

**Development of a Novel Inhibitor to the Conserved Developmental Regulator, WDR5, for
Treatment of Acute Leukemia**

By

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Dedication

To my parents, Katherine and Douglas Townsend,

For love, support and guidance through the years and for fostering my scientific curiosity.

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Abstract

A majority of cases of acute leukemia in infants, as well as a subset of secondary acute leukemia in adults is characterized by translocation of the *MLL1* gene to generate an oncogenic MLL1 fusion protein. The MLL1 fusion protein acts to abnormally recruit transcription-promoting mechanisms to target gene loci, including *HoxA9* and *Meis1*. Overexpression of MLL1 gene targets through these mechanisms results in transformation. The MLL1 fusion protein has been found to cooperate with wild-type MLL1 to promote gene expression in leukemia. Wild-type MLL1 is a histone methyltransferase with specificity for H3 at lysine 4 (H3K4). Methyltransferase activity of MLL1 is stimulated through direct association with a conserved complex of proteins, WDR5, RbBP5 and ASH2L. As H3K4 methylation is strongly correlated with transcription activation in MLL1 translocation leukemias, we sought to develop inhibitors for MLL1 methyltransferase activity based on our understanding of MLL1 complex regulation by its constituent components. H3K4 methylation by the wild-type MLL1 complex is essential for expression of *HoxA9* and *Meis1* as well as transformation in leukemia. This activity is dependent on association between MLL1 and its direct interaction partner, WDR5. To exploit the essential function of this interaction in leukemia, we have used rational design to develop cell-permeable, peptidomimetic inhibitors, **MM-102** and **MM-401**, to the MLL1:WDR5 interaction. We demonstrate that both **MM-102** and **MM-401** specifically inhibit assembly of MLL1 with WDR5, RbBP5 and ASH2L and block methyltransferase activity of the MLL1 complex. We show that mouse and human leukemia cells, transformed with MLL1 fusion

proteins are specifically targeted by **MM-401** and treatment with this compound results in differentiation or cell death. We also show that inhibition of the MLL1:WDR5 interaction by **MM-401** specifically impairs expression of *HoxA9* and *Meis1*, as well as histone methylation at these loci. These studies show that interaction between MLL1 and WDR5 is essential for expression of *HoxA9* and *Meis1* in leukemia. This also demonstrates that inhibition of the MLL1:WDR5 interaction has potential utility for treating leukemias with MLL1 rearrangement and our compound **MM-401**, is a promising lead for future drug development.