# Development of novel PROTAC Small-Molecule Degraders of MDM2 Protein and Peptidomimetic Inhibitors Targeting WDR5-MLL1 Protein-Protein Interaction

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## ABSTRACT

The transcription factor p53 plays an important role in suppression of tumor development, as it was involved in several important regulation of cell process, such as activation of DNA repair, cell cycle arrest, apoptosis and senescence. About 50% of human cancers carries mutated or deleted *TP53*, the gene coding p53 protein, which results p53 nonfunctional as a tumor suppressor. Even in the cancer cells with wild-type *TP53*, the p53 functions are inhibited by several mechanisms. Human murine double minute 2 (MDM2) protein is a primary, endogenous cellular inhibitor of the tumor suppressor p53 through their direct protein-protein interaction, which makes it an attractive cancer therapeutic target. In the past 15 years, a number of potent, selective and efficacious MDM2 inhibitors have been developed and advanced into clinical trials for cancer treatment. Recently targeting protein degradation using small molecules emerged as a novel strategy for drug development. Herein, we present our design, synthesis and evaluation of MDM2 degraders based on proteolysis targeting chimera (PROTAC) strategy. One of our most promising compound **LE-102** could effectively induce MDM2 degradation at as low as 1 nM within 0.5 hour in RS4;11 leukemia cells. **LE-102** achieves an $IC_{50}$ value of 2.3 nM in cell growth inhibition of RS4;11 with wild-type p53. It can also induce complete and durable tumor regression in vivo against RS4;11 xenograft tumors in mice and significantly extend the survival time in RS4;11 survival model. The mechanism studies have shown that **LE-102** is a highly potent and efficacious MDM2 degrader. The development of MDM2 degrader is a novel efficient strategy targeting MDM2 for cancer therapy.

Inducing MDM2 Protein degradation by PROTACs has shown its promising potential in cancer treatment. While during our study, a class of compounds based on the core structure of our MDM2 inhibitor **MI-1061**, failed to induce degradation of their consensus target, but it displayed pronounced cell proliferation inhibition effects in several cancer cell lines. The cell growth inhibition activity of **LD-277**, a compound in this class, is not related to MDM2 degradation and activation of p53 pathway but mediated through the cereblon-dependent ubiquitination and degradation of the translation termination factor GSPT1. These findings demonstrated that small modification could convert PROTACs to molecular glue with unexpected mechanism and effects. Carefully target validation and activity evaluation are required in the development of PROTACs and molecular glue.

Mixed Lineage Leukemia protein 1 (MLL1) is a histone H3 Lysine 4 methyltransferase, which is an important regulator of transcription and mediator of normal development and disease. Translocation of MLL1 has widely found in infant acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML). And persistent activation of HoxA9 and MEIS1 caused by MLL1 fusions is important for sustaining the leukemic phenotype. Although MLL1 fusion proteins are oncogenic, they require the maintenance of a wild-type allele for leukemogenesis. And WD repeat domain 5 protein (WDR5) is important for the HMT activity of wild-type MLL1 complex. Therefore, inhibition of WDR5-MLL1 protein-protein interaction could be a valid approach for the treatment of MLL-rearranged leukemias. Herein, we report herein the design, synthesis and evaluation of macrocyclic peptidomimetics that bind to WDR5 and block the WDR5-MLL protein-protein interaction. **LC-337** binds to WDR5 with Ki value << 1 nM and inhibits cell growth in MOLM13 with $IC_{50}$ value of 33 nM and is >800 times better than the previously reported compound **MM-401**.