Bruton Tyrosine Kinase Inhibition in Multiple Sclerosis

The missing link for treatment optimization?

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We explore here a novel class of disease-modifying therapies (DMTs): the Bruton tyrosine kinase inhibitors (BTKIs), which might be the missing link in the management of MS, targeting both innate and adaptive arms of the immune system. Decades of neuroimmunologic research substantiate the role of innate and adaptive immunity in the pathophysiology of relapsing and progressive courses of multiple sclerosis (MS). Immune cell numbers and locations depend on disease stage. It is now evident that the neuroimmune crosstalk in MS occurs not only across an open blood-brain barrier (BBB) but also behind a repaired barrier.[1,2] In this latter case, activated macrophages, microglia, and B lymphocytes play a central function. A previously immune-privileged central nervous system (CNS) in MS is now "hiding in plain sight."[2]

The Immune System in MS

Adaptive immunity has a fundamental role in the relapsing forms of MS. Specifically, autoreactive T cells go amiss secondary to Foxp3-T–cell dysregulation,[3] resulting in T-cell maturation and proliferation in response to unknown extrinsic or intrinsic antigens.[3] The resulting activated T lymphocytes engage B lymphocytes, which are antigen-presenting cells, to cause B-cell maturation and differentiation into antibody-secreting plasma cells. Both B and T lymphocytes cross the BBB to enter the CNS where they damage myelin, oligodendrocytes, and astroglia in the prephagocytic stage and release more proinflammatory molecules, which facilitates migration of other monocytes and macrophages from the periphery to initiate phagocytosis. Within the brain, migrant and meningeal B lymphocytes amplify inflammation via their antigen-presenting capacity and antibody production, causing cortical demyelination.[3]

In the progressive course of MS, compartmentalized inflammation likely confers resistance to highly effective DMTs available for relapsing MS.[1] Macrophages and microglia, essential players in innate immunity, are a sine qua non for disease progression.[4] Several mechanisms contribute to progression. First, slowly expanding lesions (SELs), or *smoldering plaques*, are associated with axonal damage. SELs are characterized by a thin rim of activated microglia, containing iron and myelin debris surrounding a center almost completely devoid of macrophages/microglia. Second, meningeal follicles rich in B cells are responsible for subpial cortical demyelination, which has now been observed in both early and progressive forms of MS and neuronal degeneration. Third, widespread activated microglia or microglial nodules, an early finding in nonlesional MS, are overrepresented in progressive disease and associated with increased axonal transection. Finally, immune cells in these processes release multiple neurotoxic molecules, including cytokines, proteases, free radicals, and antibodies, to cite a few. Specifically, activated macrophages and microglia release oxygen and nitrogen species that damage mitochondria, resulting in hypoxia with oligodendroglia and neuronal demise.[1-4]

BTKIs

Bruton TK (BTK) is a nonreceptor, cytoplasmic tyrosine kinase (TK) that phosphorylates tyrosine residues with a phosphate group from adenosine triphosphate (ATP) (Figure A).[5] BTK is a signal transducer of various B cell receptors (BCRs), pattern recognition receptors (PRRs), chemokines, cytokines, and Fc receptors present on all immune cells except T lymphocytes. Antigen binding to the BCR leads to BTK activation, which in turn, generates 2 pathways that regulate the gene expression required for B-cell proliferation, maturation, differentiation, and chemokine/cytokine expression. In MS, the detrimental effects of activated macrophages and microglia, such as myelin phagocytosis and cytokine secretion, are mediated via the Fc receptor and tumor necrosis factor α (TNFα) via toll-like receptor-4 (TLR-4) on the cell surface with subsequent BTK activation,[5,6] suggesting BTK inhibition as a therapeutic approach in relapsing and progressive MS.

The BTKIs ibrutinib, evobrutinib, tolebrutinib, and orelabrutinib bind BTK irreversibly by a covalent bond at cysteine 481 (C481). Others, such as fenebrutinib bind reversibly to BTK via hydrogen and ionic bonds or hydrophobic interactions.[5] Pharmacologic properties of the BTKIs being studied for potential treatment of MS are summarized in the Table.[5,7] BTKIs are lipophilic small molecules, that can be orally administered.

Ibrutinib was the first BTKI approved by the Food and Drug Administration (FDA) for the indication of chronic

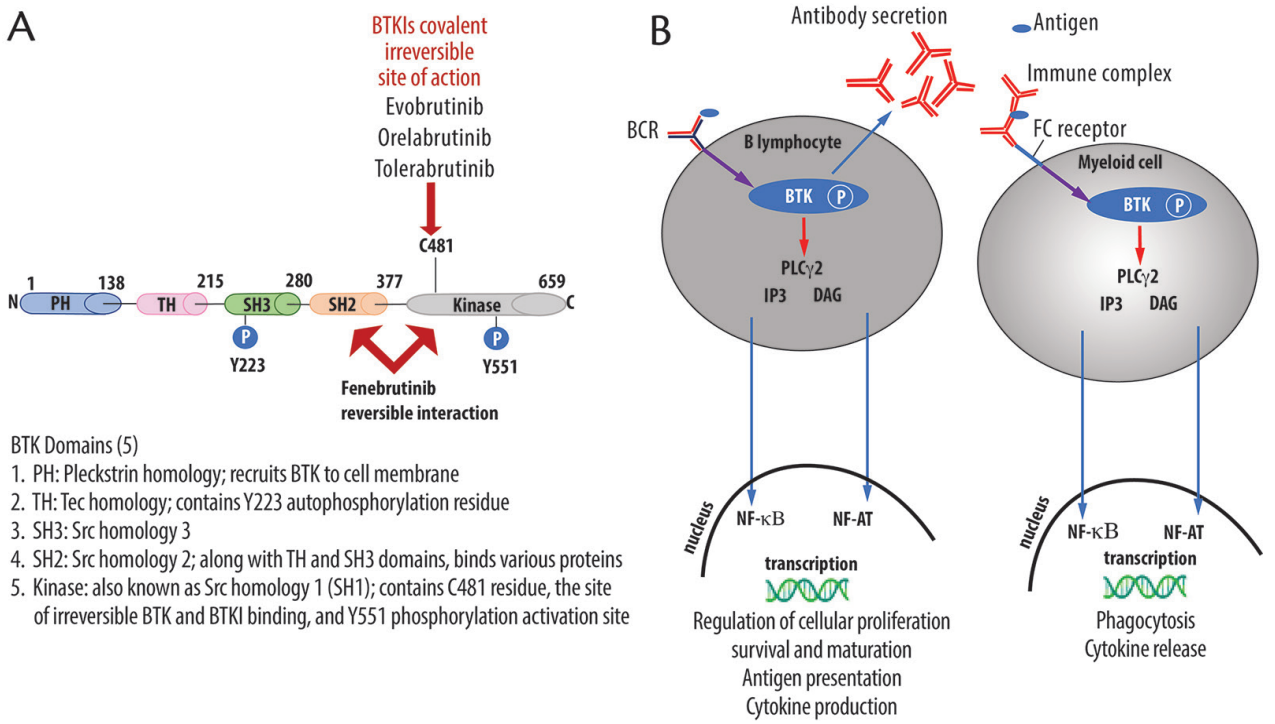


Figure. The structure and domains of BTK and binding sites for BTKIs (A) that block the mechanism (B) by which BTKs activate innate and adaptive immunity. Abbreviations: BCR, B cell receptor; BTK, Bruton tyrosine kinase; BTKI, BTK inhibitor; NF-AT, nuclear factor activated T-cell; NF-κB, nuclear factor kappa B.

lymphocytic leukemia and mantle cell lymphoma. Despite its effectiveness for B-cell malignancies, resistance and off-target inhibition of other kinases are relative drawbacks of ibrutinib.[5,7] Genetic polymorphisms in the BTK kinase domains that affect C481 or other areas of the SH1 domain (Figure) can disrupt the covalent bond between BTK and ibrutinib, conferring resistance.[5] Irreversible binding of BTKIs to other kinases with similar cysteine residues can cause off- target complications, such as cardiac arrhythmias, bleeding rash, hypertension, diarrhea, infections, and arthralgias,[7] which may be more frequent with certain comorbidities and concomitant medications.[8]

Second-generation covalent and irreversible BTKIs (eg, evobrutinib, tolebrutinib, and orelabrutinib) selectively phosphorylate BTK over other kinases. Tolebrutinib is a CNS-penetrant BTKI by design,[9] although evobrutinib and fenebrutinib also cross the BBB. The noncovalent reversible BTKI fenebrutinib is less susceptible to resistance and off-target side effects.[5]

BTKIs in Other Autoimmune Diseases

Fenebrutinib has shown clinical effectiveness in treating B-cell malignancies and rheumatoid arthritis (RA)[10] and in a phase 2 trial for chronic spontaneous urticaria.[11] Notably, evobrutinib was not shown effective in a trial for RA,[12] and neither fenebrutinib nor evobrutinib were effective for systemic lupus erythematosus (SLE), which could be due to confounding features of background immunosuppression with oral glucocorticoids[13,14] or indicate that BTKIs are not useful for SLE.

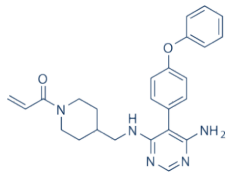
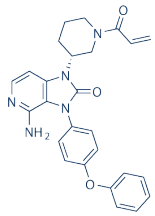
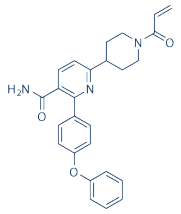
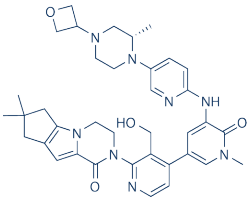
In the phase 2 trial of fenebrutinib vs placebo or adalimumab in participants with RA who had incomplete response to methotrexate, the most common adverse events with fenebrutinib were nausea, headache, anemia, and upper respiratory tract infections. The risk of infections was low and similar across all treatment groups. Transient grade 3 transaminase elevations were similar to what has been observed in other phase 2 trials of BTKIs.[10]

Preclinical Studies of BTKIs Relevant to MS

Coculture of ex vivo B lymphocytes from mice pretreated with evobrutinib and naïve T cells from a TCR transgenic mouse model specific for MOG 35-55 peptide had decreased T-cell proliferation, demonstrating that evobrutinib-treated B lymphocytes reduced encephalitogenic T cells. In addition, B cells from naïve mice pretreated with evobrutinib inhibited BCR-mediated B-cell activation and production of proinflammatory cytokines, such as interferon γ. Similar findings occurred with murine and human B lymphocytes treated with evobrutinib.[6] Tolebrutinib inhibited BTK activity in human microglia and Ramos cells (ie, B lymphocytes from Burkitt lymphoma).[9]

Several BTKIs have been tested in experimental allergic encephalomyelitis (EAE), an animal model of MS, with ben-

TABLE. COMPARISON OF BRUTON TYROSINE KINASE INHIBITOR PHARMACOLOGY

	Evobrutininb (M-251) (PRN2246)	Tolebrutinib (SAR442168)	Orelabrutinib (ICP-022)	Fenebrutinib (GDC-0853)
Structure				
Molecular weight	429.51[24]	455.51[24]	427.9[25]	664.80[24]
Chemical bond with BTK10	Covalent, irreversible	Covalent, irreversible	Covalent, irreversible	Noncovalent, reversible
Inhibition site	Kinase domain C481 residue	Kinase domain C481 residue	Kinase domain C481 residue	SH2 domain K430 residue, kinase domain M 477 and D539 residues
IC50 (nM)[a]	37.97	0.4-0.79	1.6	2.37
Inhibition of other tyrosine kinases	Minimal, targets BTK selectively[7]	Binds 12 of 250 tyrosine kinases at 1 mcMol[9]	Best selectivity, BTK only; > 90% inhibition[25]	Targets 2 of 286 kinases[7]

Abbreviations: BTK, Bruton tyrosine kinase; BTKI, BTK inhibitor; IC50, half-maximal concentration. [a]The IC50 for the BTKIs of interest vary depending on the type of used cells to determine the inhibition constant; however different papers report comparable values.

eficial results. In addition to ameliorating disease course and histologic findings, evobrutinib reduced B-cell antigen presentation (by decreasing B-cell expression of major histocompatibility complex II [MHCII]), T-lymphocyte numbers, and levels of proinflammatory cytokines, as well as encephalitogenic T cells.[6] Additionally, evobrutinib decreased B-lymphocyte maturation and differentiation in the lymph nodes without affecting their number. Evobrutinib has also been shown to promote remyelination in both a transgenic-tadpole and a mouse-derived cerebellar organotypic culture model of demyelination.[5] Tolebrutinib and fenebrutinib decreased EAE severity in mice in a dose-dependent manner.[5,15,16] Other BTKIs not currently being studied in humans have decreased microglial activation, T-cell differentiation, TNFα production, astrocyte proliferation, and myelinotoxicity in the EAE model of MS.[5]

Clinical Studies of BTKIs in MS

Evobrutinib

In a multicenter, international, double-blind, placebo-controlled phase 2 trial, participants with relapsing MS who were treated with oral evobrutinib (75 mg once daily) for 24 weeks had significantly fewer T1 gadolinium-enhancing (Gd^+) lesions over weeks 12 to 24 compared with those who received placebo. However, no significant difference in T1 Gd^+ lesions was seen with 25 mg once daily or 75 mg twice daily of evobrutinib vs placebo.[17] During an open-label extension (OLE) phase, participants were treated with 25 mg evobrutinib daily. No significant difference in annualized relapse rate (ARR) occurred with evobrutinib vs placebo at weeks 24 or 48; however, for those who received 75 mg twice daily in the double-blind portion of the trial, ARR was 0.08 and 0.11 at 24 and 48 weeks, respectively and maintained at 0.12 over 108 weeks in the OLE phase.[18] Although this trial was not designed to compare evobrutinib to the positive comparator dimethyl fumarate (DMF), 74% to 87% of participants who took evobrutinib vs 89% of those who took DMF were relapse free at 24 weeks.

In post hoc analysis of data from 166 participants, treatment with evobrutinib was associated with relative reductions in a neurofilament light chain,[19] a marker of neuronal degeneration.[20] Individuals treated with 75 mg evobrutinib once daily vs placebo had reductions of 15.4% (P=.043) and 14.1% (P=.10) at weeks 12 and 24, respectively. Treatment with evobrutinib 75 mg twice daily vs placebo was associated with relative NfL reduction by 18.9% (P=.010) and 16.8% (P=.040) at weeks 12 and 24, respectively. Post hoc analysis also showed reduced SEL volume after 48 weeks of treatment with evobrutinib vs placebo in a dose-dependent manner, with the highest effect at 75 mg twice daily (P=.047). This effect was especially apparent in a subgroup analysis of participants with higher disability.[21]

Taken together, these results suggest that evobrutinib affects acute and chronic neuroinflammation that results in worsening disability, complementing the findings from preclinical studies.

Safety and Tolerability. In the phase 2 trial,[17] the highest rate of serious adverse events (SAEs) occurred with evobrutinib 75 mg twice daily. Grade 3 or 4 adverse events were reported with either 75 mg daily or twice daily and included nasopharyngitis and increases in alanine aminotransferase

(ALT), aspartate aminotransferase (AST), and lipase levels. Liver enzyme elevation did not fulfill the Hy's Law FDA definition of drug-induced hepatotoxicity, however, and no elevation in liver enzymes was seen in the open-label phase.[22] Grade 1 and 2 lymphopenias were similar with evobrutinib or placebo, but higher with DMF. Overall, there was no increase in infections or safety issues over the 108-week study period.

Ongoing Trials. There are 2 identical multicenter randomized, double-blind phase 3 trials[a] underway comparing evobrutinib to teriflunomide in relapsing-remitting MS. Target enrollment is 930 people and the primary endpoint is ARR after 96 weeks of treatment. Time to 12- or 24-week confirmed progression on the Expanded Disability Status Scale (EDSS) over 96 weeks; the number of Gd^+ T1 lesions and new or enlarging T2 lesions at 24, 48, and 96 weeks; and change from baseline on the Patient-Reported Outcomes Measurement Information System (PROMIS) MS Physical Function and PROMIS Fatigue MS Short Form scores will also be measured.

Tolebrutinib

In an international, multicenter, double-blind, placebo-controlled crossover phase 2 study, participants were randomly assigned to 1 of 2 cohorts. Participants in each cohort were then randomly assigned to receive 1 of 4 doses of tolebrutinib (5, 15, 30, or 60 mg/day). The first cohort had 12 weeks of tolebrutinib treatment after 4 weeks of placebo, whereas the second cohort had 4 weeks of placebo and then 12 weeks of tolebrutinib.[23] On brain MRI, done every 4 weeks, there was a dose-dependent decrease in the number of Gd^+ T1 lesions and new or enlarging T2 lesions, with a relative reduction of 85% and 89%, respectively, with tolebrutinib 60 mg vs placebo. Neither clinical relapses nor disability progression were assessed because of the short duration of the trial. Headache was the most common side effect among all groups. Although a single patient was admitted to the hospital due to an MS relapse, there were no adverse events necessitating treatment discontinuation.

Ongoing Trials. There are 2 subsequent phase 3 trials comparing the effects of tolebrutinib vs teriflunomide on ARR in a combined total of 1,800 participants with relapsing-remitting MS.[b] Additionally, there are 2 double-blind placebo-controlled studies of tolebrutinib vs placebo in primary progressive[c] and nonrelapsing secondary progressive MS.[d] The primary outcome of both studies, enrolling 990 and 1290 patients, respectively, is time to 6-month clinical disability progression (CDP).

Fenebrutinib

Based on phase 2 trial data of other BTKIs in MS, there are 2 ongoing phase 3 clinical trials of fenebrutinib vs teriflunomide for relapsing-remitting and active secondary progressive MS, with 736 participants in each trial.[e] Another multicenter, randomized, double-blind, double-dummy, parallel-group trial is comparing the efficacy and safety of fenebrutinib with ocrelizumab as an active comparator in 946 participants with primary progressive MS.[f] The primary endpoint in all 3 of these trials is a 12-week composite score for confirmed disability progression (CDP-12), with a coprimary endpoint of ARR in the trials comparing fenebrutinib with teriflunomide in relapsing-remitting and active secondary progressive MS.

Orelabrutinib

A phase 2 randomized, double-blind, placebo-controlled trial comparing orelabrutinib with placebo in 160 participants with relapsing-remitting MS is also ongoing.[g] In the core portion of this trial, participants will be randomly assigned to receive placebo or 1 of 3 doses of orelabrutinib for 24 weeks followed by an open-label treatment period.

Conclusions

BTKIs are newcomers to the MS treatment landscape and might be the much-anticipated molecule with clinically meaningful results for progressive MS, targeting compartmentalized inflammation, B lymphocytes, activated macrophages/microglia, and perhaps some yet to be identified player(s) in MS pathophysiology. Knowing that cortical demyelination is an early and late finding in MS, BTKIs may be "game changers" as these molecules may be even more impactful than available DMTs for relapsing forms.[4] The field of MS has abstained from using combination therapies but, in oncology, some protocols combine BTKIs with rituximab.[5] Combination therapies with a highly effective antiinflammatory DMT and a BTKI might be the future of MS management, although such a combination might increase the risk of complications.[5] It is also possible that lessons learned from using BTKIs in cancer and MS can be used in the future to manufacture even safer or more tolerable BTKIs More than a decade of intensive research in oncology has refined the pharmacologic and structural properties of BTKIs to improve effectiveness and minimize serious adverse events. Whether the BTKIs in phase 3 trials going will hold this promise is something only time will tell.[7,8]

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a Study of evobrutinib in participants with RMS 1 and 2 (NCT04338022 and NCT04338061)
b Relapsing forms of multiple sclerosis (RMS) study of Bruton's tyrosine kinase (BTK) inhibitor tolebrutinib (GEMINI) 1 (NCT04410978) and 2 (NCT04410991)
c Primary progressive multiple sclerosis (PPMS) study of Bruton's tyrosine kinase (BTK) inhibitor tolebrutinib (PERSEUS)(NCT04458051)
d Nonrelapsing secondary progressive multiple sclerosis (NRSPMS) study of Bruton's tyrosine kinase (BTK) inhibitor tolebrutinib (HERCULES)(NCT04411641)
e Study to evaluate the efficacy and safety of fenebrutinib compared with teriflunomide in relapsing multiple sclerosis (FENhance) 1 (NCT04586023) and 2 (NCT04586010)
f A study to evaluate the efficacy and safety of fenebrutinib compared with ocrelizumab in adult participants with primary progressive multiple sclerosis (FENtrepid) (NCT04544449)
g A phase 2 study of orelabrutinib in patients with relapsing-remitting multiple sclerosis (NCT04711148)

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