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Inhibitors of BTK and ITK: State of the New Drugs for Cancer, Autoimmunity and Inflammatory Diseases

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Abstract

BTK and ITK are cytoplasmic tyrosine kinases of crucial importance for B and T cell development, with loss-of-function mutations causing X-linked agammaglobulinemia and susceptibility to severe, frequently lethal, Epstein–Barr virus infection, respectively. Over the last few years, considerable efforts have been made in order to develop small-molecule inhibitors for these kinases to treat lymphocyte malignancies, autoimmunity or allergy/hypersensitivity. The rationale is that even if complete lack of BTK or ITK during development causes severe immunodeficiency, inactivation after birth may result in a less severe phenotype. Moreover, therapy can be transient or only partially block the activity of BTK or ITK. Furthermore, a drug-induced B cell deficiency is treatable by gamma globulin substitution therapy. The newly developed BTK inhibitor PCI-32765, recently renamed Ibrutinib, has already entered several clinical trials for various forms of non-Hodgkin lymphoma as well as for multiple myeloma. Experimental animal studies have demonstrated highly promising treatment effects also in autoimmunity. ITK inhibitors are still under the early developmental phase, but it can be expected that such drugs will also become very useful. In this study, we present BTK and ITK with their signalling pathways and review the development of the corresponding inhibitors.

Introduction

BTK and ITK are TEC family kinases (TFKs) and loss-of-function mutations cause human disease

Before reviewing the newly developed inhibitors of BTK and ITK, we provide a background to these tyrosine kinases. In the first section, we discuss their identification and the effect of inactivating mutations. In the following section, we describe the intracellular signalling pathway of BTK and ITK and summarize what is known about their regulation. This is followed by the description of the inhibitors.

TFKs, consisting of BTK, BMX (ETK), ITK, TEC and TXK (RLK), form the second largest family of non-receptor kinases in humans, the largest being the SRC family. The TFK ancestor emerged already prior to the evolution of metazoans and shows evidence of differential evolutionary wiring [1–3]. BTK and ITK contain an N-terminal Pleckstrin homology (PH) domain, followed by a Tec homology (TH), Src homology (SH)-3, -2 and -1 (catalytic) domains [4–6]. As depicted in Fig. 1, the TH domain consists of an N-terminal Zn^{2+}-binding BTK motif, and one or two proline-rich motifs [4, 7–9].

All the mammalian TFKs were identified in the 1990s. ITK [10, 11] and BTK [12, 13] were each cloned independently by two groups. This family of kinases soon received wide interest, owing to the fact that *BTK* mutations cause an X-linked form of B-lymphocyte deficiency (X-linked agammaglobulinemia, XLA) in man [13–16]. Today, more than 1000 patients with known mutations exist in the BTKbase registry [17]. In mice, mutations cause the phenotypically milder X-linked immunodeficiency (Xid) [18, 19]. In mice, it seems as if the TEC kinase has a unique compensatory role, because, while the TEC kinase is also expressed in human B lymphocytes, in mice, the double knockout of BTK and TEC causes an XLA-like phenotype. In contrast, TEC single-knockout mice do not have an overt phenotype [20].

ITK deficiency in humans is much less common and was not reported until 16 years after the identification of mutations in *BTK*. Thus, *ITK* is one of the several genes in which loss-of-function mutations cause susceptibility to severe, often fatal, Epstein–Barr virus infections [21].

BTK is constitutively expressed in myeloid and lymphoid cells but absent in T cells and in mature plasma cells [22]. It is found during all stages of the B cell lineage up until the plasma-cell stage, where it is absent in the most

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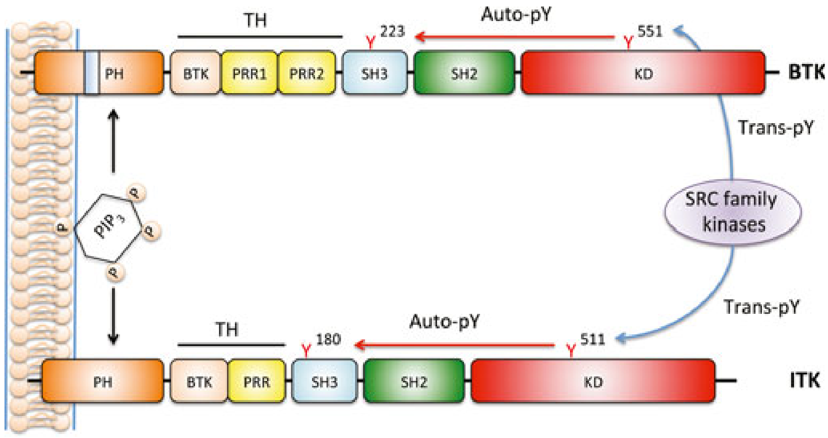


Figure 1 Schematic representation of BTK and ITK showing membrane binding and regulatory tyrosine phosphorylation sites. BTK and ITK have similar domain organization, with the difference that BTK has two proline-rich repeats (PRR) in the Tec homology domain (TH). The highly conserved BTK motif binds a Zn^{2+} ion, which stabilizes the PH domain. The PH domain binds phosphatidylinoisitol-3,4,5-trisphosphate (PIP3), which is generated by PI3K (Fig. 2). In the PH domain of BTK, there is a 27 amino acid insertion not found in ITK (marked in blue). The SH3 domain binds to proline-rich regions, while the SH2 domain interacts with phosphorylated tyrosine residues forming reversible signalling complexes. The depicted trans-phosphorylated tyrosine residue in the catalytic domain has an activating function, whereas the role of the autophosphorylated tyrosine in the SH3 domain is less defined.

mature form. Mutations cause a differentiation block at the stage of pre-B cells, with mature B cells being very few and non-responsive to foreign antigens, even if rare patients can have close to normal numbers [14, 23–25].

ITK is less widely expressed and is crucial for T-lymphocyte development, as initially shown by knocking out the gene in mice [26]. More in-depth studies revealed that both ITK-deficient mice [5, 6, 27] and the few patients with ITK mutations analysed so far show almost complete absence of invariant natural killer T cells. While several different genetic defects show susceptibility to severe EBV infection, it was recently reported that ITK deficiency is clinically distinct from both signalling lymphocyte activation molecule (SLAM)-associated protein (SAP) and X-linked inhibitor of apoptosis protein (XIAP) deficiency [28]. ITK may also be a crucial host factor needed for the development of an HIV infection [29]. Further studies have shown that ITK-deficient mice have drastically reduced lung inflammation, eosinophil infiltration and mucous production in response to ovalbumin-induced induction of allergic asthma [30]. Therefore, inhibition of T cell activation has been one of the strategies for developing immunosuppressive agents to treat autoimmune disorders and inflammation [31]. Suppression of host immune functions by blocking T cell activation is also a successful modality for preventing organ transplant rejection [32].

In contrast to BTK, ITK is not constitutively expressed. Thus, the corresponding transcript was initially identified from an IL-2-dependent mouse T cell line [11]. This means that even if BTK and ITK frequently are considered to be analogs, selectively expressed in T- and B cells, respectively, their differential expression with regard to the need for inducibility shows that this is not entirely true. This difference is also likely to influence the treatment effect of inhibitors.

Signalling pathways of BTK and ITK and target diseases

TFKs play central, but diverse, modulatory roles in various cellular processes. They participate in signal transduction in response to virtually all types of extracellular stimuli that are transmitted by growth factor receptors, cytokine receptors, G-protein-coupled receptors, antigen receptors and integrins [33, 34]. As illustrated in Fig. 2 (Step 1), following BCR, TCR stimulation, SRC family kinases are activated, leading to the phosphorylation of immunoreceptor tyrosine activation motifs (ITAMs) of the CD79 (Ig-α, Ig-β) and CD3 complex chains. Similarly, PI3K is activated to catalyse the conversion of membrane-associated PIP2 to PIP3 leading to BTK/ITK recruitment to the plasma membrane through the interaction of its PH domain with PIP3 [35]. Concomitantly, the phosphorylation of Ig-α and Ig-β ITAMs leads to the recruitment of SYK/ZAP70 kinase via SYK SH2 domains. Following activation, BTK/ITK ignites multiple downstream signals generating pleiotropic effects (Fig. 2, step 2): (1) PLCγ activation, generation of second messengers, such as inositol [1,4,5]-triphosphate (IP3), diacylglycerol (DAG) and calcium, (2) Cell proliferation, differentiation, apoptosis and survival [16, 36]. However, the molecular basis of many of these pathways is not fully understood, and many interacting molecules remain to be isolated. Using affinity purification combined with tandem mass spectrometry, we have recently characterized the interaction of BTK with an ankyrin-repeat domain protein [37] and the protein 14-3-3,

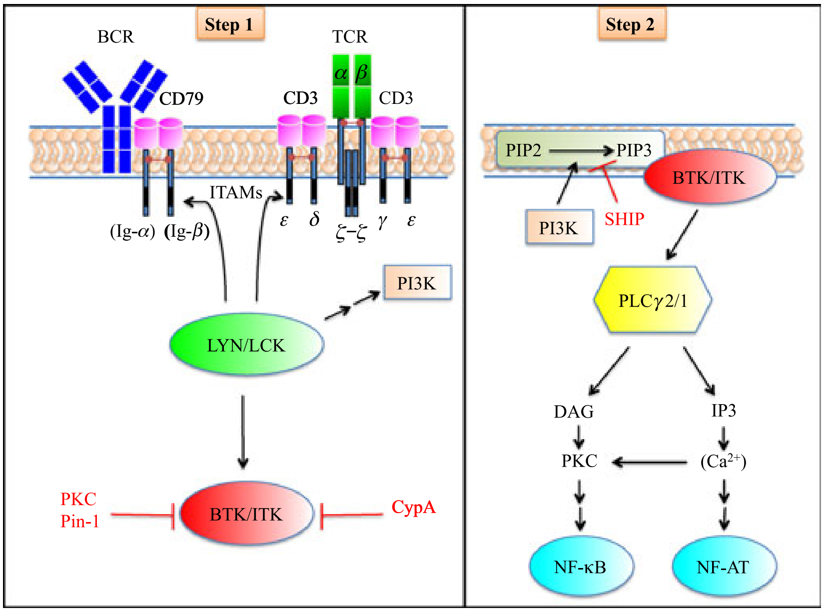


Figure 2 BTK and ITK activation. B cell receptor (BCR) and BTK signalling components are depicted to the left and T cell receptor (TCR) to the right upon engagement of BCR and TCR, SRC family kinases including LYN and LCK are activated leading to phosphorylation of immunoreceptor tyrosine activation motifs (ITAMs) (Step 1). Activated PI3K converts PtdIns-4,5-bisphosphate (PIP2) into PIP3, which tethers BTK/ITK to the membrane (Fig. 1), where they phosphorylate regulatory tyrosine residues in PLCγ2 and PLC, γ1 respectively (Step 2). The increasing PLCγ activity results in the production of the secondary messengers DAG and IP3 inducing activation of the transcription factors NF-κB (active mainly in B cells) and NF-AT (active mainly in T cells). Endogenous BTK/ITK inhibitors (PKC, Pin-1/CypA) regulate their activity affecting cellular responses such as cell survival, apoptosis, adhesion, migration and proliferation.

which regulate nucleo-cytoplasmic shuttling [38] and attenuate signalling [39].

To date, almost only B cell-derived tumours have been treated with the newly developed inhibitor for BTK, Ibrutinib (see below). The rationale is that tumours, similar to non-transformed B cells, may be dependent on BCR signalling for their survival. However, tumours with activating mutations downstream of BTK are likely to be resistant, and this also seems to be the case [40]. Although animal models of autoimmunity show very promising outcomes from Ibrutinib treatment, the drug has not yet been used in clinical studies in man. Allergies and other forms of hypersensitivity and inflammatory diseases with a strong B cell component could also become targets. Target diseases for ITK inhibitors are less defined and will await studies in relevant animal models. As always, potential side effects have to be balanced against the benefit of treatment.

BTK inhibitors

LFM-A13

The leflunomide metabolite analog (LFM-A13) (alpha-cyano-beta-hydroxy-beta-methyl-*N*-(2,5-ibromophenyl)-propen-amide) (Table 2) is one of the first rationally designed antileukemic agents targeting BTK [41, 42]. This small-molecule inhibitor binds non-covalently to the catalytic site of BTK in a reversible manner (half-maximal inhibitory concentration (IC_{50}) = 17.2 μM for human BTK *in vitro* and IC_{50} = 2.5 μM for recombinant BTK). It does not affect the enzymatic activity of other protein tyrosine kinases, including EGFR, HCK, IRK JAK1, JAK3 and IRK at concentrations of 278 μM [41]. However, even if the molecule has been described as a highly specific inhibitor of BTK, it can also efficiently affect the activity of other kinases such as the erythropoietin receptor, JAK2 and downstream molecules [43].

During the last decade, a plethora of *in vitro* and *in vivo* studies have suggested that LFM-A13 could act as a dual-function anticancer drug with apoptosis-promoting and antithrombotic properties [44–46]. In addition, LFM-A13 also exhibits antiproliferative activity against Her2/Neu-overexpressing breast cancer cells [47]. Furthermore, LFM-A13 has been reported to prevent acute fatal graft-versus-host disease in a murine model of allogeneic bone marrow transplantation [48]. In addition, LFM-A13 has been broadly used *in vitro* to inhibit BTK downstream signalling pathways (Fig. 2) and further to elucidate the role of SFKs [49–51]. On neutrophils, for example, it has been shown that LFM-A13 also negatively affects the translocation of Rac-2, RhoA, ADP ribosylation factor-1, TEC, BMX and BTK induced by fMet-Leu-Phe [52]. Moreover, LFM-A13 could block the endogenous phosphorylation of Myd88 adapter-like (Mal) on tyrosine in cells treated with macrophage-activating lipopeptide-2 or LPS [53] and LFM-A13 inhibited Heme oxygenase (HO-1) induction by the classical TLR4 ligand LPS in cell cultures [54]. Other reports show that LFM-A13 by inhibiting TFKs, rescues the suppression of TCR-induced CD25 expression in Jurkat cells [55]. In primary myeloma-bearing immunodeficient mice, LFM-A13 inhibited osteoclast activity, prevented myeloma-induced bone resorption and moderately suppressed myeloma growth [56]. Administration of LFM-A13 is not toxic to mice, rats or dogs at daily dose levels as high as 100 mg/kg [57]. However, as mentioned, rather high doses are needed for a pharmacological effect, and we are not aware of any ongoing, or planned, clinical studies.

Dasatinib

Dasatinib, BMS-354825 or Sprycel [N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide] (Table 2) is an orally available, dual ABL/SRC tyrosine kinase inhibitor (TKI), which was developed to treat patients with chronic myelogenous leukaemia (CML), who had failed, or were intolerant to, therapy with Imatinib BCR-ABL1 and SFK TKI [58, 59]. Other diseases in which bone metastases are frequent (e.g. breast or prostate tumours) could also benefit from the addition of Dasatinib to standard-of-care treatments [60, 61].

Native targets of Dasatinib in CML cells have been identified using a chemical proteomics approach [62–64]. Besides ABL and SRC kinases, BTK and TEC, but not ITK, were recognized as major binders inhibited by nanomolar concentrations. In addition, the gatekeeper residue as the critical determinant of Dasatinib susceptibility has been detected with the help of structure-based mutagenesis experiments. Mutation of Thr-474 in BTK to Ile and Thr-442 in TEC to Ile conferred resistance to Dasatinib, whereas mutation of the corresponding residue in ITK (Phe-435) to Thr sensitized the otherwise insensitive ITK [64]. Other studies have shown that Dasatinib induces apoptosis in primary chronic lymphocytic leukaemia (CLL) cells blocking LYN kinase activity [65, 66]. Moreover, Dasatinib decreased levels of the activated, phosphorylated forms of AKT, ERK1/2 and p38 and reduced the expression of the antiapoptotic proteins MCL-1 and BCL-X_L [67]. Thus, it seems that Dasatinib as a single agent has activity in relapsed and refractory CLL [66].

In line with the role of TFKs in lymphoid and myeloid cells, Dasatinib inhibited the secretion of several immunomodulators [68, 69]. The observed inhibition of TFKs predicts immunosuppressive (side) effects of this drug and may offer therapeutic opportunities for inflammatory and immunological disorders [64, 70, 71]. However, further experiments are required to describe the exact mechanism of the above-mentioned hypothesis.

In summary, this compound has been approved by the FDA for the treatment of patients with CML in all phases or Ph+-ALL, who were resistant, or intolerant, to therapy, with Imatinib. In Europe, it has been approved for therapy of patients with CML who are resistant, or intolerant, to Imatinib [59]. However, the drug has not been used to clinically interfere with TFKs.

Ibrutinib (PCI-32765)

Ibrutinib, (1-{(3R)-3-[4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl]piperidin-1-yl}prop-2-en-1-one) (Table 2), is a selective and irreversible small-molecule BTK inhibitor that inhibits BCR signalling in human B cells. It was originally named PCI-32765 and re-named Ibrutinib by the World Health Organization (WHO) and the United States Adopted Name (USAN) Council. Orally administered Ibrutinib has demonstrated to be particularly active in different B cell malignancies including CLL, mantle cell lymphoma (MCL), diffuse large B cell lymphoma (DLBCL) and multiple myeloma (MM) [72–75].

Ibrutinib inactivates BTK through covalent binding to the active site (Cys-481) in the ATP-binding domain of BTK with IC_{50} of 0.5 nmol/L [76]. Several TFKs with homology to BTK, including BMX and ITK, have similar cysteine residues that might also be irreversibly inhibited by Ibrutinib. Other kinases that can also be sensitive to Ibrutinib at nanomolar concentrations include BLK, TEC, EGFR, ERBB2, HER2, HER4 and JAK3 [72, 76, 77].

Ibrutinib as a potential drug for B cell malignancies

The use of Ibrutinib in preclinical and clinical trials appears to be a promising new strategy for treatment of B cell malignancies (Table 1). The *in vivo* effect of ibrutinib has been demonstrated in patients with CLL. Recent reports have shown that Ibrutinib inhibits CLL cell survival and proliferation as well as induces CLL apoptosis [77, 78]. In addition, treatment of CD40- or BCR-activated CLL cells with Ibrutinib results in inhibition of BTK tyrosine phosphorylation and also effectively abrogates downstream survival pathways activated by this kinase, including ERK1/2, PI3K and NF-κB [74, 77].

Ibrutinib also acts by modulating the interaction between CLL cells and their microenvironment. For example, it inhibits activation-induced proliferation of CLL cells and effectively blocks survival signals, which are provided externally to CLL cells from the microenvironment (CD40L, BAFF, IL-6, IL-4 and TNFα, fibronectin) engagement and stromal cell contact, as well as migration in response to tissue-homing chemokines (CXCL12, CXCL13) [79]. Moreover, the secretion of BCR-dependent cytokines such as CCL3 and CCL4 is effectively decreased both *in vitro* and in patients with CLL treated with Ibrutinib [78].

Table 1 Clinical trials of Ibrutinib in B cell malignancies.

Disease	Study description	Drugs and doses	Study phase	Estimated patients/age	Objective	Study duration	Clinical trials.gov identifier
CLL and SLL	CLL, SLL	Ibrutinib (420 mg)	II	30 ≥18 years	Impact on leukemia cell trafficking and death	2012–2015	NCT01752426
	ROR CLL,SLL with 17p deletion	Ibrutinib (420 mg)	II	111 ≥18 years	ORR,PFS, OS	2013–2016	NCT01744691
	CLL, SLL in patients older than 65 or have 17p deletion	Ibrutinib (420 mg)	II	86 ≥65 years and ≥ 18 year for 17p deletion	ORR, OS, PFS	2011-2015	NCT01500733
	CLL, SLL	Ibrutinib (420 mg) VS chlorambucil	III	272 ≥65 years	ORR, PFS	2013–2016	NCT01722487
	ROR CLL, SLL	Ibrutinib (420 mg) VS Ofatumumab	III	350 ≥18 years	PFS, OS, ORR	2012–2015	NCT01578707
	ROR CLL,SLL	Ibrutinib (420 mg) + Rituximab+ Bendamustine	III	580 ≥18 years	PFS,OS, ORR	2012–2018	NCT01611090
CLL, SLL, B-PLL	ROR CLL,SLL, B-PLL	Ibrutinib	II	75 ≥18 years	PFS, ORR,OS	2012–2014	NCT01589302
DLBCL	ROR DLBCL	Ibrutinib (560 mg)	II	60 ≥18 years	Efficacy and safety	2011–2014	NCT01325701
FL	Refractory FL	Ibrutinib (560 mg)	II	110 ≥18 years	ORR,OS, FPS	2013–2016	NCT01779791
MCL	MCL	Ibrutinib (560 mg)	II	110 ≥18 years	ORR, PFS, OS	2012–2015	NCT01599949
	ROR MCL	Ibruinib (560 mg) VS Temsirolimus	III	280 ≥18	PFS, OS	2012	NC2012-000601-74
	ROR MCL	Ibrutinib (560 mg) VS Temsirolimus	III	280 ≥18 years	PFS,ORR, OS	2012–2017	NCT01646021
	MCL	Ibrutinib (560 mg) + Rituximab+ Bendamustine	III	520 ≥65 years	PFS,OS, ORR	2013–2019	NCT01776840
B cell neoplasm	Recurrent mature B cell neoplasm	Ibrutinib (420, 560 mg)	I	24 ≥20 years	Safety and pharmacokinetic	2012–2014	NCT01704963
MM	Relapsed or relapsed and refractory MM	Ibrutinib (420, 560, 840 mg)	II	164 ≥18 years	Efficacy and safety	2012–2016	NCT01478581
WM	WM	Ibrutinib	II	33 ≥18 years	ORR, safety	2012–2014	NCT01614821

Follicular lymphoma (FL), overall response rate (ORR), progression-free survival (PFS), overall survival (OS), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), multiple myeloma (MM), mantle cell lymphoma (MCL), diffused large B cell lymphoma (DLBCL), B cell prolymphocytic leukemia (B-PLL), relapsed or refractory (ROR), Waldenströms macroglobulinemia (WM).

The initial phase I study of Ibrutinib enrolled patients with B cell lymphomas including CLL demonstrated that a dose of 420 mg is as efficient as 840 mg. In fact, the occupancy and inhibition of BTK were similar in both doses, so 420 mg was selected for further studies to minimize adverse effects [77]. Administration of Ibrutinib (420 mg/day) in patients with CLL induces a rapid shrinkage of enlarged lymph nodes and symptomatic improvement within the first few weeks of treatment [73]. The expected pattern of initial rapid nodal response with sometimes marked lymphocytosis was also observed. This increase in lymphocyte count was transient and could be typically resolved after the first few months of therapy [72, 77]. In the 2012 American Society of Hematology meeting, it was reported that Ibrutinib induces an overall response rate (ORR) of 68% in previously untreated CLL patients, aged 65 or older, and an ORR of 71% in previously treated patients [72].

It has been recently reported that BTK is highly expressed in malignant plasma cells from patients with MM [80, 81]. This is in contrast to the most mature normal plasma cells, where BTK is not expressed [22]. In MM models, Ibrutinib reduced osteoclast formation and bone resorption and also inhibited BTK-mediated osteoclastogenesis induced by M-CSF and RANKL [80]. The chemokine and cytokine secretion from bone marrow stromal cells (BMSCs) and osteoclasts was significantly decreased by Ibrutinib, and it blocks SDF-1-induced adhesion and migration [81]. Furthermore, it also inhibited MM cell growth triggered by IL-6 or coculture with

BMSCs *in vitro*. In addition, Ibrutinib inhibits MM cell–induced osteolysis of implanted human bone chips in SCID mice [80].

Ibrutinib also shows promising and encouraging results as a single-agent drug in MCL. Thus, Ibrutinib produced ORR of 66% in phase II study for patients receiving (560 mg/day) [82]. Co-administration of Ibrutinib with the proteasome inhibitor Bortezomib resulted in a synergistic effect and induced MCL cell-death [74].

Ibrutinib as a therapeutic agent for autoimmune diseases

Ibrutinib shows promising activity against experimental autoimmune diseases, particularly in rheumatoid arthritis (RA). In mice with collagen-induced arthritis (CIA), administration of 12.5 mg/kg effectively reduced arthritic symptoms after few days of treatment [72, 83]. These studies show that in CIA and collagen antibody-induced arthritis, Ibrutinib markedly reduced inflammation, bone resorption and cartilage destruction. Moreover, the infiltration of inflammatory cells, cytokine and chemokine levels in synovial fluid are decreased [83, 84]. In the MRL-Fas (lpr) lupus model, treatment with Ibrutinib inhibits autoantibody production and renal impairment [76].

Pharmacokinetics

Ibrutinib is administered orally and rapidly absorbed. Between 1 and 2 hours after drug administration, it reaches the mean plasma concentration. It has a short half-life of 2-3 hours, but BTK inhibition will remain for 24 hours. Generally, the drug is well tolerated, with mild adverse effects like cough, fatigue, diarrhoea and infectious complications, which could be managed easily (Table S1) [85].

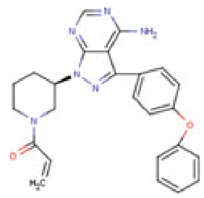
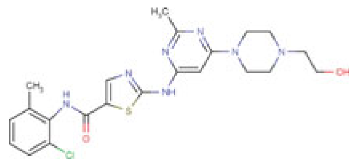
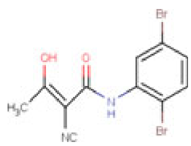
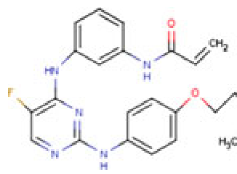
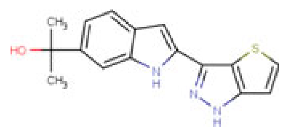
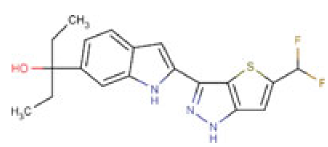
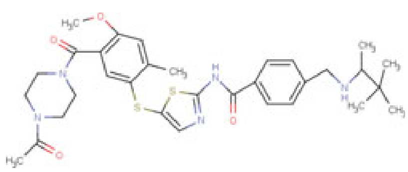
AVL-292

AVL-292 is a new orally available potent compound that selectively inhibits BTK (Table 2). It also binds to Cys-481 at the kinase domain, sustaining BTK inhibition for 24 hours. Human clinical trials with AVL-292 have demonstrated that the drug is safe and like other BCR inhibitors, the drug caused lymphocytosis within few weeks of the treatment [73, 84]. AVL-292 also showed a promising effect in animal models with rheumatoid arthritis. Currently, according to ClinicalTrials.Gov, there are two studies evaluating AVL-292, one recruiting for non-Hodgkin lymphoma, CLL and Waldenström's macroglobulinemia (WM), and a second, active, but not recruiting, studying the safety and pharmacokinetic of this drug [84].

ITK inhibitors

The crystal structure of the ITK kinase domain was elucidated for the phosphorylated and non-phosphorylated kinase domain bound to staurosporine, a potent broad-spectrum kinase inhibitor [86]. These structures are highly useful for the design of selective ITK inhibitors and provide insight into the influence of inhibitor binding and

Table 2 Chemical structure of BTK and ITK inhibitors.

Structure	Compound name
BTK inhibitors	
	Ibrutinib (PCI-32765) MW 440.5
	Dasatinib MW 488
	LFM-A13 MW 360
	AVL-292 MW 423.17
ITK inhibitors	
	Compound 3 (Sanofi) MW 297.38
	Compound 7 (Sanofi) MW 375.44
	BMS-509744 MW 623.83

phosphorylation on the conformation of ITK [86]. Together, Met-398, Phe-403, Ala-407, Met-410 and Met-411 (from the α-C-helix), Phe-374 (from the Gly-rich loop), and Val-424, Leu-433 and Lys-391 define an extensive hydrophobic pocket lying adjacent to the ITK active site [86]. The size and shape of this pocket are different in SFKs as well as in Ser/Thr kinases such as CDK2. Although residues Met-398, Phe-403 and Met-410 are conserved in ITK and BTK, Phe-435 is unique to ITK [86]. This residue is therefore identified as a gatekeeper (or blocker) of this extensive hydrophobic pocket. An inhibitor of the other TEC family members (BTK, TXK/RLK, TEC and BMX) may exploit the smaller threonine residue at this position, either via a hydrogen bonding interaction or by accessing the hydrophobic pocket [86]. A broad-spectrum inhibitor of all TFKs could be achieved using small lipophilic groups positioned close to residue Phe-435 [86] and may have a general role in non-cytotoxic immunosuppression.

A high-throughput screen campaign of a compound collection by Bristol-Myers Squibb and Boehringer Ingelheim (Table 2) led to the identification of potent ITK inhibitors (Table S2) from a distinct scaffold series of 2-aminothiazoles [87, 88] and benzimidazoles [89–91], respectively. Further development of benzimidazoles have demonstrated selective inhibition of ITK, improved the cellular and functional potency as well as the drug-like properties, with the 10n and 10o compounds as excellent agents for proof-of-concept studies [92].

Novel ITK inhibitors based on (4- or 5-aryl)pyrazolyl-indole scaffolds were also found to be selective for ITK over other kinases [93]. Molecular modelling predictions showed that pyrazolyl-indoles are inhibitors of ITK. Docking models of various pyrazolyl-indole derivatives with ITK indicated a common binding mode, which involved three hydrogen bonds in the hinge region of ITK between: (i) N–H of the pyrazole ring with the carbonyl of Glu-436 of ITK, (ii) N of pyrazole and N–H of Met-438 of ITK and (iii) N–H of the indole ring with the carbonyl of Met-438 of ITK [93]. It was also apparent from docking, that among the three series examined, 5-benzylpyrazolyl-indoles (compound 34) show the best binding to ITK, and were followed in the decreasing order by 4-phenylpyrazolyl-indoles (compound 13) and 5-phenylpyrazolyl-indoles (compound 24) (Table S2). On the other hand, compound 44, which was developed by chemical optimization of an initial high-throughput screening hit, inhibited ITK's activity with an IC_{50} in the nanomolar range [94]. Compound 44 substantially reduced pro-inflammatory immune responses *in vitro* and *in vivo* after systemic administration in two acute contact hypersensitivity models [94].

In another study, a series of ITK inhibitors, active in nanomolar concentrations, were synthesized based on indolylindazole libraries. The potential of this series of compounds was confirmed through *in vivo* tests in an anti-CD3-IL2 mouse model. The intravenous administration of highly potent ITK inhibitor 11o (Table S2) resulted in dose-dependent, efficient suppression of IL-2 at 10 mg/kg [95].

The discovery of a new series of potent and selective novel ITK inhibitors based on 3-aminopyride-2-ones has also been reported [96]. These inhibitors were identified by structure-based design, starting from a fragment generated *de novo*, the 3-aminopyrid-2-one motif. Among various derivatives, the compound 7v (Table S2) illustrates the fact that fragment-like *de novo* starting point can rapidly evolve into biologically inhibitors of ITK, with good potency and selectivity profile. Other derivatives 7w, 7x and 7y also show potent inhibition of ITK (Table S2) [96].

Another selective inhibitor, 7-benzyl-1-(3-(piperidin-1-yl)propyl)-2-(4-(pyridin-4-yl)phenyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one (CTA056), was developed through screening of 9600 compounds, followed by molecular modelling, and extensive structure–activity relationship studies [97]. CTA056 (Table S2) exhibits the highest inhibitory effects towards ITK, followed by BTK and BMX. Among the 41 cancer cell lines analysed, CTA056 selectively targets acute lymphoblastic T cell leukaemia and cutaneous T cell lymphoma [97].

Recently, novel and selective thienopyrazole inhibitors of ITK were generated by combining structure-based design and medicinal chemistry at Sanofi (Table 2) (Table S2). The most potent compounds crystallized with the target kinase ITK and also with SYK [98]. The multikinase profiling screen of tested compounds found compound 3 to be potent towards ITK, but not selective (IC_{50} = 1.9 nM ITK, IC_{50} = 4.4 nM SYK and IC_{50} = 73.8 nM TXK), while compound 7 was more selective towards ITK (IC_{50} = 0.3 nM ITK, IC_{50} = 358 nM SYK and IC_{50} = 77.5 nM TXK) [98].

Conclusions

It is evident that the BTK inhibitor Ibrutinib has demonstrated very promising results as a new treatment modality for both B cell malignancies and autoimmunity. Other BTK inhibitors are under development, and it will be very interesting to see the outcome of this collective effort. ITK inhibitors are also likely to serve as important future drugs. However, as compared with Ibrutinib, the ITK inhibitors are less mature, and more research is needed before their usefulness and safety profile can be evaluated.

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References

1 Ortutay C, Nore BF, Vihinen M, Smith CIE. Phylogeny of Tec family kinases identification of a premetazoan origin of Btk, Bmx, Itk, Tec, Txk, and the Btk regulator SH3BP5. *Adv Genet* 2008;64:51–80.

2 Nawaz HM, Kylsten P, Hamada N, Yamamoto D, Smith CIE, Lindvall JM. Differential evolutionary wiring of the tyrosine kinase Btk. *PLoS ONE* 2012;7:e35640.

3 Wu F, Zhao J, Chen L *et al.* A novel BTK-like protein involved in immune response in Lethenteron japonicum. *Immunol Lett* 2012;146:57–63.

4 Smith CIE, Islam TC, Mattsson PT, Mohamed AJ, Nore BF, Vihinen M. The Tec family of cytoplasmic tyrosine kinases: mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. *BioEssays* 2001;23:436–46.

5 Gomez-Rodriguez J, Kraus ZJ, Schwartzberg PL. Tec family kinases Itk and Rlk / Txk in T lymphocytes: cross-regulation of cytokine production and T-cell fates. *FEBS J* 2011;278:1980–9.

6 Qi Q, Kannan AK, August A. Tec family kinases: Itk signaling and the development of NKT alphabeta and gammadelta T cells. *FEBS J* 2011;278:1970–9.

7 Hyvonen M, Saraste M. Structure of the PH domain and Btk motif from Bruton's tyrosine kinase: molecular explanations for X-linked agammaglobulinaemia. *EMBO J* 1997;16:3396–404.

8 Vihinen M, Nilsson L, Smith CIE. Tec homology (TH) adjacent to the PH domain. *FEBS Lett* 1994;350:263–5.

9 Vihinen M, Nore BF, Mattsson PT *et al.* Missense mutations affecting a conserved cysteine pair in the TH domain of Btk. *FEBS Lett* 1997;413:205–10.

10 Heyeck SD, Berg LJ. Developmental regulation of a murine T-cell-specific tyrosine kinase gene, Tsk. *Proc Natl Acad Sci USA* 1993;90:669–73.

11 Siliciano JD, Morrow TA, Desiderio SV. itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2. *Proc Natl Acad Sci USA* 1992;89:11194–8.

12 Tsukada S, Saffran DC, Rawlings DJ *et al.* Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. 1993. *J Immunol* 2012;188:2936–47.

13 Vetrie D, Vorechovsky I, Sideras P *et al.* The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature* 1993;361:226–33.

14 Conley ME, Dobbs AK, Farmer DM *et al.* Primary B cell immunodeficiencies: comparisons and contrasts. *Annu Rev Immunol* 2009;27:199–227.

15 Holinski-Feder E, Weiss M, Brandau O *et al.* Mutation screening of the BTK gene in 56 families with X-linked agammaglobulinemia (XLA): 47 unique mutations without correlation to clinical course. *Pediatrics* 1998;101:276–84.

16 Lindvall JM, Blomberg KE, Valiaho J *et al.* Bruton's tyrosine kinase: cell biology, sequence conservation, mutation spectrum, siRNA modifications, and expression profiling. *Immunol Rev* 2005;203:200–15.

17 Valiaho J, Smith CIE, Vihinen M. BTKbase: the mutation database for X-linked agammaglobulinemia. *Hum Mutat* 2006;27:1209–17.

18 Rawlings DJ, Saffran DC, Tsukada S *et al.* Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science* 1993;261:358–61.

19 Thomas JD, Sideras P, Smith CIE, Vorechovsky I, Chapman V, Paul WE. Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science* 1993;261:355–8.

20 Ellmeier W, Jung S, Sunshine MJ *et al.* Severe B cell deficiency in mice lacking the tec kinase family members Tec and Btk. *J Exp Med* 2000;192:1611–24.

21 Huck K, Feyen O, Niehues T *et al.* Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* 2009;119:1350–8.

22 Smith CIE, Baskin B, Humire-Greiff P *et al.* Expression of Bruton's agammaglobulinemia tyrosine kinase gene, BTK, is selectively down-regulated in T lymphocytes and plasma cells. *J Immunol* 1994;152:557–65.

23 Nomura K, Kanegane H, Karasuyama H *et al.* Genetic defect in human X-linked agammaglobulinemia impedes a maturational evolution of pro-B cells into a later stage of pre-B cells in the B-cell differentiation pathway. *Blood* 2000;96:610–7.

24 Noordzij JG, de Bruin-Versteeg S, Comans-Bitter WM *et al.* Composition of precursor B-cell compartment in bone marrow from patients with X-linked agammaglobulinemia compared with healthy children. *Pediatr Res* 2002;51:159–68.

25 Wood PM, Mayne A, Joyce H, Smith CIE, Granoff DM, Kumararatne DS. A mutation in Bruton's tyrosine kinase as a cause of selective anti-polysaccharide antibody deficiency. *J Pediatr* 2001;139:148–51.

26 Liao XC, Littman DR. Altered T cell receptor signaling and disrupted T cell development in mice lacking Itk. *Immunity* 1995;3:757–69.

27 Hussain A, Yu L, Faryal R, Mohammad DK, Mohamed AJ, Smith CIE. TEC family kinases in health and disease–loss-of-function of BTK and ITK and the gain-of-function fusions ITK-SYK and BTK-SYK. *FEBS J* 2011;278:2001–10.

28 Linka RM, Risse SL, Bienemann K *et al.* Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* 2012;26:963–71.

29 Readinger JA, Schiralli GM, Jiang JK *et al.* Selective targeting of ITK blocks multiple steps of HIV replication. *Proc Natl Acad Sci USA* 2008;105:6684–9.

30 Mueller C, August A. Attenuation of immunological symptoms of allergic asthma in mice lacking the tyrosine kinase ITK. *J Immunol* 2003;170:5056–63.

31 Ohashi PS. T-cell signalling and autoimmunity: molecular mechanisms of disease. *Nat Rev Immunol* 2002;2:427–38.

32 Hong JC, Kahan BD. Immunosuppressive agents in organ transplantation: past, present, and future. *Semin Nephrol* 2000;20:108–25.

33 Qiu Y, Kung HJ. Signaling network of the Btk family kinases. *Oncogene* 2000;19:5651–61.

34 Nore BF, Vargas L, Mohamed AJ *et al.* Redistribution of Bruton's tyrosine kinase by activation of phosphatidylinositol 3-kinase and Rho-family GTPases. *Eur J Immunol* 2000;30:145–54.

35 Miller AT, Berg LJ. New insights into the regulation and functions of Tec family tyrosine kinases in the immune system. *Curr Opin Immunol* 2002;14:331–40.

36 Reth M, Brummer T. Feedback regulation of lymphocyte signalling. *Nat Rev Immunol* 2004;4:269–77.

37 Gustafsson MO, Hussain A, Mohammad DK *et al.* Regulation of nucleocytoplasmic shuttling of Bruton's tyrosine kinase (Btk) through a novel SH3-dependent interaction with ankyrin repeat domain 54 (ANKRD54). *Mol Cell Biol* 2012;32:2440–53.
38 Mohamed AJ, Vargas L, Nore BF, Backesjo CM, Christensson B, Smith CIE. Nucleocytoplasmic shuttling of Bruton's tyrosine kinase. *J Biol Chem* 2000;275:40614–9.
39 Mohammad DK, Nore BF, Hussain A, Gustafsson MO, Mohamed AJ, Smith EC. Dual phosphorylation of Btk by Akt/PKB Provides Docking for 14-3-3ζ, Regulates Shuttling and Attenuates both Tonic and Induced Signaling in B Cells. *Mol Cell Biol*. 2013 Jun 10. [Epub ahead of print] PMID: 23754751.
40 Yang Y, Shaffer AL 3rd, Emre NC *et al.* Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. *Cancer Cell* 2012;21:723–37.
41 Mahajan S, Ghosh S, Sudbeck EA *et al.* Rational design and synthesis of a novel anti-leukemic agent targeting Bruton's tyrosine kinase (BTK), LFM-A13 [alpha-cyano-beta-hydroxy-beta-methyl-N-(2, 5-dibromophenyl)propenamide]. *J Biol Chem* 1999;274:9587–99.
42 Ghosh S, Uckun FM. Alpha-cyano-N-(2,5-dibromophenyl)-beta-hydroxybut-2-enamide. *Acta Crystallogr C* 1999;55(Pt 8): 1364–5.
43 van den Akker E, van Dijk TB, Schmidt U *et al.* The Btk inhibitor LFM-A13 is a potent inhibitor of Jak2 kinase activity. *Biol Chem* 2004;385:409–13.
44 Uckun FM, Zheng Y, Cetkovic-Cvrlje M *et al.* In vivo pharmacokinetic features, toxicity profile, and chemosensitizing activity of alpha-cyano-beta-hydroxy-beta- methyl-N-(2,5-dibromophenyl)propenamide (LFM-A13), a novel antileukemic agent targeting Bruton's tyrosine kinase. *Clin Cancer Res* 2002;8:1224–33.
45 Uckun FM, Vassilev A, Bartell S, Zheng Y, Mahajan S, Tibbles HE. The anti-leukemic Bruton's tyrosine kinase inhibitor alpha-cyano-beta-hydroxy-beta-methyl-N-(2,5-dibromophenyl) propenamide (LFM-A13) prevents fatal thromboembolism. *Leuk Lymphoma* 2003;44:1569–77.
46 Tibbles HE, Samuel P, Erbeck D, Mahajan S, Uckun FM. In vivo toxicity and antithrombotic profile of the oral formulation of the antileukemic agent, LFM-A13-F. *Arzneimittelforschung* 2004;54:330–9.
47 Uckun FM, Dibirdik I, Qazi S *et al.* Anti-breast cancer activity of LFM-A13, a potent inhibitor of Polo-like kinase (PLK). *Bioorg Med Chem* 2007;15:800–14.
48 Cetkovic-Cvrlje M, Uckun FM. Dual targeting of Bruton's tyrosine kinase and Janus kinase 3 with rationally designed inhibitors prevents graft-versus-host disease (GVHD) in a murine allogeneic bone marrow transplantation model. *Br J Haematol* 2004;126:821–7.
49 Doyle SL, Jefferies CA, O'Neill LA. Bruton's tyrosine kinase is involved in p65-mediated transactivation and phosphorylation of p65 on serine 536 during NFkappaB activation by lipopolysaccharide. *J Biol Chem* 2005;280:23496–501.
50 Redondo PC, Ben-Amor N, Salido GM, Bartegi A, Pariente JA, Rosado JA. Ca2 + -independent activation of Bruton's tyrosine kinase is required for store-mediated Ca2 + entry in human platelets. *Cell Signal* 2005;17:1011–21.
51 Olsson S, Sundler R. Different roles for non-receptor tyrosine kinases in arachidonate release induced by zymosan and Staphylococcus aureus in macrophages. *J Inflamm (Lond)* 2006;3:8.
52 Gilbert C, Levasseur S, Desaulniers P *et al.* Chemotactic factor-induced recruitment and activation of Tec family kinases in human neutrophils. II. Effects of LFM-A13, a specific Btk inhibitor. *J Immunol* 2003;170:5235–43.
53 Gray P, Dunne A, Brikos C, Jefferies CA, Doyle SL, O'Neill LA. MyD88 adapter-like (Mal) is phosphorylated by Bruton's tyrosine kinase during TLR2 and TLR4 signal transduction. *J Biol Chem* 2006;281:10489–95.
54 Vijayan V, Baumgart-Vogt E, Naidu S, Qian G, Immenschuh S. Bruton's tyrosine kinase is required for TLR-dependent heme oxygenase-1 gene activation via Nrf2 in macrophages. *J Immunol* 2011;187:817–27.
55 Susaki K, Kitanaka A, Dobashi H *et al.* Tec protein tyrosine kinase inhibits CD25 expression in human T-lymphocyte. *Immunol Lett* 2010;127:135–42.
56 Bam R, Ling W, Khan S *et al.* Role of Bruton's tyrosine kinase in myeloma cell migration and induction of bone disease. *Am J Hematol* 2013;88:463–71.
57 Uckun FM. Clinical potential of targeting Bruton's tyrosine kinase. *Int Rev Immunol* 2008;27:43–69.
58 Lombardo LJ, Lee FY, Chen P *et al.* Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem* 2004;47:6658–61.
59 Santos FP, Cortes J. Dasatinib for the treatment of Philadelphia chromosome-positive leukemias. *Expert Opin Pharmacother* 2012;13:2381–95.
60 Somlo G, Atzori F, Strauss LC *et al.* Dasatinib plus capecitabine for advanced breast cancer: safety and efficacy in phase I study CA180004. *Clin Cancer Res* 2013;19:1884–93.
61 Araujo JC, Mathew P, Armstrong AJ *et al.* Dasatinib combined with docetaxel for castration-resistant prostate cancer: results from a phase 1–2 study. *Cancer* 2012;118:63–71.
62 Rix U, Hantschel O, Durnberger G *et al.* Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. *Blood* 2007;110:4055–63.
63 Bantscheff M, Eberhard D, Abraham Y *et al.* Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat Biotechnol* 2007;25:1035–44.
64 Hantschel O, Rix U, Schmidt U *et al.* The Btk tyrosine kinase is a major target of the Bcr-Abl inhibitor dasatinib. *Proc Natl Acad Sci USA* 2007;104:13283–8.
65 Contri A, Brunati AM, Trentin L *et al.* Chronic lymphocytic leukemia B cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. *J Clin Invest* 2005;115:369–78.
66 Amrein PC, Attar EC, Takvorian T *et al.* Phase II study of dasatinib in relapsed or refractory chronic lymphocytic leukemia. *Clin Cancer Res* 2011;17:2977–86.
67 Veldurthy A, Patz M, Hagist S *et al.* The kinase inhibitor dasatinib induces apoptosis in chronic lymphocytic leukemia cells in vitro with preference for a subgroup of patients with unmutated IgVH genes. *Blood* 2008;112:1443–52.
68 Herrmann H, Blatt K, Ghanim V *et al.* Glucocorticosteroids rescue basophils from dasatinib-augmented immunoglobulin E-mediated histamine release. *Int Arch Allergy Immunol* 2012;159:15–22.
69 Das J, Chen P, Norris D *et al.* 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1- piperazinyl)]-2-methyl-4-pyrimidinyl]amino)]-1,3-thiazole-5-carboxamide (dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. *J Med Chem* 2006;49:6819–32.
70 Sillaber C, Herrmann H, Bennett K *et al.* Immunosuppression and atypical infections in CML patients treated with dasatinib at 140 mg daily. *Eur J Clin Invest* 2009;39:1098–109.
71 Kneidinger M, Schmidt U, Rix U *et al.* The effects of dasatinib on IgE receptor-dependent activation and histamine release in human basophils. *Blood* 2008;111:3097–107.

72 Burger JA, Buggy JJ. Emerging drug profiles: Bruton tyrosine kinase (BTK) inhibitor ibrutinib (PCI-32765). *Leuk Lymphoma* 2013; doi:10.3109/10428194.2013.777837. [Epub ahead of print].

73 Buggy JJ, Elias L. Bruton tyrosine kinase (BTK) and its role in B-cell malignancy. *Int Rev Immunol* 2012;31:119–32.

74 Dasmahapatra G, Patel H, Dent P, Fisher RI, Friedberg J, Grant S. The Bruton tyrosine kinase (BTK) inhibitor PCI-32765 synergistically increases proteasome inhibitor activity in diffuse large-B cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) cells sensitive or resistant to bortezomib. *Br J Haematol* 2013;161:43–56.

75 Pan Z, Scheerens H, Li SJ *et al.* Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem* 2007;2:58–61.

76 Honigberg LA, Smith AM, Sirisawad M *et al.* The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci USA* 2010;107:13075–80.

77 Brown JR. Ibrutinib (PCI-32765), the first BTK (Bruton's tyrosine kinase) inhibitor in clinical trials. *Curr Hematol Malig Rep* 2013;8:1–6.

78 Ponader S, Chen SS, Buggy JJ *et al.* The Bruton tyrosine kinase inhibitor PCI-32765 thwarts chronic lymphocytic leukemia cell survival and tissue homing in vitro and in vivo. *Blood* 2012;119:1182–9.

79 Herman SE, Gordon AL, Hertlein E *et al.* Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* 2011;117:6287–96.

80 Tai YT, Chang BY, Kong SY *et al.* Bruton tyrosine kinase inhibition is a novel therapeutic strategy targeting tumor in the bone marrow microenvironment in multiple myeloma. *Blood* 2012;120:1877–87.

81 Edwards CM. BTK inhibition in myeloma: targeting the seed and the soil. *Blood* 2012;120:1757–9.

82 Brett LK, Williams ME. Current and Emerging Therapies in Mantle Cell Lymphoma. *Curr Treat Options Oncol* 2013;14:198–211.

83 Chang BY, Huang MM, Francesco M *et al.* The Bruton tyrosine kinase inhibitor PCI-32765 ameliorates autoimmune arthritis by inhibition of multiple effector cells. *Arthritis Res Ther* 2011;13:R115.

84 Robak T, Robak E. Tyrosine kinase inhibitors as potential drugs for B-cell lymphoid malignancies and autoimmune disorders. *Expert Opin Investig Drugs* 2012;21:921–47.

85 Advani RH, Buggy JJ, Sharman JP *et al.* Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol* 2013;31:88–94.

86 Brown K, Long JM, Vial SC *et al.* Crystal structures of interleukin-2 tyrosine kinase and their implications for the design of selective inhibitors. *J Biol Chem* 2004;279:18727–32.

87 Das J, Liu C, Moquin RV *et al.* Discovery and SAR of 2-amino-5-[(thiomethyl)aryl]thiazoles as potent and selective Itk inhibitors. *Bioorg Med Chem Lett* 2006;16:2411–5.

88 Das J, Furch JA, Liu C *et al.* Discovery and SAR of 2-amino-5-(thioaryl)thiazoles as potent and selective Itk inhibitors. *Bioorg Med Chem Lett* 2006;16:3706–12.

89 Snow RJ, Abeywardane A, Campbell S *et al.* Hit-to-lead studies on benzimidazole inhibitors of ITK: discovery of a novel class of kinase inhibitors. *Bioorg Med Chem Lett* 2007;17:3660–5.

90 Winters MP, Robinson DJ, Khine HH *et al.* 5-Aminomethyl-1H-benzimidazoles as orally active inhibitors of inducible T-cell kinase (Itk). *Bioorg Med Chem Lett* 2008;18:5541–4.

91 Moriarty KJ, Winters M, Qiao L *et al.* Itk kinase inhibitors: initial efforts to improve the metabolical stability and the cell activity of the benzimidazole lead. *Bioorg Med Chem Lett* 2008;18:5537–40.

92 Riether D, Zindell R, Kowalski JA *et al.* 5-Aminomethylbenzimidazoles as potent ITK antagonists. *Bioorg Med Chem Lett* 2009;19:1588–91.

93 Velankar AD, Quintini G, Prabhu A *et al.* Synthesis and biological evaluation of novel (4 or 5-aryl)pyrazolyl-indoles as inhibitors of interleukin-2 inducible T-cell kinase (ITK). *Bioorg Med Chem* 2010;18:4547–59.

94 von Bonin A, Rausch A, Mengel A *et al.* Inhibition of the IL-2-inducible tyrosine kinase (Itk) activity: a new concept for the therapy of inflammatory skin diseases. *Exp Dermatol* 2011;20:41–7.

95 Herdemann M, Weber A, Jonveaux J, Schwoebel F, Stoeck M, Heit I. Optimisation of ITK inhibitors through successive iterative design cycles. *Bioorg Med Chem Lett* 2011;21:1852–6.

96 Charrier JD, Miller A, Kay DP *et al.* Discovery and structure-activity relationship of 3-aminopyrid-2-ones as potent and selective interleukin-2 inducible T-cell kinase (Itk) inhibitors. *J Med Chem* 2011;54:2341–50.

97 Guo W, Liu R, Ono Y *et al.* Molecular characteristics of CTA056, a novel interleukin-2-inducible T-cell kinase inhibitor that selectively targets malignant T cells and modulates oncomirs. *Mol Pharmacol* 2012;82:938–47.

98 McLean LR, Zhang Y, Zaidi N *et al.* X-ray crystallographic structure-based design of selective thienopyrazole inhibitors for interleukin-2-inducible tyrosine kinase. *Bioorg Med Chem Lett* 2012;22:3296–300.

99 Lin TA, McIntyre KW, Das J *et al.* Selective Itk inhibitors block T-cell activation and murine lung inflammation. *Biochemistry* 2004;43:11056–62.

100 Cook BN, Bentzien J, White A *et al.* Discovery of potent inhibitors of interleukin-2 inducible T-cell kinase (ITK) through structure-based drug design. *Bioorg Med Chem Lett* 2009;19:773–7.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Pharmacokinetics of Ibrutinib.

Table S2. An overview over drug-like compounds under development for ITK inhibition. Cell lines, Jurkat and DT40 are used to measure cellular responses, either interleukin production or inhibition of kinase activity.