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A Moving Target: Inactivating BTK Mutations as Drivers of Follicular Lymphoma

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SUMMARY

Drugs that target Bruton tyrosine kinase (BTK) have been highly successful and changed the landscape of therapies in B-cell lymphomas. However, their lower rates of effectiveness in follicular lymphoma are unexplained. Recent work describes inactivating BTK mutations that show that at least some follicular lymphomas do not require BTK.

See related article by Hu et al., p. 2301

In this issue of *Clinical Cancer Research*, Hu and colleagues report on the functional properties of recently described Bruton tyrosine kinase (BTK) mutations in follicular lymphoma (1). Such mutations were first described in 2017 by Krysiak and colleagues (2) in a genomic survey of 105 primarily treatment-naïve patients with follicular lymphoma. In that study, recurrent BTK mutations were observed in seven of 105 individuals (∼7%), alongside recurrent mutations in other members of the B-cell receptor signaling pathway, such as CARD11 and CD79B; however, the functional roles of these mutations were not tested. In the current report, the investigators sequenced all exonic regions of the *BTK* gene in 150 cases of untreated follicular lymphoma by Sanger sequencing. Even though Sanger sequencing has a sensitivity of detection of around 15%–20% variant allele frequency (compared with 1%–2% for next-generation sequencing), they found an identical percentage of BTK mutations ($n = 10/150$, 7%). Of note, none of the BTK mutations described between the two articles overlapped. This suggests no specific hotspots exist and more mutations are likely to be identified.

To study the functional properties of the *BTK* variants they observed, the authors expressed them in HEK293T cells and four lymphoid cell lines. Surprisingly, they found that eight of nine tested mutations led to destabilization of the BTK protein and five of nine resulted in inactivation of its kinase function. Given that BTK is being studied as a drug target in follicular lymphoma in several clinical trials of BTK inhibitors, this unexpected finding argued against the *a priori* hypothesis that these mutations must be activating downstream of the B-cell receptor (as is the case for CD79B and CARD11 mutations). But why are these mutations selected for in untreated follicular lymphoma? Luckily, these investigators did not stop there. They proceeded to discover that while signaling through other downstream targets of the B-cell receptor decreased or was unchanged, AKT signaling increased across all of the mutants. Furthermore, direct testing on primary samples from patients with follicular lymphoma revealed AKT activation to be a hallmark of follicular lymphoma cells with or without BTK mutations. BTK immunoprecipitation followed by mass spectrometry did not reveal any interaction between the BTK variants and regulators of AKT. In addition, chemical BTK inhibitors did not induce AKT activation, while proteolysis targeting chimera (PROTAC)-mediated degradation did. These findings suggest it is the absence of BTK, and not its kinase function, that mediates compensatory AKT activation.

The above findings have both bedside-to-bench mechanistic impact upon our understanding of B-cell receptor signaling and potential bench-to-bedside implications for targeting BTK in follicular lymphoma and other lymphomas. While the link between decreased BTK protein and increased AKT signaling is a novel finding, it is important to point out the authors did not mechanistically link the two and AKT activation was seen across follicular lymphomas they tested independent of BTK mutation status. The authors show that pretreating the cell lines expressing the BTK variants with the PI3Kδ-specific inhibitor, idelalisib (CAL-101), almost completely blocked the AKT activation seen, while there was no difference in surface immunoglobulin expression that could explain the enhanced AKT signaling. As suggested by the authors, this hints at a mechanism whereby in the absence of BTK protein, AKT is brought to the membrane to interact with phospho-inositol triphosphate (see **Fig. 1**). However, given that AKT activation is seen in follicular lymphomas without BTK mutations, there may be complementary pathways activating PI3K–AKT signaling that are also active in BTK mutants. Regardless, PI3Kδ inhibition resulted in decreased AKT signaling in BTK-mutant cell lines and could potentially have activity against BTK-mutated follicular lymphomas. Further in-depth studies of this approach should be investigated perhaps beginning with analysis of follicular lymphoma samples taken from participants in clinical trials of PI3K-specific inhibitors.

Another interesting translational aspect of this work is the potential implications for BTK-targeted therapy development in follicular lymphoma. Mutations occurring in BTK were first described in the setting of chronic lymphocytic leukemia resistant to the covalent BTK inhibitor, ibrutinib (3). These mutations are acquired under the selective pressure of irreversible kinase inhibition and result in a change of the cysteine amino acid at position 481, thereby preventing binding of the drug. Importantly, the BTK mutations reported in the current study were all discovered in patients who had not received any therapy, including BTK inhibitors. Nonetheless, two of the nine mutations tested caused ibrutinib resistance when overexpressed *in vitro* (Y315N and P597S). Presumably, this resistance is mediated by alternative mechanisms than C481 mutations, as there is emerging evidence revealing some non-C481 mutations associated with clinical ibrutinib resistance. The clinical significance of BTK mutations in untreated follicular lymphoma remains unclear and requires further

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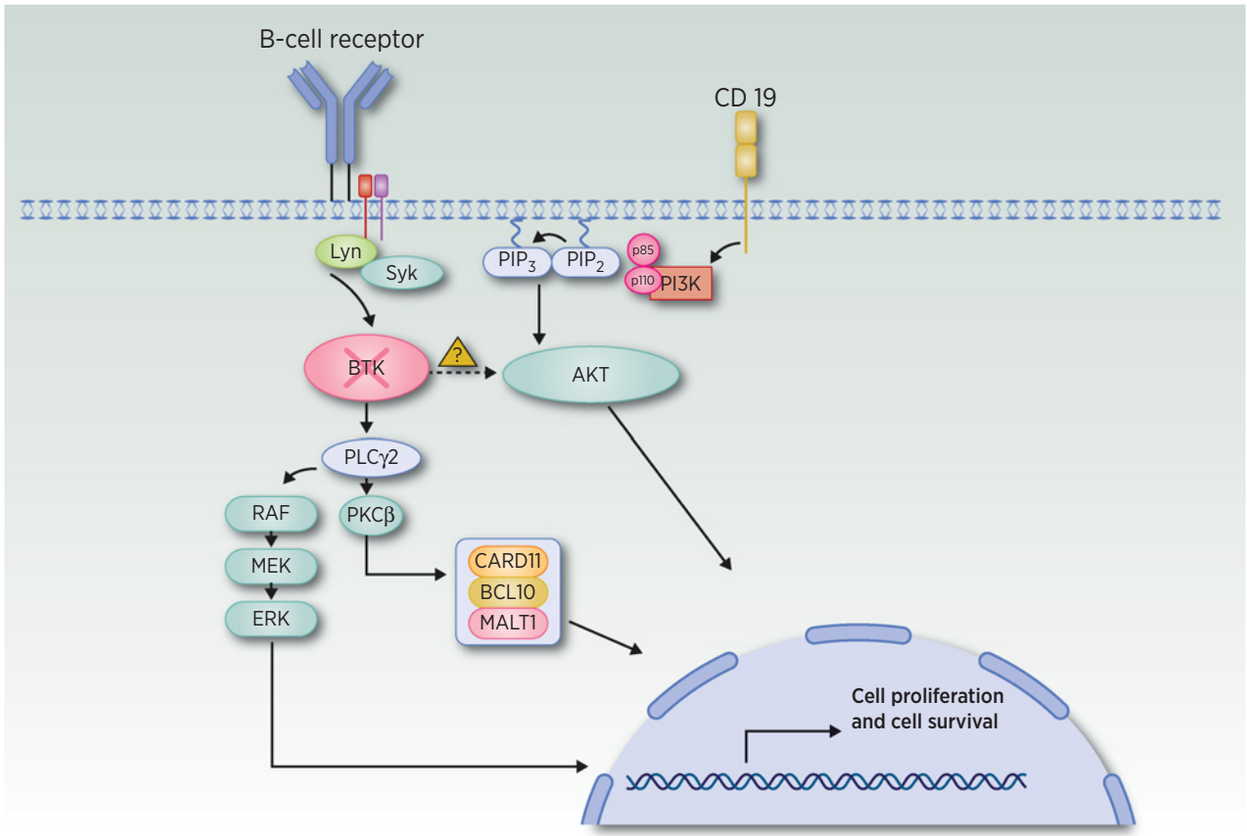

Figure 1.

Effect of BTK mutations on B-cell receptor signaling in follicular lymphoma. Mutations occurring in BTK in treatment-naïve follicular lymphoma were found to cause decreased protein stability or loss of kinase activation, leading to loss of protein function. These loss-of-function mutations and experiments using PROTACs to degrade BTK resulted in activation of AKT through still yet not understood mechanisms. AKT, protein kinase B; BCL10, B-cell lymphoma/leukemia 10; CARD11, caspase recruitment domain-containing protein 11; CD 19, B-lymphocyte antigen; Lyn, Src family tyrosine kinase; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKCβ, protein kinase C beta; PLCγ2, phospholipase C gamma-2; RAF, proto-oncogene serine/threonine-protein kinase; Syk, spleen tyrosine kinase.

investigation of specific mutations to determine whether they may serve as predictors of response or resistance to targeted therapies. However, noncovalent BTK inhibitors and BTK-degrading PROTACs are concomitantly being developed and the influence of BTK mutations on response to these drugs remains to be seen. In the case of PROTACs, this article suggests that this strategy might result in increased AKT activation, potentially acting as a therapeutic escape mechanism.

Finally, this study uncovered a still yet to be defined link between BTK mutations and increased AKT activation that was abrogated by PI3Kδ inhibition. The PI3Kδ-specific inhibitor, idelalisib, is FDA approved for use in relapsed follicular lymphoma based on a 54% overall response rate (4). In addition, dual and pan-PI3K isoform inhibitors have been approved in relapsed follicular lymphoma. As mentioned before, early trials of BTK inhibitors in relapsed follicular lymphoma did not meet primary efficacy endpoints, but about 21% of patients responded with 11% complete responses (5). This leaves the door open to the possibility that patients could be preselected for likelihood of response to targeted therapies based on molecular testing. The study by Hu and colleagues in this issue highlights the importance for functional testing of variants detected by genomic approaches.

Authors' Disclosures

No disclosures were reported.

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