



# Bruton's Tyrosine Kinase Inhibition as an Emerging Therapy in Systemic Autoimmune Disease

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Accepted: 16 August 2021 / Published online: 5 October 2021  
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## Abstract

Systemic autoimmune disorders are complex heterogeneous chronic diseases involving many different immune cells. A significant proportion of patients respond poorly to therapy. In addition, the high burden of adverse effects caused by “classical” anti-rheumatic or immune modulatory drugs provides a need to develop more specific therapies that are better tolerated. Bruton's tyrosine kinase (BTK) is a crucial signaling protein that directly links B-cell receptor (BCR) signals to B-cell activation, proliferation, and survival. BTK is not only expressed in B cells but also in myeloid cells, and is involved in many different signaling pathways that drive autoimmunity. This makes BTK an interesting therapeutic target in the treatment of autoimmune diseases. The past decade has seen the emergence of first-line BTK small-molecule inhibitors with great efficacy in the treatment of B-cell malignancies, but with unfavorable safety profiles for use in autoimmunity due to off-target effects. The development of second-generation BTK inhibitors with superior BTK specificity has facilitated the investigation of their efficacy in clinical trials with autoimmune patients. In this review, we discuss the role of BTK in key signaling pathways involved in autoimmunity and provide an overview of the different inhibitors that are currently being investigated in clinical trials of systemic autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus, as well as available results from completed trials.

## Key Points

Bruton's tyrosine kinase (BTK) is a crucial signaling protein that links signals from the B-cell antigen receptor to the activation, proliferation, and survival of B cells.

Together with the emergence of very specific small-molecule BTK inhibitors, this makes BTK an interesting therapeutic target in the treatment of autoimmune diseases.

Here, we discuss the role of BTK in autoimmunity and the current status of BTK inhibition in clinical trials of systemic autoimmune diseases.

## 1 Introduction

Autoimmune disorders are driven by a loss of self-tolerance, often involving aberrant selection and activation of autoreactive B cells and subsequent auto-antibody production [1]. Each B cell harbors a unique antigen receptor, the B-cell receptor (BCR), that can recognize a specific antigen, thereby activating the B cell. Bruton's tyrosine kinase (BTK) is a crucial signaling protein that directly links BCR signals to B-cell proliferation and survival [2] (Fig. 1). The gene encoding BTK was discovered as the gene affected in X-linked agammaglobulinemia (XLA) patients [3, 4]. Because of the lack of functional BTK protein, mature B cells and antibodies are almost completely absent in these patients. The importance of BCR signaling in autoimmunity is underlined by studies in animal models showing that single-gene defects and changes in expression of BCR signaling molecules or inhibitory co-receptors can lead to autoimmunity [5, 6]. Furthermore, in human autoimmune diseases, genetic susceptibility loci include genes encoding BCR signaling molecules, for example *LYN*, *BLK*, *BANK1*, *PTPN22*, and *PXK* [7–12]. The efficacy of B-cell-depleting therapy in autoimmune patients has prompted the search for more

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specific B-cell-targeting therapies, leading to the development of small-molecule inhibitors of BCR signaling molecules including BTK.

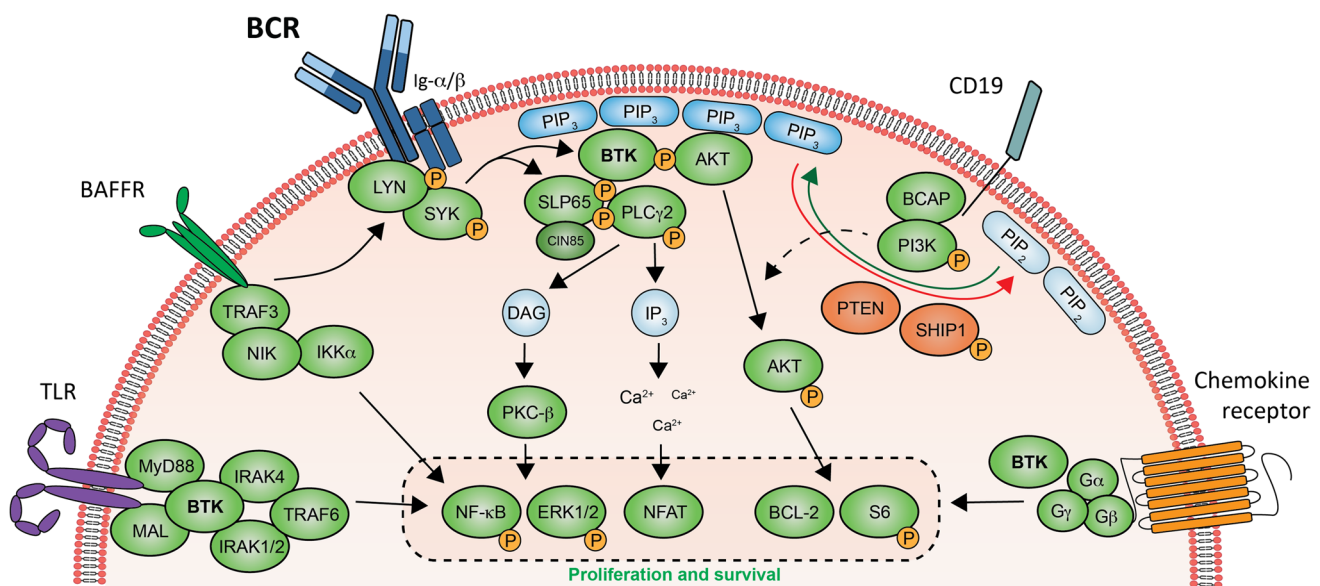
In addition to B cells, BTK is expressed by most hematopoietic cells, including the monocyte/macrophage/dendritic cell lineage, granulocytes, mast cells, platelets, osteoclasts, and erythroid cells [3, 4, 13–20]. Although rarely BTK expression has been reported in mouse T and natural killer (NK) cells [21, 22], it is generally accepted that these cells do not express BTK protein (search BTK at <http://rstats.immgen.org/Skyline/skyline.html>) [2–4, 20]. BTK also plays a role in many other key immune signaling pathways, including B-cell-activating factor receptor (BAFFR), Toll-like receptor (TLR), chemokine receptor, and Fc receptor (FcR) signaling (Fig. 1) [20].

The first-in-kind inhibitor of BTK kinase activity to reach the clinic was ibrutinib (Imbruvica, PCI-32765), which obtained US Food and Drug Administration (FDA) approval in 2013 for mantle cell lymphoma (MCL) patients, and subsequently for chronic lymphocytic leukemia (CLL) patients upon successful phase II trials [23, 24]. The efficacy of ibrutinib in CLL may not only be attributed to inhibition

of BTK, affecting survival and homing of CLL cells, as changes in the tumor microenvironment through off-target effects, such as reducing T-cell exhaustion and promoting an anti-tumor Th1 phenotype, might contribute to its efficacy [25]. Off-target kinase inhibition may not be beneficial in autoimmunity and may be associated with significant side effects, making ibrutinib less suitable for treatment of chronic autoimmune diseases. These findings prompted the development of more specific BTK inhibitors with a favorable safety profile for the treatment of autoimmune diseases.

The first BTK inhibitor reported for the treatment of a chronic inflammatory disorder was evobrutinib (M2951), in a clinical trial with active relapsing-remitting multiple sclerosis (MS) patients [26]. At a dose of 75 mg once daily, this BTK inhibitor significantly reduced the number of enhancing lesions from 12 through 24 weeks of treatment compared to placebo. Efficacy was maintained for 2 years with a reduction in annual relapse rate and increased time until first relapse [27].

A large number of experimental models support the efficacy of BTK inhibition in MS. In experimental autoimmune encephalomyelitis (EAE) mouse models of MS, evobrutinib



**Fig. 1** Role of BTK in B-cell signaling. Overview of BCR signaling and other important signaling modules for B cells. Upon BCR engagement, LYN will activate and phosphorylate Ig- $\alpha$  and Ig- $\beta$ , subsequently activating SYK. Together with CD19-mediated activation of PI3K, this leads to the activation of SLP65, BTK, and PLC $\gamma$ 2. This in turn activates downstream signaling pathways crucial for proliferation and survival, including engagement of ERK, NF- $\kappa$ B, and downstream mediators of AKT like S6, and anti-apoptotic proteins like BCL-2. Signaling downstream of TLRs and BAFFR also involves BTK phosphorylation, leading to activation of these same proliferation and survival factors. Other receptor signaling pathways like chemokine receptor signaling also contribute to migration, proliferation, and survival of B cells. BTK Bruton's tyrosine kinase, BCR

B-cell receptor, Ig immunoglobulin, PTPN22 protein tyrosine phosphatase non-receptor type 22, SYK spleen tyrosine kinase, PI3K phosphoinositide 3-kinase, SLP65 Src homology 2 domain-containing leukocyte adaptor protein of 65 kDa, CIN85 Cbl-interacting protein of 85 kDa, PLC $\gamma$ 2 phospholipase C $\gamma$ 2, DAG diacylglycerol, IP $_3$  inositol triphosphate, PKC- $\beta$  protein kinase C  $\beta$ , TRAF3 TNF receptor-associated factor 3, NIK NF- $\kappa$ B-inducing kinase, IKK $\alpha$  inhibitor of NF- $\kappa$ B kinase, MyD88 myeloid differentiation factor 88, MAL MyD88 adaptor-like, IRAK2 interleukin-1 receptor-associated kinase 2, ERK extracellular signal-related kinase, NFAT nuclear factor of activated T cells, BCAP B-cell adaptor for PI3K, PTEN phosphatase and tensin homolog, SHIP1 SH-2 containing inositol 5' polyphosphatase 1, BCL-2 B-cell lymphoma-2

prophylactically reduced disease severity [28], and evobrutinib treatment in mice with established symptoms resulted in reduced leptomeningeal inflammation [29]. Hereby, the numbers of meningeal B cells were reduced, but myeloid cell infiltrates appeared to persist. BTK is expressed in microglia and astrocytes, and treatment with evobrutinib resulted in a significant reduction in astrocytosis in the cortex surrounding the areas of meningeal inflammation [29]. Likewise, ibrutinib treatment decreased the proliferative response of microglia cells upon injection of anti-myelin oligodendrocyte glycoprotein monoclonal antibodies in mice [30]. BTK inhibition resulted in improved remyelination in vitro in demyelinated cerebellar slices, as well as in vivo in a transgenic *Xenopus laevis* model [31]. Furthermore, evobrutinib was shown to inhibit activation of B cells, reducing pro-inflammatory cytokine RNA expression, and limiting expression of proteins involved in antigen presentation [28]. However, T cells were also indirectly affected, and evobrutinib reduced disease severity in a B-cell independent MS mouse model [32]. In addition, GM-CSF-induced M1 macrophage differentiation and cytokine expression were reduced in vitro [33]. Taken together, these findings suggest that the therapeutic efficacy of BTK inhibition in MS patients may partly be due a substantial impact on the myeloid compartment, and thus beyond effects on B-cell activity.

The positive results of BTK inhibition obtained in MS patients, together with the promising efficacy in pre-clinical animal models of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [34, 35], make BTK inhibition an interesting therapeutic option in systemic autoimmune disorders. This notion is further supported by various human in vitro studies. In this review, we discuss the role of BTK in key signaling pathways involved in autoimmunity, as well as the various inhibitors that are currently being investigated in clinical trials in patients with systemic autoimmune disease.

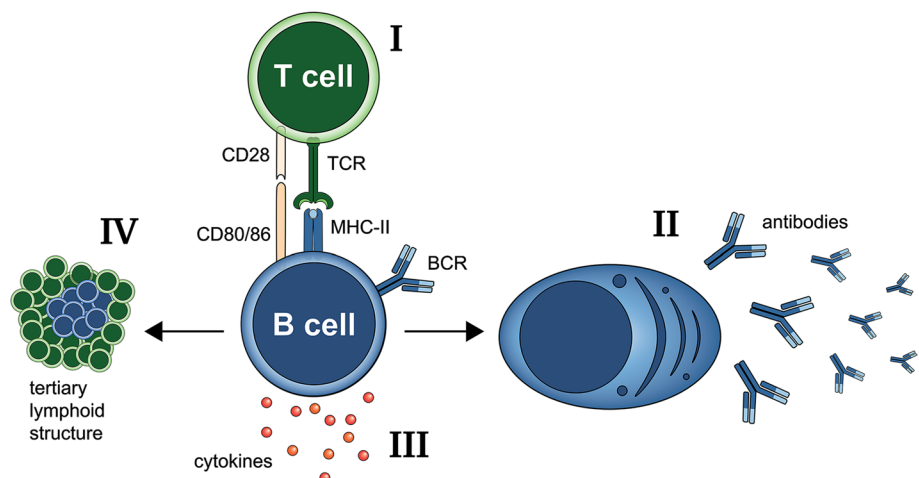
## 2 Bruton's Tyrosine Kinase (BTK) in Signaling Pathways

### 2.1 BTK in B-Cell Receptor Signaling

In the context of autoimmune disease, B cells can have various pathogenic characteristics (Fig. 2). Autoreactive B cells can initiate and/or enhance the autoimmune response by presenting auto-antigens on major histocompatibility complex II (MHC-II) molecules, and by providing activating signals through co-stimulatory factors, such as CD80 and CD86, to autoreactive T cells. In turn, B cells can differentiate, in a T-dependent or -independent manner, into plasma cells that produce autoreactive antibodies. B cells can be the source of large quantities of cytokines, including the pro-inflammatory cytokine IL-6. These support an inflammatory and autoimmune environment by activating surrounding cells and stimulating proliferation and survival. Finally, B cells are involved in stimulating the development and maintenance of tertiary lymphoid structures by producing soluble factors, such as lymphotoxin  $\alpha 1\beta 2$  [36, 37]. These structures may contain germinal centers and are often found in the affected organs of autoimmune disease patients.

B cells are generated in the bone marrow, where gene rearrangement of immunoglobulin V, D, and J elements in developing B cells leads to the expression of a unique BCR. Only those B cells expressing a functional BCR survive and subsequently undergo negative selection as they are checked for autoreactivity. This selection mainly takes place during development in the bone marrow and during the transitional stage in the circulation and is dependent on BCR signaling strength [38]. The BCR converts extracellular activation by cognate antigen into intracellular signals, mostly dominated by phosphorylation of protein kinases (Fig. 1). Following antigen binding to the BCR,

**Fig. 2** The four main pathogenic roles of B cells in the context of systemic autoimmune disease. (I) Initiation or enhancement of autoimmune responses by presenting auto-antigens to T cells and concomitantly providing co-stimulatory signals. (II) B cells can differentiate into autoantibody-producing plasma cells. (III) B cells can produce pro-inflammatory cytokines. (IV) B cells are involved in stimulating the development and maintenance of these tertiary lymphoid structures



the recruitment of the Src-family tyrosine protein kinase Lck/yes novel tyrosine kinase (LYN) and phosphorylation of the IgM-associated heterodimers Ig $\alpha$  and Ig $\beta$  are early events in this cascade, leading to the phosphorylation of spleen tyrosine kinase (SYK) [39]. BTK is then recruited to the cell membrane by phosphoinositide 3-kinase (PI3K) through the formation of phosphatidylinositol-3,4,5-triphosphate (PIP<sub>3</sub>) [40]. This recruitment enables SYK to phosphorylate BTK at tyrosine Y551 in its kinase domain [41–45]. SYK concomitantly phosphorylates the B-cell linker (BLNK) protein, also known as SLP65, which serves as a scaffolding protein by interacting with various signaling molecules including BTK and PLC $\gamma$ 2 [46]. Upon BCR engagement, multiple SLP65 molecules are recruited to CIN85 trimers, resulting in the assembly of large CIN85-SLP65 macromolecular signaling complexes [47]. As a result, BTK is fully activated and autophosphorylation at Y223 follows, although this event does not appear to be crucial for BTK function in mouse B cells [48]. Active BTK in turn phosphorylates phospholipase C $\gamma$ 2 (PLC $\gamma$ 2) [49], leading to cytoplasmic calcium influx and the activation of several downstream signaling pathways and transcription factors, important for survival, proliferation, and differentiation, including nuclear factor (NF)- $\kappa$ B [50, 51].

The rapid and stronger response of IgG-expressing memory B cells is thought to be, at least partially, dependent on the presence of the immunoglobulin tail tyrosine (ITT) motif in the cytoplasmic tail of membrane IgG [52]. The ITT motif can be phosphorylated by SYK, resulting in the recruitment of BTK via the adaptor protein Grb2. The dual activation pathway of BTK is thought to result in an amplification of IgG-induced calcium mobilization in memory B cells.

## 2.2 BTK and B-Cell-Activating Factor Receptor (BAFFR) Signaling

Survival and homeostatic proliferation of mature B cells are dependent on tonic BCR signaling and B-cell activating factor (BAFF), which is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family [53, 54]. BAFF can bind three receptors: B-cell maturation antigen (BCMA), transmembrane activator and CAML interactor (TACI), and BAFF receptor (BAFFR). In addition to activating the non-canonical NF $\kappa$ B pathway, the latter transduces survival signals via the canonical NF- $\kappa$ B pathway by co-opting the BCR signaling pathway through SYK and BTK (Fig. 1) [55–57]. Activation of these downstream signaling pathways fuels B-cell survival in a direct and indirect fashion: directly by enhancing the expression of pro-survival factors and dampening the expression of pro-apoptotic factors [58, 59], and indirectly by driving the expression of the receptors for

BAFF and their downstream signaling substrates [60–62]. In addition, during B-cell activation BAFF supports class switching and differentiation into plasma cells [63].

Murine studies have shown that a disbalance in either of these signals can cause an altered B-cell selection and can thereby result in an autoimmune phenotype. For example, overexpression of BAFF in mice leads to the development of a Sjögren/SLE-like phenotype [64]. Similarly, B-cell-specific overexpression of BTK leads to a systemic autoimmune phenotype in mice [6]. In human systemic autoimmune disease, increased BAFF levels in serum and enhanced BCR signaling in circulating B cells are also observed [63, 65], possibly allowing the escape of autoreactive B cells from negative selection. Increased levels of BAFF may rescue autoreactive B cells from anergy and promote the formation of a mature follicular dendritic cell network, allowing subsequent affinity maturation and differentiation in germinal centers (GC) [66, 67].

## 2.3 BTK in Other Signaling Pathways

Next to its well described role in BCR signaling, BTK is also involved in signaling downstream of TLRs (Fig. 1), specifically TLR3, 4, 7, 8, and 9 both in B cells and in other hematopoietic cell types [68]. Although evidence exists for the involvement of BTK kinase activity in downstream TLR signaling [69], BTK is mainly thought to function as a linker protein for the recruitment of myeloid differentiation primary response 88 (MyD88) and MyD88 adaptor-like (MAL) to the receptor [70]. TLR stimulation leads to the activation of interferon-regulatory factors and/or the NF- $\kappa$ B pathway, resulting in expression of co-stimulatory receptors and adhesion molecules, the production of pro-inflammatory cytokines and antigen presentation. In B cells, TLR activation leads to B-cell survival by similar mechanisms as previously described for BCR and BAFF signaling [71]. Therefore, TLR signaling can lead to synergistic effects when B cells are simultaneously stimulated by the BCR [72].

BTK is also involved in FcR signaling [73, 74]. The activation of the Fc $\epsilon$ R promotes histamine release in mast cells and Fc $\gamma$ R activation leads to pro-inflammatory cytokine production and enhances antigen presentation by myeloid cells [75]. BTK links RANK (receptor activator of NF- $\kappa$ B) activation together with co-stimulation, for example by FcRs, to drive osteoclast differentiation [76]. This can play a pathogenic role in systemic autoimmune diseases such as RA [76, 77]. BTK is involved in downstream signaling of several chemokine receptors, including CXCR4 [78], and in the intracellular signal transduction required for integrin activation and upregulation [79–81]. Therefore, in leukocytes it is important for the attraction towards and infiltration into the site of inflammation. BTK may also play an antibody-independent role in autoimmunity through its

interaction with transcription factor Bright/ARID3a, which is implicated in Ig heavy chain transcription [82]. Mice overexpressing *Arid3a* display autoimmunity, and SLE patients show increased *ARID3a* expression in B cells, although this did not correlate with autoantibody production [83–85]. In this context, it is of note that both ARID3a and interferon (IFN) $\alpha$  were induced in B cells upon TLR9 stimulation [86].

Taken together, the wide expression and functional profile of BTK implies that modulating BTK activity could be of benefit by restraining these inflammatory and autoimmune processes by targeting multiple pathways in multiple cell types simultaneously.

## 2.4 BTK Structure and Inhibitor Target Sites

BTK is a member of the highly conserved family of cytoplasmic TEC kinases, which also includes tyrosine kinase expressed in hepatocellular carcinoma (TEC), interleukin-2 inducible T-cell kinase (ITK), bone marrow-expressed kinase (BMX), and resting lymphocyte kinase (RLK) [19].

BTK consists of 659 amino acids and contains five domains with two phosphorylation sites. It is transiently recruited to the cell membrane upon BCR activation through the activity of PI3K, which results in the generation of PIP<sub>3</sub>, which interacts with the N-terminal pleckstrin homology (PH) domain of BTK. In addition, it contains a Tec homology (TH), a SRC homology 3 (SH3), and an SH2 domain, as well as a C-terminal kinase domain. The proline-rich TH domain is important for optimal activity and stability of the protein, but also has the capacity to bind other kinases including LYN. The SH3 domain contains the Y223 autophosphorylation site and interacts with various signaling molecules via binding to proline-rich regions. The SH2 domain has the capacity to bind to phosphorylated tyrosine residues of signaling molecules, in particular SLP65.

Because many human diseases, particularly malignancies, involve dysregulation of one of the 518 identified protein kinases [87], these enzymes have become important drug targets. Nearly all currently FDA-approved small-molecule kinase inhibitors have a competitive mechanism of action. Such reversible inhibitors are classified as type I-V, based on their interaction with the ATP-binding pocket of an active or inactive kinase conformation or binding to a site distinct from the active site [88]. In contrast, many BTK inhibitors, including the prototypic irreversible inhibitor ibrutinib [34, 35], are classified as type VI, and form a covalent bond with Cys-481 within the ATP-binding site of the kinase domain. They generally have a high specificity, because the Cys-481 amino acid residue is poorly conserved among kinases (Table 1). More recently developed inhibitors, such as acalabrutinib, poseltinib, spebrutinib, and tirabrutinib, also interact irreversibly with Cys-481 [89] (Table 1). Although

rilzabrutinib (PRN-1008) forms a covalent bond with Cys-481, inhibition is reversible [90].

Other reversible BTK inhibitors, including RN486, CGI1746, and fenebrutinib [75, 91, 92], are classified as type II inhibitors, because they stabilize an inactive non-phosphorylated form of BTK [93]. These inhibitors target the H3 pocket (a network of three hydrogen bonds involving the residues K430, G414, and T474), which is immediately adjacent to the region occupied by ATP. Thereby, these inhibitors sequester the Y551 residue and prevent its phosphorylation [94]. Interestingly, the ability to sequester Y551 was shown to determine the potency for inhibition of Fc $\gamma$ R and Fc $\epsilon$ R signaling, but not of BCR signaling, perhaps providing an added benefit in autoimmune and allergic indications, respectively [94]. Very recently, another BTK inhibitor was developed, remibrutinib (LOU064), which also stabilizes an inactive BTK conformation by selectively targeting the H3 pocket by forming a covalent bond [95]. An obvious important benefit of covalent inhibitors is their ability to reach higher and more sustained BTK occupancy, compared with reversible inhibitors that need to be present in the circulation at high levels to allow for effective inhibition.

It has been well documented that many CLL patients treated with ibrutinib develop drug resistance due to a C481S mutation that disrupts the binding of this BTK inhibitor to the cysteine residue [96]. Several BTK inhibitors can effectively counteract ibrutinib-resistant C481S-BTK. These include the type II inhibitors CGI-1746 and fenebrutinib, as well as XMU-MP-3, which has a unique binding mode and interacts with the M477, E445, and S538 residues of BTK. Likewise, the irreversible inhibitor ZYBT1 inhibits both wild-type and C481S-BTK but binds to the same site as ibrutinib [75, 92, 97, 98]. Altogether, the current number of BTK inhibitors and the obtained knowledge on their biological activity is overwhelming, given that already by the end of February 2017 almost 1,500 small-molecule inhibitors had been developed, involving over 300 BTK-inhibitor-related patents [89].

## 3 Evidence For a Critical Role of BTK in Autoimmunity

### 3.1 Animal Models

Many animal studies have indicated a critical role of BTK in autoimmunity, in both B cells and myeloid cells. Btk-deficient *Xid* mice are protected against the development of (full blown) autoimmunity in several disease models, including collagen-induced arthritis (CIA) for RA and the NZB $\times$ NZW model for SLE [20, 99, 100]. Vice versa, overexpression of BTK specifically in murine B cells is sufficient to induce a spontaneous autoimmune phenotype resembling human

**Table 1** Characteristics of the Bruton's tyrosine kinase (BTK) inhibitors first to be developed and in autoimmune disease clinical trials

Inhibitor (other names)	Interaction type	Interaction site	Other targets	References
Ibrutinib (Imbruvica/PCI-32765)	Irreversible, covalent	Cys481 $IC_{50}$ = 0.5 nM	BMX $IC_{50}$ = 0.8 nM EGFR $IC_{50}$ = 5.6 nM ITK $IC_{50}$ = 11 nM TEC $IC_{50}$ = 78 nM LYN $IC_{50}$ = 200 nM	[35]
Acalabrutinib (Calquence/ACP-196)	Irreversible, covalent	Cys481 $IC_{50}$ = 3 nM	ERBB4 $IC_{50}$ = 16 nM BMX $IC_{50}$ = 46 nM TEC $IC_{50}$ = 126 nM TXK $IC_{50}$ = 368 nM	[160]
BMS-986142	Reversible, covalent	Cys481 $IC_{50}$ < 0.5 nM	TEC $IC_{50}$ = 10 nM ITK $IC_{50}$ = 15 nM BLK $IC_{50}$ = 23 nM TXK $IC_{50}$ = 28 nM BMX $IC_{50}$ = 32 nM	[105]
Branebutinib (BMS-986195)	Irreversible, covalent	Cys481 $IC_{50}$ = 0.1 nM	TEC $IC_{50}$ = 0.9 nM BMX $IC_{50}$ = 1.5 nM TXK $IC_{50}$ = 5.0 nM ITK $IC_{50}$ = 100 nM	[139]
Elsubrutinib (ABBV-105)	Irreversible, covalent	Cys481 $IC_{50}$ = 180 nM	BLK $IC_{50}$ = 5940 nM JAK-3 $IC_{50}$ = 8640 nM TXK $IC_{50}$ = 9180 nM EGFR $IC_{50}$ = 14400 nM	[125]
Evobrutinib (M2951)	Irreversible, covalent	Cys481 $IC_{50}$ = 9 nM	BMX $IC_{50}$ = 20 nM TEC $IC_{50}$ = 7300 nM	[126]
Fenebrutinib (G DC-0853/RG7845)	Reversible, non-covalent	K430, M477, and D539 $IC_{50}$ = 2.3 nM	SRC $IC_{50}$ = 302 nM BMX $IC_{50}$ = 351 nM FGR $IC_{50}$ = 387 nM	[92]
Orelabrutinib (ICP-022)	Irreversible, covalent	Cys481 $IC_{50}$ = 1.6 nM	*	[161]
Poseltinib (HM71224/LY-3337641)	Irreversible, covalent	Cys481 $IC_{50}$ = 1.95 nM	BMX $IC_{50}$ = 0.64 nM TEC $IC_{50}$ = 4.57 nM TXK $IC_{50}$ = 4.62 nM EGFR $IC_{50}$ = 4.96 nM BLK $IC_{50}$ = 13.5 nM	[103]
Remibrutinib (LOU064)	Irreversible, covalent	Cys481 $IC_{50}$ = 1.3 nM	*	[95]
Rilzabrutinib (PRN1008)	Reversible, covalent	Cys481 $IC_{50}$ = 1.3 nM	TEC $IC_{50}$ = 0.8 nM BMX $IC_{50}$ = 1.0 nM RLK $IC_{50}$ = 1.2 nM BLK $IC_{50}$ = 6.3 nM	[132]
Spebrutinib (CC-292/AVL292)	Irreversible, covalent	Cys481 $IC_{50}$ < 0.5 nM	BMX $IC_{50}$ = 0.7 nM TEC $IC_{50}$ = 6.2 nM TXK $IC_{50}$ = 8.9 nM ITK $IC_{50}$ = 36 nM	[104]
TAS5315	Covalent*	Cys481 $IC_{50}$ < 0.15 nM	*	[141, 142]
Tirabrutinib (ONO/GS-4059)	Irreversible, covalent	Cys481 $IC_{50}$ = 2 nM	BMX $IC_{50}$ = 6 nM TEC $IC_{50}$ = 48 nM TXK $IC_{50}$ = 92 nM BLK $IC_{50}$ = 300 nM	[162]

*BLK* B lymphocyte kinase, *BMX* bone-marrow expressed kinase, *Cys481* cysteine 481, *EGFR* epidermal growth factor receptor, *ERBB4* Erb-B2 receptor tyrosine kinase 4, *FGR* Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog,  $IC_{50}$  half maximal inhibitory concentration, *ITK* interleukin-2 inducible T-cell kinase, *JAK-3* Janus kinase-3, *LYN* Lck/yes novel tyrosine kinase, *RLK* resting lymphocyte kinase, *SRC* proto-oncogene tyrosine-protein kinase Src, *TEC* tyrosine kinase expressed in hepatocellular carcinoma, *TXK* TXK tyrosine kinase

\*No data available

SLE and Sjögren's syndrome (SjS), featuring spontaneous GC and plasma cell formation, autoantibody production and immune infiltration in lungs, kidneys, and salivary glands [6]. This phenotype depends on B–T cell interaction and TLR signaling, and can be ameliorated by *in vivo* Btk inhibition [6, 101, 102].

In murine CIA, Btk inhibition *in vivo* reduces disease severity in a dose-dependent fashion, even after onset of arthritis, comparable to standard-of-care therapeutics such as dexamethasone (Table 2) [35, 92, 103, 104]. Furthermore, Btk inhibition enhances the effects of standard-of-care therapeutics when administered together [105]. Inhibitors may target multiple cell types involved in the pathogenesis of disease, including B cells, myeloid cells, and osteoclasts, thereby reducing autoantibody formation, pro-inflammatory cytokine production and bone destruction. Similar to CIA, Btk inhibition also protects against severe progressive disease in a number of murine lupus models, including mice with a mutation in Fas (MRL/lpr) [2]. The efficacy depends on inhibition of both B cells and myeloid cells. Interestingly, lupus nephritis or kidney damage is often reduced, despite persistence of autoantibodies in the serum, particularly anti-RNA antibodies [106].

Other non-autoimmune disease models provide evidence of a role for BTK in cell types and pathogenic mechanisms that may be relevant in autoimmune diseases. For example, Btk is essential in the crosstalk between TLR4 and FcγRIIIa signaling leading to activation of alveolar neutrophils in an LPS/immune complex-mediated model of acute respiratory distress syndrome (ARDS) [107]. Furthermore, Btk inhibition inhibited neutrophil extracellular trap release into the lungs upon influenza infection in mice [108]. BTK may also be protective in some diseases. In bone marrow-derived macrophages in mice (and monocytes in humans), Btk deficiency or Btk inhibition leads to increased NLR family pyrin domain-containing 3 (NLRP3) activation, increasing susceptibility for inflammatory bowel disease (IBD) [109]. Together these studies emphasize the therapeutic potential of BTK inhibition in autoimmunity and may explain side effects observed in patients treated with BTK inhibitors.

### 3.2 Human Studies

BTK protein and phosphorylated BTK levels are increased in circulating B cells of RA, SjS, and granulomatosis with polyangiitis (GPA) patients [65, 110]. This increase was already evident in new emerging B cells, *i.e.*, transitional and naïve B cells, and was associated with active disease. BTK protein and phosphorylation levels correlated with autoantibodies and with numbers of pathogenic T cells in the circulation, as well as with immune infiltrates in the salivary glands of SjS patients [110, 111].

*In vitro* treatment of macrophages in synovial explants of RA and psoriatic arthritis patients with RN486, a highly selective, reversible inhibitor of BTK, showed that BTK is required for cytokine production in macrophages upon FcR or CD40 stimulation [112]. Enhanced BTK expression, through BTK-encoding adenovirus transduction of macrophages, increased IL-6 production upon CD40 or FcγR stimulation [112]. In a co-culture system of activated platelets with primary human fibroblast-like synoviocytes (FLS), cytokine production was inhibited by RN486 in a dose-dependent manner [113].

Similarly, pro-inflammatory cytokine production by effector B cells of systemic sclerosis (SSc) patients is inhibited by ibrutinib treatment *in vitro*, as is anti-topoisomerase autoantibody production [114]. In B cells from GPA patients, *in vitro* BTK inhibition with BMS-986142 also reduced BCR-mediated cytokine production, but appeared to be less effective in B cells from patients with active GPA, compared to B cells from patients in remission and healthy controls [65]. Furthermore, *in vitro* memory B cell and plasma cell formation and antibody production were inhibited in patients in remission, but not in patients with active GPA. These findings indicate that although BTK inhibition can inhibit effector functions of B cells in autoimmune patients, B cells from patients with active disease may be less susceptible to BTK inhibition.

Based on the observation that BTK inhibition protects against fatal lung injury in acute respiratory distress syndrome mouse models [107, 108], as well as the rather mild course of Coronavirus disease 2019 (COVID-19) in XLA patients and BTK inhibitor-treated leukemia patients [115–119], acalabrutinib was administered in a prospective study in COVID-19 patients. Clinical recovery of COVID-19 was seen and inflammatory markers normalized, indicating that BTK inhibition has the capacity to correct the dysregulated and damaging inflammatory response by macrophages [120]. Although a randomized phase II clinical trial of acalabrutinib in hospitalized patients (NCT04346199, available at [clinicaltrials.gov](https://clinicaltrials.gov)) failed to meet the primary endpoint according to an Astra Zeneca press release on 12 November 2020, other BTK inhibitors, including abivertinib (NCT04528667; NCT04440007) and ibrutinib (NCT04439006), are currently under investigation in clinical trials. Interestingly, very recently, evidence was provided that during severe COVID-19, the SYK inhibitor fostamatinib can counteract the critical pro-inflammatory role of aberrantly glycosylated anti-spike IgG that activates macrophages via FcγRs [121]. Blocking signaling of the FcγRIIIa-SYK axis in platelets using fostamatinib may additionally prevent platelet aggregation and vascular complications in COVID-19 patients [122]. These findings may have implications for the treatment of autoimmune disorders,

Table 2 Preclinical data for Bruton's tyrosine kinase (BTK) inhibitors evaluated in clinical trials for autoimmune disorders

Animal models	Human in vitro studies	References
<i>Acalabrutinib</i>		
–	B cells: Inhibition of B-cell activation (CD69 expression)	[160]
<i>BMS-986142</i>		
CAIA: Treatment from the start, dose-dependent reduction of clinical scores	GPA: Reduced cytokine expression, but not memory cell/ plasma cell formation and antibody production in B cells from active patients. In B cells from patients in remission, these effector functions were inhibited by BMS-986142	[65, 105]
CIA (mice): Treatment prior to disease onset, dose-dependent reduction of disease severity; treatment start at time of boost (day 21), dose-dependent reduction of disease severity and joint destruction. Enhances efficacy of standard-of-care agents		
<i>Brexbutinib</i>		
CIA (mice): Treatment at immunization, dose-dependent reduction of clinical scores, reduced joint destruction, almost complete protection at highest dose	Healthy B cells/PBMC: Inhibits BCR-mediated proliferation and activation of B cells, FcR-mediated TNF $\alpha$ production by PBMC	[139]
NZB/W: Reduced mortality, dose-dependent reduction of proteinuria, anti-dsDNA, reduced glomerulonephritis (comparable to prednisolone)		
<i>Elsubrutinib</i>		
NP-immunizations: NP-LPS: no significant effect on antibody development; NP-Ficol: reduced IgM and IgG3; NP-KLH: reduced IgM after boost, high dose reduced IgG1	Healthy B cells/PBMC/eosinophils: Inhibits BCR-mediated B-cell activation, IgE-mediated histamine release by eosinophils and FcR- and TLR-mediated cytokine production by PBMC	[125]
Vaccine response (pneumococcal, Prevnar): no significant effect on antibody levels		
CIA (rats): Treatment at onset disease, dose-dependent reduction in clinical score, bone loss		
IFN- $\alpha$ -accelerated NZB/W FI: Treatment prior to onset proteinuria, dose-dependent reduction in mortality, proteinuria, and anti-dsDNA (only high doses)		
<i>Evo Brutinib</i>		
CIA (mice): Treatment before onset of disease, dose-dependent reduction of incidence and severity of arthritis, and histopathological scores of joint inflammation/destruction	Healthy B cells: Inhibition of BCR-induced B-cell activation (comparable to MS B cells), inhibition of BCR- and TLR9-induced cytokine production	[28, 126, 163]
CIA (rats): treatment at onset of disease, dose-dependent reduction of clinical and histopathological score	MS B cells: BTK protein levels increased in memory CD27+ B cells, no differences in BTK phosphorylation with healthy B cells.	
IFN- $\alpha$ -accelerated NZB/W FI: Dose-dependent reduction of proteinuria and kidney damage, reduced plasma cell numbers and B- and T-cell activation		
EAE: Before onset: Dose-dependent reduction of disease severity, cytokine expression, reduced B- and T-cell infiltration in CNS, reduced Ag-dependent B-cell differentiation, antigen presentation		
<i>Fenebrutinib</i>		
CIA (rats): Treatment started after onset of arthritis, dose-dependent reduction of ankle thickness, inflammation in the joints and joint destruction	Healthy B cells: Inhibits anti-IgM-induced BCR signaling, anti-IgM- and CD40L-induced proliferation	[92]
	Healthy CD14+ monocytes: prevents FcR-mediated TNF $\alpha$ production	
<i>Orelabrutinib</i>		
CIA (rats): Dose-dependent reduction in disease score, proinflammatory cytokine production, and histopathological score		¥
MRL/lpr: Increased survival of treated mice, reduction in anti-dsDNA antibodies and IFN $\alpha$ levels in serum, urine protein levels		
<i>Poseltinib</i>		
CIA (mice): Treatment started 10 days after boost, reduced weight loss and severity of arthritis, reduced IL-6 and IgG serum levels and reduced bone erosion/bone loss	Healthy B-cells: Inhibits signaling and activation	[103, 130]
MRL/lpr: Treatment started at 8 weeks of age, reduced B-cell activation, reduced anti-DNA IgG, reduced skin lesions, improved renal function	Healthy CD14+ Monocytes: dose-dependent inhibition of cytokine production upon FcR and TLR stimulation. Induction of osteoclast formation inhibited.	
NZB/W FI: Treatment started at 18 weeks of age, reduced GC B-cell/plasma cell formation, improved renal function, increased survival at higher doses		



Table 2 (continued)

Animal models	Human in vitro studies	References
<i>Remibrutinib</i>		
SRBC Immunization (rats): treatment at time of immunization, dose-dependent reduction in Ag-specific IgM antibodies	Blood B cells: Reduced BCR-mediated CD69 expression Blood basophils: Reduced FcεR-mediated CD63 expression Human monocyte cell line: Reduced FcγR-mediated IL-8 production	[95]
CIA (rats): Treatment after onset of disease, reduction of clinical score and joint inflammation and destruction		
<i>Rilzabrutinib</i>		
Passive arthus reaction (rats): Dose-dependent reduction of intradermal dye extravasation	B cells: Reduced BCR-mediated activation and proliferation, reduced CD40/IL-2/IR- or TLR9-mediated antibody production	[132, 164]
Anti-GBM nephritis: Dose-dependent reduction of kidney inflammation and improvement of kidney function	Monocytes: Reduced FcγR-mediated TNFα production Basophils/mast cells: Reduced FcεR-mediated degranulation and CD63 expression	
Anti-CD41-mediated ITP: Pretreatment dose dependently reduced platelet loss		
CIA (rats): Treatment at disease onset, dose-dependent reduction of clinical and histopathological scores; treatment after disease onset, reversal of disease		
Canine pemphigus: Immediate and rapid clinical improvement		
<i>Spebrutinib</i>		
CIA (mice): Treatment started after onset of arthritis, dose-dependent reduction of disease severity, weight loss, and inflammatory cytokines in serum, reduced inflammation and bone destruction in joints	B cells: Reduced BCR/TLR-mediated activation, proliferation, cytokine production, plasma cell differentiation, and antibody production Macrophages: Reduced FcγR-mediated TNFα production Monocyte-derived DC: Reduced TLR9 mediated CD86 expression Basophils: Reduced FcεR-induced degranulation Osteoclast: Reduced osteoclastogenesis	[104, 134]
<i>TAS5315</i>		
CIA (mice): Treatment started after onset, dose-dependent reduction in arthritis scores, inflammation, pannus formation, cartilage and bone damage, induction of bone repair	Monocytes: Dose-dependent reduction of TNFα and IL-6 production Osteoclasts: Reduced osteoclast differentiation and bone resorption	[141, 143, 144]
MRL/lpr: Reduced glomerulonephritis, serum BUN, and anti-dsDNA antibody levels		
<i>Tirabrutinib</i>		
CIA (mice): Start treatment at time of boost, dose-dependent reduction in disease score and joint damage, reduction of MIP-1α, IL-1β, KC, IL-6, RANKL, and MMP-3 production in joints of arthritic mice	Monocytes: Reduced FcγR- and TLR9-mediated TNFα and IL-6 production Osteoclast (precursors): Reduced M-CSF- and RANK-L-mediated osteoclast differentiation, reduced MIP-1α and RANTES production in bone marrow cultures	[135–138]
NZW/B F1: Start treatment at 12 weeks (before onset), reduced proteinuria, reduced GC B cells and plasma cells, reduced anti-dsDNA antibodies in serum		

*anti-dsDNA* anti-double strand DNA, *anti-GBM* anti-glomerular basal membrane, *BCR* B-cell receptor, *BUN* blood urea nitrogen, *CAIA* collagen antibody-induced arthritis, *CIA* collagen-induced arthritis, *CNS* central nervous system, *EAE* experimental autoimmune encephalomyelitis, *FcR* Fc receptor, *GC* germinal center, *GPA* granulomatosis with polyangiitis, *IFNα* interferon α, *ITP* immune thrombocytopenia, *KC* keratinocyte-derived chemokine, *M-CSF* macrophage-colony-stimulating factor, *MIP-1α* macrophage inflammatory protein α, *MRL/lpr* Murphy Roths Large lymphoproliferative, *MS* multiple sclerosis, *NP-KLH* 4-hydroxy-3-nitrophenylacetyl-keyhole limpet, *NP-LPS* 4-hydroxy-3-nitrophenylacetyl-lipopolysaccharide, *PBMC* peripheral blood mononuclear cell, *RANKL* receptor-activator of nuclear factor kappa B ligand, *RANTES* Regulated upon Activation Normal T Cell Expressed and Presumably Secreted, *SRBC* sheep red blood cell, *TLR* toll-like receptor, *TNFα* tumor necrosis factor α, *NZB/W* New Zealand black × New Zealand white

\*No data available

‡Unpublished data from company presentation (<https://www.innocarepharma.com/media/1419/innocare-pharma-investor-presentation-2019.pdf>)

since changes in antibody glycosylation are also observed during active autoimmune disease [123] and BTK inhibition abolishes Fc $\gamma$ RIII-induced TNF $\alpha$ , IL-1 $\beta$ , and IL-6 production in macrophages in the context of myeloid cell-mediated RA [75].

## 4 BTK Inhibitors in Clinical Trials of Systemic Autoimmune Diseases

Although several trials with BTK inhibitors in autoimmune diseases have been completed, few have been published in research articles. When no publications were available, the data in this review were largely obtained from clinicaltrials.gov (search for stated NCT number) or from published abstracts of scientific meetings.

### 4.1 Acalabrutinib

Acalabrutinib, an irreversible, covalent inhibitor of BTK, was the second BTK inhibitor to reach the clinic for B-cell malignancies [124]. This inhibitor has a much higher specificity for BTK than the first-in-kind inhibitor ibrutinib, making it more suitable for autoimmune patients. A clinical trial was conducted with RA patients with stable methotrexate (MTX) treatment, but to date results have not been published (NCT02387762).

### 4.2 BMS-986142

This reversible, covalent BTK inhibitor reduced FcR-mediated cytokine production by healthy human peripheral blood mononuclear cells (PBMCs) and BCR-induced cytokine production by healthy human B cells *in vitro* (Table 2) [105]. As described above, in GPA, BMS-986142 inhibited plasma cell differentiation and antibody production *in vitro*, although B cells from patients with active disease appeared less susceptible to treatment than B cells from remission patients or healthy individuals. In animal models of RA, this inhibitor has shown promising efficacy by dose dependently reducing joint inflammation and destruction, synergistically with standard-of-care treatment [105]. However, in a clinical trial with active RA patients, no significant improvement of symptoms was observed after 12 weeks of combined treatment with BMS-986142 and MTX compared to placebo and MTX (NCT02638948) (Table 3). A monotherapy study with primary SjS patients was terminated due to inability to reach protocol objectives (NCT02843659).

### 4.3 Elsubrutinib

In animal models of autoimmunity, this irreversible, non-covalent BTK inhibitor showed dose-dependent

improvement in disease severity, organ damage, and mortality [125]. Importantly, antibody formation following model antigen immunization or vaccination was not hampered in mice, suggesting that normal antibody responses can be mounted upon infection [125]. *In vitro*, elsubrutinib inhibited activation and pro-inflammatory cytokine production by B cells and PBMCs. However, in a clinical trial with RA patients, elsubrutinib did not enhance the efficacy of the JAK-1 inhibitor upadacitinib, and appeared to have limited efficacy as monotherapy (NCT03682705). A phase II clinical trial with SLE patients is currently recruiting patients (NCT03978520; long-term extension study NCT04451772).

### 4.4 Evobrutinib

This irreversible covalent BTK inhibitor showed promising results in animal models for RA, SLE, and MS [28, 126]. In addition, it showed clinical efficacy in MS patients, as described above [26]. Nevertheless, evobrutinib did not appear to improve symptoms in RA patients on stable MTX or with inadequate response to MTX and SLE patients (NCT02784106, NCT03233230, and NCT02975336).

### 4.5 Fenebrutinib

Fenebrutinib is a highly specific, reversible, non-covalent inhibitor of BTK. It was effective in CIA in mice and inhibits BCR and CD40L-mediated signaling and activation of B cells [92]. In a cohort of RA patients with an inadequate response to MTX, fenebrutinib increased the proportion of patients with an American College of Rheumatology (ACR) 50 response (50% improvement of disease symptoms defined by the ACR [127]) after 12 weeks of treatment with a dose of 150 mg daily compared to placebo, and reached an effectiveness comparable to adalimumab treatment with a high dose of 200 mg twice daily [128]. In addition, at this high dose, it increased the ACR50 response rate in another patient cohort, characterized by an inadequate response to TNF $\alpha$  inhibition, although not significantly. However, ACR20 response and changes in disease activity score 28-C-reactive protein (DAS28-CRP; a disease activity score including 28 swollen joint counts and CRP levels [129]) at 12 weeks compared to baseline were significant in these patients [128]. All doses were well tolerated, with (serious) adverse events occurring in fenebrutinib-treated patients at similar rates as in adalimumab-treated patients. Fenebrutinib moderately reduced total IgM, IgG, and rheumatoid factor-IgM in the serum of patients in a dose-dependent fashion, and in the second cohort it also reduced serum anti-citrullinated protein antibody (ACPA)-IgG. Despite the high efficacy in RA patients, a similar study in moderate to severe SLE patients did not improve symptoms (NCT02908100; long-term extension study NCT03407482).

**Table 3** Ongoing and completed clinical trials (phase II or III) with Bruton's tyrosine kinase (BTK) inhibitors in systemic autoimmune diseases

Trial no. and type	Disease	Treatment arms	Treatment duration	Primary outcome	Results
<i>Acalabrutinib</i>					
NCT02387762 phase IIa, R, DB, PC	Active RA background MTX	<ul style="list-style-type: none"> <li>• Placebo daily + stable MTX (7.5–25 mg) weekly</li> <li>• ACA 15mg daily + stable MTX (7.5–25 mg) weekly</li> </ul>	4 weeks	DAS28-CRP at 4 weeks	No results reported
<i>BMS-986142</i>					
NCT02638948 phase II, R, DB, PC	Active RA with inadequate response to MTX	<ul style="list-style-type: none"> <li>• Placebo daily + MTX</li> <li>• BMS 100 mg daily + MTX</li> <li>• BMS 200 mg daily + MTX</li> </ul>	12 weeks	% ACR20/ACR70 response at 12 weeks	No significant difference in % ACR20 or ACR70 response at 12 weeks in both treatment arms compared to placebo <sup>#</sup>
NCT02843659 phase II, R, DB, PC	Moderate to severe primary SjS	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• BMS 350 mg daily</li> </ul>	12 weeks	Mean change ESSDAI from baseline at 12 weeks	Terminated due to inability to meet protocol objectives
<i>Branebrutinib</i>					
NCT04186871 phase II, R, DB, PC	Active SLE, primary SjS or RA	<ul style="list-style-type: none"> <li>• SLE/SjS: <ul style="list-style-type: none"> <li>• Placebo daily<sup>†</sup></li> <li>• BRA daily</li> </ul> </li> <li>• RA: <ul style="list-style-type: none"> <li>• Placebo daily, followed by abatacept</li> <li>• BRA daily followed by abatacept</li> </ul> </li> </ul>	*	<ul style="list-style-type: none"> <li>• SLE: % <math>\geq</math> 50% reduction mCLASI from baseline at 24 weeks</li> <li>• SjS: % changes in composite score from baseline at 24 weeks</li> <li>• RA: % ACR50 response at 12 weeks</li> </ul>	Currently recruiting
<i>Elsbrutinib</i>					
NCT03682705 phase II, R, DB, PC	Active RA with inadequate response/ intolerance to DMARD	<ul style="list-style-type: none"> <li>• Placebo + placebo daily</li> <li>• ELS 60mg + upadacitinib 15 mg daily</li> <li>• ELS 60 mg + placebo daily</li> <li>• ELS 20 mg + placebo daily</li> <li>• ELS 5 mg + placebo daily</li> <li>• Placebo + 15 mg upadacitinib daily</li> <li>• Placebo + placebo daily<sup>†</sup></li> <li>• ELS + upadacitinib daily</li> <li>• ELS + placebo daily</li> <li>• Placebo + upadacitinib daily</li> <li>• Placebo + placebo daily<sup>†</sup></li> <li>• ELS + upadacitinib A daily</li> <li>• ELS + upadacitinib B daily</li> <li>• ELS + placebo daily</li> </ul>	12 weeks	Change in DAS28-CRP from baseline At 12 weeks	Significant effect of combined ELS + UPA treatment compared to placebo, but no difference compared to UPA monotherapy <sup>#</sup>
NCT03978520 phase II, R, DB, PC	Moderate to severely active SLE	<ul style="list-style-type: none"> <li>• Placebo + placebo daily</li> <li>• Placebo + placebo daily<sup>†</sup></li> <li>• ELS + upadacitinib daily</li> <li>• ELS + placebo daily</li> <li>• Placebo + upadacitinib daily</li> <li>• Placebo + placebo daily<sup>†</sup></li> </ul>	*	Achievement of SRI-4 and steroid dose $\geq$ 10 mg prednisone equivalent at 24 weeks	Currently recruiting
NCT04451772 LTE of NCT03978520	Moderate to severely active SLE	<ul style="list-style-type: none"> <li>• Placebo + placebo daily</li> <li>• ELS + upadacitinib A daily</li> <li>• ELS + upadacitinib B daily</li> <li>• ELS + placebo daily</li> </ul>	Up to 56 weeks	Number of participants with adverse events through week 104	Currently recruiting

Table 3 (continued)

Trial no. and type	Disease	Treatment arms	Treatment duration	Primary outcome	Results
<i>Evo Brutinib</i>					
NCT02784106 phase IIa, R, DB, PC	RA with stable MTX treatment	<ul style="list-style-type: none"> <li>• Placebo daily + MTX</li> <li>• EVO 2 × 50 mg daily + MTX</li> </ul> Extension period from 12–26 weeks both arms with EVO 2 × 50 mg daily	12 weeks (extension through 26 weeks)	% ACR20 response at 12 weeks	No significant difference in ACR20 % between placebo and treatment <sup>#</sup>
NCT03233230 phase IIb, R, DB, PC	RA with inadequate response to MTX	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• EVO 25 mg daily</li> <li>• EVO 75 mg daily</li> <li>• EVO 2 × 50 mg</li> </ul>	12 weeks	% ACR20 response using hsCRP at 12 weeks	No significant difference in ACR20 % between placebo and treatment arms <sup>#</sup>
NCT02975336 phase II, R, DB, PC	Active SLE	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• EVO 25 mg</li> <li>• EVO 75 mg daily</li> <li>• EVO 2 × 50 mg daily</li> </ul> Extension period from 52–104 weeks both arms with EVO 2 × 50 mg daily	52 weeks (extension through 104 weeks)	SRI-4 response at 52 weeks	No significant difference in SRI-4 response between placebo and treatment arms <sup>#</sup>
<i>Fenebrutinib</i>					
NCT02833350 phase II, R, DB, PC	Active RA with inadequate response to MTX (cohort 1) or TNF inhibition (cohort 2)	Cohort 1: <ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• FEN 50 mg daily</li> <li>• FEN 150 mg daily</li> <li>• FEN 2 × 200 mg daily</li> </ul> Cohort 2: <ul style="list-style-type: none"> <li>• Adalimumab 40 mg/2 weeks all arms + MTX and folic acid</li> <li>• Placebo daily</li> <li>• FEN 2 × 200 mg daily</li> </ul> Both arms: + MTX and folic acid	12 weeks	% ACR50 response at 12 weeks	Cohort 1: ACR50 response rate was significantly higher in 150 mg (28%), 2 × 200 mg (35%) and ADA (36%)-treated patients compared to placebo (15%). [128] Cohort 2: ACR50 response rate was increased in 2 × 200 mg treated patients (25%) compared to placebo (15%), although not significantly ( $p = 0.07$ ) [128]
NCT02983227 LTE of NCT02833350	RA with inadequate response to MTX (cohort 1) or TNF inhibitors (cohort 2)	<ul style="list-style-type: none"> <li>• 2 × 200 mg FEN daily</li> </ul>	52 weeks	% adverse events by week 60 % ACR50 response at 52 weeks	Cohort 1: 60.2% reported AE, 57.1% ACR50 response <sup>#</sup> Cohort 2: 57% reported AE, 50% ACR50 response
NCT02908100 phase II, R, DB, PC	Moderate to severe SLE	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• FEN 150 mg daily</li> <li>• FEN 2 × 200 mg daily</li> </ul> All arms + standard care	48 weeks	SRI-4 response at week 48	No significant difference in SRI-4 response between placebo and treatment arms <sup>#</sup>

Table 3 (continued)

Trial no. and type	Disease	Treatment arms	Treatment duration	Primary outcome	Results
NCT03407482 LTE of NCT02908100 <i>Poseltinib</i>	SLE	<ul style="list-style-type: none"> <li>• FEN 2 × 200 mg daily</li> </ul>	*	% adverse events by week 8	Terminated due to lack of efficacy in parent study
NCT02628028 phase II, R, DB, PC	RA with mildly active (part A) or moderately to severely (part B) disease	<p>Part A:</p> <ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• POS 5 mg daily</li> <li>• POS 10 mg daily</li> <li>• POS 30 mg daily</li> </ul> <p>Part B:</p> <ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• POS 5 mg daily</li> <li>• POS 10 mg daily</li> <li>• POS 30 mg daily</li> </ul>	<p>Part A: 4 weeks</p> <p>Part B: 12 weeks</p> <p>+ 52 weeks extension</p>	<p>Part A: Number of treatment emergent/serious adverse events or adverse event of special interest</p> <p>Part B: % patients with ACR20 response at 12 weeks</p>	<p>Part A: No safety signals precluded start of part B. [131]</p> <p>Part B: Interim analysis indicated no statistical difference in ACR20 rate, trial was discontinued [131]</p>
<i>Orelabrutinib</i> NCT04305197 phase Ib/IIa, R, DB, PC	Mild to moderate SLE	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• ORE 50 mg daily</li> <li>• ORE 80 mg daily</li> <li>• ORE 100 mg daily</li> </ul>	12 weeks	% patients with (serious) adverse events	Currently recruiting
<i>Remibrutinib</i> NCT04035668 phase II, R, DB, PC	Active primary SJS	<ul style="list-style-type: none"> <li>• Placebo<sup>§</sup></li> <li>• REM low dose</li> <li>• REM intermediate dose</li> <li>• REM high dose</li> <li>• REM high dose 2</li> </ul>	*	Changes in ESSDAI from baseline at 24 weeks	Currently recruiting
<i>Rilzabrutinib</i> NCT02704429 phase II, open label	Newly diagnosed or relapsed pemphigus vulgaris with moderate-severe disease	<p>Part A:</p> <ul style="list-style-type: none"> <li>• RIL 2 × 400–600 mg daily</li> </ul> <p>Part B:</p> <ul style="list-style-type: none"> <li>• 24 weeks RIL + 4 weeks follow-up</li> </ul>	<p>Part A: 12 weeks + 12 weeks follow-up</p> <p>Part B: 24 weeks + 4 weeks follow-up</p>	<p>Part A: Incidence of adverse events at 4 weeks; % control of disease activity (CDA) at week 4</p>	<p>Part A: 74% treatment emergence adverse events</p> <p>52% CDA at 4 weeks [133]</p>
NCT03762265 phase III, R, DB, PC <i>Spebrutinib</i>	Newly diagnosed or relapsing moderate to severe pemphigus vulgaris or foliaceus	<ul style="list-style-type: none"> <li>• Placebo + ≤ 5 mg prednisone daily</li> <li>• RIL + ≤ 5 mg prednisone daily</li> </ul>	68 weeks + 48 weeks extension	% complete remission at week 37	Active, not recruiting

Table 3 (continued)

Trial no. and type	Disease	Treatment arms	Treatment duration	Primary outcome	Results
NCT01975610 phase IIa, R, DB, PC	RA (female) with stable MTX treatment	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• SPE 375 mg daily</li> </ul>	4 weeks	% ACR20 response at 4 weeks	10/24 treated patients reached ACR20 by 4 weeks, compared to 5/23 placebo-treated patients ( $p = 0.25$ ) [134]
TAS5315	RA with inadequate response to MTX	<ul style="list-style-type: none"> <li>• Placebo<sup>‡</sup></li> <li>• TAS low dose</li> <li>• TAS high dose</li> </ul> All arms + MTX; after 12 weeks, placebo group is randomized into low or high dose	12 or 36 weeks	% ACR20 response at 12 weeks	Completed, no data reported
<i>Tirabrutinib</i>					
NCT03100942 phase II, R, DB, PC	Active SjS	<ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• TIR 40 mg daily</li> <li>• Lanraplenib 30 mg daily</li> <li>• Filgotinib 200 mg daily</li> </ul> After 24 weeks, placebo group is randomized into other groups through 48 weeks	TIR: 49.4 weeks LAN: 50.4 weeks FIL: 50.3 weeks	% patients fulfilling protocol-specified response at 12 weeks	No significant difference in improvement of clinical symptoms at 12 weeks was found for any treatment arm compared to placebo <sup>#</sup>
NCT02626026 # phase I, R, DB, PC	Healthy individuals (cohort 1) and active RA (cohort 2)	Cohort 1, part A: <ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• TIR 2 × 10 mg TIR daily</li> </ul> Cohort 1, part B: <ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• TIR 20 mg daily</li> </ul> Cohort 2, part B: <ul style="list-style-type: none"> <li>• Placebo daily</li> <li>• TIR 20 mg daily</li> </ul>	Part A: 1 week Part B: 4 weeks	% treatment-emergent adverse events and % treatment-emergent laboratory abnormalities at 7 days (part A) or 59 days (part B)	Part A: No apparent differences in adverse events or laboratory abnormalities <sup>#</sup> Part B: No apparent differences in adverse events, but more laboratory abnormalities in treated patients

ACA acalabrutinib, ACR20 American College of Rheumatology 20, BMS BMS-986142, BRA branebrutinib, CDA control of disease activity, DAS28-CRP disease activity score 28-C-reactive protein, DB double blind, DMARD disease-modifying anti-rheumatic drug, ELS elsubrutinib, ESSDAI EULAR Sjögren's syndrome disease activity index, EVO evobrutinib FEN fenebrutinib, FIL filgotinib, hsCRP high sensitive CRP, LAN lanraplenib, mCLASI modified Cutaneous Lupus Erythematosus Disease Area and Severity Index, MTX methotrexate, ORE orelabrutinib, PC placebo-controlled, POS poseltinib, R randomized, RA rheumatoid arthritis, REM remibrutinib, RIL rilzabrutinib, SJS Sjögren's syndrome, SLE systemic lupus erythematosus, SPE spebrutinib, SRI-4 SLE responder index-4, TAS TAS5315, TIR tirabrutinib, TNF tumor necrosis factor, UPA upadacitinib

<sup>#</sup>Unpublished data obtained from clinicaltrials.gov

\*Information on treatment duration not available

<sup>‡</sup>Information on dose not available

#### 4.6 Poseltinib

Poseltinib, an irreversible, non-covalent BTK inhibitor, reduced disease severity in several animal models of autoimmunity after onset of disease [103, 130]. Safety assessment in RA patients with mildly active disease showed favorable results [131]. However, the second part of this trial, with moderately to severely active RA patients, was discontinued when interim analysis showed no significant improvement of symptoms after 12 weeks of treatment with any of the three doses tested [131].

#### 4.7 Rilzabrutinib

Rilzabrutinib is a reversible, covalent BTK inhibitor that potently inhibits B-cell activation and proliferation upon BCR stimulation, and antibody production upon CD40/IL-21R or TLR9 stimulation *in vitro* [132]. In addition, it showed efficacy in a wide range of (auto)immune animal models [132]. Of note, in contrast to first-line BTK inhibitors, rilzabrutinib does not interfere with normal platelet aggregation. An open-label study with pemphigus vulgaris patients yielded promising results, with 52% and 70% of patients showing control of disease activity at 4 weeks and 8 weeks, respectively, which further increased and was maintained after treatment was stopped [133]. A phase III, placebo-controlled trial is currently ongoing (NCT03762265).

#### 4.8 Spebrutinib

Spebrutinib can effectively inhibit the activation of several immune cells through various receptors and signaling pathways *in vitro* and reduces disease severity in CIA in mice [104]. In a clinical trial with female RA patients on stable MTX, naïve B cells increased in the circulation of treated patients, and transitional B cells decreased. In addition, levels of CTX-1, a collagen-degradation product, were decreased, suggesting reduced osteoclastogenesis, as were CXCL13 levels [134]. However, the ACR20 response rate was not significantly increased in treated patients versus placebo-treated controls at 4 weeks.

#### 4.9 Tirabrutinib

This irreversible, covalent inhibitor of BTK showed significant effectiveness in the inhibition of human osteoclasts *in vitro* and in CIA *in vivo* [135–138]. Nevertheless, in a clinical trial with SjS patients, no clinical improvement was observed (NCT03100942).

#### 4.10 BTK Inhibitors in Clinical Trials of Autoimmune Diseases with Results Pending

Branebrutinib is an irreversible, covalent inhibitor of BTK that has shown efficacy in RA and SLE mouse models, reducing clinical signs of disease dose dependently [139]. This inhibitor was well tolerated in a phase I study with healthy individuals [140], and these promising results have led to a clinical trial with active SLE, primary SjS, and RA patients, which is currently recruiting patients (NCT04186871).

Orelabrutinib is an irreversible, covalent inhibitor of BTK that was reported to be effective in animal models of RA and SLE, and is currently in a clinical trial with SLE patients (NCT04305197).

Remibrutinib is a highly specific, irreversible, non-covalent BTK inhibitor that reduced antigen-specific IgM formation in rats upon sheep red blood-cell immunization and reduced arthritis development and joint destruction in CIA in rats [95]. A clinical trial with primary SjS patients is currently recruiting (NCT04035668).

TAS5315 is a covalent inhibitor of BTK that showed high selectivity and promising efficacy in animal models of RA and SLE [141–144]. A clinical trial with RA patients with inadequate response to MTX has been completed, but no data have been reported yet (NCT03605251).

### 5 Concluding Remarks and Future Perspectives

The last decade has seen the emergence of a large number of small-molecule inhibitors targeting BTK, many of which demonstrated promising efficacy in preclinical studies with autoimmune animal models. However, in contrast to the convincing clinical efficacy of BTK inhibitors in patients with B-cell malignancies, many clinical trials conducted in autoimmune patients to date have reported negative results, indicating that BTK inhibition as monotherapy may not be sufficient in (many of) these patients. However, the observed reduction of enhancing lesions in evobrutinib-treated patients with multiple sclerosis is an important and very promising exception. Differences in the pathogenic mechanisms of disease and cell types involved may underlie the differences in efficacy. For example, it is of note that BTK is expressed in astrocytes and microglia cells and that animal models for MS have demonstrated that BTK inhibition affects the numbers of astrocytes and microglia cells in the brain and supports remyelination [30, 31].

The reasons for a limited efficacy of BTK inhibition in systemic autoimmunity remain largely unknown, but several mechanisms may contribute to this phenomenon. First, BTK inhibitor treatment in humans may be associated with

low BTK occupancy. Effects of BTK inhibition in affected organs in humans are mostly studied in vitro, and BTK occupancy is often not measured in vivo, even in preclinical models. For example, in vitro experiments have shown an effect of BTK inhibition on synovial macrophages and the platelet–fibroblast-like synoviocyte interaction in RA [112, 113], but whether these effects also occur in vivo remains unclear. For MS, in vivo data in humans are limited, but in EAE mice high BTK occupancy following evobrutinib treatment was observed in the brain [145]. Limited BTK occupancy might also be supported by the observation that there is no profound loss of circulating B cells even after prolonged inhibitor treatment, in contrast to the severe B-cell deficiency found in patients with XLA [146].

Secondly, it is conceivable that BTK inhibition affects critical immunosuppressive capacities of B cells. For example, BTK inhibition in vitro inhibited the differentiation of B cells with a regulatory function and their production of IL-10 and IL-35, which are essential mediators of immunosuppressive B cells [147]. However, small doses of ibrutinib were shown to preserve IL-10 production, while reducing the profibrotic cytokines TNF $\alpha$  and IL-6 in B cells of patients with SSc [114].

Thirdly, BTK inhibitor efficacy may be related to differential involvement of BTK in other signaling pathways in various immune cells. As mentioned above, BTK inhibition can be particularly effective in specific cells types, for example strongly limiting the activity of astrocytes and microglia cells. BTK is required for TLR-induced IL-10 production in macrophage and DC populations [148, 149]. In this context, it was shown that BTK-deficient DCs or inhibitor-treated DCs gain a more mature phenotype with a stronger T-cell-stimulatory ability than wild-type cells [149, 150]. Furthermore, the absence of BTK in XLA patients is associated with impaired TLR9-induced production of IL-6, IL-12, TNF- $\alpha$ , and IL-10, whereby it is of note that TLR9 is protective in autoimmune diseases such as SLE [151, 152].

Finally, in particular signaling pathways BTK functions as a linker molecule, as was shown for interaction with Myd88 and MAL [70], which would render kinase inhibition ineffective. Efficacy may also be hampered by the induction of rewiring of signaling pathways by BTK inhibition in B cells. In this context, we recently showed that upon BTK inhibition in mice, phosphorylation of several proximal BCR signaling molecules, such as CD79a, Syk, and PI3K, as well as the key Btk-effector PLC $\gamma$ 2 and the more downstream kinase extracellular signal-regulated kinase (Erk) were significantly increased [153].

Taken together, these findings suggest that the suppressive activity of BTK inhibition in autoreactive B cells might partly be counteracted by inhibitor-induced activation of autoreactive T cells or proinflammatory myeloid cells. On the other hand, it is possible that the high specificity of

second-generation BTK inhibitors, while greatly improving their safety profile, is accompanied by a loss of beneficial off-target effects that may well be essential in complex autoimmune diseases. To improve the effectiveness of BTK inhibitors, therapeutic strategies should be explored that combine BTK inhibition with other highly specific inhibitors, thereby retaining the favorable safety profile.

Selection or outgrowth of resistant clones is a major problem in clinical management of tumors. As most small-molecule inhibitors have fewer side effects than chemotherapy, treatment with multiple small-molecule inhibitors combined with B-cell-depleting agents is gaining broad interest [154]. Combinations explored in this field may also be of use in autoimmunity. Some strategies aim to target several signaling proteins in order to breach the microenvironmental niche involved in CLL disease. Treatment with the dual SYK/JAK inhibitor cerdulatinib showed induction of apoptosis in CLL cells and reduced capacity to produce CCL3 and CCL4 [155, 156], two chemokines that are induced upon interaction with nurse-like cells, which are supporting tumor-associated macrophages in CLL. It is also attractive to explore combination therapies of BTK inhibitors with histone deacetylase inhibitors (HDACis), as they have demonstrated potential in autoimmune animal models, and use of isoform selective HDACis may overcome adverse effects [157]. Combination therapy with ibrutinib and HDACis affected the survival of CLL cells in the *E $\mu$ TCL-1* CLL mouse model and induced synergistic cytotoxicity in primary cells from CLL patients [158]. Treatment with CUDC-907, which is a dual inhibitor of both PI3K and HDAC activity, resulted in increased apoptosis of MCL cells [159].

BTK inhibition may also enhance the effect of standard-of-care anti-rheumatic drugs, as shown with BMS-986142 in CIA [105]. However, this synergistic effect was not observed in a trial with combined treatment with the BTK inhibitor elsubrutinib and JAK-1 inhibitor upadacitinib in RA patients (NCT03682705). A similar study in SLE patients is currently ongoing (NCT03978520). Future studies investigating combination strategies with different BTK inhibitors and other specific therapeutics may lead to combination therapies that induce long-term remission of disease and facilitate personalized medicine.

## Declarations

**Funding** This project was supported by grants from the Target2B! consortium, The Dutch Arthritis Foundation (grant no. 19-1-201) and Erasmus MC MRace.

**Conflict of Interest** The authors declare no conflicts of interest.

**Ethics Approval** Not applicable.

**Consent to Participate** Not applicable.



**Consent for Publication** Not applicable.

**Availability of Data and Material** Not applicable.

**Code Availability** Not applicable.

**Author Contributions** SFHN, JR, RW, and OBJC wrote the manuscript.

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