C-Myc Targeting by Degradation: Novel Dual c-Myc/GSPT1 Degrader GT19715 Exerts Profound Cell Kill in vitro and in vivo in Acute Myeloid Leukemia and Lymphomas

Yuki Nishida1, Darah A. Scruggs2, Edward Ayoub3, Tallie Patishileva1, Lauren B. Ostermann1, Vivian R. Ruvolo1, Po Yee Mak1, Bing Z. Carter1, Steffen Boettcher2, Abhishek Maiti3, Qianxiang Zhou4, Zhaohui Yang4, Honghua Yan4, Liandong Ma4 and Michael Andreeff1

1Section of Molecular Hematology and Therapy, Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX 4 Kintor Pharmaceutical Ltd., Suzhou, China. * Corresponding Authors

Abstract

Objectives: The oncoprotein c-Myc governs epigenome and transcriptome and is deregulated in 70% of all human cancers. MYC is highly expressed in TP53 mutant or venetoclax (ven) resistant AML (Nishida, Blood 2021, Sallman, Blood 2021). However, targeting c-Myc or the MYC pathway has not met with success. Targeting oncoproteins utilizing cereblon E3 ligase modulators (CELMoDs) are attractive modulators to specifically target thritthundr undegradable oncoproteins.

Design and Setting: We developed the first c-Myc degrader GT19630 (GT19715, the salt form of GT19630). We tested it in cell-free, cellular assays and in animal studies.

Results: GT19630 effectively degraded oncogenic c-Myc protein (IC50 = 1.5 nM) in HL-60 cells. C-Myc was effectively pulled down by biotinylated GT19630 in a cell-free, in vitro affinity purification assay; and a prosasome inhibitor isoxaomb completely blocked c-Myc degradation. IC50 of GT19715 in HL-60 cells was 1.8 nM, being considerably lower than 40.2 nM, an IC50 of normal myeloid progenitors in CFU assay, suggesting a therapeitic window. GT19630 shares chemical properties with other CELMoDs and proteomic analyses revealed degradation of translation turnover factor G1 to S phase transition proteins (GSPT1), an important factor in LSC survival (Surka et al. Blood 2021). Indeed, GT19630 effectively degrades GSPT1 along with complete degradation of c-Myc in a xenograft model with HL-60 cells, and inhibits tumor growth at a dose as low as 0.3 mg/kg/d. GT19715 has no effect on normal myeloid lineages in rats at 6 mg/kg. GT19715 eliminates circulating blasts and prolongs survival in the c-Myc-driven systemic Daudi leukemia/lymphoma model. Importantly, GT19715 induces cell-killing independent of TP53 status, and baseline c-Myc protein levels significantly correlated with sensitivity to GT19715 in MOLM-13 cells with CRISPR engineered knockout or mutations of TP53 (R2 = 0.86, P = 0.02). We found that MV4;11 ven resistant (VR) cells demonstrated elevated protein levels of c-Myc, and GSPT1 and exhibited greater sensitivity to GT19715 compared to ven-sensitive parental cells. Finally, GT19715 significantly reduced human CD45+ AML blasts compared to TP53 mutant AML cells while sparing CD34+ normal BM cells.

Conclusion: First results with the novel dual c-Myc/GSPT1 degrader GT19630/GT19715binds c-Myc and degraded c-Myc and GSPT1 utilizing the ubiquitin proteasome system. GT19630/GT19715 demonstrates promising preclinical anti leukemia efficacy, providing rationale for its clinical development.

GT19715 Reduces c-Myc and GSPT1 Protein and Induces Cytoeduc tion in vitro and in vivo

GT19715 Exerts Greater Sensitivity in Immature CD34+ Primary AML Cells While Sparing CD34+ Normal BM Cells

GT1975 Exerts Immature CD34+ Cell Death in MYC-driven HL-60 Xenograft Model

Summary

- First c-Myc/GSPT1 dual degrader GT19630/GT19715binds c-Myc and degraded c-Myc and GSPT1 utilizing the ubiquitin proteasome system.
- GT19715 induced tumor reduction in MYC-driven HL-60 xenograft model.
- TP53 mutant AML samples exhibited elevated expression levels of MYC compared to TP53 wild-type ones and c-Myc protein levels correlated with sensitivity to GT19715 in TP53 mutant AML cells.
- Ven-resistant AML cells with elevated c-Myc protein levels responded to GT19715 better than Ven-sensitive AML cells.
- Immature CD34+ AML cells were more sensitive to GT19715 than more mature CD34- AML cells. Notably, normal bone marrow CD34+ cells were less sensitive to GT19715 than CD34+ AML cells, suggesting a therapeutic window.
- GT19715 demonstrated promising anti-leukemia efficacy in MYC-driven Daudi systemic leukemia and PDX AML models.

COI and Funding

- Qianxiang Zhou, Zhaohui Yang, Honghua Yan and Liandong Ma are employees of Kintor Pharmaceutical.
- Funding source: Research funding (Kintor Pharmaceutical), The Paul and Mary Hazas Chair in Genetics (MA); Leukemia Specialized Programs of Research Excellence (CA100632) (MA); Cancer Prevention Research Institute of Texas (RP130397) (MA); National Institutes of Health Cancer Center Support Grant (P30CA016672) (MA).

Contact: mandreef@mdanderson.org

Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX, Kintor Pharmaceutical Ltd., Suzhou, China. * Corresponding Authors

1Abstract

GT19630 Binds c-Myc Protein and Induces Proteasome-Dependent c-Myc Degradation in MYC-driven HL-60 Cells

GT19630 IC50 of normal myeloid progenitors in CFU assay, suggesting a therapeutic window. GT19630 shares chemical properties with other CELMoDs and proteomic analyses revealed degradation of translation turnover factor G1 to S phase transition proteins (GSPT1), an important factor in LSC survival (Surka et al. Blood 2021). Indeed, GT19630 effectively degrades GSPT1 along with complete degradation of c-Myc in a xenograft model with HL-60 cells, and inhibits tumor growth at a dose as low as 0.3 mg/kg/d. GT19715 has no effect on normal myeloid lineages in rats at 6 mg/kg. GT19715 eliminates circulating blasts and prolongs survival in the c-Myc-driven systemic Daudi leukemia/lymphoma model. Importantly, GT19715 induces cell-killing independent of TP53 status, and baseline c-Myc protein levels significantly correlated with sensitivity to GT19715 in MOLM-13 cells with CRISPR engineered knockout or mutations of TP53 (R2 = 0.86, P = 0.02). We found that MV4;11 ven resistant (VR) cells demonstrated elevated protein levels of c-Myc, and GSPT1 and exhibited greater sensitivity to GT19715 compared to ven-sensitive parental cells. Finally, GT19715 significantly reduced human CD45+ AML blasts compared to TP53 mutant AML cells while sparing CD34+ normal BM cells.

Conclusion: First results with the novel dual c-Myc/GSPT1 degrader GT19630/GT19715binds c-Myc and degraded c-Myc and GSPT1 utilizing the ubiquitin proteasome system. GT19630/GT19715 demonstrates promising preclinical anti leukemia efficacy, providing rationale for its clinical development.