ ΣΤΗΝ ΠΟΛΛΑΠΛΗ ΣΚΛΗΡΥΝΣΗ

Ελευθερία Κοροπούλη[1], Ιωάννης Τζάρτος[1], Δημήτριος Τζανετάκος[1], Δημήτριος Κίτσος[1], Μαρία Χονδρογιάννη[1], Άννα Κεραμιδά[1], Βασίλειος Γιαννόπαπας[1,2], Μαρία Μαΐλη, Κωνσταντίνος Μελάνης[1], Μαρία-Ιωάννα Στεφάνου, Σωτήριος Γιαννόπουλος[1], Γεώργιος Τσιβγούλης[1]

[1] Β' Πανεπιστημιακή Νευρολογική Κλινική, Σχολή Ιατρικής, Εθνικού και Καποδιστριακού Πανεπιστημίου Αθηνών, Πανεπιστημιακό Γενικό Νοσοκομείο «Αττικόν», Αθήνα, Ελλάδα
[2] Τμήμα Φυσιοθεραπείας, Πανεπιστήμιο Δυτικής Αττικής, Αθήνα, Ελλάδα

Περίληψη
Η Πολλαπλή Σκλήρυνση (ΠΣ) είναι μία χρόνια φλεγμονώδης απομυελινωτική νόσος του κεντρικού νευρικού συστήματος με έκτοπη ενεργοποίηση του ανοσοποιητικού συστήματος. Αυτό οδηγεί σε φλεγμονώδεις και εκφυλιστικές διεργασίες που προκαλούν υποτροπές της νόσου και προοδευτικότητα και διαμεσολαβούνται από τα Τ και τα Β λεμφοκύτταρα και τα μικρογλοιακά κύτταρα. Τα Β λεμφοκύτταρα έχουν αναδειχθεί ως κεντρικοί ρυθμιστές τόσο των υποτροπιαζόντων όσο και των προοδευτικών μορφών της νόσου και η στόχευσή τους έχει αποδειχθεί εξαιρετικά αποτελεσματική για την πολλαπλή σκλήρυνση με ενεργότητα (υποτροπιάζουσα ΠΣ & πρωτοπαθώς προϊούσα ΠΣ με ενεργότητα). Οι θεραπείες που στοχεύουν τα Β λεμφοκύτταρα περιλαμβάνουν τους παράγοντες που στρέφονται έναντι του κυτταρικού αντιγόνου επιφανείας των Β λεμφοκυττάρων CD20, οι οποίοι έχουν εγκριθεί για τη θεραπεία της πολλαπλής σκλήρυνσης, και παράγοντες που στρέφονται εναντίον της τυροσινικής κινάσης του Bruton που βρίσκονται στο στάδιο αξιολόγησης σε κλινικές μελέτες. Οι παράγοντες αυτοί φαίνεται ότι στοχεύουν τόσο τις φλεγμονώδεις διεργασίες που σχετίζονται με τις υποτροπές όσο και τις φλεγμονώδεις διεργασίες που σχετίζονται με την προοδευτικότητα της νόσου, αν και η αποτελεσματικότητά τους στις προοδευτικές μορφές πολλαπλής σκλήρυνσης χρειάζεται να εκτιμηθεί περαιτέ-

ρω. Η παρούσα ανασκόπηση παρέχει μία σύντομη αναφορά στο ρόλο που παίζουν τα Β λεμφοκύτταρα στην Πολλαπλή Σκλήρυνση καθώς και στην ασφάλεια και αποτελεσματικότητα των θεραπειών που στοχεύουν τα Β λεμφοκύτταρα στην Πολλαπλή Σκλήρυνση. Επίσης αναφέρονται πρακτικές συστάσεις για τη χρήση των παραγόντων που στοχεύουν τα Β λεμφοκύτταρα σε διάφορες μορφές πολλαπλής σκλήρυνσης.

Λέξεις Ευρετηρίου: *Πολλαπλή σκλήρυνση, λεμφοκύτταρα, Β κύτταρα, μονοκλωνικά αντισώματα, αναστολείς της τυροσινικής κινάσης του Bruton*

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by demyelination and neuronal degeneration. It can follow various patterns of CNS inflammation in space and in time affecting multiple brain and spinal cord regions. MS may present with a relapsing-remitting course (RRMS) or present either at the beginning or later in its course with clinical and/or imaging signs of constant progression, adopting a primary progressive phenotype (PPMS), a secondary progressive phenotype (SPMS) or a progressive relapsing phenotype (PRMS). Inflammation in MS, originally thought of to be driven primarily by T cells, has been shown to be mediated also by B lymphocytes (also known as B cells) and cells of the innate immunity including microglia, which are the CNS-resident macrophages. In particular, B lymphocytes have emerged as central regulators of the immune processes that lead to the emergence and perpetuation of inflammation in MS and mediate a large part of inflammatory injury in the CNS of MS-affected individuals[1]. Of note, B cells is the hallmark cellular constituent of ectopic lymphoid follicles seen mostly in progressive MS[2]. MS-associated immune activation causes the acute focal inflammatory lesions that underlie disease relapses resulting in relapse-associated worsening (RAW), and constant chronic inflammation that leads to a slowly progressive course with subsequent signs of neurodegeneration causing loss of neural tissue, disability accumulation and progression independent of relapse activity (PIRA)[3]. The latter is reflected in the significant brain volume decrease observed in MS patients suffering from the disease for several years[4], attributed to axonal damage and neuron death[5]. Whether inflammatory and degenerative mechanisms intersect and the mechanisms by which they do so remain for the most part unknown.

The course of MS reflects the entire disease complexity that includes relapses resulting from focal inflammatory lesions, chronic inflammation in distinct CNS compartments, inflammation-mediated neural degeneration, primary degenerative processes as well as processes of neuronal repair and remyelination[6–8] (Table 1). In this narrative review, we highlight the roles of B lymphocytes in both inflammatory and degenerative pathways activated in MS and discuss the current B cell-targeting therapies in MS including the approved anti-CD20 monoclonal antibodies, directed against the cluster of differentiation 20 (CD20), and the currently evaluated in randomized-controlled clinical trials (RCTs) Bruton's tyrosine kinase (BTK) inhibitors. We also provide a summary of the effects of these compounds on MS-associated neuroinflammation and neurodegeneration. Finally, we also provide practical recommendations regarding the use of B cell-targeting therapies in different MS subtypes.

Table 1. Cellular processes in the course of Multiple Sclerosis. The course of multiple sclerosis is characterized of discrete relapses due to focal inflammatory lesions visualized with magnetic resonance imaging, long-standing slowly progressive inflammation that leads to degeneration in the absence of detectable focal lesions, inflammation-independent degeneration and sparse remyelination consisting of thin incomplete myelin sheaths surrounding degenerating axons. These processes are differentially activated in time and in space in the course of MS.

Cellular processes in the course of Multiple Sclerosis and their anatomical correlates	
Relapses (inflammation-mediated)	Acute focal inflammatory lesions
Inflammation-mediated progression	Compartmentalized CNS inflammation Slowly expanding lesions
Neurodegeneration-mediated progression	Loss of CNS volume (brain and spinal cord atrophy)
Remyelination	Oligodendrocyte clusters around lesions Thin/incomplete myelin sheaths

Functions and areas of residency of B cells in multiple sclerosis

B lymphocytes, the mediators of humoral adaptive immunity, and their differentiated counterparts plasma cells that produce antibodies directed against select antigens, constitute a significant part of anatomical MS lesions and pursue a lot of immune responses aberrantly activated in MS. B lymphocytes have been postulated to drive inflammation in MS, possibly in antibody-dependent and antibody-independent mechanisms[9]. The latter comprise antigen presentation to and activation of T cells as well as release of inflammatory mediators including cytokines, chemokines and cytotoxic factors that lead to inflammation-dependent and inflammation-independent injury that may be culminated in cell death of neuronal and non-neuronal cells[10,11]. There is apparently a reciprocal interaction between B cells and T cells in MS in that T cells enhance the clustering and function of B cells at niches of inflammation within the CNS[12]. Of note, B cells that reside in the CNS of MS-affected individuals have altered molecular identity compared to B cells in non-diseased individuals. In particular, they may carry hypermutations and synthesize antibodies with their constituent domains rearranged[13]. The main roles of B lymphocytes in immune diseases, which may also apply in the CNS of MS-affected individuals, are summarized in Table 2.

B cell localization in the CNS of patients suffering from MS is important for understanding the exact roles that these cells play in the pathogenesis of the disease. B cells infiltrate the meninges and form aggregates arranged in a follicular pattern. These B cell aggregates are known as B cell follicles and localize in the vicinity of active inflammatory lesions. They are thought to play important roles in the induction and maintenance of inflammation within the adjacent cortical tissue, specifically in cases with SPMS but not in PPMS[14], in accordance with the role of such ectopic lymphoid clusters in other chronic inflammatory diseases[15]. CD20+ B cells have also been detected in white matter perivascular spaces, suggesting that these regions serve as areas of active ongoing inflammation. Moreover, cerebrospinal fluid contains B cells and plasma cells which contribute to the intrathecal synthesis of antibodies[12]. Therefore, it has been hypothesized that B cells exert both local effects by acting in clusters at juxtacortical compartments and systemic effects by secreting circulating antibodies and/or by being disseminated throughout the CNS by means of CSF[9]. These observations have expanded our view of the role of B lymphocytes in CNS inflammatory disorders and have revived the interest for targeting B cell-dependent pathways in MS.

Table 2. Known roles of B-cells in autoimmune diseases that may apply in Multiple Sclerosis (MS) pathogenesis. The various roles of B lymphocytes, presented at the top part of the table, reflect their complex contribution in MS pathophysiology. The localization areas of B cells, presented at the bottom part of the table, point to their role in regulating disease activity.

B cell functions that may apply in Multiple Sclerosis
Antigen presentation
Activation of T lymphocytes
Antibody production
Secretion of inflammatory mediators
Secretion of cytotoxic factors and induction of cell death
Host cells for demyelination-inducing viruses
B cell localization in Multiple Sclerosis
Meningeal lymphoid follicles
Perivascular spaces (mostly periventricular)
Active lesions
Slowly expanding lesions
Cerebrospinal fluid

Therapies directed against B lymphocytes in multiple sclerosis

Therapies that target and deplete B lymphocytes in multiple sclerosis include anti-CD20 monoclonal antibodies and Bruton's tyrosine kinase (BTK) inhibitors.

Anti-CD20 monoclonal antibodies

CD20 is a transmembrane protein expressed in a selective pattern on the cell surface of B lymphocytes. Monoclonal antibodies directed against CD20 have been developed as potent and selective inhibitors of CD20-expressing B lymphocytes and are thereafter referred to as anti-CD20 monoclonal antibodies. Four anti-CD20 monoclonal antibodies, Rituximab, Ocrelizumab, Ofatumumab and Ublituximab are currently in use in MS, and are directed against adjacent or partially overlapping epitopes on CD20 extracellular domains[16,17] (Table 3).

General considerations:

The administration of anti-CD20 monoclonal antibodies has been associated with infusion-related reactions (short-term effects) and with long-term effects that include infections, malignant disease activation and potential teratogenic effects in pregnant women [rituximab: US Food and Drug Administration (FDA) pregnancy category: C; ocrelizumab: US FDA pregnancy category: Not assigned; ofatumumab: US FDA pregnancy category: Not assigned; Ublituximab: US FDA pregnancy category: Not assigned]. Therefore, prior to the initiation of treatment with an anti-CD20 monoclonal antibody, screening for active infections, screening for active malignant disease, sufficient vaccination for select pathogens and pregnancy programming in women of child-bearing age is recommended. Infectious diseases routinely screened include hepatitis B, hepatitis C, human immunodeficiency virus infection and tuberculosis. Contraindications for the administration of this class of drugs include life-threatening infusion-related reaction and active HBV infection.

Vaccination against common pathogens is strongly recommended and completed prior to treatment. All immunizations should be performed according to the immunization guidelines, at least 4 weeks for live and live-attenuated vaccines and at least 2 weeks for non-live vaccines, before the initiation of treatment. Screening for malignant diseases is guided by patient's history and age and may include

Table 3. Anti-CD20 monoclonal antibodies in different forms of multiple sclerosis. Anti-CD20 monoclonal antibodies (Anti-CD20 mAb) with efficacy against different forms of MS are presented with respect to their composition, route of administration, administration scheme and forms of MS for which they have a formal approval based on randomized-controlled clinical trials (Ocrelizumab, Ofatumumab, Ublituximab) or they have proven efficacy without approval (Rituximab, off-label use). Abbreviations: RMS, relapsing multiple scleroris; PPMS, primary progressive multiple sclerosis; SPMS, secondary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis.

Anti-CD20 mAb	mAb composition	Administration route	Administration scheme	Use in Multiple Sclerosis types
Rituximab	Chimeric	Intravenous infusion	4 weekly infusions; subsequently every 6 months	PPMS with activity SPMS
Ocrelizumab	Humanized	Intravenous infusion	2 initial doses, 300 mg each, with a 2-week interval; subsequently, 600 mg every 6 months	RMS PPMS with activity
Ofatumumab	Human (100%)	Subcutaneously	3 initial doses, 20 mg each, at weeks 0, 1, 2; subsequently monthly (starting at week 4)	RMS with active disease
Ublituximab	Chimeric, glycoengineered	Intravenous infusion	2 initial doses: 1st, 150 mg; 2nd, 450 mg; subsequently 450 mg every 6 months	RMS: RRMS, active SPMS

routine or more rigorous diagnostic approaches. Active infections and active malignant diseases are contraindications for initiating treatment with anti-CD20 monoclonal antibodies. Nevertheless, if successfully treated, such conditions do not prevent the patient from receiving anti-CD20 therapy at a later time point. To minimize the risk of short-term reactions related to the infusion of rituximab, ocrelizumab and ublituximab special precautions are taken with administration of preparative medications including corticosteroids, antihistamines and paracetamol. However, this premedication scheme does not rule out the possibility of allergy and the need for a close follow-up of the patient during and shortly after infusion. Anti-CD20 antibodies should be administered under the supervision of an experienced physician or nurse, and at a health center where resuscitation facilities are readily available.

Of particular importance is the alertness for progressive multifocal leukoencephalopathy (PML) in patients who present with a rapid and severe deterioration, given that PML associated with anti-CD20 antibodies has been reported in isolated cases[18].

The four available anti-CD20 monoclonal antibodies are briefly presented below:

Rituximab:

It is a mouse/human chimeric IgG1 antibody directed against the CD20 transmembrane protein expressed on B cell surface. Rituximab has been approved for autoimmune disorders including rheumatoid arthritis. Rituximab has shown efficacy in the treatment of RRMS in phase II RCTs[19,20], and it has been used for years as an off-label therapy for MS in particular for cases refractory to other MS treatments or in MS patients with concomitant systemic autoimmune disorders[21].

Administration: It is administered in 4 weekly intravenous infusions at a dose of 375 mg/m^2 of body surface area each, and thereafter every 6 months.

Side-effects: The most common are infusion-related reactions and infections.

Contraindications: Life-threatening hypersensitivity, active infection, severely immunocompromised state.

Ocrelizumab

It is a humanized IgG1 monoclonal antibody directed against CD20+ B cells. It has been approved by FDA and EMA for adult patients with RMS and active PPMS according to the results of OPERA I/II and ORATORIO trials, respectively[22,23].

Administration: It is administered by intravenous infusion. The initial dose is 600 mg administered as two separate infusions, 300 mg each, with a 2-week interval. Subsequent doses, 600 mg each, are administered every 6 months, with the first one of these being administered 6 months after the first initial dose.

Side-effects: The most common are infusion-related reactions and infections.

Contraindications: Life-threatening hypersensitivity, active infection, severe immunocompromise, active malignancy.

Ofatumumab

It is a 100% human IgG1 antibody directed against CD20 and subsequently against CD20-expressing B lymphocytes. It has been approved by FDA and EMA for use in adult patients with relapsing forms of MS, including RRMS or a secondary progressive course with disease activity, based on the results of the ASCLEPIOS I and II trials[24].

Administration: It is administered by subcutaneous injection containing 20 mg of ofatumumab at weeks 0, 1 and 2, and thereafter monthly starting at week 4. The first dose should be administered under the care of a physician or specialized nurse. Subsequent doses can be self-administered at home.

Side-effects: The most common are local and systemic reactions associated with the infusion and infections.

Contraindications: Life-threatening hypersensitivity, active hepatitis B infection.

Ublituximab

It is a glycoengineered chimeric monoclonal antibody directed against the CD20 transmembrane protein expressed on B lymphocytes. It has been approved by the Food and Drug Administration (FDA, USA) and the European Medicines Agency (EMA, EU) for use in adult patients with relapsing forms of MS, including RRMS and SPMS with activity, based on the results of ULTIMATE I/II trials[25,26].

Administration: It is administered by intravenous infusion. The administration scheme of ublituximab includes two initial doses and subsequently, one dose every six months (two infusions per year), as follows: first infusion, 150 mg IV over 4 hours; second infusion, 2 weeks after the first infusion, 450 mg IV over 1 hour; subsequent infusions (starting 6 months after the first infusion), 450 mg IV every 24 weeks (6 months) over 1 hour.

Side-effects: The most common are infusion-associated reactions and infections.

Contraindications: Active hepatitis B infection, life-threatening infusion reaction.

Bruton's tyrosine kinase inhibitors

Bruton's tyrosine kinase (BTK) is expressed in B

lymphocytes and constitutes an integral component of B cell receptor signaling playing important roles in regulating the survival, proliferation, maturation and function of B lymphocytes. It is also required for the proper function of microglia[27]. BTK inhibition disrupts B cell receptor signaling pathways critical to B lymphocyte survival and function and leads to B cell dysfunction and ultimately apoptosis[28]. Owing to their ability to target B lymphocytes, BTK inhibitors are being tested for their efficacy in a variety of B cell disorders including MS. In MS, BTK inhibitors are being tested both with respect to their ability to prevent relapses and with respect to their effectiveness in halting disease progression independent of relapses. BTK inhibitors have a low molecular weight that allows them to pass the blood-brain barrier and enter the CNS. As such, their therapeutic potential may extend beyond reducing relapses to halting disease progression seen particularly in progressive MS[29,30]. There are currently five BTK inhibitors, Evobrutinib, Tolebrutinib, Remibrutinib, Orelabrutinib and Fenebrutinib, which are being evaluated for their efficacy in relapsing MS (RMS) including relapsing remitting and progressive MS with activity.

Different members of the BTK inhibitor family differ in their affinity to bind and inhibit BTK, their pharmacokinetics, their ability to cross the blood-brain barrier and their risk to provoke side-effects related to their on- and/or off-target effects (Table 4). However, it seems that all BTK inhibitors may cause derangements in liver function tests, which although asymptomatic and reversible, have raised serious concerns about their safety and are the cause of placing a partial or complete hold in some of the ongoing randomized-controlled clinical trials preventing them from recruiting more patients. Whether there are predisposing factors for the development of this side effect is currently under study.

When to target B lymphocytes in multiple sclerosis

MS displays multi-level heterogeneity that results from the variability of affected areas of the CNS, the pathophysiology of lesions among different patients and in the same patient, the temporal course (rate of progression, sequence of relapses) and the response to treatment[7]. There are apparently differential contributions of immune system components in different subsets of patients, as proven by the higher or lower efficacy of the same MS medications in patients with seemingly the same MS course. The landscape of MS therapeutics is further complicated by comorbidities and the side effects of the medications that affect the selection of the most appropriate drug. These factors make evident the need for personalized therapeutic approaches, which may now become at least to some extent feasible

Table 4. Bruton's tyrosine kinase inhibitors currently under evaluation in multiple sclerosis. Five Bruton's tyrosine kinase inhibitors are being evaluated in phase III randomized-controlled clinical trials for their efficacy and safety in relapsing MS. Please note that results from the EVOLUTION clinical trials showed evobrutinib did not meet its primary endpoint of annualized relapse rate for up to 156 weeks compared to oral teriflunomide in both studies. https://www.merckgroup.com/en/news/evobrutinib-phase-lll.html

BTK inhibitor	Binding	MS subtypes in clinical trials TRIAL NAME	Phase	Administration route	Company
Evobrutinib	Covalent, irreversible	Relapsing MS EVOLUTION	III	Oral	Merck
Tolebrutinib	Covalent, irreversible	RRMS GEMINI I, II Relapsing SPMS HERCULES PPMS PERSEUS	III III III	Oral	Sanofi-Genzyme
Orelabrutinib	Covalent, irreversible	RRMS	II	Oral	InnoCare Pharma
Remibrutinib	Covalent, irreversible	Relapsing MS (RRMS, active SPMS) REMODEL I, II	III	Oral	Novartis
Fenebrutinib	Non-covalent, reversible	Relapsing MS FENhance, FENhance 2 PPMS FENtrepid	III III	Oral	Genentech

thanks to the growing list of available MS drugs. At present, MS medications for individual patients may be selected by certain experienced MS physicians based on the clinical course and the lesions visualized in MRI of the CNS, as well as on the therapeutic criterion as evident by the response of the patient to previous immune treatments (e.g. plasmapheresis, corticosteroids). Given this complexity and our inability to dissect the exact roles that B lymphocytes play in individual MS patients, the clinical course of MS is probably the most important criterion in making a decision as to which medication is the most appropriate for individual patients. Based on available evidence[16], we suggest some clinical scenarios in MS where B cell-depleting therapies may be considered:

- Progressive MS, including primary progressive MS, secondary progressive MS, and progressive relapsing MS.
- RRMS with high disease activity that does not respond to other potent agents (e.g. Natalizumab).
- Refractory or recurrent optic neuritis, in particular if it has sufficiently responded to plasmapheresis.
- Overlapping syndromes characterized by the co-occurrence of multiple sclerosis with a systemic autoimmune disorder, such as Sjögren syndrome, rheumatoid arthritis or systemic lupus erythematosus (off-label use).
- CNS demyelination as a result of a systemic autoimmune disease (off-label use).

Effects of B cell-targeting therapies on multiple sclerosis-associated neuroinflammatory and neurodegenerative pathways

MS leads to progressive disability by distinct relapses resulting from new focal CNS lesions and by slow progressive worsening resulting from chronic inflammation in distinct CNS compartments that is complicated by concurrent degenerative processes[3,6]. The slowly progressive pathways that lead to insidious cumulative disability in MS have been thus far difficult to hamper because of lack of effectiveness of the available therapeutic compounds on the molecular and cellular substrates of these processes[31]. This section focuses on the cellular and molecular evidence for the effects of B cell-depleting therapies in MS signaling pathways and/or on MS-related pathology.

Anti-CD20 monoclonal antibodies may disrupt B cell clusters localized at the meninges in SPMS[32]. Further, BTK inhibitors, as potent B cell modulators, may have the potential to halt disease progression through their robust effects on multiple stages of B cell cycle and function[29]. Indeed, BTK inhibition has been shown to downregulate B cell-dependent immune responses[33] and recent evidence demonstrates high efficacy of investigational BTK inhibitors in halting MS progression in a mouse model of SPMS[34], suggesting an effect of these compounds in disease-associated inflammation and progressive degeneration. However, the mechanisms by which they exert such effects are not completely understood given that B cell-depleting therapies may not affect the formation of ectopic lymphoid follicles, which are thought to be critical for inflammation maintenance and additive disability in progressive MS[35]. In consistency with preclinical data, RCTs have provided preliminary evidence regarding the efficacy of BTK inhibitors in relapsing MS.[36,37] However, it should be noted that results from the EVOLUTION clinical trials showed evobrutinib did not meet its primary endpoint of annualized relapse rate for up to 156 weeks compared to oral teriflunomide in both studies[38].

Conclusions

B cells, the cellular substrate of humoral adaptive immunity, reside in multiple areas of the MS-affected CNS including the CSF and various areas of the parenchyma. Their broad localization pattern reflects their multifaceted roles in inducing and maintaining MS-related pathology by the exertion of local and systemic effects resulting in activation of a multitude of immune signaling mediators and effectors. In particular, their detection in areas of rapidly progressive and slowly progressive inflammatory lesions points toward a role of these cells in both relapsing and progressive forms of MS.

In support of the central regulatory role of B cells in MS, B cell-targeting or depleting drugs have emerged as powerful tools for various forms of MS including refractory RRMS, progressive MS or overlapping syndromes of MS with a systemic autoimmune disorder. After the advent and establishment of efficacy of the first anti-CD20 monoclonal antibodies, there is an ever-growing list of compounds that deplete B cells and/or abrogate their function and are highly effective in RCTs or have already been approved for active MS. This has demonstrated that even if B cells are not the only players in MS signaling cascades, they apparently constitute the cornerstone of inflammation-mediated injury that occurs in MS.

B cell-targeting therapies are currently being expanded by the advent of the new anti-CD20 monoclonal antibodies Ofatumumab and Ublituximab and the currently tested in clinical trials BTK inhibitors. Halting the relentlessly progressive course of MS reflected in brain and spinal cord atrophy observed in chronic MS-affected individuals remains a challenge and is a parameter for success for the new drugs being added in the armamentarium of MS thera-

peutics. Of note, BTK inhibitors have broader effects on the immune system by affecting both adaptive and innate immunity, which raises promise for progressive MS types that have been difficult to treat. The efficacy of the new B cell-targeting therapies in slowing down MS-associated neurodegeneration requires a long-term follow-up after treatment initiation, with more sophisticated biochemical and/or imaging approaches of the CNS (volume loss quantification of the entire brain, spinal cord or select CNS structures, markers of neurodegeneration) that will allow the in vivo study of the effects of these drugs on MS-related degenerative processes.

References

[1] Comi G, Bar-Or A, Lassmann H, et al. Role of B Cells in Multiple Sclerosis and Related Disorders. *Ann Neurol*. 2021;89(1):13-23. doi:10.1002/ana.25927

[2] Bell L, Lenhart A, Rosenwald A, Monoranu CM, Berberich-Siebelt F. Lymphoid Aggregates in the CNS of Progressive Multiple Sclerosis Patients Lack Regulatory T Cells. *Front Immunol*. 2020;10(January):1-18. doi:10.3389/fimmu.2019.03090

[3] Lublin FD, Häring DA, Ganjgahi H, et al. How patients with multiple sclerosis acquire disability. *Brain*. 2022;145(9):3147-3161. doi:10.1093/brain/awac016

[4] De Stefano N, Giorgio A, Battaglini M, et al. Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes. *Neurology*. 2010;74(23):1868-1876. doi:10.1212/WNL.0b013e3181e24136

[5] Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal Transection in the Lesions of Multiple Sclerosis. *N Engl J Med*. 1998;338(5):278-285. doi:10.1056/nejm199801293380502

[6] Giovannoni G, Popescu V, Wuerfel J, et al. Smouldering multiple sclerosis: the 'real MS.' *Ther Adv Neurol Disord*. 2022;15(X):1-18. doi:10.1177/17562864211066751

[7] Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. *Ann Neurol*. 2000;47(6):707-717. doi:10.1002/1531-8249(200006)47:6<707::AID-ANA3>3.0.CO;2-Q

[8] Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. A study of 113 cases. *Brain*. 1999;122(12):2279-2295. doi:10.1093/brain/122.12.2279

[9] Cencioni MT, Mattoscio M, Magliozzi R, Bar-Or A, Muraro PA. B cells in multiple sclerosis — from targeted depletion to immune reconstitution therapies. *Nat Rev Neurol*. 2021;17(7):399-414. doi:10.1038/s41582-021-00498-5

[10] Wang J, Jelcic I, Mühlenbruch L, et al. HLA-DR15 Molecules Jointly Shape an Autoreactive T Cell Repertoire in Multiple Sclerosis. *Cell*. 2020;183(5):1264-1281.e20. doi:10.1016/j.cell.2020.09.054

[11] Duddy M, Niino M, Adatia F, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J Immunol*. 2007;178(10):6092-6099. doi:10.4049/jimmunol.178.10.6092

[12] Schafflick D, Xu CA, Hartlehnert M, et al. Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis. *Nat Commun*. 2020;11(1):1-14. doi:10.1038/s41467-019-14118-w

[13] Ramesh A, Schubert RD, Greenfield AL, et al. A pathogenic and clonally expanded B cell transcriptome in active multiple sclerosis. *Proc Natl Acad Sci U S A*. 2020;117(37):22932-22943. doi:10.1073/pnas.2008523117

[14] Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain*. 2007;130(4):1089-1104. doi:10.1093/brain/awm038

[15] Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol*. 2006;6(3):205-217. doi:10.1038/nri1786

[16] Graf J, Mares J, Barnett M, et al. Targeting B Cells to Modify MS, NMOSD, and MOGAD: Part 1. *Neurol Neuroimmunol neuroinflammation*. 2021;8(1):1-11. doi:10.1212/NXI.0000000000000918

[17] Margoni M, Preziosa P, Filippi M, Rocca MA. Anti-CD20 therapies for multiple sclerosis: current status and future perspectives. *J Neurol*. 2022;269(3):1316-1334. doi:10.1007/s00415-021-10744-x

[18] Patel A, Sul J, Gordon ML, et al. Progressive Multifocal Leukoencephalopathy in a Patient with Progressive Multiple Sclerosis Treated with Ocrelizumab Monotherapy. *JAMA Neurol*. 2021;78(6):736-740. doi:10.1001/jamaneurol.2021.0627

[19] Salzer J, Svenningsson R, Alping P, et al. Rituximab in multiple sclerosis. *Neurology*. 2016;87(20):2074-2081. doi:10.1212/WNL.0000000000003331

[20] Hauser SL, Waubant E, Arnold DL, et al. B-Cell Depletion with Rituximab in Relapsing–Remitting Multiple Sclerosis. *N Engl J Med*.

2008;358(7):676-688. doi:10.1056/nejmoa0706383
[21] Chisari CG, Sgarlata E, Arena S, Toscano S, Luca M, Patti F. Rituximab for the treatment of multiple sclerosis: a review. *J Neurol*. 2022;269(1):159-183. doi:10.1007/s00415-020-10362-z
[22] Riederer F. Ocrelizumab versus placebo in primary progressive multiple sclerosis. *J fur Neurol Neurochir und Psychiatr*. 2017;18(1):30-31. doi:10.1056/nejmoa1606468
[23] Hauser SL, Bar-Or A, Comi G, et al. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. *N Engl J Med*. 2017;376(3):221-234. doi:10.1056/nejmoa1601277
[24] Hauser SL, Bar-Or A, Cohen JA, et al. Ofatumumab versus Teriflunomide in Multiple Sclerosis. *N Engl J Med*. 2020;383(6):546-557. doi:10.1056/nejmoa1917246
[25] Steinman L, Fox E, Hartung H-P, et al. Ublituximab versus Teriflunomide in Relapsing Multiple Sclerosis. *N Engl J Med*. 2022;387(8):704-714. doi:10.1056/nejmoa2201904
[26] Lee A. Ublituximab: First Approval. *Drugs*. 2023;83(5):455-459. doi:10.1007/s40265-023-01854-z
[27] Shinners NP, Carlesso G, Castro I, et al. Bruton's tyrosine kinase mediates NF-κB activation and B cell survival by B cell-activating factor receptor of the TNF-R family. *J Immunol*. 2007;179(9):6369-6369. doi:10.4049/jimmunol.179.9.6369-a
[28] Ghosh S, Mohammed Z, Singh I. Bruton's tyrosine kinase drives neuroinflammation and anxiogenic behavior in mouse models of stress. *J Neuroinflammation*. 2021;18(1):1-26. doi:10.1186/s12974-021-02322-9
[29] Geladaris A, Torke S, Weber MS. Bruton's tyrosine kinase inhibitors in multiple sclerosis: pioneering the path towards treatment of progression? *CNS Drugs*. 2022;36(10):1019-1030. doi:10.1007/s40263-022-00951-z
[30] Krämer J, Bar-Or A, Turner TJ, Wiendl H. Bruton tyrosine kinase inhibitors for multiple sclerosis. *Nat Rev Neurol*. 2023;19(5):289-304. doi:10.1038/s41582-023-00800-7
[31] Faissner S, Plemel JR, Gold R, Yong VW. Progressive multiple sclerosis: from pathophysiology to therapeutic strategies. *Nat Rev Drug Discov*. 2019;18(12):905-922. doi:10.1038/s41573-019-0035-2
[32] Roodselaar J, Zhou Y, Leppert D, Hauser AE, Urich E, Anthony DC. Anti-CD20 disrupts meningeal B-cell aggregates in a model of secondary progressive multiple sclerosis. *Neurol Neuroimmunol NeuroInflammation*. 2021;8(3):1-10. doi:10.1212/NXI.0000000000000975
[33] 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(4):535-548. doi:10.1007/s00401-020-02204-z
[34] Evonuk KS, Wang S, Mattie J, et al. Bruton's tyrosine kinase inhibition reduces disease severity in a model of secondary progressive autoimmune demyelination. *Acta Neuropathol Commun*. 2023;11(1):1-19. doi:10.1186/s40478-023-01614-w
[35] Brand RM, Friedrich V, Diddens J, et al. Anti-CD20 Depletes Meningeal B Cells but Does Not Halt the Formation of Meningeal Ectopic Lymphoid Tissue. *Neurol Neuroimmunol NeuroInflammation*. 2021;8(4):1-10. doi:10.1212/NXI.0000000000001012
[36] Reich D, Arnold D, Vermersch P, et al. Safety and efficacy of tolebrutinib, an oral brain-penetrant BTK inhibitor, in relapsing multiple sclerosis: A phase 2b, randomized, double-blind, placebo-controlled trial by Daniel S Reich et Al. *Mult Scler Relat Disord*. 2023;77(9):729-738. doi:10.1016/j.msard.2023.104850
[37] 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(25):2406-2417. doi:10.1056/nejmoa1901981
[38] https://www.merckgroup.com/en/news/evobrutinib-phase-lll.html

Disclosures

JT and GT report unrestricted grant support from Roche Hellas.