LIST ALL AUTHORS and AFFILIATIONS
Kartika Venugopal (1), Daphné Dupéré-Richer(2), Jonathan D. Licht(2), Olga A. Guryanova(1)
(1) Department of Pharmacology & Therapeutics, and (2) Department of Medicine, University of Florida

TITLE
Targeting DNA replication as a therapeutic strategy for Acute Myeloid Leukemia with DNMT3A mutations

HYPOTHESIS:
Targeting cells with DNMT3A mutations by pharmacologic agents that stall DNA replication such as nucleoside analogues may offer enhanced therapeutic benefit. We propose that using these already approved nucleoside analogues alone, due to the aforementioned sensitivity of DNMT3A mutant class of AML patients, at more tolerable doses, would be a good therapeutic strategy for this subtype of AML.

BACKGROUND/AIMS:
Acute myeloid leukemia (AML) is an aggressive malignancy of the blood system, wherein somatic mutations in epigenetic modifier and chromatin remodeling genes are common. Recurrent somatic mutations in the DNA methyltransferase 3 alpha (DNMT3A) gene are detected in about 30% of AML cases. More than half of DNMT3A alterations are hot-spot mutations affecting arginine at position 882 (R882) and predict poor outcomes in patients treated with standard induction chemotherapy, subsequently leading to early disease relapse. Additionally, this induction chemotherapy used to treat AML, is highly toxic and is poorly tolerated by most patients. Therefore, identifying a class of patients that can respond to drug treatment even at lower toxicity would be ideal.

We and others have performed DNA methylation profiling in both primary AML samples and in animal models carrying a DNMT3A mutation. We observed modest DNA hypomethylation that only partially explained how mutant DNMT3A contributes to AML pathogenesis. Recent studies have uncovered disordered chromatin remodeling in response to DNA topological stress in DNMT3A-mutant cells. These changes were accompanied by altered cell cycle-related gene expression signatures and attenuated CHK1 signaling, implicated in DNA damage response and replication fork integrity.

METHODS:
In order to study the sensitivity of cells with DNMT3A mutations to replication stress, we performed a drug dose response viability assay, comparing wildtype and mutant DNMT3A cell lines treated with different replication stalling agents such as nucleoside analogues cytarabine (Ara-C), fludarabine, and cladribine. Next, we investigated increased apoptosis and analyzed DNA damage signaling proficiency in response to cytarabine. A similar drug sensitivity study was performed in murine Dnmt3a-mutant bone marrow cells treated with cytarabine, using clonogenic survival assay as a readout. We are now using CRISPR/Cas9 technology to generate isogenic cell lines with differing DNMT3A status that will serve as a tool for subsequent mechanistic studies.

RESULTS & CONCLUSIONS
Our drug dose response studies show increased sensitivity to cytarabine, fludarabine and cladribine in leukemia cell lines harboring DNMT3A mutations. Analysis of apoptosis by Annexin V staining assay revealed a higher proportion of cells undergoing apoptosis after treatment with AraC for the DNMT3A mutant cell lines. Higher levels of expression of the DNA damage and apoptosis markers, like cl-PARP and γH2AX was observed in the DNMT3A mutant cell lines. A similar drug sensitivity trend was observed in murine Dnmt3a-mutant bone marrow cells treated with cytarabine, using clonogenic survival in MethoCult as a readout. These studies will shed light on the mechanism of a potential vulnerability in DNMT3A-mutant leukemia cells and may lead to a more effective treatment strategy for this common subtype of AML.