



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

**Arginine Methylation of BRD4 By CARM1 Regulates Its Function in AML**

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Acute myeloid leukemia (AML) is generally an aggressive and lethal malignancy characterized by the accumulation of immature myeloid cells in the bone marrow and peripheral blood. We have previously shown the dependence of AML, but not normal hematopoiesis, on the presence of coactivator associated arginine methyltransferase 1 (CARM1), which suggests that targeting CARM1 could be useful in the treatment of AML. In preclinical studies, we and others have shown promising effects of CARM1 inhibitors on the growth of hematologic malignancies<sup>1</sup>. In order to unearth a potential mechanism for the strong efficacy of CARM1 inhibition or knockdown (KD) in the AML1-ETO (AE+) driven AML mouse model, we employed the BioID system in an AE+ human cell line, SKNO-1, to identify novel substrates and interacting partners of CARM1 that could drive the pathogenesis of the disease. We identified over 500 interactors of CARM1, many of which are novel, including one particularly promising therapeutic target, bromodomain-containing protein 4 (BRD4).

When examining the mechanism of action of CARM1 inhibitors, we observed significant overlap between the gene expression changes induced by CARM1 inhibition or KD and BRD4 inhibition or KD. Given evidence that both the histone reader, BRD4, and a histone writer, CARM1, play active roles in AML generation, we examined the relationship between these two proteins in AML. We observed a direct physical interaction between these proteins, using immunoprecipitation and two AE+ AML cell lines. Furthermore, using an overexpression system and tagged fragments of BRD4, we identified the particular interacting regions of BRD4 with CARM1. We also identified BRD4 as a substrate of CARM1 and used mass spectrometry to map the sites of CARM1-dependent BRD4 asymmetric dimethylation, implicating four specific arginine residues in the C-terminus of the protein. This crucial information led us to generate antibodies towards these specific CARM1-dependent methylated arginine residues. Using these novel antibodies, we have shown that small molecule inhibition or KD of CARM1 reduces the methylation of BRD4 while also affecting its intracellular localization. In AE+ leukemia cells, exposure to CARM1 inhibitor results in the reduced chromatin localization of BRD4, while in normal CD34+ HSPCs, under the same conditions, we do not see this effect. These findings suggest a selective effect of CARM1 inhibitors for AML therapy, that may not significantly impair normal hematopoiesis. To identify which BRD4-dependent genes are affected by CARM1 inhibition we utilized chromatin-immunoprecipitation and sequencing (ChIP-seq) coupled with RNA-seq and found the loss of BRD4 from promoters and intragenic regions of several oncogenes, together with loss of RNA expression.

Given the importance of CARM1 and BRD4 in the development of AML, we are currently investigating the biological and transcriptional activities present in BRD4 mutants that cannot be methylated by CARM1. While methylation of the N-terminus of BRD4 by PRMTs has been reported<sup>2</sup>; we have focused our efforts on the C-terminus of the protein, given the re-localization of BRD4 that occurs when CARM1 is inhibited or knocked down. We continue to define the biological effects of a CARM1-BRD4 signaling axis, to assess the importance of this axis in the pathogenesis of oncogene-driven AML. This study also suggests that inhibiting bromodomain containing proteins like BRD4 can be accomplished by targeting the enzymes that post-translationally modify their function. Further insights will be provided by pre-clinical animal and other studies that are ongoing.

1. Greenblatt SM, Man N, Hamard PJ, et al. CARM1 is essential for myeloid leukemogenesis but dispensable for normal hematopoiesis. *Cancer Cell*. 2019;35(1):156. doi:10.1016/j.ccell.2018.12.008

2. Liu L, Lin B, Yin S, et al. Arginine methylation of BRD4 by PRMT2/4 governs transcription and DNA repair. *Sci Adv*. 2022;8(49):eadd8928. doi:10.1126/sciadv.add8928

**Disclosures** No relevant conflicts of interest to declare.

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