

Resistance Mechanisms for the Bruton's Tyrosine Kinase inhibitor Ibrutinib. Woyach *et al.* The New England Journal of Medicine 2014 Epub

B-cell receptor ligation in normal B cells induces signals for proliferation, energy, migration and apoptosis. Markers of these pathways are also evident to varying degrees in CLL (Figure 1). Therefore it has been hypothesised for a number of years that B cell receptor (BCR) signalling plays a prominent role in driving proliferation and accumulation of chronic lymphocytic leukaemia (CLL) cells in patients. Although the antigen is currently unknown evidence for both antigen or autoantigen driven BCR ligation have been hypothesised, but it is still unclear which if any drives BCR signalling in CLL.

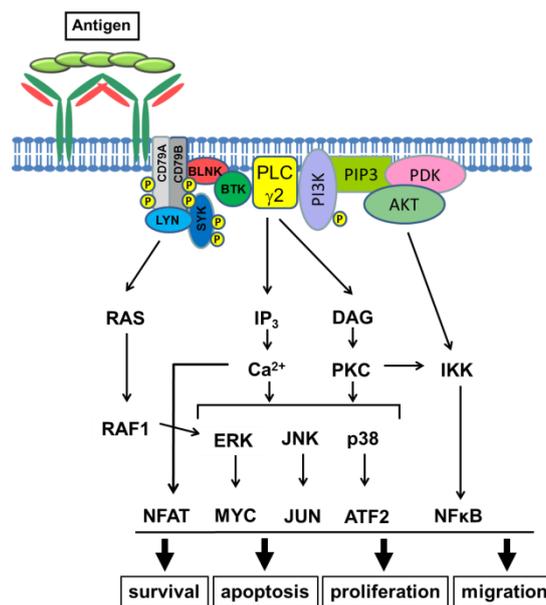


Figure 1. B cell receptor signalling pathways in CLL.

In the last 18 months kinase inhibitors which target proteins within the BCR signalling pathway such as Phosphoinositide 3-kinase (PI3K) by idelalisib and Bruton's tyrosine kinase (BTK) by ibrutinib have shown fantastic results in phase III clinical trials which have led to a therapeutic advance in the treatment of CLL. This paper review will summarise recent findings showing the emergence of resistant mechanisms in patients treated with ibrutinib (388-868 days).

The BTK gene is not recurrently mutated in CLL, but BTK is transcriptionally upregulated in CLL and is constitutively active (probably through BCR signalling). Ibrutinib irreversibly binds BTK at the cysteine 481 (C481) residue which inhibits its kinase activity and subsequently cellular proliferation and downstream signalling in vitro. It has been reported that seventy one percent of patients treated with ibrutinib have an objective complete or partial response with an estimated 75% progression free survival. Few patients have relapsed on ibrutinib but we need to understand this resistance mechanism if we are to understand which patients may or may not respond to ibrutinib.

The findings in this study revolved around the finding that six patients treated with ibrutinib became resistance to the drug. All 6 patients had high risk cytogenetic features including del(11q22.3), del(17p13.1) or a complex karyotype. Five of the six patients showed a mutation at position 481 from a cysteine to a serine (C481S), which is the residue to which ibrutinib binds. The sixth patient

had an arginine to tryptophan mutation in PLC γ 2 at residue 665 (R665W). Interestingly one patient had both a low frequency BTK mutation (C481S) and 3 distinct PLC γ 2 mutations in R665W, L845F and S707Y identified by whole-exome and Ion Torrent sequencing. All sequencing was confirmed by sanger sequencing and no patient showed evidence of mutation at baseline by whole exome sequencing.

Functional studies in this paper showed that the affinity of ibrutinib for the BTK C481 residue was reduced 52-fold in the mutated (C481S) compared to wild type samples. This resulted in a significantly less effective blocking of BTK autophosphorylation and subsequent downstream signalling. Furthermore the C481S mutation prevented irreversible binding of the drug to BTK as seen in the wild type samples and allowed only reversible binding of ibrutinib to the protein.

The S707Y PLC γ 2 mutation is known to have a gain-of-function effect in other systems so the paper concentrated on PLC γ 2 mutations in R665W and L845F. Both mutations were introduced to HEK cells or the chicken B cell line DT40. Both mutations resulted in a gain of function following BCR ligation with anti-IgM, enabling BCR signalling which was independent of BTK. Thus the effect of ibrutinib on inhibiting BCR signalling and PLC γ 2 phosphorylation were also reduced in these cell lines. These assays were repeated in CLL patient samples and the same effect on signalling was observed in samples with the PLC γ 2 mutation.

In the one sample that had both BTK and PLC γ 2 mutations ibrutinib was unable to inhibit phosphorylation of either protein, therefore was unable to inhibit BCR signalling in these samples.

The study also mentions that treatment of patients with ibrutinib usually results in a lymphocytosis, which appears to be due to cells leaving the lymph nodes and entering the periphery (blood). In many cases this lymphocytosis resolved within 8 months, however in this study they found a group of patient where the lymphocytosis persisted for more than 12 months. They investigated this group of patients to see whether the extended lymphocytosis was due to the known mutations in BTK and PLC γ 2 described above. Ion Torrent was used to assess the mutations to a sequencing depth of 700 reads. None of the 9 patients investigated showed evidence of the known resistant mutations described above. However the authors suggested that this did not rule out the possibility of mutations because of the presence of a large number of non-mutant clones which may mask small clones within the samples.

The study postulated that because these BTK and PLC γ 2 mutations have arisen in patients with increased genetic instability including del(11q22.3), del(17p13.1) or complex karyotype, that this group of patients may be more susceptible to relapse on ibrutinib. They therefore concluded that it is rational to treat this group of patients with combination therapies in order to avoid the development of ibrutinib resistance.

Finally they did mention that samples with longer lymphocytosis or ibrutinib resistance without the known mutations (above), did not rule out resistance through mutations in other coding genes, non-coding RNA, epigenetic activation or silencing, or gene amplification.

Therefore a better understanding of these resistant mechanisms is still required and will hopefully lead to the development of combination therapies that will prevent or treat resistant disease.

Andrew Steele, University of Southampton, June 11th 2014.