

## Bankhead-Coley Cancer Research Program

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**Project Title:** Myelodysplastic Syndrome, Dnmt3 and Azacitidine

**Project Summary:** Myelodysplastic syndrome (MDS) is the most common hematological disease among the elderly, characterized by varying degrees of cytopenias and dysplastic changes of marrow stem cells. MDS is considered to be a pre-malignant status, which often transforms into acute leukemia. MDS patients can receive chemotherapy and bone marrow transplant; however, most elderly patients are ineligible and treatment side effects can be deadly. Presently, MDS treatment has focused on the use of azacitidine, which delays clonal evolution to acute leukemia, improves blood counts, and lengthens patient survival. However, how the drug works remains unsolved. Although azacitidine has been reported to demethylate genes in MDS patients, it is still controversial whether DNA demethylation is responsible for the observed clinical effect of the drug. Azacitidine becomes incorporated into genomic DNA, and forms adducts with DNA methyltransferase (Dnmt). This covalent and irreversible binding of the enzyme to drug-substituted DNA, rather than secondary DNA demethylation due to enzyme depletion, is believed to be the principal cause for cytotoxicity. Recently, this research team determined that cellular sensitivity to azacitidine is predominately mediated by the expression status of Dnmt3. Dnmt3 expression is known to be low or undetectable in most adult tissues. However, in MDS and other hematopoietic malignancies, aberrantly high expression levels of Dnmt3 are observed, which might underlie the effectiveness of azacitidine. This research initially focuses on the hypothesis that Dnmt3 expression facilitates azacitidine susceptibility in hematopoietic cells as well (Specific Aim 1). The obtained results will lead the way to further conducting clinical studies using patients' samples. Ultimately, the research team will examine whether higher Dnmt3 expression levels at diagnosis correlates with higher efficacy of the drug in MDS patients. Results from these studies are expected to help predict which MDS patients may be responsive to the drug. Preliminary data from this research also indicate that azacitidine induces cellular apoptosis only when Dnmt3 is expressed in the cells. The team will, therefore, define the mechanism by which the azacitidine can induce apoptosis of hematopoietic cells (Specific Aim 2). Since this preliminary data indicate that azacitidine induces DNA breakage, p53 activation and cellular apoptosis, all of which depends on Dnmt3 expression, the hypothesis will be tested that p53 may be an important downstream factor critical for Dnmt3/azacitidine-mediated cell death. Accordingly, azacitidine may be less effective in cells harboring p53 mutations, even when they express Dnmt3. This question is important for further defining which MDS patients would be most responsive to the drug. Finally, another intriguing fact about Dnmt3 in MDS is that high expression levels of Dnmt3 frequently accompany the disease. It has not been studied, to the best of this team's knowledge, if this high expression of Dnmt3 could be a predisposition to the disease. The team's preliminary studies indicate that the constitutive overexpression of Dnmt3 induces aberrant differentiation of hematopoietic stem cells (HSCs). It is possible that DNA hypermethylation caused by aberrant Dnmt3 expression may disturb normal hematopoiesis, thus becoming a predisposition to myelodysplasia and subsequent

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malignancies. This hypothesis will be tested by overexpressing Dnmt3 in murine HSCs (Specific Aim 3). In this Grant period, the focus will be on isolation of HSCs and lentiviral transduction of vectors expressing Dnmt3 and GFP into HSCs. By demonstrating successful lentiviral transduction into HSCs, the team can move forward to the transplantation experiments proposed in the federal grant. Ultimately, this study direction will elucidate if aberrant de novo DNA methylation disturbs normal hematopoiesis, and potentially provide a novel animal model for studying MDS. These studies are expected to lead the way in developing novel therapeutic strategies for MDS treatment and in gaining meaningful insight into the etiology of the disease.