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Comment on Jebaraj et al, page 859

Overcoming resistance hurdles

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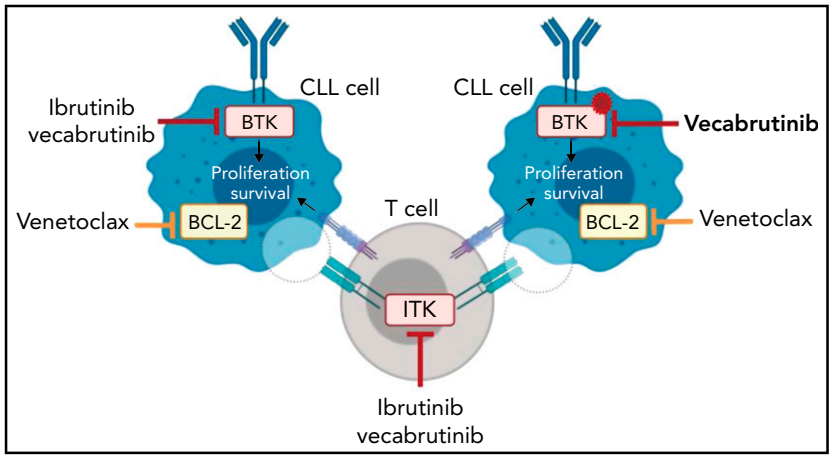
In this issue of *Blood*, Jebaraj et al[1] characterize a novel Bruton tyrosine kinase (BTK) inhibitor, vecabrutinib, that targets mutant BTK C481S and wild-type BTK in preclinical models. This is the first BTK inhibitor that also inhibits interleukin-2–inducible T-cell kinase (ITK) and can still bind to mutant BTK.

Clinical outcomes in chronic lymphocytic leukemia (CLL) have greatly improved over the last 2 decades with the development of regimens combining cytotoxic drugs with anti-CD20 monoclonal antibodies,[2] but outcomes vary significantly. For instance, patients in genetic high-risk groups, such as those harboring *TP53* mutations, continue to have suboptimal outcomes despite treatment with the best available therapy.[3] Similarly, patients who relapse early or are refractory to chemotherapy have an unfavorable outcome.[4] These areas of ongoing unmet clinical need lend impetus to developing truly novel targeted approaches to treating high-risk B-cell leukemias. Among the most promising classes of targeted therapies in CLL are inhibitors of BTK (BTKi's). BTK plays a prominent role in the BCR signaling pathway. Clinical activity of ibrutinib (the first US Food and Drug Administration–approved BTKi) is attributed to attenuated homing and retention of CLL cells to the microenvironment as a result of impaired BCR-controlled integrin-mediated adhesion and chemokine-controlled migration.[5]



The noncovalent BTK/ITK inhibitor vecabrutinib can block wild-type and C481S-mutant BTK in preclinical models. Vecabrutinib has immunomodulatory effects similar to those of ibrutinib in the murine Eμ-TCL1 model. Treatment with a combination of vecabrutinib and venetoclax leads to prolonged survival of Eμ-TCL1 mice. Illustration created with BioRender.com.

Microenvironmental crosstalk plays an important role in CLL pathogenesis and progression. CLL cells are strongly dependent on interactions with other immune cells, thus shaping a highly orchestrated network: the tumor microenvironment.[6] The inhibitory effects of ibrutinib on microenvironment homing and adhesion correlate with its clinical efficacy because ibrutinib treatment causes a rapid reduction of the lymph node size followed by a prolonged lymphocytosis.[7] This prolonged lymphocytosis resulting from treatment with kinase inhibitors seems to have no clinical disadvantage.[7] However, it could enhance the possibility of resistant clones accumulating. Acquired resistance to ibrutinib was reported in ~80% of patients as a result of mutations in BTK itself or the downstream kinase phospholipase Cγ2 (PLCγ2).[8] The BTK C481, most commonly serine, mutation confers resistance by preventing the covalent binding of ibrutinib to its target cysteine 481 in BTK (C481S).[8]

To date, 5 noncovalent BTKi's that can inhibit the kinase in the presence of a BTK C481 mutation[9] have entered clinical trials. In their article, Jebaraj et al characterized the noncovalent BTKi vecabrutinib (SNS-062) and demonstrated binding in the adenosine triphosphate binding pocket of BTK independent of the C481 residue (see figure). Vecabrutinib inhibited BCR signaling in wild-type and BTK C481S–mutant cells as measured by calcium flux. Furthermore, adoptive Eμ-TCL1 mice treated with vecabrutinib have increased survival compared with vehicle control (median survival, 35 vs 28 days).

Interestingly, like ibrutinib, vecabrutinib is also a potent ITK inhibitor (50% inhibitory concentration,14 nM). ITK is downstream of the T-cell receptor. The T cells present in the tumor microenvironment function as supporters of CLL, and it has also become clear that CLL cells actively recruit supportive regulatory T cells.[6] It has been reported that ibrutinib can reverse defects in T cells.[10] So the authors investigated whether vecabrutinib, like ibrutinib, has immunomodulatory effects. Treatment with either ibrutinib or vecabrutinib reduced the number of immunosuppressive $CD4^+$ regulatory T cells in Eμ-TCL1 mice, which suggests that vecabrutinib can reduce the supportive functions found in the microenvironment (see figure).

Because ibrutinib and vecabrutinib showed only a limited cytotoxic effect in CLL cells, combination with a drug that induces rapid apoptosis would be favorable. Venetoclax directly targets BCL-2 (a key regulator of programmed cell death) and is highly expressed in CLL cells. Jabaraj et al demonstrated that vecabrutinib, similar to ibrutinib, primes CLL cells to BCL-2 dependency (see figure). Subsequently, treatment with the combination of vecabrutinib and venetoclax resulted in prolonged survival of Eμ-TCL1 mice.

Approved BTKi-based regimens combined with BCL-2 inhibitor–based regimens are now well advanced in clinical trials,[9] and evaluation is needed to determine whether such combination therapies reduce the development of BTK and PLCγ2-mutated clones. Combining vecabrutinib with venetoclax could overcome the hurdle of development of BTK mutations and hopefully achieve long-term remissions, an essential step toward improving outcomes for patients with CLL.

Conflict-of-interest disclosure: R.T. is an employee of the Walter and Eliza Hall Institute, which receives milestone and royalty payments related to venetoclax. ■

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Comment on Johnston et al, page 889

GEP: time for prospective study in HL?

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In this issue of *Blood*, Johnston et al[1] use gene expression profiling (GEP) to investigate the tumor microenvironment in biopsies from children diagnosed with classical Hodgkin lymphoma (cHL). The study includes a comparison with an adult cohort and the development of a pediatric prognostic model.

From the late 1980s, GEP has been used by researchers to identify the cell of origin to better understand lymphoma pathology. Such studies were initially limited to the expression of a single gene but were soon expanded to a large panel of genes with improvements in the technologies used. The ability to use formalin-fixed, paraffin-embedded tissues enabled the use of GEP in the clinical setting. Two specific clinical domains were investigated: improved classification and subclassification of lymphomas and identification of new prognostic markers (see figure). The first approach was very successful for non-Hodgkin lymphomas (NHLs). Our understanding of rare lymphomas such as gray zone lymphoma, primary B-cell mediastinal lymphoma, or peripheral T-cell lymphomas, for instance, was greatly improved. Moreover, subclassification of more frequent lymphomas, like diffuse large B-cell lymphoma or follicular lymphoma, is now recognized as a clinically meaningful method to predict the outcome and development of targeted treatment.[2,3]

In cHL, one difficulty was the paucity of tumor cells. The first studies were done on cell lines or dissected cells in order to confirm the B-cell origin of cHL and how the pathology and GEP impact outcome.[4,5] However, the cells in the microenvironment are now recognized to play a major role in HL pathology and outcome.[6] Thus, GEP study of the clinical biopsy, not just the tumor cells, is needed.

Johnston et al first demonstrate significant differences in gene expression between pediatric and adult cHL with an enrichment of eosinophils, B cells, and mast-cells signatures in children, while macrophage and stromal cells signatures were more prominent in adults. This is a major point, even if it is still difficult to confirm that HL is intrinsically different