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Monograph

Tolebrutinib

Rec INN; USAN

Bruton tyrosine kinase (BTK) inhibitor
Treatment of multiple sclerosis

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Summary

Despite the demonstrated efficacy of B cell-depleting treatments in patients with multiple sclerosis (MS), concern has emerged regarding long-term safety and tolerability of such therapies, in terms of increased risk of immune functioning impairment. Hence, the need for safer and more sustainable treatments has fostered the search of different approaches for B-cell targeting, the most promising of which being the inhibition of Bruton tyrosine kinase (BTK). BTK is a crucial enzyme for the signaling pathways that regulate B-cell and myeloid cell (including microglia) activation, proliferation and homeostasis. Several studies have shown that BTK inhibitors (BTKi) are effective treatments in vitro and in animal models of MS. Tolebrutinib, a small, orally available, irreversible BTKi, is a promising molecule as it exerts its function both in the periphery and the central nervous system, thus having the potential to address new pathogenetic targets such as chronic active lesions. A phase I study on healthy volunteers has shown good tolerability of the treatment and only mild adverse events. These data have been confirmed by a phase II trial on relapsing MS patients, which also showed a dose-dependent reduction in new gadolinium-enhancing lesions at MRI scans. Confirmatory larger phase III trials are ongoing.

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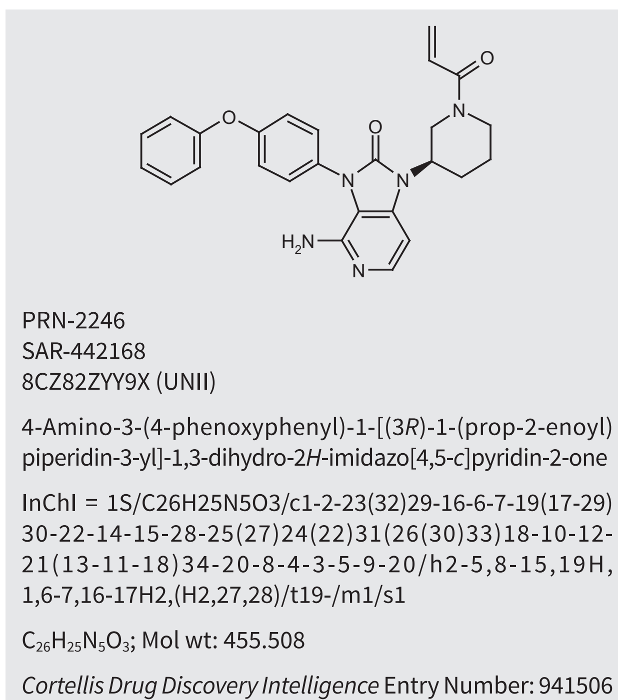


PRN-2246
SAR-442168
8CZ82ZYY9X (UNII)

4-Amino-3-(4-phenoxyphenyl)-1-[(3*R*)-1-(prop-2-enoyl)piperidin-3-yl]-1,3-dihydro-2*H*-imidazo[4,5-*c*]pyridin-2-one

InChI = 1S/C26H25N5O3/c1-2-23(32)29-16-6-7-19(17-29)30-22-14-15-28-25(27)24(22)31(26(30)33)18-10-12-21(13-11-18)34-20-8-4-3-5-9-20/h2-5,8-15,19H,1,6-7,16-17H2,(H2,27,28)/t19-/m1/s1

$C_{26}H_{25}N_5O_3$; Mol wt: 455.508

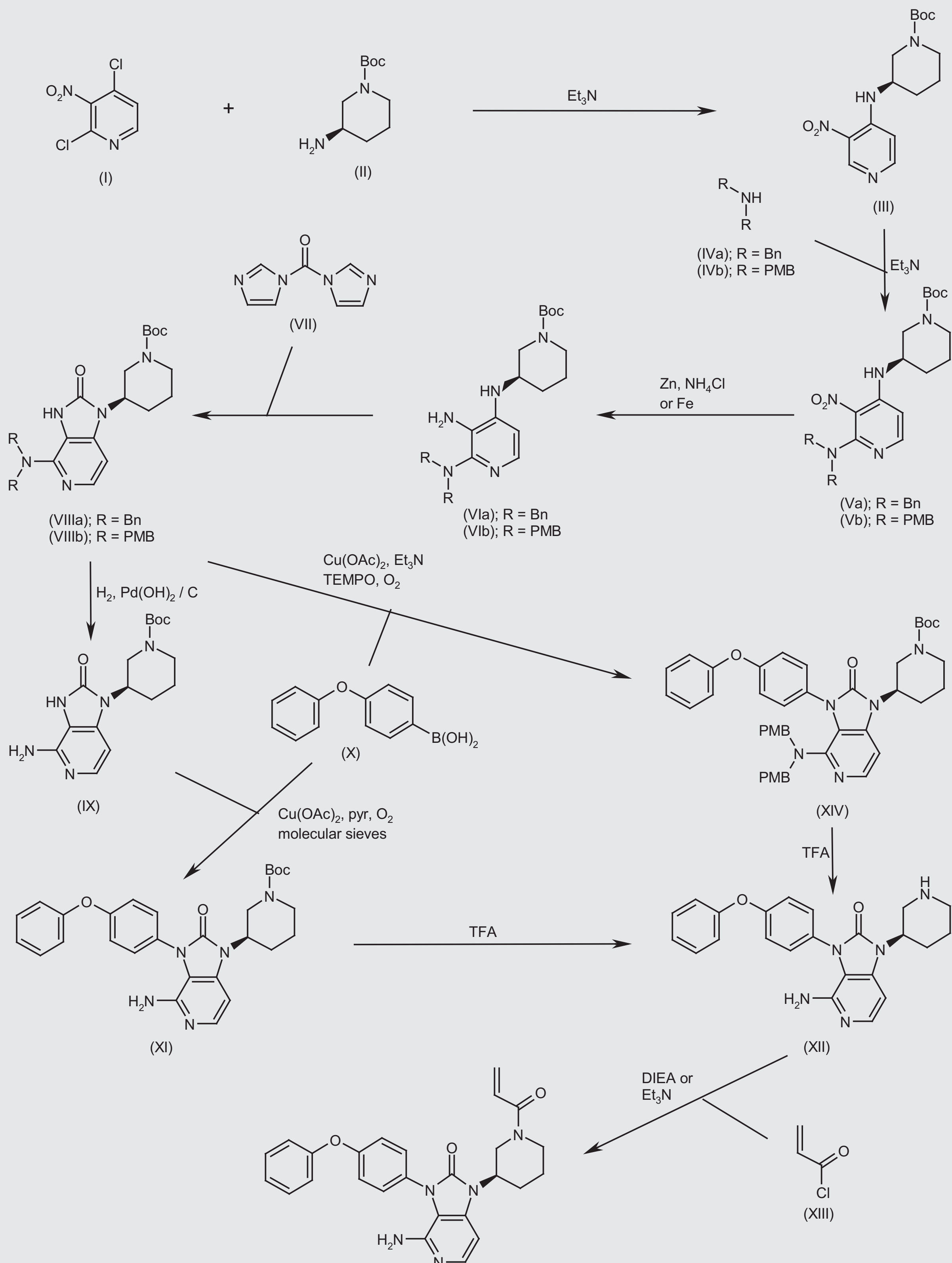
Cortellis Drug Discovery Intelligence Entry Number: 941506

Key words: Tolebrutinib – PRN-2246 – SAR-442168 – Bruton tyrosine kinase – Multiple sclerosis

Synthesis*

Tolebrutinib (PRN-2246, SAR-442168) can be produced by coupling of 2,4-dichloro-3-nitropyridine (I) with 1-Boc-3(*R*)-aminopiperidine (II) using Et_3N in DMF to give secondary amine (III) (1, 2), which upon coupling with dibenzylamine (IVa) (1) or *N*,*N*-bis(4-methoxybenzyl)amine (IVb) (2) in the presence of Et_3N in *i*-PrOH at 95 °C or acetonitrile affords the respective tertiary amines (Va) (1) or (Vb) (2). Reduction of dibenzyl 3-nitro-2-pyridinamine derivative (Va) with Zn and NH_4Cl in MeOH at 50 °C (1) or bis-PMB pyridinamine derivative (Vb) by means of Fe in AcOH/MeOH (2) provides 3-aminopyridine derivative (VIa) (1) or (VIb) (2), which are then cyclized with carbonyldiimidazole (VII) in acetonitrile or THF to generate 1,3-dihydroimidazo[4,5-*c*]pyridin-2-one derivatives (VIIIa) (1) or (VIIIb) (2). Debenzylation of

Scheme 1. Synthesis of Tolebrutinib



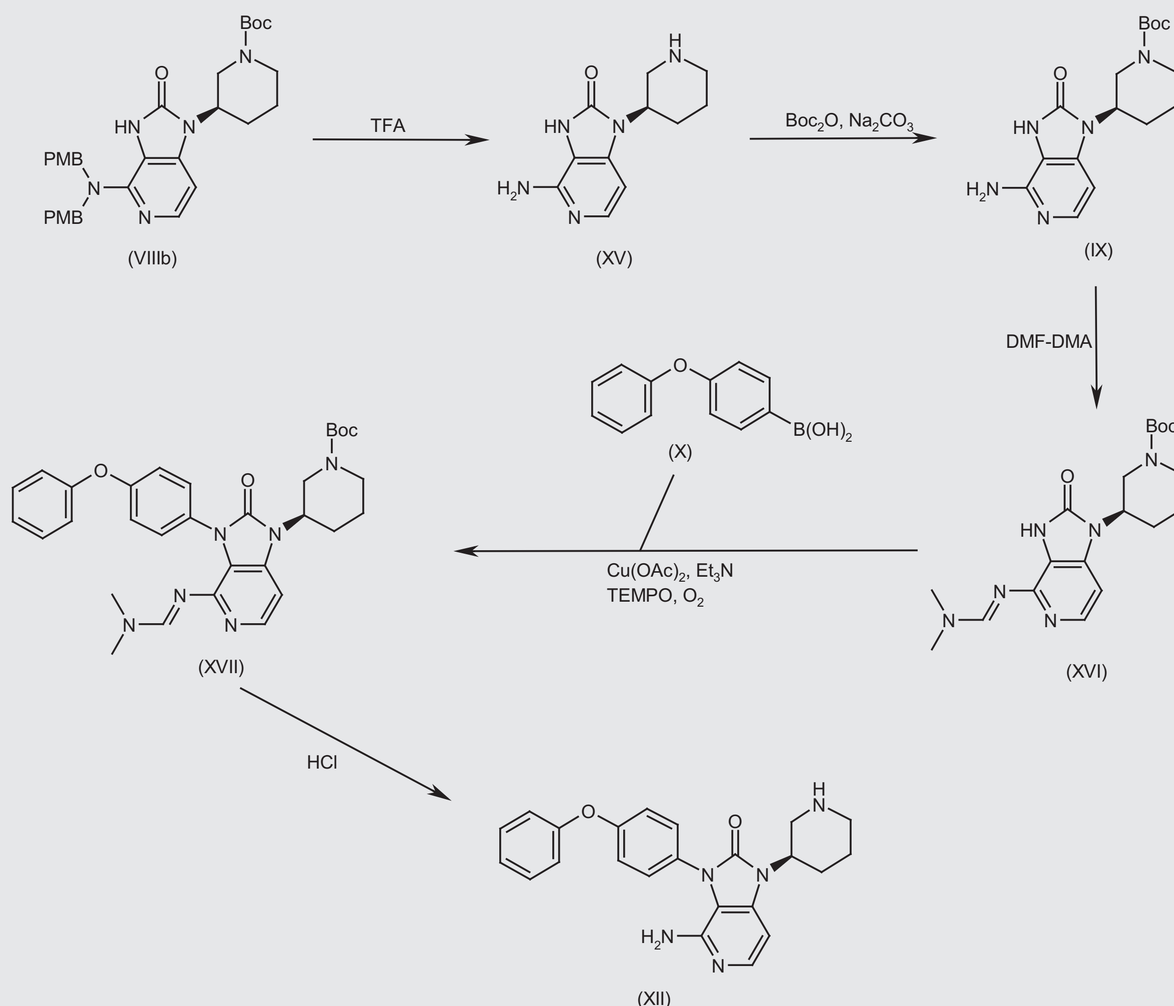
compound (VIIIa) using H_2 over $Pd(OH)_2/C$ in EtOAc at 60 °C yields amide (IX), which upon Chan-Lam coupling with 4-phenoxyphenylboronic acid (X) by means of $Cu(OAc)_2$, pyridine, atmospheric O_2 and molecular sieves in DMF generates compound (XI). N-Deprotection of carbamate (XI) using TFA in CH_2Cl_2 gives piperidine derivative (XII) (1). Alternatively, intermediate (II) can be also produced by Chan-Lam coupling of 1,3-dihydroimidazo[4,5-*c*]pyridin-2-one (VIIIb) with 4-phenoxyphenylboronic acid (X) by means of $Cu(OAc)_2$, Et_3N, TEMPO and O_2 in CH_2Cl_2 to provide 3-(4-phenoxyphenyl)imidazo[4,5-*c*]pyridin-2-one derivative (XIV), which upon deprotection using TFA in CH_2Cl_2 at 50 °C affords the corresponding amine (XII). Finally, intermediate (XII) is subjected to N-acylation with acryloyl chloride (XIII) in the presence of DIEA in CH_2Cl_2 or Et_3N in CH_2Cl_2/MeOH at 0 °C (1-3). Scheme 1.

Alternative synthesis of intermediate (XII)

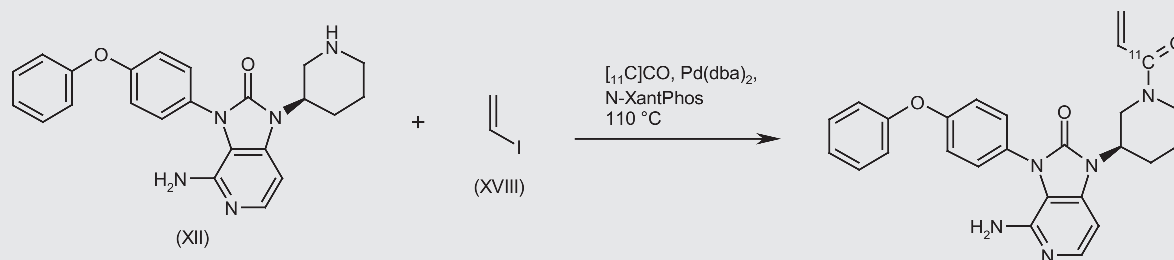
N-Deprotection of 1,3-dihydroimidazo[4,5-*c*]pyridin-2-one derivative (VIIIb) by means of TFA in CH_2Cl_2 gives 4-amino-1-[3(*R*)-piperidyl]-imidazo[4,5-*c*]pyridin-2-one (XV), which upon N-protection with Boc_2O in the presence of Na_2CO_3 in dioxane/H_2O affords carbamate (IX). Coupling of amine (IX) with DMF-DMA yields the corresponding imine (XVI), which is subjected to Chan-Lam coupling with 4-phenoxyphenylboronic acid (X) by means of $Cu(OAc)_2$, Et_3N, TEMPO and O_2 in CH_2Cl_2 to yield 3-(4-phenoxyphenyl)-imidazo[4,5-*c*]pyridin-2-one derivative (XVII). N-Deprotection of compound (XVII) by means of HCl in dioxane at 50 °C furnishes the corresponding amine (XII) (2). Scheme 2.

On the other hand, [^{11}C]tolebrutinib is synthesized by radiocarbonylation of 4-amino-3-(4-phenoxyphenyl)-1-[3(*R*)-

Scheme 2. Synthesis of Intermediate (XII)



Scheme 3. Synthesis of [^{11}C]Tolebrutinib



piperidyl]imidazo[4,5-*c*]pyridin-2-one (XII) with iodoethylene (XVIII) and [^{11}C]CO (obtained by reduction of [^{11}C]CO_2, produced in a MC17 cyclotron, over molybdenum) by means of Pd(dba)$_2$ and *N*-XantPhos in THF 110 °C (4). Scheme 3.

Background

Multiple sclerosis (MS) is an immune-mediated, chronic disease of the central nervous system (CNS) whose pathogenetic hallmarks are inflammation, demyelination and neurodegeneration, including neuronal damage and axonal transection (5). The disease course is variable over time, with the most frequent clinical phenotype at onset being relapsing–remitting MS (RRMS, 85% of cases), characterized by monophasic acute-subacute neurological episodes lasting at least 24 h (relapses) and suggestive of an inflammatory event of the CNS. The neuropathological counterpart of a relapse is the evidence of new or expanding demyelinating lesions in the CNS. Relapses can evolve toward complete recovery, but they can also leave permanent disability. A variable proportion of patients with RRMS ranging from 20% to 75% develop progressive neurologic decline later in the disease and transition to secondary progressive MS (SPMS). Around 10-15% of individuals show progression since the disease onset (primary progressive MS, PPMS) (6), with accumulation of neurological disability that evolves independently from possible relapses.

Although the precise triggers driving the disease are still undetermined, it is widely accepted that MS is a complex disorder, in which environmental, genetic and immunological factors are deeply intertwined (7). Particularly, in recent years the role of both the innate and adaptive arms of the immune system has been extensively investigated in preclinical and clinical studies, and the immunopathogenetic model of MS has dramatically evolved over the last 10-15 years. The classical view that MS is associated with an inflammatory T-helper cell profile—inherited from animal models of demyelinating disorders such as experimental autoimmune encephalomyelitis (EAE) (8)—has been challenged by the remarkable results of the novel selective B cell-targeting therapies, thus suggesting a role for these cells in MS pathogenesis.

Several aberrant B-cell responses have been identified in patients with MS (pwMS) in recent years. The presence of an enhanced immunoglobulin G (IgG) intrathecal synthesis in the form of oligoclonal bands (OCBs) is a pivotal finding in pwMS, and it is included in the most recent revision of the McDonald diagnostic criteria for MS (9). The finding of lipid-specific IgM OCB is a predictor of a worse disease course (10). B cells could also exert their pathogenic role in MS through an antibody-independent mechanism, which is related to the production of proinflammatory cytokines (i.e., interleukin-6 [IL-6], granulocyte-macrophage colony-stimulating factor, tumor necrosis factor and lymphotoxin-α) and to the deficient release of regulatory cytokines as IL-10. The aberrant cytokine response could boost Th_1, Th_{17} and myeloid cell responses toward the cellular immune cascade involved in the genesis of demyelinating plaques and clinical relapses (11). B lymphocytes are also potent antigen-presenting cells that can enhance T-cell-mediated autoimmunity, due to the recognition of specific protein antigens for which they express surface Ig receptors (12). Moreover, neuropathological studies have shown that B cells infiltrate CNS lesions in MS: They appear in the perivascular cuff as well as in meninges; particularly, meningeal inflammation has been associated with a gradient of neuronal, astrocyte and oligodendrocyte loss from the surface inwards in cortical gray matter (13). B cells can organize in meningeal lymphoid follicle-like structures in patients with SPMS, and tend to be associated with a worse disease course (14).

In the last 10-15 years, several therapeutic options that target B cells have emerged for MS; all of them are monoclonal antibodies (MAbs) that address the B-cell antigen CD20. The chimeric antibody rituximab, the humanized ocrelizumab and the fully human ofatumumab share a similar mechanism of action, and they all ultimately induce a sustained B-cell lineage depletion, which turns in suppression of

relapses and of radiological disease activity, as well as in potentially slowing down disease progression (15).

Despite these outstanding achievements, concerns have emerged on the use of B cell-depleting agents for lifetime treatment, particularly regarding the long-term impairment of immune system functioning. Indeed, many studies have highlighted the progressive reduction of circulating antibodies, probably related to the impairment of de novo development of antibody-producing cells (16, 17). Hence, in recent years the need for new B-cell-targeted treatments that are more sustainable over time and with reversible effects has emerged. In this context, one of the most promising approaches is the therapeutic inhibition of Bruton tyrosine kinase (BTK), which plays a crucial role in B-cell receptor (BCR) signaling and, consequently, in B-cell activation (18). Several clinical trials on BTK inhibitors for MS treatment are ongoing, including evobrutinib, fenebrutinib and tolebrutinib. The present review briefly summarizes the structural and functional features of BTK as well as the immunological and clinical implications of its therapeutical inhibition, and it focuses on the preclinical and clinical studies involving the BTK inhibitor tolebrutinib in MS treatment.

BTK Structure and Functions

Tyrosine kinases

Tyrosine kinases (TKs) are a family of intracellular enzymes that catalyze ATP-mediated tyrosine phosphorylation in target proteins that are involved in specific signal transduction pathways. Reversible phosphorylation is a key mechanism for many cellular processes that control cell proliferation, differentiation, migration, survival and apoptosis. TKs are classified as receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). RTKs encompass an extracellular domain that binds to a specific ligand, a transmembrane domain, and an intracellular catalytic domain, which phosphorylates different substrates leading to signal transduction (19). NRTKs are intracellular TKs that do not show a direct function in sensing extracellular signals, as they lack receptor-like features; however, they are critical components in the regulation of the immune system, particularly of B and T cells (20).

In 1952, American pediatrician Ogden Bruton described X-linked agammaglobulinemia (XLA), an inherited condition characterized by the absence of antibodies that led to recurrent bacterial infections and sepsis in early childhood. The gene responsible for XLA was identified in 1993, and it was named Bruton's tyrosine kinase. BTK was found to be an NRTK that is crucial for B-cell signaling (21), as it regulates B-cell survival, activation, proliferation and differentiation to antibody-producing plasma cells.

BTK structure

BTK is a member of the TEC (TK expressed in the hepatocellular carcinoma) family of kinases, comprising 5 TKs, all belonging to the NRTK group. The expression of these kinases is mainly related to the hematopoietic system (22). BTK is a 659-amino-acid enzyme encompassing 5 protein domains from the N-terminal: pleckstrin homology domain (PH), TEC homology domain (TH), SRC homology domains 2 (SH2) and 3 (SH3), and the catalytic kinase C-terminal domain (also known as SRC homology domain 1 or SH1). Its role in BCR signaling and in other fundamental signaling cascades of the immune system is described below.

BTK function in B-cell receptor signaling

BCR is an antigen-specific receptor expressed on the B-cell surface; it displays a membrane-bound antibody and Igα/Igβ heterodimers that are responsible for cytosolic signal transduction via their immunoreceptor tyrosine-based activation motif (ITAM) residues, a component of the cytosolic tail of Igα/Igβ heterodimers. The binding of specific antigens to BCR complex—together with the presence of an adequate co-stimulatory signal—activates the signaling cascade through SRC family kinase LYN-mediated phosphorylation of ITAMs, followed by the activation of SYK. LYN also phosphorylates the tyrosine residue of the intracellular tail of coreceptor CD19, thus activating phosphatidylinositol 3-kinase (PI3K) and allowing it to bind to the B-cell adapter (BCAP) (18). BTK is hence moved to the plasma membrane from the cytosol by phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) through the PH domain; SYK is subsequently able to activate BTK via phosphorylation of BLNK, a B-cell linker protein that bridges BTK to phospholipase C-γ2 (PLCγ2). Activation of PLCγ2 results in further downstream signaling that results in the cleavage of the plasma membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG subsequently activates protein kinase C, the pathways of mitogen-associated protein kinase (MAPK) and nuclear factor κB (NF-κB), while IP_3 mediates a calcium release from the endoplasmic reticulum which allows the translocation of nuclear factor of activated T cells (NFAT) into the nucleus. NFAT and NF-κB are key regulators of gene expression related to B-cell survival and proliferation, and cytokine and chemokine expression.

Other BTK functions in the innate and adaptive immune system

BTK plays a crucial role in the signaling cascade following the activation of chemokine receptors, particularly CXCR4 and CXCR5 expressed on B cells surface and related to trafficking and homing of these lymphocytes. BTK is responsible for chemokine-induced migration mediated by integrins; hence, its inhibition may impair chemokine receptor expression and, consequently, B-cell migration and homing

(23). BTK it is also involved in the cascade pathways of Toll-like receptors (TLR), a group of transmembrane proteins that recognize conserved molecules from microorganisms; the BTK-mediated activation of this cascade is related to B-cell activation, proliferation, antibody secretion, class switch and proinflammatory cytokine secretion (24).

Moreover, BTK also exerts its signaling function through IgG-specific Fc receptors (FcγR) in myeloid cells (macrophages and microglia); it is implicated in integrin activation and in the interaction between B cells and APCs (24). BTK is expressed in dendritic cells, mast cells: It shows high affinity for IgE receptor in mast cells, thus promoting secretion of proinflammatory cytokines as well as degranulation and histamine release (25); ultimately, it has been shown that BTK is an essential player in NK cell activation (26).

BTK Inhibitors

The pivotal role of BTK in the immune system homeostasis and pathology has prompted the arrangement of small-molecule BTK inhibitors (BTKi) for B-cell malignancies and chronic autoimmune disorders. The first BTKi available for clinical use were antileukemic agents: Ibrutinib was approved by the U.S. Food and Drug Administration (FDA) in 2013 for mantle cell lymphoma and chronic lymphatic leukemia, and later for Waldenström macroglobulinemia. Its remarkable therapeutic results fostered the search for other—more selective and better tolerated—BTKi.

BTKi are classified into 2 groups according to their mode of binding to BTK: covalent irreversible inhibitors and noncovalent reversible inhibitors. Irreversible inhibitors covalently bind to Cys481 in the ATP-binding pocket of BTK. Reversible inhibitors mainly bind to a specific pocket of the inactive conformation of BTK by weak, reversible forces. Irreversible inhibitors have longer-lasting inhibitory activity compared to noncovalent BTKi (27). On the contrary, they sometimes display higher toxicity and risks related to chronic use. Most of the BTKi under investigation in clinical trials for MS are irreversible BTKi.

BTKi in animal models of multiple sclerosis

Several studies have reported the beneficial effects of BTKi in animal models of MS (i.e., EAE). Particularly, 3 different tyrosine kinase inhibitors—sorafenib, imatinib and GW-2580—proved to be effective in preventing the development of EAE and in treating the disease in mice inoculated with an encephalitogenic peptide of myelin oligodendrocyte glycoprotein (MOG) (28). Accordingly, evobrutinib was shown to improve clinical and histopathological features of EAE in mice, via the inhibition of B-cell antigen presentation, activation and differentiation mediated by T-cell receptor (29). More recently, it was demonstrated that evobrutinib reduces meningeal contrast enhancement in brain MRI scans of mice with EAE (30). Ultimately, tyrphostin AG126, a member of a family of TK inhibitors, was shown to mitigate the clinical symptoms of EAE and to reduce encephalitogenic Th17 differentiation, inflammation in brain and spinal cord and microglia activation (31).

Tolebrutinib Chemical Structure and Preclinical Studies

As BTK signaling pathways involve resident immune cells of the CNS—i.e., microglia, which is crucially implicated in MS disease activity (32)—a BTKi with the potential of crossing the blood–brain barrier (BBB) could target adaptive (B-cell activation) and innate (microglial) immune cells both in the periphery and in the CNS. Tolebrutinib (also known as BTK'168, PRN-2246 or SAR-442168) is a small molecule with molecular weight of 455.2 Da; it is a potent and selective oral BTKi designed to penetrate the BBB in order to target BTK-expressing cells within the CNS.

Tolebrutinib belongs to the family of irreversible BTKi, as it covalently binds to cysteine-481 in the kinase domain of BTK (33). The drug combines long duration of action and favorable pharmacokinetic properties to achieve efficacy at low doses. Tolebrutinib demonstrated excellent selectivity for BTK over several receptors, enzymes and transporters in enzyme activity assays (4). In vitro, tolebrutinib showed a strong inhibition of BTK in Ramos B cells (IC_{50} of 0.4 nM) and in microglia (IC_{50} = 0.7 nM). It also blocked the BCR-mediated activation (IC_{50} = 10 nM) and Fc receptor activation (IC_{50} = 166 and 9.6 nM for FcεR and FcγR, respectively) of immune cells, and it inhibited microglial FcγR activation (IC_{50} = 157 nM) through durable occupancy of BTK. Moreover, in a mouse model of EAE, tolebrutinib achieved a dose-dependent protection from disease induction, and its BTK occupancy was confirmed in both the periphery and the CNS, suggesting that the drug could effectively target the central pathways involved in MS pathogenesis (34). These results have recently paved the way for clinical application of tolebrutinib, which has currently been tested in 1 phase I and 1 phase II MS clinical trials.

Clinical Studies

Phase I clinical trial

A first-in-human, randomized, double-blind, placebo-controlled phase I trial on healthy volunteers (HV) receiving tolebrutinib or placebo has been recently concluded (35). The study was comprised of 3 parts: Part A included 5 arms (8 HV per cohort, 6 active and 2 placebo) of single ascending doses (SAD) of 5, 15, 30, 60 and 120 mg of tolebrutinib. The 60-mg cohort was administered a second 60-mg dose with a moderate fat meal after a 6-day washout. Part B included 5 arms (10 HV per cohort, 8 active and 2 placebo) of multiple ascending doses (MAD) of 7.5, 15, 30, 60 and 90 mg given once daily for 10 days. Part C was a single open-label dose of 120 mg given to 4 HV. Overall, 94 HV were enrolled

in the study (74 active, 20 placebo). The primary endpoint was the evaluation of safety and tolerability, including the assessment of physical examinations, ECGs, vital signs, clinical laboratory results and adverse events (AEs). Secondary endpoints included assessment of pharmacokinetics and a pharmacodynamic evaluation of peripheral BTK occupancy. Part C subjects underwent lumbar puncture 2 h after a single dose administration to assess cerebrospinal fluid (CSF) exposure to the drug.

Tolebrutinib was well tolerated for 10 days by all participating subjects; mild drug-related AEs were reported only in part B cohorts, with diarrhea and headache being the most common manifestations (> 10% of subjects). No serious AEs or deaths were observed.

After oral administration under fasting conditions, tolebrutinib showed rapid absorption with a median time to maximum concentration (t_{max}) of approximately 1 h, and a mean half-life of 1.5-2 h. Binding to plasma proteins was ~93.5%. Drug exposure was generally dose-proportional; there was a modest food effect for the drug administration, as exposure mildly increased when tolebrutinib was administered after a moderately fat meal to the 60-mg SAD cohort after 6-day washout. Active HV in MAD cohorts displayed low drug accumulation after 10 consecutive days of exposure, as expected given the rapid clearance of tolebrutinib. The mean CSF concentration was 1.87 ng/mL (4.1 nM), exceeding mean maximum plasma concentration at 15-mg dose. Moreover, CSF exposure was 2 times higher than the IC_{90} of 2.0 nM obtained in a competitive binding assay in Ramos B cells, thus suggesting that tolebrutinib could effectively achieve significant CNS target engagement.

The study showed a dose-dependent increase in BTK occupancy measured at 4, 12 and 24 h after dosing in SAD cohorts and at day 1 of the MAD portion. Food intake did not impact on the pharmacodynamic profile. Maximal mean BTK occupancy was reached after administration of single 60- and 120-mg doses. Within MAD cohorts, BTK occupancy increased with sequential doses and returned toward baseline at day 7 after dosing in all cohorts; complete inhibition was reached with doses ≥ 15 mg. Flow cytometry analysis of samples from the MAD cohorts did not show depletion of B cells; on the contrary, increase in B-cell count (both naive and memory B cells) was observed at day 4 and 10 in the MAD cohorts.

Phase II clinical trial

A phase IIb randomized, double-blind, placebo-controlled, crossover trial on tolebrutinib has been conducted globally (40 centers in Europe and North America) and the results have been published recently (36). This multicenter study (ClinicalTrials.gov Identifier NCT03889639) enrolled pwMS aged 18-55 years, fulfilling 2017 McDonald's criteria (5), with an expanded disability status scale (EDSS) < 5.5 and affected by relapsing MS—either RRMS or relapsing SPMS according to Lublin's 2013 revised classification of clinical courses (6). Moreover, patients had to meet 1 or more of the following eligibility criteria: ≥ 1 relapse in the year before baseline screening and/or ≥ 2 relapses within the previous 2 years and/or at least 1 active gadolinium-enhancing (GdE) brain lesion in the 6 months before screening. Patients with PPMS or nonactive SPMS, or with a relapse occurring within 30 days of random allocation to treatment were excluded. A total of 130 enrolled pwMS were randomly assigned to 2 cohorts (1:1, 64 subjects in cohort 1 and 66 in cohort 2), and those in each cohort were subsequently assigned (1:1:1:1) to 1 of 4 tolebrutinib dosage groups (33 patients to 5 mg, 32 to 15 mg, 33 to 30 mg and 32 to 60 mg). Duration of the study was 16 weeks for both cohorts. Patients assigned to cohort 1 were treated with tolebrutinib for the first 12 weeks and with placebo for the remaining 4 weeks to mask the treatment assignment and to provide additional safety data. Patients in cohort 2 underwent placebo for the first 4 weeks and then crossed over to tolebrutinib for 12 weeks. Each patient took the medication orally once daily with or without food (the same dietary regimen was maintained in each participant).

The primary outcome was to determine the dose–response relationship for tolebrutinib to reduce the number of new active brain MRI lesions. MRI scans were performed at baseline and every 4 weeks. Particularly, the primary endpoint was the comparison between GdE lesions at MRI scan performed after 12 weeks of tolebrutinib treatment (i.e., at week 12 for cohort 1 and week 16 for cohort 2) and those detected 4 weeks previously. Secondary endpoints were the number of new/enlarging T2 lesions and the total number of GdE lesions at the end of 12 weeks of tolebrutinib treatment, AEs and serious AEs. The trial also addressed several exploratory endpoints, the most relevant of which being the volume of slowly expanding lesions by dose group and the number of paramagnetic rim lesions evaluated through susceptibility-weighted imaging (SWI) sequences in all active treatment groups combined.

Of 130 enrolled subjects, 128 were RRMS patients, while 2 were diagnosed with active SPMS; 129 pwMS completed the treatment regimen. Mean exposure time to tolebrutinib was homogenous among the different dosage groups; mean exposure time to placebo was 28 days.

A statistically significant dose–response relationship between tolebrutinib and the new GdE lesions was observed, with maximal effect being shown with the 60-mg dosage, corresponding to an 85% adjusted relative reduction in new GdE lesions (95% confidence interval [CI], 28-97%) versus placebo. The group treated with tolebrutinib 60 mg had a mean number of lesions of 0.13 (SD 0.43) compared to 1.03 (SD 2.50) of those under placebo. After 12 weeks of treatment, 90% of tolebrutinib-treated patients displayed no new GdE lesions compared to 75% of participants in the cohort 2 placebo period observed at week 4.

Accordingly, a dose–response relationship was also shown for the number of new/enlarging T2 lesions, with their maximal relative reduction (89%, 95% CI, 68-96%) observed with the 60-mg dose compared to placebo. New/enlarging T2 lesions were absent in 87% of patients in the 60-mg tolebrutinib-treated group compared to 66% of pwMS under placebo, and the mean number of such lesions was 0.23 (SD 0.62) in the former group versus 2.12 (SD 5.16) in the latter.

Patients under the 60-mg tolebrutinib regimen showed an approximately 2.5 times greater plasma concentration compared to the 30-mg group over the 12 weeks of treatment. It was also observed that exposure of tolebrutinib was 2 times higher in patients taking the drug during a meal, and that greater exposure (> 40 ng·h/mL) was associated with fewer GdE lesions and new/enlarging T2 lesions.

In exploratory analysis, the volume of slowly expanding lesions was lower with 60 mg tolebrutinib compared with lower doses and placebo. Among 16 patients with available SWI sequences showing paramagnetic rim lesions at baseline, the number of these alterations remained stable overall during subsequent assessments. These findings are partly contrasting, as it is supposed that paramagnetic rim lesions tend to expand over time and could be one of the major players in clinical progression; hence, longer course of treatment is mandatory to assess whether BTKi-mediated modulation of CNS-resident immune cells could translate into clinical benefit related to slowing disability in progressive MS.

Overall, the results of this phase II trial suggest that 60 mg tolebrutinib once daily could be the most efficacious treatment regimen for further evaluation of the drug in relapsing MS. The best exposure could be ensured by the administration of tolebrutinib with food.

Tolebrutinib showed a good safety profile, with a homogenous distribution of AEs among all treated groups. No AE led to drug discontinuation, and no treatment-related deaths were observed. Most of the AEs were mild, with headache being the most frequent across all treatment arms during weeks 1-4. Elevated alanine aminotransferase (ALT) was observed in 3 subjects under 12-week tolebrutinib regimen, 2 of whom had concentrations > 3 times higher than the upper limit of normal. All of them completed the treatment. The only reported serious AE was a severe MS relapse in a subject undergoing the 60-mg regimen, that evolved toward recovery and did not need treatment discontinuation. These data confirm the good safety profile suggested by the phase I trial, although confirmation of these data in larger phase III trials is mandatory.

Ongoing clinical trials

The encouraging results shown by the phase II trial combined with the promising safety profile of tolebrutinib have supported the rationale to pursue phase III studies of tolebrutinib in relapsing–remitting and progressive forms of MS, that are ongoing (summarized in Table I).

The phase II study described above continued in an ongoing long-term safety study (NCT03996291) (37) which has enrolled 125 patients to assess the long-term safety and tolerability of tolebrutinib in relapsing MS. As a secondary endpoint, the study evaluates the drug efficacy on disease activity, assessed by clinical and imaging methods.

The 2 phase III randomized, triple-blind, active-comparator trials GEMINI 1 and GEMINI 2 (NCT04410978 and NCT04410991, respectively) started in June 2020 and are currently recruiting patients with relapsing forms of MS to assess the efficacy of daily tolebrutinib compared to a daily dose of teriflunomide 14 mg measured by annualized relapse rate (ARR) (38, 39). Secondary outcomes include the comparison of the efficacy of tolebrutinib versus teriflunomide on disability progression, MRI lesions, cognitive performance and quality of life; safety and tolerability evaluation; assessment of population pharmacokinetics of tolebrutinib and relevant metabolites and its relationship to efficacy and safety.

Another phase III randomized, triple-blinded, placebo-controlled trial known as HERCULES (NCT04411641) started in September 2020 and is recruiting patients affected by nonrelapsing secondary progressive multiple sclerosis (estimated enrollment: 1,290 participants) to assess the efficacy of tolebrutinib compared to placebo in delaying disability progression. Moreover, the study aims at evaluating the efficacy of the drug on clinical endpoints, MRI lesions, cognitive performance, physical function and quality of life. The trial also will evaluate safety, tolerability, pharmacokinetics and pharmacodynamics of tolebrutinib (40).

Ultimately, a phase III study in patients with progressive MS (PERSEUS, NCT04458051) is recruiting participants (estimated enrollment: 990 patients) to assess the efficacy of tolebrutinib in delaying disability progression in PPMS (41). The trial also aims at evaluating secondary endpoints similar to the ones defined for HERCULES trial.

Conclusions

Over the last 15 years, B cell-targeting therapies have dramatically changed the treatment approach to MS, as depleting MAbs such as rituximab, ocrelizumab or ofatumumab proved to be highly effective in reducing clinical relapses, radiological disease activity and, to a lesser extent, disease progression. However, the need for long-term B-cell depletion—especially in young patients potentially undergoing several years of treatment—has raised much concern on the progressive decline of the adaptive immune system functions, particularly regarding humoral

Table I. Ongoing clinical trials for tolebrutinib.

Compound and ClinicalTrials.gov Identifier	Responsible party	Study design	Indication	Stage of development
Tolebrutinib Oral NCT03996291	Sanofi	Phase II clinical trial Start date: September 23, 2019 Allocation: N/A Intervention model: single group assignment Masking: none (open label) Primary purpose: treatment Primary objective: safety and tolerability of tolebrutinib Secondary objectives: efficacy of tolebrutinib on disease activity	Relapsing MS	Enrollment: 125 subjects Recruitment completed, ongoing
Tolebrutinib Oral NCT04742400	National Institute of Neurological Disorders and Stroke (NINDS)|National Institutes of Health Clinical Center (CC)	Phase II clinical trial Start date: April 15, 2021 Allocation: nonrandomized Intervention model: parallel assignment Masking: none (open label) Primary purpose: treatment Primary objective: to evaluate the effects of 48 weeks of tolebrutinib treatment on the paramagnetic rim of chronically inflamed white matter lesions, as seen on 7-tesla MRI. Secondary objectives: 1) To assess safety and tolerability of 96 weeks of treatment with tolebrutinib following intravenous anti-CD20 antibody therapy. 2) To assess the possible repair of chronically inflamed white matter lesions in which inflammation at the lesion edge has been modulated by tolebrutinib	MS	Estimated enrollment: 30 subjects Recruitment status: active, recruiting
Tolebrutinib Oral NCT04410978 GEMINI 1	Sanofi	Phase III clinical trial Start date: June 30, 2020 Allocation: randomization Control group: active comparator (teriflunomide) Intervention model: parallel assignment Masking: triple (participant, investigator, outcomes assessor) Primary purpose: treatment (efficacy and safety) Primary objective: ARR Secondary objectives: EDSS, MRI (new or GdE) lesions and safety	Relapsing MS	Estimated enrollment: 900 subjects Recruitment status: active, recruiting
Tolebrutinib Oral NCT04410991 GEMINI 2	Sanofi	Phase III clinical trial Start date: June 11, 2020 Allocation: randomization Control group: active comparator (teriflunomide) Intervention model: parallel assignment Masking: triple (participant, investigator, outcomes assessor) Primary purpose: treatment (efficacy and safety) Primary objective: ARR Secondary objectives: EDSS, MRI (new or GdE) lesions and safety	Relapsing MS	Estimated enrollment: 900 subjects Recruitment status: active, recruiting

(Continued)

Table I. *Ongoing clinical trials for tolebrutinib. (Cont.)*

Compound and ClinicalTrials.gov Identifier	Responsible party	Study design	Indication	Stage of development
Tolebrutinib Oral NCT04411641 HERCULES	Sanofi	Phase III clinical trial Start date: June 11, 2020 Allocation: randomization Control group: placebo Intervention model: parallel assignment Masking: triple (participant, investigator, outcomes assessor) Primary purpose: treatment Primary objective: efficacy of tolebrutinib in delaying disability progression Secondary objectives: efficacy of tolebrutinib vs. placebo on clinical endpoints, MRI (new or GdE) lesions, cognitive performance, physical function and quality of life; safety, pharmacokinetics and pharmacodynamics endpoints	Nonrelapsing secondary progressive MS	Estimated enrollment: 1,290 subjects Recruitment status: active, recruiting
Tolebrutinib Oral NCT04458051 PERSEUS	Sanofi	Phase III clinical trial Start date: August 13, 2020 Allocation: randomization Control group: placebo Intervention model: parallel assignment Masking: triple (participant, investigator, outcomes assessor) Primary purpose: treatment Primary objective: efficacy of tolebrutinib vs. placebo on clinical endpoints, MRI (new or GdE) lesions, cognitive performance, physical function and quality of life; safety, pharmacokinetics and pharmacodynamics endpoints	Primary progressive MS	Estimated enrollment: 990 subjects Recruitment status: active, recruiting

MS, multiple sclerosis; MRI, magnetic resonance imaging; ARR, annualized relapse rate; EDSS, expanded disability status scale; GdE, gadolinium-enhancing.

response and protection by vaccinations. These issues have specifically emerged during the COVID-19 pandemic, with concerns on the risk of worse disease course and hospitalization in SARS-CoV-2-infected pwMS on rituximab or ocrelizumab (42). In this context, targeting the function of B lymphocytes and of other myeloid cells (i.e., microglia) potentially involved in MS pathogenesis with nondepleting drugs could be a valid approach to reach clinical efficacy without the limitations of the current therapeutic options. BTKi such as tolebrutinib could therefore provide attractive treatment benefits for MS as well as for other autoimmune diseases; of note, BTKi are currently the only therapeutic strategy that strongly bridges innate and adaptive immunity. Notwithstanding the promising results in terms of reduction of radiological activity displayed by tolebrutinib in the phase II trial, it is still unclear whether this and other BTKi will display a comparable efficacy to the one achieved by biological compounds, hence raising questions on the correct placement of tolebrutinib in the therapeutic spectrum of MS. A recent paper has suggested that a sequential approach of initial depletion and subsequent BTK inhibition may allow B-cell repopulation while controlling pathogenic B-cell function, thus achieving long-term disease control while maintaining normal numbers of circulating B cells (29). However, only phase III trials and—possibly—head-to-head drug comparisons will properly address these issues.

Another significant therapeutic goal of tolebrutinib is the ability of reaching CNS with good CSF exposure, thus inhibiting microglial activation related to chronic active lesions and disease progression. Contrasting results about tolebrutinib's effect on the radiological equivalent of chronic active lesions have emerged from the phase II trial; hence, longer phase III trials are likely to be necessary to assess if tolebrutinib is effective in reducing disability progression.

Tolebrutinib showed good safety profile in the phase I and phase II clinical trials, with only minor drug-related AEs being reported. If confirmed in larger and longer phase III trials, these findings could be an important step forward compared to the first-generation BTKi such as ibrutinib, whose severe AEs such as cardiac arrhythmias, diarrhea, bleeding, infection, arthralgias and hypertension precluded its use in MS and other autoimmune disorders (43).

In conclusion, tolebrutinib is a promising and exciting therapeutic approach for MS; ongoing longer and larger trials are needed to determine the real efficacy and risks of this new molecule.

Disclosures

The authors state no conflicts of interest.

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