Bruton’s Tyrosine Kinase Inhibitors in Multiple Sclerosis

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B cells play a central role in the pathogenesis of multiple sclerosis (MS), as demonstrated through the success of various B cell-depleting monoclonal antibodies. Bruton’s tyrosine kinase (BTK) is a critical molecule in intracellular signaling from the receptor of B cells and receptors expressed in the cells of the innate immune system. BTK inhibitors may be a non-cell-depleting alternative to B cell modulation.

Bruton’s tyrosine kinase | multiple sclerosis | B cells | BTK inhibitors

1. Introduction

Multiple sclerosis (MS) has classically been considered an autoimmune disease of the central nervous system (CNS) with a degenerative component that becomes increasingly more evident as the disease progresses. Both the adaptive and the innate arms of the immune system are involved in the pathogenesis of MS. Even though immunoglobulin abnormalities found in the cerebrospinal fluid (CSF) are the most conspicuous immunological finding, it was still believed that anomalies of the humoral response did not have the same pathogenetic relevance as those of the cellular response. Knowledge derived from experimental autoimmune encephalomyelitis (EAE) has also contributed to the view of MS as a T-cell disease. Strong support against this view was provided by the results of a phase II clinical trial with rituximab, a monoclonal antibody depleting CD20+ B cells in patients with relapsing-remitting MS who showed a rapid and profound response in clinical and magnetic resonance imaging (MRI) parameters [1]. However, another anti B-cell trial using a fusion protein, atacicept—which binds to the cytokines, BlyS and APRIL, and is involved in B-cell maturation, differentiation, and survival—had to be prematurely terminated due to disease reactivation. This failure revealed the complexity of the pathogenic role of B cells in MS [2]. Two phase III trials with ocrelizumab and ofatumumab further confirmed the beneficial effect of B cell depletion with anti-CD20+ monoclonal antibodies [3][4].

B cells are potent APCs that can drive T-cell mediated autoimmunity [5]. In EAE, they have a critical pathogenetic role, dependent on MHC II, and independent of their humoral response [6]. Memory B cells from MS patients elicited CD4+ T cell proliferation in response to myelin basic protein (MBP) and myelin-oligodendrocyte glycoprotein (MOG), a finding not reproduced with memory B cells from healthy donors [7]. B cells produce pro-inflammatory cytokines, such as lymphotoxin alpha, IL6, IL2, IL17, and tumor necrosis factor-alpha (TNF-α) [8]. B cells from MS patients secrete more IL6 than healthy controls [9] and abnormally secrete TNF-α and lymphotoxin when activated with interferon-γ or the pathogen-associated CPG-DNA [10]. On the other hand, B cells can act as regulators in autoimmunity by different anti-inflammatory cytokines, mostly IL10 [11]. Mice with IL10-deficient B cells

develop a more severe EAE [12]. In MS patients, B cells produce a decreased average secretion of IL10 [13]. Ectopic follicle-like aggregates containing B cell and plasma cells may appear in meninges from patients with secondary progressive MS [14]; however, early MS [15] can also be associated with cortical demyelination [15][16]. In the CNS of progressive MS, antigen-experienced B cell clones obtained from meninges were related to clones that were isolated from inflammatory infiltrates, normal-appearing white matter, and CSF [17]. Importantly, the immunological abnormalities of B cells can be detected on both sides of the BBB—as demonstrated by the sequencing of IgG heavy chain variable region genes (IgG-VH) of samples of CSF and blood processed in parallel—indicating the exchange of these cells across the BBB [18].

Currently, B cells are considered fundamental players in the pathogenesis of MS [19].

Based on the fundamental role of B cells in MS pathogenesis, and considering the profound therapeutic impact of anti-CD20+ monoclonal antibodies, other anti-B cell alternatives have been explored to circumvent the problems associated with chronic B-cell depletion, such as humoral deficiency [20]. Bruton’s tyrosine kinase (BTK) has emerged in recent decades as a critical target for diseases in which B cells have a major involvement, as is the case for several hematologic malignancies. Additionally, experimental models of human autoimmune diseases have revealed that BTK is a target of the utmost importance to cancel B cell pro-inflammatory functions without the risks associated with cell depletion.

2. General Review

Studies in mouse models, known as xid (X-linked immunodeficiency), revealed the central role of BTK in the BCR signaling, differentiation, and survival of B cells [21]. Further experiments with mature B cells using BTK inhibitors demonstrated the involvement of BTK in B cell malignancies and models of autoimmune diseases [22].

In addition to its capital role in B cells, BTK is also involved in the functions of other cell lineages that are pathogenetically relevant for MS, such as monocytes and macrophages, dendritic cells, and microglia.

Macrophages present antigen to T cells and have critical phagocytic capacity and cytokine secretion.BTK inhibition by ibrutinib suppressed the FcγR-mediated secretion of TNFα but did not interfere with phagocytosis [23]. In monocytes from healthy volunteers, the irreversible inhibitor, evobrutinib (used in clinical trials for MS), skewed macrophage polarization towards the M2 anti-inflammatory phenotype [24].

Dendritic cells are APCs to T lymphocytes, along with B cells and macrophages. They represent a bridge between innate and adaptive immunity, have a pathogenetic role in immune diseases, such as MS, and are being used for tolerizing approaches in MS [25]. BTK is involved in the activation and differentiation of dendritic cells; however, compared to B cells, BTK functions are not as well-defined [21].

Microglia are phagocytic cells which reside in the CNS, derived from progenitor cells in the embryonic yolk sac that migrate into the CNS. They constitute a fundamental part of the innate immunity in the CNS and express BTK [26].

BTK modulates microglial phagocytosis in murine models of Alzheimer’s disease [27]. Targeting microglia with small-molecule BTK inhibitors (BTKi) could develop into a therapeutic tool of great interest for MS and neurological disorders in which microglia have a pathogenic implication

BTK inhibitors

BTKi are small-molecule agents. Depending on the binding to BTK, inhibitors are classified as irreversible or reversible. Irreversible inhibitors usually bind to cysteine residue 481 of the kinase domain through covalent bonds. As cysteine-481 is critical for ATP binding, which is required for catalytic activity, the union with inhibitors determines the suppression of signaling downstream of BTK [28]. Reversible inhibitors non-covalently bind to different BTK-specific pockets with hydrogen bonds, ionic bonds, or hydrophobic forces [28]. Weak binding decreases potency, but also toxicity and risks associated with chronic use.

Currently, several BTKi are in various stages of development for all the clinical forms of multiple sclerosis. Three covalent, irreversible inhibitors are being assayed in clinical trials, either in phase III (tolebrutinib and evobrutinib) or in phase II (orelabrutinib). Fenebrutinib is a non-covalent reversible inhibitor assayed in phase III trials.

TABLE . Bruton’s tyrosine kinase inhibitors currently assayed in clinical trials for multiple sclerosis

Product	Type of BTKi	Sponsor	ClinicalTrials Gov Identifier	Phase	Type of Trial	Patients	Start Date	Estimated Completion Date
Fenebrutinib	Non-covalent, reversible	Hoffmann-La Roche	NCT04544449	III RDB	Fenebrutinib (or placebo) vs. ocrelizumab (or placebo) 1:1 (FENtrepid)	946 PPMS	2020	2028
Fenebrutinib	Non-covalent, reversible	Hoffmann-La Roche	NCT04586023	III RDB	Fenebrutinib vs. teriflunomide 1;1 (FENhance)	734 RMS	2021	2024

Fenebrutinib	Non-covalent, reversible	Hoffmann-La Roche	NCT04586010	III RDB	Fenebrutinib vs. teriflunomide 1;1 (FENhance)	734 RMS	2021	2024
Tolebrutinib	Covalent, Irreversible	Sanofi/Principia	NCT04458051	III RDB	SAR442168 (tolebrutinib) vs. placebo (PERSEUS)	990 PPMS	2020	2024
Tolebrutinib	Covalent, Irreversible	Sanofi/Principia	NCT04410978	III RDB	SAR442168 (tolebrutinib) vs. teriflunomide GEMINI1	900 RMS	2020	2023
Tolebrutinib	Covalent, irreversible	Sanofi/Principia	NCT04410991	III RDB	SAR442168 (tolebrutinib) vs. teriflunomide GEMINI2	900 RMS	2020	2023
Tolebrutinib	Covalent, irreversible	Sanofi/Principia	NCT04411641	III RDB	SAR442168 (tolebrutinib) vs. placebo (HERCULES)	1290 SPMS	2020	2024
Evobrutinib	Covalent, irreversible	Merck KGaA	NCT04338022	III RDB	Evobrutinib vs. teriflunomide (EvolutionRMS 1)	930 RMS	2020	2026

Evobrutinib	Covalent, irreversible	Merck KGaA	NCT04338022	III RDB	Evobrutinib vs. teriflunomide (EvolutionRMS 2)	930 RMS	2020	2026
Orelabrutinib	Covalent, irreversible	Beijing InnoCare Pharma Tech Co., Ltd.	NCT04711148	II RDB	Orelabrutinib, three doses vs. placebo at 1:1:1:1 ratio	160 RRMS	2021	2024

3. Current Insights

For B cell malignancies, BTK inhibition by small molecules represents a major therapeutic advance. The enthusiasm for these types of drugs has expanded toward the field of autoimmune diseases with B cell-dependent pathogenesis. Experience with BTKi has developed with experimental models of several human autoimmune diseases, but clinical advances have been much more limited. For the time being, clinical trials with different BTKis are in various phases of development, but none of them has gained the approval of regulatory agencies.

As shown with in vitro and in vivo experiments, the inhibition of BTK affects nuclear factors that are essential for maturation, proliferation, and cytokine production by B cells. In EAE, B cell–T cell interactions are affected, leading to a strong reduction in the ability to activate naïve T cells that promote encephalitogenic T cells, and a marked decrease in pro-inflammatory cytokine secretion, as clearly demonstrated with evobrutinib [29]. Interference with B cell maturation may determine a diminished generation of new pathogenic B cells. In MS, it remains to be seen if BTKis serve to eliminate autoreactive B cells, more dependent on BTK for survival than normal B cells [30], and the extent to which they can modify the different B cell types, particularly memory B cells, which are considered the major targets for immunotherapy [31].

Alteration in the downstream signals generated from the BCR appears to be the most important mechanism of action for the BTKi in B cells. In addition to the BCR, interference with the signaling of other receptors present in B cells and cells of the innate immune system may significantly contribute to the therapeutic effect. Chemokine receptors CXCR4 and CXCR5, related to migration and homing, may be a contributing factor. The inhibition of signaling from TLR, FcγR, GM-CSFR, the control of adhesion, or the effect on the NLRP3 inflammasome are also factors to be explored in depth. In MS and other autoimmune diseases, the impact of BTKi on those receptors poses many questions on the pathogenic role of the innate immune system [32]. The expression of BTK in microglial cells, experimentally demonstrated [26][27], and the permeability of the BBB to small molecule inhibitors [33] raises the possibility of effectively modulating the activated microglia in MS patients.

Since the introduction of ibrutinib, safety has been an essential concern for developing new BTKi. In theory, non-covalent reversible inhibitors would be more appropriate for autoimmune disorders, at the cost of lower efficacy. Published experience in a phase II trial with the irreversible inhibitor, evobrutinib, suggested that it has an acceptable safety profile. Only the long-term exposure of MS patients to both types of BTKis in phase III clinical trials will provide a larger perspective concerning safety and tolerance.

In animal models of autoimmune diseases, including MS, results with different BTKis have raised expectations, and time will show whether the efficacy of those models translates to human diseases [34]. In EAE, experience with evobrutinib revealed the drug’s central mechanism of action, inhibiting the activation of pathogenic T cells, a mechanism shared by anti CD20+ monoclonal antibodies [29]. It is inevitable to comment on the differential effects of those antibodies versus BTKi in MS. A phase II trial with rituximab showed an almost immediate and robust cancellation of CNS inflammation, detected as soon as four weeks after the first dose, with a marked effect on relapses [1]; in contrast, only one dose of evobrutinib achieved a significant reduction on MRI lesions from weeks 12 to 24, with no effect on secondary outcomes [35]. Compared to evobrutinib, and even though direct comparisons are not feasible, evidence indicates that the anti-CD20+ monoclonal antibodies exert a level of disease control that is not reached by evobrutinib. However, regarding efficacy against MS, the available information is preliminary, making it difficult to draw conclusions on BTKi. We will only know the real impact of those compounds on MS after the completion of the currently ongoing phase III clinical trials. BTKi offers a series of good points for long-term therapy: ease of administration and access to the CNS for the effective control of inflammation and the possibility of utilization in conjunction with anti-CD20+ antibodies, not as combined therapy, as is used in B cell malignancies —in which different clinical protocols associate BTKi with rituximab (or other anti-CD20+ antibodies) [36]—but in sequence, as suggested by Torke et al. [29] Sequential use after B cell depletion with these antibodies would have the advantage of rapid inflammatory control, followed by drugs that inactivate B cells without significant effects on immunoglobulin levels.

4. Conclusions

Over the last three decades, immune therapy for MS has passed important milestones, effectively controlling clinical and MRI activity, even in patients with significantly active disease, slowing the accumulation of disability and delaying entry into the secondary progressive phase [37][38]. Despite modest advances with SPMS or PPMS, progression has been a stumbling block for therapy. Progression in MS is complex and is thought to depend on different factors, some of which are not well understood. Persistent inflammation within the CNS stands out as one of the candidate factors. Inflammation accompanies myelin damage alongside the evolution of the disease; the composition of the inflammatory infiltrate includes CD8+ T lymphocytes, B cells, activated microglia, and macrophages [39]. In chronic stages, BBB permeability is restored, preventing the entry of therapeutic agents. Compartmentalized or “trapped” inflammation [40] in the CNS has become an important challenge for therapy, and it may be a major contributor to myelin and axonal damage. Additionally, the presence of ectopic B cell follicle-like structures in the meninges are related not only to cortical damage [15][16] but also to spinal cord pathology [41].

For MS, the availability of a family of new drugs that can reach therapeutic concentrations inside the CNS, counteract the inflammation driven by B cells, and modulate the critical players in innate immunity, such as macrophages and microglia, is a therapeutic promise, because other disease-modifying drugs do not fulfill those aims. If these inhibitors effectively control persistent CNS inflammation, this could represent a great step toward the prevention of MS progression. We are currently in the preliminary—albeit exciting—stage of development, with many ongoing clinical trials hoping that the promise of BTKi will turn into reality. Intense clinical research will follow in the coming years.

References

1. Hauser, S.L.; Waubant, E.; Arnold, D.L.; Vollmer, T.; Antel, J.; Fox, R.J.; Bar-Or, A.; Panzara, M.; Sarkar, N.; Agarwal, S.; et al. B-Cell Depletion with Rituximab in Relapsing–Remitting Multiple Sclerosis. N. Engl. J. Med. 2008, 358, 676–688.
2. Kappos, L.; Hartung, H.-P.; Freedman, M.S.; Boyko, A.; Radü, E.W.; Mikol, D.D.; Lamarine, M.; Hyvert, Y.; Freudensprung, U.; Plitz, T.; et al. Atacicept in Multiple Sclerosis (ATAMS): A Randomised, Placebo-Controlled, Double-Blind, Phase 2 Trial. Lancet Neurol. 2014, 13, 353–363.
3. Hauser, S.L.; Bar-Or, A.; Comi, G.; Giovannoni, G.; Hartung, H.-P.; Hemmer, B.; Lublin, F.; Montalban, X.; Rammohan, K.W.; Selmaj, K.; et al. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N. Engl. J. Med. 2017, 376, 221–234.
4. Hauser, S.L.; Bar-Or, A.; Cohen, J.A.; Comi, G.; Correale, J.; Coyle, P.K.; Cross, A.H.; de Seze, J.; Leppert, D.; Montalban, X.; et al. Ofatumumab versus Teriflunomide in Multiple Sclerosis. N. Engl. J. Med. 2020, 383, 546–557.
5. Serreze, D.V.; Fleming, S.A.; Chapman, H.D.; Richard, S.D.; Leiter, E.H.; Tisch, R.M. B Lymphocytes Are Critical Antigen-Presenting Cells for the Initiation of T Cell-Mediated Autoimmune Diabetes in Nonobese Diabetic Mice. J. Immunol. Baltim. Md. 1950 1998, 161, 3912–3918.
6. Molnarfi, N.; Schulze-Topphoff, U.; Weber, M.S.; Patarroyo, J.C.; Prod’homme, T.; Varrin-Doyer, M.; Shetty, A.; Linington, C.; Slavin, A.J.; Hidalgo, J.; 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, 2921–2937.
7. Harp, C.T.; Ireland, S.; Davis, L.S.; Remington, G.; Cassidy, B.; Cravens, P.D.; Stuve, O.; Lovett-Racke, A.E.; Eagar, T.N.; Greenberg, B.M.; et al. Memory B Cells from a Subset of Treatment-Naïve Relapsing-Remitting Multiple Sclerosis Patients Elicit CD4+ T-Cell Proliferation and IFN-γ Production in Response to Myelin Basic Protein and Myelin Oligodendrocyte Glycoprotein. Eur. J. Immunol. 2010, 40, 2942–2956.

8. Bao, Y.; Cao, X. The Immune Potential and Immunopathology of Cytokine-Producing B Cell Subsets: A Comprehensive Review. J. Autoimmun. 2014, 55, 10–23.
9. Barr, T.A.; Shen, P.; Brown, S.; Lampropoulou, V.; Roch, T.; Lawrie, S.; Fan, B.; O’Connor, R.A.; Anderton, S.M.; Bar-Or, A.; et al. B Cell Depletion Therapy Ameliorates Autoimmune Disease through Ablation of IL-6-Producing B Cells. J. Exp. Med. 2012, 209, 1001–1010.
10. Bar-Or, A.; Fawaz, L.; Fan, B.; Darlington, P.J.; Rieger, A.; Ghorayeb, C.; Calabresi, P.A.; Waubant, E.; Hauser, S.L.; Zhang, J.; et al. Abnormal B-Cell Cytokine Responses a Trigger of T-Cell-Mediated Disease in MS? Ann. Neurol. 2010, 67, 452–461.
11. Chekol Abebe, E.; Asmamaw Dejenie, T.; Mengie Ayele, T.; Dagnew Baye, N.; Agegnehu Teshome, A.; Tilahun Muche, Z. The Role of Regulatory B Cells in Health and Diseases: A Systemic Review. J. Inflamm. Res. 2021, 14, 75–84.
12. Fillatreau, S.; Sweenie, C.H.; McGeachy, M.J.; Gray, D.; Anderton, S.M. B Cells Regulate Autoimmunity by Provision of IL-10. Nat. Immunol. 2002, 3, 944–950.
13. Duddy, M.; Niino, M.; Adatia, F.; Hebert, S.; Freedman, M.; Atkins, H.; Kim, H.J.; Bar-Or, A. Distinct Effector Cytokine Profiles of Memory and Naive Human B Cell Subsets and Implication in Multiple Sclerosis. J. Immunol. Baltim. Md. 1950 2007, 178, 6092–6099.
14. Serafini, B.; Rosicarelli, B.; Magliozzi, R.; Stigliano, E.; Aloisi, F. Detection of Ectopic B-Cell Follicles with Germinal Centers in the Meninges of Patients with Secondary Progressive Multiple Sclerosis. Brain Pathol. Zur. Switz. 2004, 14, 164–174.
15. Lucchinetti, C.F.; Popescu, B.F.G.; Bunyan, R.F.; Moll, N.M.; Roemer, S.F.; Lassmann, H.; Brück, W.; Parisi, J.E.; Scheithauer, B.W.; Giannini, C.; et al. Inflammatory Cortical Demyelination in Early Multiple Sclerosis. N. Engl. J. Med. 2011, 365, 2188–2197.
16. Howell, O.W.; Reeves, C.A.; Nicholas, R.; Carassiti, D.; Radotra, B.; Gentleman, S.M.; Serafini, B.; Aloisi, F.; Roncaroli, F.; Magliozzi, R.; et al. Meningeal Inflammation Is Widespread and Linked to Cortical Pathology in Multiple Sclerosis. Brain J. Neurol. 2011, 134, 2755–2771.
17. Lovato, L.; Willis, S.N.; Rodig, S.J.; Caron, T.; Almendinger, S.E.; Howell, O.W.; Reynolds, R.; O’Connor, K.C.; Hafler, D.A. Related B Cell Clones Populate the Meninges and Parenchyma of Patients with Multiple Sclerosis. Brain J. Neurol. 2011, 134, 534–541.
18. Von Büdingen, H.-C.; Kuo, T.C.; Sirota, M.; van Belle, C.J.; Apeltsin, L.; Glanville, J.; Cree, B.A.; Gourraud, P.-A.; Schwartzburg, A.; Huerta, G.; et al. B Cell Exchange across the Blood-Brain Barrier in Multiple Sclerosis. J. Clin. Invest. 2012, 122, 4533–4543.
19. Comi, G.; Bar-Or, A.; Lassmann, H.; Uccelli, A.; Hartung, H.; Montalban, X.; Sørensen, P.S.; Hohlfeld, R.; Hauser, S.L.; Expert Panel of the 27th Annual Meeting of the European Charcot Foundation. Role of B Cells in Multiple Sclerosis and Related Disorders. Ann. Neurol. 2021, 89, 13–23.

20. Sacco, K.A.; Abraham, R.S. Consequences of B-Cell-Depleting Therapy: Hypogammaglobulinemia and Impaired B-Cell Reconstitution. Immunotherapy 2018, 10, 713–728.
21. Rip, J.; Van Der Ploeg, E.K.; Hendriks, R.W.; Corneth, O.B.J. The Role of Bruton’s Tyrosine Kinase in Immune Cell Signaling and Systemic Autoimmunity. Crit. Rev. Immunol. 2018, 38, 17–62.
22. Honigberg, L.A.; Smith, A.M.; Sirisawad, M.; Verner, E.; Loury, D.; Chang, B.; Li, S.; Pan, Z.; Thamm, D.H.; Miller, R.A.; et al. The Bruton Tyrosine Kinase Inhibitor PCI-32765 Blocks B-Cell Activation and Is Efficacious in Models of Autoimmune Disease and B-Cell Malignancy. Proc. Natl. Acad. Sci. USA 2010, 107, 13075–13080.
23. Spaargaren, M.; Beuling, E.A.; Rurup, M.L.; Meijer, H.P.; Klok, M.D.; Middendorp, S.; Hendriks, R.W.; Pals, S.T. The B Cell Antigen Receptor Controls Integrin Activity through Btk and PLCγ2. J. Exp. Med. 2003, 198, 1539–1550.
24. Alankus, Y.; Grenningloh, R.; Haselmayer, P.; Bender, A.; Bruttger, J. BTK Inhibition Prevents Inflammatory Macrophage Differentiation: A Potential Role in MS. Mult. Scler. J. 2018, 24 (Suppl. S2), 264.
25. Raïch-Regué, D.; Grau-López, L.; Naranjo-Gómez, M.; Ramo-Tello, C.; Pujol-Borrell, R.; Martínez-Cáceres, E.; Borràs, F.E. Stable Antigen-Specific T-Cell Hyporesponsiveness Induced by Tolerogenic Dendritic Cells from Multiple Sclerosis Patients. Eur. J. Immunol. 2012, 42, 771–782.
26. Martin, E.; Aigrot, M.-S.; Grenningloh, R.; Stankoff, B.; Lubetzki, C.; Boschert, U.; Zalc, B. Bruton’s Tyrosine Kinase Inhibition Promotes Myelin Repair. Brain Plast. 2020, 5, 123–133.
27. Keaney, J.; Gasser, J.; Gillet, G.; Scholz, D.; Kadiu, I. Inhibition of Bruton’s Tyrosine Kinase Modulates Microglial Phagocytosis: Therapeutic Implications for Alzheimer’s Disease. J. Neuroimmune Pharmacol. 2019, 14, 448–461.
28. Liang, C.; Tian, D.; Ren, X.; Ding, S.; Jia, M.; Xin, M.; Thareja, S. The Development of Bruton’s Tyrosine Kinase (BTK) Inhibitors from 2012 to 2017: A Mini-Review. Eur. J. Med. Chem. 2018, 151, 315–326.
29. Torke, S.; Pretzsch, R.; Häusler, D.; Haselmayer, P.; Grenningloh, R.; Boschert, U.; Brück, W.; Weber, M.S. Inhibition of Bruton’s Tyrosine Kinase Interferes with Pathogenic B-Cell Development in Inflammatory CNS Demyelinating Disease. Acta Neuropathol. 2020, 140, 535–548.
30. Crofford, L.J.; Nyhoff, L.E.; Sheehan, J.H.; Kendall, P.L. The Role of Bruton’s Tyrosine Kinase in Autoimmunity and Implications for Therapy. Expert Rev. Clin. Immunol. 2016, 12, 763–773.
31. 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.

32. Weber, A.N.R.; Bittner, Z.; Liu, X.; Dang, T.-M.; Radsak, M.P.; Brunner, C. Bruton’s Tyrosine Kinase: An Emerging Key Player in Innate Immunity. Front. Immunol. 2017, 8, 1454.
33. Smith, P.F.; Owens, T.D.; Langrish, C.L.; Xing, Y.; Francesco, M.R.; Shu, J.; Hartmann, S.; Karr, D.; Burns, R.; Quesenberry, R.; et al. Phase 1 Clinical Trial of PRN2246 (SAR441268), a Covalent BTK Inhibitor Demonstrates Safety, CNS Exposure and Therapeutic Levels of BTK Occupancy. Mult. Scler. J. 2019, 5, 52.
34. Ruderman, E.M.; Pope, R.M. More than Just B-Cell Inhibition. Arthritis Res. Ther. 2011, 13, 125.
35. Montalban, X.; Arnold, D.L.; Weber, M.S.; Staikov, I.; Piasecka-Stryczynska, K.; Willmer, J.; Martin, E.C.; Dangond, F.; Syed, S.; Wolinsky, J.S. Placebo-Controlled Trial of an Oral BTK Inhibitor in Multiple Sclerosis. N. Engl. J. Med. 2019, 380, 2406–2417.
36. Burger, J.A. Bruton Tyrosine Kinase Inhibitors: Present and Future. Cancer J. 2019, 25, 386–393.
37. Trojano, M.; Pellegrini, F.; Fuiani, A.; Paolicelli, D.; Zipoli, V.; Zimatore, G.B.; Di Monte, E.; Portaccio, E.; Lepore, V.; Livrea, P.; et al. New Natural History of Interferon-β-Treated Relapsing Multiple Sclerosis. Ann. Neurol. 2007, 61, 300–306.
38. University of California, San Francisco MS-EPIC Team; Cree, B.A.C.; Hollenbach, J.A.; Bove, R.; Kirkish, G.; Sacco, S.; Caverzasi, E.; Bischof, A.; Gundel, T.; Zhu, A.H.; et al. Silent Progression in Disease Activity–Free Relapsing Multiple Sclerosis. Ann. Neurol. 2019, 85, 653–666.
39. Frischer, J.M.; Bramow, S.; Dal-Bianco, A.; Lucchinetti, C.F.; Rauschka, H.; Schmidbauer, M.; Laursen, H.; Sorensen, P.S.; Lassmann, H. The Relation between Inflammation and Neurodegeneration in Multiple Sclerosis Brains. Brain J. Neurol. 2009, 132, 1175–1189.
40. Lassmann, H.; van Horssen, J.; Mahad, D. Progressive Multiple Sclerosis: Pathology and Pathogenesis. Nat. Rev. Neurol. 2012, 8, 647–656.
41. Reali, C.; Magliozzi, R.; Roncaroli, F.; Nicholas, R.; Howell, O.W.; Reynolds, R. B Cell Rich Meningeal Inflammation Associates with Increased Spinal Cord Pathology in Multiple Sclerosis. Brain Pathol. 2020, 30, 779–793.