Structural Studies of DNMT1-DNA Complexes with a Reversible Series of Dicyanopyridine Containing Selective, Non-Nucleoside Inhibitors

John Horton[1], Sarath Pathuri[2], Xing Zhang[3], Xiaodong Cheng[4]

[1]MD Anderson Cancer Center of The University of Texas [2]Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, [3]The University of Texas -M.D. Anderson Cancer Center, [4]The University of Texas -M.D. Anderson Cancer Center

jrhorton@mdanderson.org

DNA methylation, a key epigenetic driver of transcriptional silencing, is universally dysregulated in cancer. The methyltransferase DNMT1 maintains the parental DNA methylation pattern on newly replicated hemimethylated DNA. Thus, DNMT1 preferentially binds to and methylates DNA containing a hemi-methylated CpG dinucleotide. In cancers, DNA methylation is universally dysregulated leading to focal hypermethylation and subsequent transcriptional silencing of CpG island-associated gene promoters. Thus, targeting DNMT1 is an attractive strategy for the treatment of cancer.

The Cancer Epigenetics Research Unit of GlaxoSmithKline has discovered a series of DNMT1-selective, reversible, non-nucleoside inhibitors that bear a core 3,5-dicyanopyridine moiety which induces robust loss of DNA methylation, transcriptional activation, and cancer cell growth inhibition in vitro leading to superior tumor regression and survival in vivo in mouse models of acute myeloid leukemia due to superior in vivo tolerability compared to cytidine analog drugs.

We have structurally characterized a set of these newly discovered inhibitors to better understand their mechanism of inhibition. All dicyanopydridine-containing inhibitors examined intercalate into the hemi-methylated DNA between two CpG base pairs through the DNA minor groove, resulting in conformational movement of the DNMT1 active-site loop. One of the surprising findings of our study was the binding of one of the compounds, GSK3735967, in two additional sites in addition to the more canonical site observed with the other compounds. The second site is formed, in part by the displacement of the DNMT1-active site loop due to the binding of the inhibitor to the canonical site. The binding of GSK3735967 to the third site mimics the binding of H4K20me3 in the BAH1 domain of DNMT1.

The discovery and characterization of these first-in-class reversible DNMT1-selective inhibitors may provide a unique therapeutic approach for treating cancers in which traditional cytidine analog hypomethylation agents have shown limited activity and high toxicity. Furthermore, our structural findings reveal specific avenues for improving the potency and selectivity of DNMT1 inhibitors.

For Further Information:

Horton, J. R., et al. (2022). "Structural characterization of dicyanopyridine containing DNMT1-selective, non-nucleoside inhibitors." Structure (in press but online at https://authors.elsevier.com/sd/article/S0969-2126(22)00090-9).

Pappalardi, M. B., et al. (2021). "Discovery of a first-in-class reversible DNMT1-selective inhibitor with improved tolerability and efficacy in acute myeloid leukemia." Nat Cancer 2(10): 1002-1017.