Targeting B-cell signaling pathways: a central role for Bruton's tyrosine kinase

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B-cell cancers constitute a large group of diseases with diverse clinical and pathological characteristics that arise from the B (bursal- or bone marrow-derived) lymphocytes of the immune system. B cells are involved in humoral immunity as part of the adaptive immune response. They display a unique B-cell receptor (BCR) on their surface which binds to a specific antigen. Antigen-binding activates the process of clonal expansion, during which the B cell reproduces to form an army of clones that secrete the same antibody. These antibodies then bind to the target antigen on foreign cells and initiate a range of immune responses that ultimately lead to the destruction of that cell.

B cells arise in the bone marrow from hematopoietic stem cells and pass through several stages of development, culminating in the formation of mature B cells in the spleen. During each stage of B-cell development, the components of the BCR are assembled and the heavy and light chains of the antibody portion of the BCR undergo genetic rearrangements to allow the production of a huge range of unique antibodies.

Both B-cell development and the clonal expansion process are prone to errors that, if uncorrected, can result in the formation of oncogenic alterations that have an impact on B-cell proliferation, apoptosis, and differentiation and can lead to the development of B-cell lymphomas and leukemias.[1-3]

Historically, B-cell malignancies have been treated with cytotoxic chemotherapies and, more recently, with targeted immunotherapies, including monoclonal antibodies, which aim to direct an immune response against cancer cells by targeting specific antigens on the surface of tumor cells. Despite significant therapeutic advances, most mature B-cell malignancies remain incurable and there is a need for new therapies. In recent years, there has been remarkable progress in understanding the cellular signaling pathways that drive B-cell malignancies. Combined with advances in medicinal chemistry, researchers have begun to develop small molecule inhibitors that specifically target B-cell signaling pathways, culminating in successful approval by the US Food and Drug Administration for several agents. Here, we discuss the therapeutic landscape that is being carved out as a result of these scientific advancements.

Targeting B-cell signaling pathways

Toll-like receptor pathway

One signaling pathway that has been implicated in the development of B-cell malignancies is the toll-like receptor (TLR) pathway. TLRs have emerged as important regulators of immunity; they are responsible for initiating nonspecific immune responses through the recognition of pathogen-associated molecular patterns, expressed by invading pathogens, and danger-associated molecular patterns, released by damaged or dying cells. The 11 human TLRs are expressed by a variety of different cells, including B cells, and each TLR specifically binds different pathogenic molecules. The signaling pathways downstream of TLRs are conducted by various cytoplasmic adaptor molecules, most prolific among them is MyD88 (myeloid differentiation primary response protein 88), which is activated by all TLRs, except TLR3, and acts as a scaffold for several downstream kinases, including Bruton's tyrosine kinase (BTK). These signaling cascades ultimately lead to the activation of mitogen-activated protein kinases (MAPKs) and the transcription factor nuclear factor kappa B (NFκB; Figure 1).[4-6]

TLRs have been shown to be involved in B-cell proliferation and survival, which led to the suggestion that inhibition of TLR signaling could temper inappropriate B-cell proliferation and survival signals in B-cell malignancies. Furthermore, many TLRs have also been found to be highly expressed on B-cell malignancies, for example, TLR9 on acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), and chronic lymphoblastic leukemia (CLL) and TLR4 on ALL and mantle cell lymphoma (MCL). The best-studied TLRs with respect to B-cell cancer are TLR9, which binds unmethylated cytosine-phosphate-guanine (CpG) motifs in bacterial and DNA viruses and is preferentially expressed on B cells, and TLR4,

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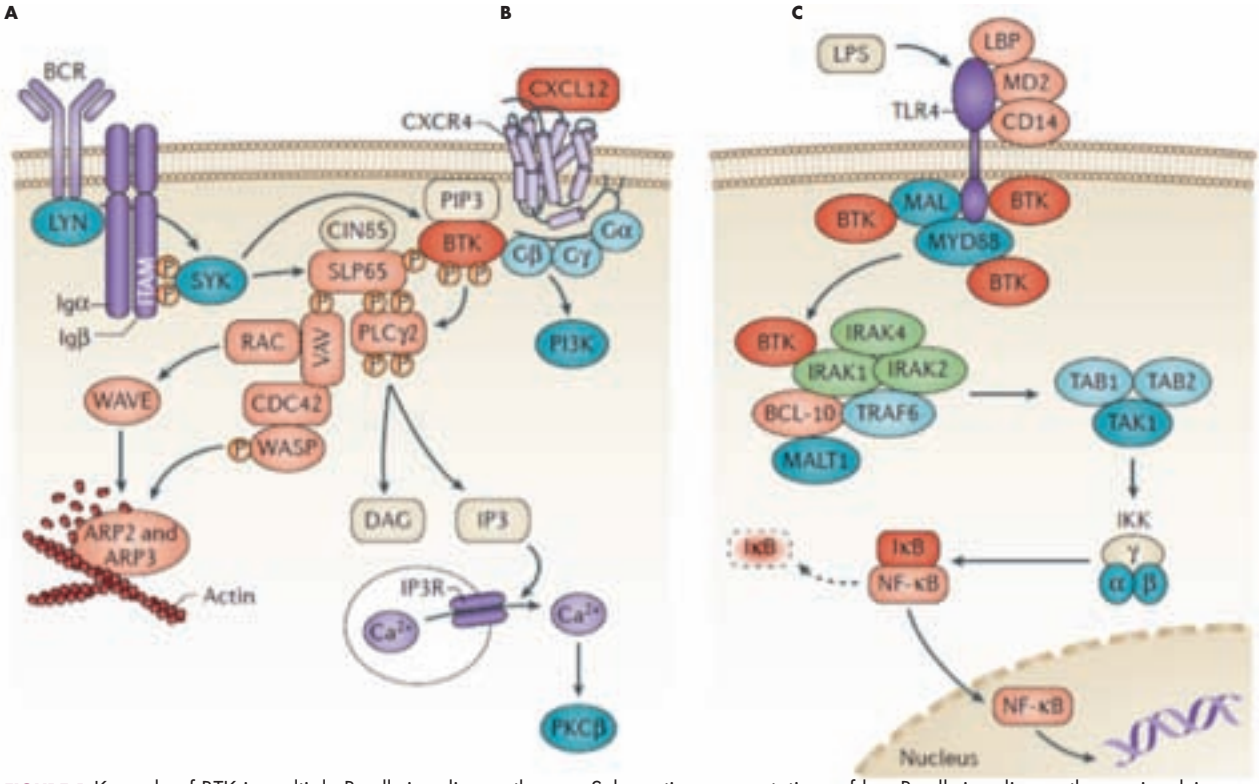

FIGURE 1 Key role of BTK in multiple B-cell signaling pathways. Schematic representations of key B-cell signaling pathways involving the BCR, chemokine receptors and TLRs; Bruton's tyrosine kinase is an important downstream signaling node in all 3 pathways. A, BCR is the main activator of signaling cascades that promote B-cell proliferation and differentiation as part of a normal immune response. Following antigen binding to the BCR the B cell is activated and downstream signaling cascades are triggered involving kinases such as Lyn, Syk, and BTK. B, B cells predominantly express the CXCR4 and CXCR5 chemokine receptors, which regulate B-cell homing and migration to and within the bone marrow and lymphoid tissue. BTK is central to downstream signaling cascades activated by CXCR4/5. C, TLRs initiate nonspecific immune responses and are involved in B-cell proliferation and survival. MyD88 is a central adaptor in TLR signaling pathways and acts as a scaffold for downstream kinases, including BTK. Source: Hendriks RW, Yuvaraj S, Kil LP. Targeting Bruton's tyrosine kinase in B-cell malignancies. Nature Rev Cancer. 2014;14:219-232. Reproduced with permission.

BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; TLR, toll-like receptor;

which binds to lipopolysaccharides (LPS).[4-6]

A range of therapeutic agents targeting various TLRs and mostly composed of nucleic acid-derived immunoregulatory sequences, have been investigated over the past decade. Currently, few of these agents are being investigated in B-cell malignancies (Table 1) and their success has been tempered by the complexity in targeting TLRs, which have been shown to both promote and inhibit cancer progression.[6] The exception is IMO-8400. This synthetic oligonucleotide-based antagonist of TLR7, 8, and 9 is in preclinical development in B-cell malignancies. Data presented at the 2014 American Association of Cancer Research annual meeting demonstrated that IMO-8400 is able to inhibit the survival and proliferation of B-cell lymphoma cells that harbor a mutation in MyD88.[7] IMO-8400 is in preclinical development for the treatment of DLBCL and Waldenström's macroglobulinemia.

Chemokine receptor pathway

Chemokines are a large family of chemotactic cytokines that are implicated in an array of important biological processes. They are grouped into 4 families, based on the spacing of cytosine residues close to the end of their protein sequence; C, CC, CXC, and CX3C. The cell surface chemokine receptors to which chemokines bind are 7-transmembrane domain-containing G protein-coupled receptors. B cells predominantly express the CXCR4 and CXCR5 chemokine receptors,[8] which have been shown to play an important role in B-cell homing to and movement within the bone marrow and lymphoid tissue. Increased expression of chemokine receptors has been observed in a variety of B-cell malignancies. As such, CXCR4 and CXCR5 have also been the subject of intense research efforts in the development of anticancer agents (Table 1). The success of these agents has been limited, and current development is mostly in other diseases such as rheumatoid arthritis and

TABLE 1 Examples of agents targeting components of B-cell signaling pathways

Agent (brand name/s)	Sponsor	Mechanism of action	Examples of ongoing clinical trials (clinicaltrials.gov identifiers)
IMO-8400	Idera Pharmaceuticals	TLR7, 8 and 9 antagonist; synthetic oligonucleotide	Preclinical
SMP-105	Dainippon Sumitomo Pharma	TLR2 agonist; autoclaved mycobacteria	Preclinical
ANA773	Anadys Pharma	TLR7 agonist; small molecule ssRNA	Preclinical
852A	3M Pharmaceuticals	TLR7 agonist; small molecule ssRNA	Phase 1/2 trials in hematologic malignancies completed
BMS-936564	Bristol-Myers Squibb	CXCR4 antibody	Phase 1 multiple myeloma and other B-cell cancers (NCT01359657, NCT01120457)
P505-15 (PRT062607)	Proteon Therapeutics	Syk inhibitor	Preclinical
PRT318	Proteon Therapeutics	Syk inhibitor	Preclinical
Bafetinib (INNO-406/NS-187)	CytRx	Lyn kinase inhibitor	Completed phase 1/2 in CLL and ALL (NCT00352677, NCT01144260)
Dasatinib (Sprycel)	Bristol-Myers Squibb	Multikinase inhibitor; originally designed to target Bcr-Abl; other targets include Src, BTK, and has been shown to inhibit CXCR4/5	FDA approved for Philadelphia chromosome-positive CLL; various ongoing trials, including phase 2 DLBCL (NCT00918463) and NHL (NCT01609816)
Idelalisib (GS-1101)	Gilead Sciences	PI3Kδinhibitor	Various ongoing trials, including phase 3 CLL and NHL (NCT01732913, NCT01569295); phase 1/2 low-grade lymphoma (NCT01306643)
IPI-145	Infinity Pharmaceuticals	PI3Kδinhibitor	Various ongoing trials, including phase 3 CLL/SLL (NCT02049515); phase 3 vs ofatumumab relapsed/refractory CLL/SLL (NCT02004522); phase 2 indolent NHL (NCT01882803)
Temsirolimus (Torisel; CCI-779)	Pfizer	mTOR inhibitor	Various ongoing trials, including phase 1/2 MCL, DLBCL, FL (NCT00117598, NCT01078142, NCT01653067)
Everolimus (Affinitor; RAD-001)	Novartis	mTOR inhibitor	Various ongoing trials, including phase 3 DLBCL (NCT00790036) and phase 2 MCL (NCT00516412)
Bortezomib (Velcade)	Millennium Pharmaceuticals	Proteasome inhibitor	FDA approved for multiple myeloma and MCL; various ongoing trials, including phase 1 CLL/SLL (NCT00963274) and phase 2 DLBCL (NCT01965977)
Carfilzomib (Krypolis)	Onyx Pharmaceuticals	Proteasome inhibitor	FDA approved for multiple myeloma; various ongoing trials, including phase 2 MCL (NCT02042950) and phase 1/2 DLBCL (NCT01959698)

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TABLE 1 *continued*

Agent (brand name/s)	Sponsor	Mechanism of action	Examples of ongoing clinical trials (clinicaltrials.gov identifiers)
PS-1145	Millennium Pharmaceuticals	IKK inhibitor	Preclinical
BMS-345541	Bristol-Myers Squibb	IKK inhibitor	Preclinical
ML120B	Millennium Pharmaceuticals	IKK inhibitor	Preclinical

ALL, acute lymphoblastic leukemia; CLL, chronic lymphoblastic leukemia; DLBCL, diffuse large B-cell lymphoma; FDA, Food and Drug Administration; MCL, mantle cell lymphoma; ss, single-stranded; SSl, small lymphocytic leukemia; TLR, toll-like receptor

HIV. However, the CXCR4 antibody BMS-936564 is currently in phase 1 trials in patients with B-cell malignancies (Table 1).[9-11]

BCR pathway

Every B cell has a unique receptor composed of an antibody portion coupled to a heterodimer of CD79A and CD79B, which contain signaling modules called immunoreceptor tyrosine-based activation motifs (ITAMs). The ITAMs contain tyrosine residues that, upon antigen-induced activation of the BCR, are phosphorylated by downstream Src-family kinases, including Lyn kinase, and other tyrosine kinases such as Syk (spleen tyrosine kinase) and BTK.[12-14] This cascade of activated kinases provides an array of targets amenable to therapeutic intervention and various Lyn, Syk, and Src kinase inhibitors are in preclinical and early clinical development (Table 1). Fostamitinib disodium was the most advanced Syk inhibitor in clinical trials, but development in cancer was terminated in 2013. Although the majority of Lyn kinase inhibitors are in preclinical development, it is interesting to note that Lyn kinase mutations may be a driver of resistance to Bcr-Abl inhibitors, such as imatinib, in CML and that bafetinib (Table 1) is actually a dual inhibitor of Bcr-Abl and Lyn kinase.[15]

TABLE 2 Ongoing phase 3 clinical trials of ibrutinib

Trial name (clinicaltrials.gov identifier)	Description
RESONATE (NCT01578707)	Ibrutinib vs ofatumumab in relapsed/refractory CLL/SLL
RESONATE-2 (NCT01722487)	Ibrutinib vs chlorambucil in patients ≥ 65 y with treatment-naïve CLL/SLL
SHINE (NCT01776840)	Ibrutinib plus bendamustine and rituximab vs bendamusine, rituximab, and placebo in newly diagnosed MCL
HELIOS (NCT01611090)	Ibrutinib plus bendamustine and rituximab vs bendamustine, rituximab, and placebo in relapsed/refractory CLL
NCT01886872	3-arm trial of ibrutinib alone and in combination with rituximab vs rituximab plus bendamustine in previously untreated CLL
NCT01974440	Ibrutinib with either bendamustine and rituximab or R-CHOP in previously treated indolent NHL
NCT02048813	Ibrutinib and rituximab vs fludarabine phosphate, cyclophosphamide, and rituximab in untreated CLL/SLL
NCT01804686	Long-term extension study
NCT01973387	Ibrutinib vs rituximab in relapsed/refractory CLL/SLL
NCT01646021	Ibrutinib vs temsirolimus in previously treated relapsed/refractory MCL
NCT01855750	Ibrutinib with R-CHOP in newly diagnosed nongerminal center B-cell DLBCL
NCT01724346	Open-label extension study in patients ≥ 65 y with CLL/SLL who participated in RESONATE-2

CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; R-CHOP, rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone; SLL, small lymphocytic leukemia

TABLE 3 Inhibitors of Bruton's tyrosine kinase

Agent (brand name/s)	Sponsor	Mechanism of action	Stage of clinical testing (clinical trials.gov identifiers)
Ibrutinib (Imbruvica)	Pharmacyclics/Janssen Biotech	BTK inhibitor	FDA approved for treatment of MCL; designated breakthrough therapy for CLL/SLL patients with 17p deletion and in Waldenström macroglobulinemia; phase 3 CLL/SLL and MCL (NCT01578707, NCT01722487, NCt01611090, NCT01776840)
CC-292	Celgene	BTK inhibitor	Phase 1/1b in DLBCL, CLL/SLL and other B-cell lymphomas (NCT02031419, NCT01744626, NCT017328611)
ONO-4059	ONO Pharmaceuticals	BTK inhibitor	Phase 1 in NHL, CLL/SLL and B-cell lymphomas (NCT01659255)
CGI-1746	CGI Pharmaceuticals	BTK inhibitor	Preclinical
RN486	F Hoffmann-La Roche	BTK inhibitor	Preclinical
LFM-A13	Paradigm Pharmaceuticals	BTK inhibitor	Preclinical
CNX-774	Avila Therapeutics	BTK inhibitor	Preclinical

BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FDA, Food and Drug Administration; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; SLL, small lymphocytic leukemia

The PI3K/Akt/mTOR (phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin) pathway already has an established role in the development of many different types of cancer and has been shown to be activated downstream of the BCR (Figure 1). Although mutations in these genes are rarely observed in lymphoid malignancies, activation of the PI3K/Akt/mTOR pathway is often associated with aberrant activation of the BCR pathway. As such, PI3K and mTOR inhibitors are being explored for the treatment of B-cell malignancies (Table 1). There are 4 isoforms of the class I PI3K subunit and the gamma and delta isoforms have been shown to be primarily expressed in lymphocytes; amplified levels of PI3Kδ in particular are often observed in B-cell malignancies.[12-14]

Idelalisib (GS-1101) is a PI3Kδ-specific inhibitor currently under development. This agent has already reached phase 3 trials in CLL in combination with the anti-CD20 antibody rituximab. The trial was stopped early when the combination showed a significant increase in progression-free survival, with median PFS not yet reached, compared with 5.5 months for the placebo and rituximab combination. Overall response rates (ORRs) were 81%, compared with 13% in the placebo–rituximab group, and overall survival at 12 months was 92% and 80%, respectively.[16] Idealisilib and a number of other PI3Kδ/ϒ isoform-specific inhibitors are also being evaluated in other B-cell malignancies (Table 1).

As with both the chemokine receptor and TLR pathways, the net result of the BCR pathway is activation of the transcription factor NFκB (Figure 1), and a number of NFκB-targeting strategies are also being evaluated in B-cell malignancies. These include inhibitors of IKK (IκB kinase); the inhibitor of kappa B (IκB) is a protein that keeps NFκB in an inactive state in the cytoplasm, whereas IKK is the kinase that targets IκB for degradation, thereby activating NFκB. A variety of IKK inhibitors are in preclinical development (Table 1). Proteasomal inhibitors have also been developed, including bortezomib and carfilzomib, since the 26S proteasome is required for degrading IκB. Both of the aforementioned inhibitors are FDA-approved for the treatment of multiple myeloma, and bortezomib is also approved for MCL. Only mild activity has been demonstrated in other B-cell malignancies thus far.[12-14]

BTK and its central role in new approved therapeutic options

One kinase in particular has proven central to many B-cell signaling pathways. BTK is a nonreceptor tyrosine kinase that is expressed on a range of hematopoietic cells, including macrophages and neutrophils, though not on T cells or normal plasma cells. However, its best understood role is in the BCR pathway, where it is recruited to the cell membrane upon BCR activation and phosphorylated by a number of different kinases. In turn BTK phosphorylates a range of other proteins downstream in the BCR pathway, ultimately activating NFκB, which orchestrates an array of vital B-cell processes. Inappropriate activation of BTK has been demonstrated to be involved in the maintenance

of a number of B-cell malignancies, particularly CLL and ALL.[17-19]

The recognition of the key role of BTK in normal and oncogenic B-cell signaling pathways has driven the development of several small molecule inhibitors of this kinase. Central among them is ibrutinib, which irreversibly inhibits BTK enzymatic activity by binding to a cysteine in the tyrosine kinase domain. Ibrutinib was recently approved by the FDA for the treatment of MCL based on the results of an international, multicenter, single-arm trial of 111 MCL patients, in which it demonstrated an ORR of 65.8%, with 17% complete response (CR) rate and 49% partial response (PR) rate, and a median duration of response of 17.5 months.[17-19] Ibrutinib has also been approved for the treatment of patients with CLL who have received at least 1 previous therapy, based on phase 2 data in 48 patients demonstrating an ORR of 58%.[20] Furthermore, it has been designated as breakthrough therapy as monotherapy in patients with CLL or SLL with 17p deletion and in Waldenström's macroglobulinemia and it continues to be evaluated in a number of other trials (Table 2, p. 225).

Two other BTK inhibitors are currently in clinical development (Table 3): CC-292 and ONO-4059. Both are at the phase 1 stage, and preliminary results for 2 trials of ONO-4059 were reported at recent conferences. In patients with relapsed or refractory CLL or SLL, the best ORR across 20-320 mg doses of ONO-4059 was 70%, while in patients with B-cell lymphoma across doses of 40, 80, and 160 mg, the best ORR was 42%.[21,22]

Treatment strategies for B-cell malignancies continue to evolve, and the recent addition of novel targeted agents to the therapeutic complement have shown considerable promise. With a number of these agents entering late stage clinical trials and the potential for synergistic activity between these targeted agents and other treatments, the stage is set for a significant improvement in patient outcomes for this group of cancers.

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