Abstract: 3802

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

Mahesh Basyal[1], Weiguo Zhang[1], Nalini B Patel[2], Sujan Piya[1], Hongying Zhang[3], William E. Fogler[4], William G. Rice[3], John L. Magnani[4], Gautam Borthakur[1,5] and Michael Andreeff[1,5]

[1]Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Texas; [2]Flow Cytometry Core, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas; [3]Aptose Biosciences, San Diego, California; [4]GlycoMimetics, Inc. Rockville, MD; [5]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

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 (Pal Singh et al., 2018). Inactivation of BTK signaling with the small molecule inhibitor ibrutinib 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 to ibrutinib is observed (Chiron et al., 2014; Wang et al., 2013). One of the molecular mechanisms of acquired resistance is the development of BTK^{C481S} 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 (Balsas et al., 2017; Weis and Cheresh, 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 (Cao 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-kinase inhibitor CG-806 was provided by Aptose Biosciences; E-selectin antagonist was provided by GlycoMimetics Inc.; BTK inhibitor Ibrutinib, ULK1 inhibitor SBI-0206965 and putative autophagy inhibitor Chloroquine (CQ) were purchased from Selleckchem. Mantle cell lymphoma cell lines z138, MIMO, Jeko-1, JVM2, and HUVEC endothelial cells were from ATCC. MSC were derived from normal bone marrow donor.

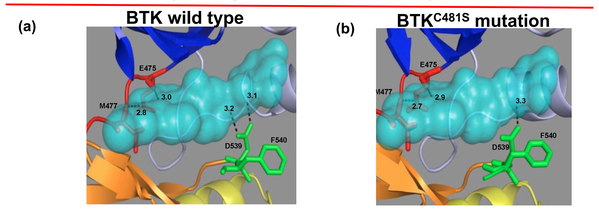
IC_{50}s and EC_{50}s: The 50% inhibitory concentration (IC_{50}) for cell growth inhibition (using Trypan blue dye exclusion method) and the 50% effective concentration (EC_{50}) for apoptosis induction (using FACS for measuring annexin V positivity) were calculated using CalcuSyn (BioSoft, Cambridge, UK).

Immunoblot Assays: MCL cells were treated with indicated concentrations of drugs and collected for immunoblot analysis.

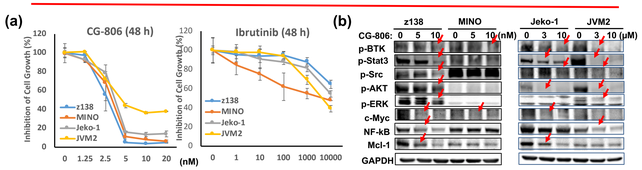
Flow cytometry: MCL cells were exposed in CG-806 for 24 h and E-selectin and CXCR4 levels were measured with FACS Caliburs by using the Cell Quest program.

Results

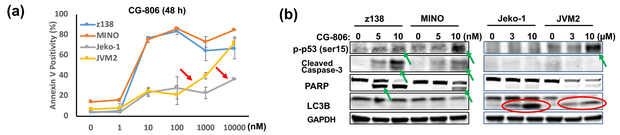
CG-806 Exerts Extremely Low IC_{50}s (< 5nM) for Suppressing BTK Activity by Non-covalently Binding BTK^{C481} Mutant and Wild Type Proteins



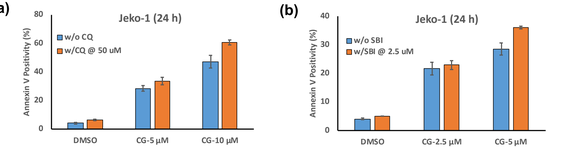
CG-806 Demonstrates Superior Anti-lymphoma Effects Compared with Ibrutinib



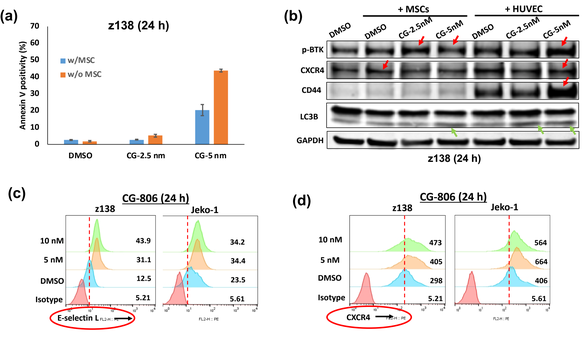
Autophagy is Associated with Resistance to CG-806-induced Apoptosis Induction in Certain MCL Cells



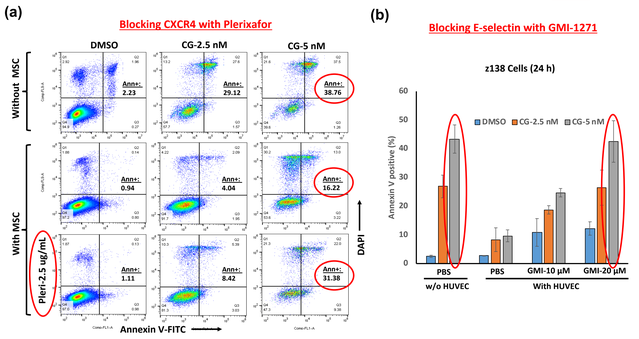
Blockade of Autophagy with CQ or SBI-0206965 Re-sensitizes CG-806-induced Pro-apoptotic Effects in the Resistant MCL Cells



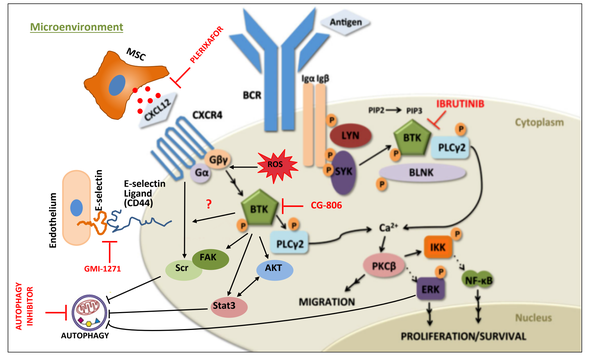
MSC- and ECs-mediated Protection from BTK Inhibition is Associated with Overexpression of Phospho-BTK, CXCR4, and E-selectin Ligands



Blockade of CXCR4/E-selectin Abrogates MSC-/ECs-mediated Protection of MCL Cells During BTK-targeted Treatment



Graphical abstract:



Conclusions

- **CG-806 exerts potent cell growth inhibitory effects in ibrutinib-resistant MCL cells.**
- **CG-806 suppresses phospho-BTK, -Stat3, -AKT, -ERK, -Src, NF-kB, and the anti-apoptotic protein Mcl1 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.**

* H. Zhang and W. Rice are employees of Aptose Biosciences; W. Fogler and J. Magnani are employees of GlycoMimetics; M. Andreeff serves on Aptose Biosciences SAB.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center Making Cancer History®

APTOSE BIOSCIENCES

GlycoMimetics, Inc.