**PI3K in CLL: are two isoforms better than one?**

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**In this issue of *Blood* Dong *et al*1 present a series of experiments with the novel B cell receptor kinase inhibitor IPI-145 (PI3Kγ/δ inhibitor) and show CLL samples resistant to ibrutinib remain sensitive to killing by this agent.**

In normal healthy B cells, the B cell receptor (BCR) detects and responds to antigens displayed on pathogens. Upon engagement it activates two key processes; first, triggering the formation of the “signalosome”, a complex of kinases and proteins leading to downstream signal transduction consequent to apoptosis, proliferation and/or migration ***(Figure 1)***; second, internalising the captured antigen for processing and subsequent presentation to T cells.

Strong evidence now exists to suggest that BCR signalling has a major role in regulating the behaviour of chronic lymphocytic leukemia (CLL) cells, and roles in the development, progression and clinical response to treatment have all been proposed.2 These observations provide a strong rationale for targeting the BCR in CLL. BCR kinase inhibitors for the treatment of CLL have shown impressive clinical responses, which has led to significant therapeutic advances in the treatment of difficult to treat and otherwise refractory patients. Two inhibitors of the BCR pathway have recently been approved by the FDA for various cohorts of CLL; the Bruton’s tyrosine kinase (BTK) inhibitor Ibrutinib and the phosphatidylinositide 3-kinase delta (PI3Kδ) inhibitor idelalisib. Both drugs were hypothesised to exert their therapeutic effects by inhibiting CLL ingress into and/or promoting efflux out of the lymph nodes into the blood, where they no longer receive proliferative and pro-survival signalling. However there is data to suggest BCR kinase inhibitors can also exert direct cytotoxicity3,4 and more so in lymph nodes.4 Unfortunately, resistance to these drugs is already emerging in patients with high risk cytogenetic features (those patients carrying del(11q22.3), del(17p13.1) or a complex karyotype).5 Therefore it is importance to fully understand how these inhibitors work and to demonstrate whether, once resistance emerges, alternative BCR pathway inhibitors can be utilised. Moreover it is still too early to tell whether these agents with long term use will be curative or will just keep the disease supressed. Therefore there is clearly a need to develop alternative BCR signalling inhibitors including PI3K inhibitors with greater efficacy.

Class 1 PI3K signalling is pivotal for cell survival in numerous cancers and is known to be overactive in CLL, with unmutated IGHV patients having increased PI3K expression/activity compared to patients with mutated IGHV.6 The Class 1 PI3K comprises Class1A (PI3Kα, PI3Kβ and PI3Kδ) and Class1B (PI3Kγ) isoforms. Whilst PI3Kδ and PI3Kγ expression is largely restricted to leukocytes, PI3Kα and PI3Kβ are generally ubiquitous. PI3Kδ and PI3Kγ are pivotal for a number of leukocyte functions including proliferation, antibody secretion, survival and migration. Whilst PI3Kβ has recently been described to be involved in neutrophil activation and generation of reactive oxygen species in response to immune complexes.7 There is also evidence of functional redundancy since multiple PI3K isoforms require inhibition to fully reverse the neutrophil survival induced by GM-CSF.8 Generally the more PI3K isoforms that are inhibited the greater the toxicity to CLL cells. However the caveat is that there is more off target toxicity to other non-haematological tissues if PI3Kα and PI3Kβ are also inhibited.

In this issue of blood Dong *et al.1* investigate *in vitro* an alternative PI3K inhibitor IPI-145, which is currently in Phase III clinical trial as a monotherapy for CLL (NCT02004522). Unlike idelalisib, IPI-145 targets both δ and γ isoforms of PI3K that are expressed in leukocytes. *In vitro,* IPI-145 induced up to ~30% apoptosis of primary CLL cells in a dose and time dependent manner, which is similar to published data with idelalisib.3 Importantly, samples that were resistant to ibrutinib were still susceptible to killing from IPI-145 even though BTK exerts its effect both upstream and downstream of PI3K, suggesting PI3K may compensate following BTK/PLCγ2 mutations/disruption. IPI-145 significantly inhibited signalling with anti-IgM at much lower concentrations than idelalisib,1,9 however whilst phosphorylated AKT(T308) and ERK1/2(T202/Y204) were completely abrogated and confirm PI3K inhibition, phosphorylated AKT(S473) was only partially inhibited. This may suggest that AKT(S473) is regulated by another pathway or that the known positive feedback mechanism induced by mTORC2 and observed in other haematological malignancies10 is active. Furthermore although IP-145 was not toxic to normal control T and B cells, CLL T, B and NK cells were more sensitive to this agent. But further work is required to understand this mechanism and what it will mean to patients.

Whilst these data confirm BCR signalling was inhibited by IPI-145, there are still a significant number of unanswered questions. Will IPI-145 inhibit CXCR4/CXCL12 signalling and subsequent migration, T cell (CD40L and IL-4), stromal (BAFF, IL-6) and toll-like receptor mediated signalling as previously shown with idelalisib? Will IPI-145 be superior to idelalisib in patients? Will IPI-145 need to be combined with another agents such as the BH3-mimetics or monoclonal antibody (mAb) therapies to obtain greater efficacy? In theory, both should synergise well with IPI-145 through the aforementioned mechanism of retaining CLL cells in the blood, both reducing the anti-apoptotic signalling and providing easy access for mAb. Finally, is inhibiting two isoforms better than one? Might the inhibition of the γ isoform of PI3K, induced by IPI-145 but not idelalisib, in fact lead to more tumour toxicity in vivo? Similarly, might it reduce the effector functions of various immune effectors when used in combination with mAb? For these answers we eagerly await the results of the up-coming trials and further *in vitro* experimentation.

Despite these unresolved questions, this paper provides exciting new data and insight into the biology of the PI3K signalling pathway in CLL cells, which will enable the development of more effective drugs for the treatment of this currently incurable disease. Moreover, the more inhibitors we have to the BCR signalling pathway, the more tools we will have to further dissect its critical signalling functions in malignant cells and the more opportunities we will have to explore rational combinations for improved therapeutic efficacy in the future.

*Conflict of interest disclosure: the author declares no competing financial interests*

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***Figure 1.*** Binding of antigen/autoantigen to the BCR results in formation of the signalosome that includes SYK, BTK and PI3K, all of which have been targeted by kinase inhibitors which have shown promise for the treatment of CLL. However the PI3K complex also regulates signalling from IL-4, CD40L, CXCL12, BAFF and the toll-like receptor (TLR). A number of signalling pathways are activated downstream of these kinases which result in the elevation of NFAT, MYC, JUN, NFκB, S6, Bim, Noxa and β-catenin which are involved in survival, apoptosis, proliferation and migration. mTOR immediately downstream of PI3K/AKT signalling is the catalytic subunit of the mTORC1 and mTORC2 complexes. mTORC2 is capable of inducing a positive feedback loop which leads to the phosphorylation of serine 473 (P S473) and reactivation of AKT downstream signalling.