Abstract: Synergistic Targeting of BTK and E-Selectin/CXCR4 in the Microenvironment of Mantle Cell Lymphomas


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Background

Mantle cell lymphoma (MCL) is a rare subtype of aggressive B-cell non-Hodgkin lymphoma that is incurable with standard therapy. Overexpression of B-cell receptor signaling through Bruton’s tyrosine kinase (BTK) is a hallmark of MCL (Pat Singh et al., 2019). Inactivation of BTK signaling with the small molecule inhibitoribrutinib is currently the most broadly used treatment for B cell lymphoma. However,ibrutinib only induces a minimal degree of B cell apoptosis in vitro at clinically achievable concentrations. Frequently, primary and acquired resistance toibrutinib is observed (Chiron et al., 2014; Wang et al., 2013). One of the molecular mechanisms of acquired resistance is the development of BTK mutations (Martin et al., 2016). In addition, the tumor microenvironment (TME), in which mesenchymal stroma cells (MSC) and vascular endothelial cells (ECs) are specialized components, has increasingly been recognized as a central determinant of drug resistance, subclonal evolution, and late progression/ transformation of B-cell lymphomas (Balsal et al., 2017; Weis and Cheson, 2011). Although the pro-tumoral ecosystem that supports MCL is still poorly understood, it has been reported that MCL cells express high levels of functional CXCR4 and CXCR5 chemokine receptors, and VLA-4 adhesion molecules (Kurtova et al., 2009). Lymphoma cells also display high levels of CD44, one of the E-selectin ligands, in co-culture with ECs (Chiron et al., 2014). These findings strongly suggest an association between acquired BTK mutations and the TME-mediated resistance in BTK-targeted therapy of MCL. Therefore, we hypothesized that the disruption of crosstalk between MCL cells and the TME (i.e., by blocking CXCR4/CXCL12 or E-selectin/CD44) could enhance BTK-targeted therapy against MCL.

Materials and Methods

Drugs & Cell Lines: Multi-knase inhibitor CG-806 was provided by Aptose Biosciences. E-selectin antagonists were provided by GlycoMimetics Inc. BTK inhibitor Bicalutin, U4411 inhibitor S183-20965 and putative antiproliferative and proapoptotic effect of CG-806 on BCL-1 and HUV/EC endothelial cells were from ATCC. MSC were derived from normal bone marrow donors.

Results

CG-806 Exerts Extreme Low IC50 (< 1 nM) for Suppressing BTK Activity by Non-covalently Binding BTK(743,744)-Mutant and BTK Type Proteins

Graphical abstract:

Conclusions

CG-806 exerts potent cell growth inhibitory effects inibrutinib-resistant MCL cells.

CG-806 suppresses phospho-BTK, -Stat3, -AKT, -ERK, -Src, NF-kB, and the anti-apoptotic protein MCL-1 while upregulating p53.

CG-806 increases autophagy in MCL cells, which may be associated with resistance to CG-806-mediated apoptosis. Inhibition of autophagy re-sensitizes MCL cells to CG-806-induced apoptosis.

CG-806 treatment upregulates CXCR4/E-selectin levels in MCL cells.

The TME (i.e., MSC and HUVEC cells) protects MCL cells from CG-806-induced apoptosis, which is partially abrogated by CXCR4/E-selectin antagonists to enhance CG-806-induced MCL cell killing.

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