ABSTRACT I

Phosphatiylinositide-3-kinase (PI3K) and Bruton tyrosine kinase (BTK) are relevant kinases involved in the pathogenesis of chronic lymphocytic leukemia (CLL). Aberrant B cell receptor (BCR) signaling with up-regulated BTK activity and constitutively activated PI3K/AKT pathway is a driving force for apoptosis resistance of CLL cells. Pharmacological inhibition of PI3Kδ and BTK by small molecule inhibitors holds great promise for treating CLL. However, the ideal balance of PI3K isoforms to be targeted in order to achieve optimal clinical efficacy still remains to be defined. Therefore potential benefits of dual inhibition of PI3Kα and PI3Kδ by copanlisib compared to single inhibition of PI3Kδ by idelalisib were subjected to a pairwise comparison in vitro. Copanlisib reduced the survival of CLL cells more than 10 times more effectively than idelalisib, but the cytotoxic effects of both PI3K inhibitors and of the covalent and reversible BTK inhibitors ibrutinib and dasatinib were low compared to their clinical efficacy. Inhibition of migration at clinical achievable concentrations of these inhibitors proved their impact on cell functions with importance for the crosstalk of tumor cells with the microenvironment. Taken together, in-vitro assessment of kinase inhibitors (KI) revealed stronger effects on the dialogue of malignant B cells with their micro-environment rather than on survival. Concluding from enhanced direct cytotoxicity against malignant B cells and more efficient disturbance of their migration by copanlisib and duvelisib, targeting more than one PI3K isoforms could be of advantage also in a clinical setting.

ABSTRACT II

Clinical success of small molecule inhibitors targeting BTK in the treatment of B cell malignancies has placed them in the spotlight of clinical research. However, the contribution of BTK to disease pathogenesis has not yet been completely unraveled. Ibrutinib a covalent, irreversible BTK inhibitor inhibits enzyme activity by binding to Cys481 (C481) in the ATP binding site of BTK. Acquired resistance to ibrutinib is a growing concern in CLL therapy and due to mutation of C481 in 7%-10% of cases. In this study, the clinically used KIs ibrutinib and dasatinib and corresponding BTK mutants with altered binding pocket served as tools to dissect the contributions of BTK activity to different cell

functions. Expression of the inhibitor resistant BTK C481S and –T474I mutants in malignant B cell line models maintained BTK auto-phosphorylation under treatment with ibrutinib or dasatinib, respectively, and permitted to assess the reversal of BTK-dependent inhibitor effects on cell functions. Comparison of inhibitor effects on cells expressing wild type versus mutant BTK showed that BTK is not functionally involved in cell survival, but in CXCL12 regulated chemotaxis and secretion of the chemokines CCL3 and CCL4. According to chemical genetic analysis, inhibition of BTK activity did not directly affect the survival of malignant B cells but functions that facilitate the dialogue within the malignant cells and their micro-environment.