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*Short communication*

# Targeting protein–protein interactions for precision cancer therapy: mechanisms, structures, and inhibitor

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## Introduction

Protein–protein interactions (PPIs) regulate essential cellular processes, and their dysregulation drives many diseases, including cancer. Once considered "undruggable," PPIs are now therapeutically targetable due to genetic and technological advances, leading to FDA-approved drugs (Camps-Fajol et al., 2025).

## PPIs in transcriptional and epigenetic control

Genetic and epigenetic alterations promote cancer by disrupting chromatin regulation and gene expression. In MLL/KMT2A-rearranged leukemias, fusion proteins recruit histone methyltransferase DOT1L to target genes, increasing H3K79 methylation and sustaining HOXA9/MEIS1-driven leukemogenic programs. Thus, DOT1L is an attractive therapeutic target, and several strategies have been developed to inhibit its enzymatic activity (Yi et al., 2022).

*Targeting the DOT1L recruitment:* Our approach focused on targeting PPIs that mediate DOT1L-dependent epigenetic regulation in MLL/KMT2A-rearranged leukemia. We defined the mechanistic basis of DOT1L recruitment by the MLL fusion partners AF9 and ENL and mapped the interaction to a conserved DOT1L motif, residues 865–874 (LPISIPLSTV) (Shen et al., 2013). Disrupting this motif impaired DOT1L recruitment, reduced H3K79 methylation, and suppressed leukemogenic genes Hoxa9 and Meis1, demonstrating the essential role of the DOT1L–AF9/ENL interaction in MLL-fusion–driven leukemogenesis. We further showed that blocking DOT1L recruitment suppresses leukemic growth and self-renewal while preserving normal hematopoiesis, suggesting improved selectivity over catalytic DOT1L inhibition (Grigsby et al., 2021). Building on this validation, we developed low-nanomolar peptidomimetic inhibitors that disrupt the DOT1L–AF9/ENL interaction, establishing it as a pharmacologically tractable target (Du et al., 2018). The DOT1L–AF9/ENL interaction is mediated by the intrinsically disordered C-terminal ANC1 homology domain (AHD), which folds upon binding to DOT1L and other partners. Using protein engineering, including MBP as a crystallization chaperone, we determined the first X-ray structures of AF9 AHD bound to peptidomimetic inhibitors (Yang et al., 2024). These studies define the molecular basis of DOT1L–AF9/ENL inhibition and support further development of selective therapeutics for MLL/KMT2A-rearranged leukemia.

## Targeting PPI networks in apoptosis regulation

Apoptosis is a form of programmed cell death controlled by Bcl-2 family PPIs in the intrinsic mitochondrial pathway. Anti-apoptotic proteins, including Bcl-2, Bcl-xL, Mcl-1, Bfl-1, and Bcl-w, promote survival by sequestering pro-apoptotic Bax, Bak, and BH3-only proteins, preventing mitochondrial permeabilization and caspase

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activation. In cancer, these proteins are often overexpressed, enabling tumor cells to evade apoptosis and resist therapy. Thus, small-molecule inhibitors that disrupt Bcl-2 family interactions and restore apoptosis remain important therapeutics, exemplified by FDA-approved Bcl-2 inhibitor venetoclax.

*Selective Mcl-1 and dual Mcl-1/Bfl-1 BH3 Mimetics:* Mcl-1 is frequently amplified or overexpressed in cancer, promoting tumor survival and chemoresistance. Bfl-1, a related anti-apoptotic protein, is also emerging as a therapeutic target. Selective or dual inhibition of Mcl-1 and Bfl-1 can restore apoptosis, overcome resistance, and benefit a variety of malignancies (Kump and Nikolovska-Coleska 2020; Tantawy et al., 2023). Using integrated high-throughput and computational screening, we identified selective Mcl-1 inhibitors based on an N-(4-hydroxynaphthalen-1-yl) arylsulfonamide scaffold that bind the Mcl-1 BH3 groove via Arg263 hydrogen bonding and hydrophobic interactions (Abulwerdi et al., 2014a). Medicinal chemistry optimization efforts produced potent analogues, including UMI-77 and compound 21, which induced Bax/Bak-dependent apoptosis and reduced leukemia and lymphoma cell viability. UMI-77 further demonstrated on-target Mcl-1 inhibition and *in vivo* efficacy in pancreatic cancer models (Abulwerdi et al., 2014b). Building on this work, we used structure-based design to develop selective nanomolar dual Mcl-1/Bfl-1 inhibitors based on a 2,5-substituted benzoic acid scaffold. These compounds selectively killed Mcl-1- or Bfl-1-dependent lymphoma cells (Kump et al., 2020). Further optimization of dual Mcl-1/Bfl-1 inhibitors will provide valuable tools for cancers driven by Mcl-1/Bfl-1-mediated pathogenesis, including leukemia, lymphoma, and melanoma.

## PPIs role in precision oncology

Functional assays can advance precision oncology by identifying tumor-specific actionable PPI dependencies. BH3 profiling is a functional assay that measures mitochondrial “priming” and defines dependence on specific anti-apoptotic Bcl-2 proteins, identifying tumors sensitive to BH3 mimetics and confirming on-target activity (Certo et al., 2006). Using this approach, we identified heterogeneous Mcl-1/Bcl-2 dependence in AML, preferential Bcl-2 dependence in B-cell lymphomas, and Mcl-1/Bfl-1 codependence in T-cell lymphomas, consistent with selective Bfl-1 expression in T-cell tumors (Kump et al., 2025). Given that NPM-ALK rearrangements in anaplastic large T-cell lymphoma can drive Bfl-1 expression, the findings support the development of selective Bfl-1 or dual Mcl-1/Bfl-1 inhibitors. These findings illustrate how functional understanding of PPIs can guide the discovery and development of precision cancer therapies.

## References


Abulwerdi, F.A. et al., 2014a. 3-Substituted-N-(4-hydroxynaphthalen-1-yl)arylsulfonamides as a novel class of selective Mcl-1 inhibitors: structure-based design, synthesis, SAR, and biological evaluation. J. Med. Chem. 57, 4111-33.

Abulwerdi, F. et al., 2014b. A novel small-molecule inhibitor of Mcl-1 blocks pancreatic cancer growth. Mol. Cancer Ther. 13, 565-75. Camps-Fajol, C. et al., 2025. Targeting PPIs in drug discovery: Modulators approved or in clinical trials for cancer treatment. Pharmacol Res. 211, 107544.

Certo, M. et al., 2006. Mitochondria primed by death signals determine cellular addiction Cancer Cell 9, 351-365.

Du, L. et al., 2018. Peptidomimetics for Targeting PPIs between DOT1L and MLL Onco-fusion Proteins AF9 and ENL. ACS Med Chem Lett. 9, 895-900. Grigsby, S.M. et al., 2021. Elucidating the Importance of DOT1L Recruitment in MLL-AF9 Leukemia and Hematopoiesis. Cancers (Basel) 13, 642.

Kump, K.J., Nikolovska-Coleska, Z., 2020. Discovery and Development of Mcl-1 Inhibitors as Anti-cancer Therapeutics: Hit to Clinical Candidate Optimization, in: Roy S., Fu H. (Eds.), The Royal Society of Chemistry, pp. 171-208.

Kump, K.J. et al., 2020. Discovery and Characterization of 2,5-Substituted Benzoic Acid Dual Inhibitors of the Anti-apoptotic Mcl-1 and Bfl-1 Proteins. J. Med. Chem. 63, 2489-2510.

Kump, K.J. et al., 2025. Understanding the Functional Dependence and Inhibition of the Bcl-2 Proteins in a Wide Spectrum of Cancers toward Precision Medicine. ACS Pharmacol. Transl. Sci. 8, 2922-2935.

Shen, C., et al., 2013. Targeting recruitment of disruptor of telomeric silencing 1-like (DOT1L): characterizing the interactions between DOT1L and mixed lineage leukemia (MLL) fusion proteins. J Biol Chem. 288, 30585-30596.

Tantawy, S.I. et al., 2023. Targeting MCL-1 protein to treat cancer: opportunities and challenges. Front Oncol. 13, 1226289. Yang, Y. et al., 2024. Structural studies of intrinsically disordered MLL-fusion protein AF9 in complex with peptidomimetic inhibitors. Protein Sci. 33, e5019.

Yi, Y., Ge, S., 2022. Targeting the histone H3 lysine 79 methyltransferase DOT1L in MLL-rearranged leukemias. J. Hematol. Oncol. 15, 35.