VOLUME 31 · NUMBER 1 · JANUARY 1 2013

JOURNAL OF CLINICAL ONCOLOGY

UNDERSTANDING THE PATHWAY

Targeting B-Cell Receptor Signaling for Anticancer Therapy: The Bruton's Tyrosine Kinase Inhibitor Ibrutinib Induces Impressive Responses in B-Cell Malignancies

Adrian Wiestner, *National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD*

See accompanying article on page 88

Ibrutinib (PCI-32765) is an orally active inhibitor of Bruton's tyrosine kinase (BTK) that covalently binds to the cysteine Cys-481 of BTK and thereby irreversibly inactivates the kinase.[1,2] In the report accompanying this article, the results of the first clinical study of ibrutinib in patients with relapsed/refractory B-cell malignancies are presented.[3] Objective responses were observed in an impressive 60% of patients, including 16% complete responses, and adverse effects were minimal. This article provides an overview of the role of BTK for B-cell receptor (BCR) signaling and the importance of the BCR in B-cell malignancies.

BTK is a cytoplasmic tyrosine kinase of the Tec family that is essential for BCR signaling.[1] Loss-of-function BTK mutations cause X-linked agammaglobulinemia, which is characterized by the virtual absence of B cells and immunoglobulins and results in recurrent bacterial infections.[4] BTK is expressed in B cells and myeloid cells but not in plasma cells or T lymphocytes. Moreover, its essential functions seem to be limited to B cells. BTK is required for BCR-induced calcium release, cell proliferation, and activation of the nuclear factor κB (NF-κB) pathway.[1,4]

The BCR consists of a surface transmembrane immunoglobulin (Ig) receptor associated with the Igα (CD79A) and Igβ (CD79B) chains.[5] The BCR serves as the receptor for antigen and promotes cell growth, proliferation, and survival of normal and malignant B cells.[6,7] On antigen binding, the tyrosine kinases LYN and SYK initiate a signal transduction cascade that involves several kinases, adapter molecules, and the generation of second messengers (Fig 1). Of the surface Ig isotypes, IgM is of particular importance for antigen-dependent signaling and is the isotype expressed by most mature B-cell malignancies.[6]

BCR signaling is increasingly implicated in the pathogenesis of some B-cell malignancies. Definitive experimental evidence comes from studies in the activated B-cell–like (ABC) subtype of diffuse large

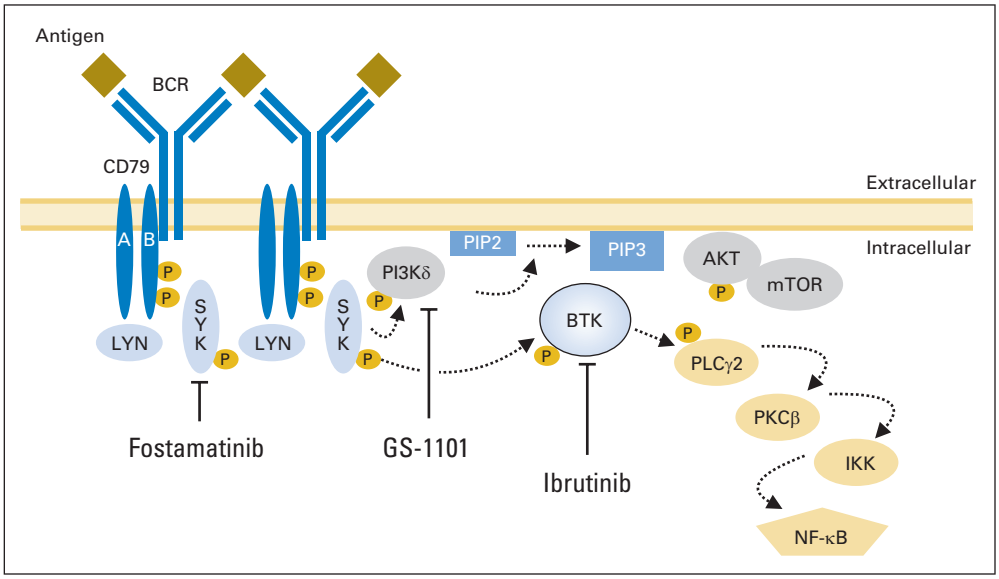

Fig 1. Antigen-dependent B-cell receptor (BCR) signaling and its targeting by small-molecule inhibitors. Antigen binding induces the aggregation of the BCR with its coreceptors CD79A and B, which become phosphorylated by the tyrosine kinases LYN and SYK. SYK activates phosphoinositide 3-kinase (PI3Kδ), which in turn converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate PIP3. PIP3 serves as a docking site for the cytoplasmic kinases Bruton's tyrosine kinase (BTK) and AKT. BTK phosphorylates and thereby activates phospholipase C gamma 2 (PLCγ2), which in turn generates a set of second messengers to activate protein kinase C beta (PKCβ). PKCβ phosphorylates IκB kinase (IKK) to activate nuclear factor κB (NF-κB) transcription factors that regulate gene expression of several survival factors. The kinases inhibited by small molecules with promising clinical activity are indicated.

B-cell lymphoma (DLBCL). A subset of ABC-DLBCL displays chronic active BCR signaling, resulting in constitutive activation of the NF-κB and phosphoinositide 3-kinase (PI3K) pathways.[8] These lymphomas die after knockdown of the BCR components IgM, CD79A, and CD79B and of kinases that transmit the BCR signal, including SYK, PI3Kδ, BTK, and protein kinase C beta.[6,8] Over 20% of primary ABC-DLBCLs carry mutations in CD79A and CD79B that enhance the cellular response to BCR activation. However, these mutations are insufficient to initiate BCR signaling, suggesting that antigen-dependent activation is also required.[8]

Chronic stimulation of B cells by microbial or viral antigens contributes to the oncogenesis of some lymphomas. Classic examples include mucosa-associated lymphoid tissue lymphoma and splenic marginal zone lymphoma arising in response to infections with *Helicobacter pylori* or hepatitis C, respectively.[9,10] In some cases, eradication of the infection leads to regression of the lymphoma, indicating an antigen-dependent state. In contrast, in chronic lymphocytic leukemia (CLL), antigenic drive seems to be provided by autoantigens.[11,12] A role of antigen is inferred from the observations that CLL cells use a restricted repertoire of Ig heavy chain variable (*IGHV*) genes[13,14] and that in many cases of CLL, virtually identical BCRs—so-called stereotyped BCRs, which could recognize shared antigens—are expressed.[15,16] Ongoing antigen-dependent BCR signaling as a mechanism of disease progression is suggested by the recent demonstration of inducible activation of the BCR and NF-κB pathways in CLL cells in the lymph node.[17] The specific antigens recognized by the BCR expressed on CLL cells remain incompletely defined, but in many cases, they may be autoantigens expressed by dying cells.[18] Most recently, a bias for certain *IGHV* genes and the expression of stereotypic receptors has also been appreciated in mantle-cell lymphoma (MCL), suggesting that response to antigen may also play a role in the pathogenesis of MCL.[19,20]

Clinical trials with the BTK inhibitor ibrutinib and the SYK inhibitor fostamatinib report objective responses across different B-cell malignancies.[3,21] Interestingly, CLL is the entity with the highest response rates at 79% and 55%, respectively. Follicular lymphoma and MCL are also highly responsive to ibrutinib but not to fostamatinib. Response rates in DLBCL were 28% with ibrutinib and 22% with fostamatinib. A possible explanation for the lower response rate in DLBCL is the inclusion of patients irrespective of molecular subtype. However, only the ABC subtype depends on chronic active BCR signaling and is expected to respond to these inhibitors.[8,22] Conceptually, B-cell malignancies that depend on BCR signaling can be targeted by ibrutinib, whereas tumors that rely on other, BTK-independent pathways or have genetic lesions that activate the BCR signal transduction cascade downstream of BTK are likely to be resistant.

Whether all the therapeutic effects of ibrutinib can be attributed to the inhibition of BCR signaling is impossible to ascertain, given that many pathways with important roles in B-cell biology, including B-cell activating factor, CD40, Toll-like receptors, and several cytokines and chemokines, can activate BTK.[4,23] Regardless, it may be that BTK inhibition is efficacious exactly because BTK is involved in many pathways that promote B-cell survival.[24,25] Similar considerations apply to the PI3Kδ inhibitor GS-1101, which reportedly also has significant clinical activity.[26-28]

Prolonged use of ibrutinib might interfere with normal B-cell function and lead to hypogammaglobulinemia. Although this aspect needs to be further explored, the absence of a decrease in IgG levels during administration of ibrutinib, as reported by Advani et al,[3] is good news. Equally encouraging is the median progression-free survival of 13.6 months. However, progression-free survival seems short in DLBCL, which could be the result of activation of BTK-independent survival pathways. Thus, the most effective use of ibrutinib may differ between different B-cell malignancies. In CLL, for example, chronic suppression of the disease may be possible and sufficient, especially for elderly or frail patients. In contrast, combination with chemotherapy to enhance cytotoxicity may be particularly warranted for ABC-DLBCL, which has a high failure rate with conventional chemotherapy and in which there is a strong biologic rationale for the use of targeted agents that inhibit BCR and NF-κB pathways.[8,22,29,30] Although much remains to be learned about the use of kinase inhibitors in B-cell malignancies, the good news is that potent novel treatment options targeting pathogenic mechanisms in these diseases have arrived.

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

REFERENCES

1. Buggy JJ, Elias L: Bruton tyrosine kinase (BTK) and its role in B-cell malignancy. Int Rev Immunol 31:119-132, 2012
2. 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 U S A 107:13075-13080, 2010
3. Advani R, Buggy JJ, Sharman JP, et al: The Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol 31:88-94, 2013
4. Hendriks RW, Bredius RG, Pike-Overzet K, et al: Biology and novel treatment options for XLA, the most common monogenetic immunodeficiency in man. Expert Opin Ther Targets 15:1003-1021, 2011
5. Dal Porto JM, Gauld SB, Merrell KT, et al: B cell antigen receptor signaling 101. Mol Immunol 41:599-613, 2004
6. Rui L, Schmitz R, Ceribelli M, et al: Malignant pirates of the immune system. Nat Immunol 12:933-940, 2011
7. Stevenson FK, Krysov S, Davies AJ, et al: B-cell receptor signaling in chronic lymphocytic leukemia. Blood 118:4313-4320, 2011
8. Davis RE, Ngo VN, Lenz G, et al: Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 463:88-92, 2010
9. Hermine O, Lefrère F, Bronowicki JP, et al: Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med 347:89-94, 2002
10. Wotherspoon AC, Doglioni C, Diss TC, et al: Regression of primary low-grade B-cell gastric lymphoma of mucosa- associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 342:575-577, 1993
11. Ghia P, Chiorazzi N, Stamatopoulos K: Microenvironmental influences in chronic lymphocytic leukaemia: The role of antigen stimulation. J Intern Med 264:549-562, 2008
12. Rosén A, Murray F, Evaldsson C, et al: Antigens in chronic lymphocytic leukemia: Implications for cell origin and leukemogenesis. Semin Cancer Biol 20:400-409, 2010
13. Fais F, Ghiotto F, Hashimoto S, et al: Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 102:1515-1525, 1998
14. Tobin G, Thunberg U, Karlsson K, et al: Subsets with restricted immunoglobulin gene rearrangement features indicate a role for antigen selection in the development of chronic lymphocytic leukemia. Blood 104:2879-2885, 2004
15. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al: Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: A molecular classification with implications for targeted therapies.

Blood 119:4467-4475, 2012

16. Messmer BT, Albesiano E, Efremov DG, et al: Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. J Exp Med 200:519-525, 2004

17. Herishanu Y, Pérez-Galán P, Liu D, et al: The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood 117:563-574, 2011

18. Chu CC, Catera R, Hatzi K, et al: Chronic lymphocytic leukemia antibodies with a common stereotypic rearrangement recognize nonmuscle myosin heavy chain IIA. Blood 112:5122-5129, 2008

19. Hadzidimitriou A, Agathangelidis A, Darzentas N, et al: Is there a role for antigen selection in mantle cell lymphoma? Immunogenetic support from a series of 807 cases. Blood 118:3088-3095, 2011

20. Pérez-Galán P, Dreyling M, Wiestner A: Mantle cell lymphoma: Biology, pathogenesis, and the molecular basis of treatment in the genomic era. Blood 117:26-38, 2011

21. Friedberg JW, Sharman J, Sweetenham J, et al: Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood 115:2578-2585, 2010

22. Staudt LM, Dunleavy K, Buggy JJ, et al: The Bruton's Tyrosine Kinase (Btk) Inhibitor PCI-32765 Modulates Chronic Active BCR Signaling and Induces Tumor Regression in Relapsed/Refractory ABC DLBCL. Blood 118, 2011 (abstr 2716)

23. Mackay F, Figgett WA, Saulep D, et al: B-cell stage and context-dependent requirements for survival signals from BAFF and the B-cell receptor. Immunol Rev 237:205-225, 2010

24. 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 117:6287-6296, 2011

25. 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 119:1182-1189, 2012

26. Burger JA: Inhibiting B-cell receptor signaling pathways in chronic lymphocytic leukemia. Curr Hematol Malig Rep 7:26-33, 2012

27. Coutre SE, Byrd JC, Furman RR, et al: Phase I study of CAL-101, an isoform-selective inhibitor of phosphatidylinositol 3-kinase P110d, in patients with previously treated chronic lymphocytic leukemia. J Clin Oncol 29:451s, 2011 (suppl; abstr 6631)

28. Woyach JA, Johnson AJ, Byrd JC: The B-cell receptor signaling pathway as a therapeutic target in CLL. Blood [epub ahead of print on June 19, 2012]

29. Chen L, Monti S, Juszczynski P, et al: SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma. Blood 111:2230-2237, 2008

30. Dunleavy K, Pittaluga S, Czuczman MS, et al: Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood 113:6069-6076, 2009

DOI: 10.1200/JCO.2012.44.4281; published online ahead of print at www.jco.org on October 8, 2012

