BTK T474I with enhanced kinase activity confers growth advantage over BTK-L528W with kinase deficiency in B malignant cells

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Abstract Content:

Background: Covalent BTKi have transformed the therapeutic landscape for chronic lymphocytic leukemia (CLL). However, acquired mutations in BTK, often occurring at C481, lead to drug resistance and disease progression. While non-covalent BTKi has demonstrated promising efficacy in CLL patients with BTK C481X mediated resistance, non-C481 mutations within BTK kinase domain confer ineffectiveness to cBTKi and ncBTKi as well. Those BTK mutations most commonly involve BTK T474I and L528W. To compare their role in promoting B malignant cell growth, we developed clonal competition models by co-culturing isogenic B malignant cells harboring BTK T474I and L528W and monitor the clonal dynamics in long term culture.

Methods: The T474I and L528W were introduced to BTK allele of B malignant cell line TMD8. BTK downstream signaling in BTK wildtype or mutant cells with or without anti-IgM stimulation was assessed by western blot. In vitro and in vivo pooled clone models containing both TMD8 wildtype and mutant cells were employed to investigate the clonal competition. The dynamics of individual clones were monitored by measuring the changes of BTK variant allele frequency (VAF) via amplicon sequencing.

Results: Hyperactivation of phosphorylated BTK Y223 in TMD8 BTK T474I cell and very limited BTK Y223 phosphorylation in TMD8 BTK L528W upon IgM stimulation was observed. Enhanced B-cell receptor (BCR) downstream signaling indicative of pPLCγ2, pERK and pAKT were noted in BTK T474I cells compared to those expressing BTK L528W. Those findings suggest that BTK T474I encodes a kinase exhibiting enhanced activity while BTK L528W results in a catalytically inactive form. Clone competition was evaluated using co-culture experiments containing TMD8 BTK T474I, BTK L528W and WT cells in medium with various FBS concentration. T474I clones were significantly expanded while L528W clones were declined in 4 weeks culture period. The outgrowth advantage of BTK T474I was particularly pronounced when cultured in medium with low FBS concentration, which simulated a relatively harsh condition for cell growth. Similar results were observed when those clonal pools were grown as xenografts. An increase in T474I VAF and a decrease in L528W VAF were noted during tumor growth in subcutaneous models incorporating TMD8 wildtype and mutant cells.

Conclusion: Our in vitro and in vivo clonal competition assays provided novel insights into the impact of BTK T474I and L528W on growth fitness. These findings suggested that BTK T474I, characterized by enhanced kinase activity, confers a growth advantage over the kinase deficient form BTK L528W.