CG’806, a Novel Pan-FLT3/BTK Multi-kinase Inhibitor, Induces Cell Cycle Arrest, Apoptosis, or Autophagy in AML Cells Depending on FLT3 Mutational Status

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Abstract: Gain-of-function mutations of the fms-like tyrosine kinase 3 (FLT3) play a pivotal role in hematopoietic malignancies (Gilliland et al., 2002). Therefore, FLT3 kinase inhibitors are an important component in the treatment of acute myeloid leukemia (AML). However, relapse/resistance to these inhibitors is frequent because of secondary acquired mutations in FLT3 gene. Recently, we reported on a novel small molecular multi-kinase inhibitor, CG’806, which showed promising effects in AML harboring FLT3 internal tandem deletion (ITD) mutations, tyrosine kinase domain (TKD) mutations, or both by inhibiting FLT3/Bruton’s tyrosine kinase (BTK)/aurora kinase (AuroK) activation. We observed an impressive inhibition of leukemic cell proliferation (i.e., IC50s of sub-nanomolar or low nanomolar concentrations, Zhang, et al., AACR, Hematological Malignancies, 2017).

To further characterize the mechanisms underlying this anti-leukemia effect, we investigated the impact of CG’806 on cell cycle progression. Measuring BrdU incorporation by flow cytometry, CG’806 triggered profound G1 cell cycle arrest in FLT3-ITD-mutated cells. Interestingly, CG’806 triggered G2/M phase arrest in FLT3 wild type (WT) cells. Immunoblotting demonstrated that the G1 arrest was mediated by downregulation of signaling associated with p-FLT3/ITD, the p-AKT/p-mTOR/cyclin D1-Rb axis, and that of cyclin B1/A2, cdk1/cdc2, and cdk4 in FLT3-ITD-mutated cells. This was not observed in FLT3 WT cells. In addition, c-Myc, a primary regulator of the G1/S transition, was downregulated in FLT3-mutated but not in WT cells treated with CG’806. In support of this finding, knockdown of c-Myc with siRNA increased the G1-arrested population in FLT3-mutated cells, suggesting a critical role of c-Myc in the CG’806-induced G1 arrest. However, only suppression of p-BTK and p-AuroK, but not p-FLT3, and no modulation of G1-arrested proteins were observed in FLT3 WT cells.

Interestingly, we observed autophagy induction in FLT3 WT cells compared to the induction of apoptosis in FLT3 mutated cells (as evidenced by modulated LC3II and cleaved-caspase 3 levels, respectively) after exposure to CG’806 for 24 hours. Inhibiting autophagy with 3-methyladenine (3-MA) partially reversed CG’806-induced G2/M arrest in FLT3 WT cells, suggesting that autophagy induction may also be involved in G2/M arrest in addition to suppression of AuroK and BTK in FLT3 WT cells. Next, we investigated if nucleoside analogues and intercalating agents enhance activity of CG’806, and observed that combination of CG’806 with conventional chemotherapeutics cytarabine or idarubicin profoundly enhanced pro-apoptotic effects.

Conclusions: CG’806 exerts profound suppression of cell proliferation by arresting cell cycle progression at G1 phase in FLT3-mutant AML cells, which is associated with inhibition of mutant FLT3 and downstream p-AKT/p-mTOR/cyclin D1-Rb signaling axis. CG’806 exerts a G2/M arrest in FLT3 WT cells, which is associated with inhibition of AuroK and downstream cyclin B1/CDK1 signaling pathway. MSC/hypoxia induce autophagy of FLT3-ITD mutated cells, which can be abrogated by chloroquine and therefore enhances CG’806-induced pro-apoptotic effect. CG’806 sensitizes AML to standard chemotherapeutic agent-mediated cytotoxicity.

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