

Two novel DNMT1 inhibitors: 4'-Thio-2'-Deoxycytidine (TdCyd) and 5'-aza-4'-Thio-2'-Deoxycytidine (aza-TdCyd), for the treatment of MEN1 tumors in preclinical study. Ziqiang Yuan¹, Juliet Gardiner¹, Asha Adem¹, Daniel Slegowski¹, Zoya Gauhar², Michael Difilippantonio³, Steven K Libutti¹. ¹Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey; ²Princeton University, Princeton, New Jersey; ³Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland.

Introduction: Human cancer genomes are characterized by widespread aberrations in DNA methylation patterns. Our previous studies have demonstrated that inactivation of menin (the protein product of MEN1) increases activity of DNMT1 and mediates DNA hypermethylation in the development of multiple endocrine neoplasia type 1 (MEN1) syndrome. Recently, our collaborators at the National Cancer Institute (NCI) developed two novel DNMT1 inhibitors: 4'-Thio-2'-Deoxycytidine (TdCyd) and 5'-aza-4'-Thio-2'-Deoxycytidine (aza-TdCyd), that inhibit aberrant DNMT1 activity in tumor cells. In the present study, we explored the anti-neoplastic activity and the molecular mechanism of these novel DNMT1 inhibitors in the inhibition of DNMT1 enzyme activity, reduction of tumor proliferation, and induction of apoptosis by using our novel NET mouse models. **Materials and Methods:** First, we determined the safety and effect of DNMT1 inhibitors in the treatment of neuroendocrine tumors using MEN1 KO mice with NETs at 12 months of age. Fifteen MEN1 KO mice with pancreatic neuroendocrine tumors (PNETs) and hyperinsulinemia were divided into three groups and were injected intraperitoneally with TdCyd (100 μ l, 2mg/kg), aza-TdCyd (100 μ l, 1 mg/kg), or PBS (100 μ l) as a control, respectively. Second, we determined if DNMT1 inhibitors can prevent NET transformation using younger MEN1 KO mice at 3 months of age. Fifteen MEN1 KO mice were divided into three groups and injected ip with TdCyd, aza-TdCyd, or PBS control, respectively. The agents were given in 21-day cycles. The agents were administered to the mice once a day during week 1 for 5 days. Then for two days mice were not treated. Agents were then administered for 5 days during week two. No agents were administered during week 3. **Results:** We evaluated the anti-tumor activity of both DNMT1 inhibitors in MEN1 KO mice with insulinomas by measuring the serum level of insulin, which we have previously validated as a tumor biomarker and surrogate measurement for tumor response to therapy. By one month after administration, mice treated with both DNMT1 inhibitors had a statistically significant decrease in serum insulin levels compared to the mice treated with PBS control ($P < 0.001$). We also observed an enhanced survival rate of 100% (5/5) for mice treated with Td-Cyd and 80% (4/5) for mice treated with Aza-TdCyd at 4 months post treatment versus 0% (0/5) in the PBS control treatment group ($P < 0.001$). DNMT1 enzyme activity in tumors decreased in response to both DNMT1 inhibitors compared with the PBS control group ($P < 0.01$). Mice were evaluated for toxicity of the DNMT1 inhibitors by monitoring mouse weight and behavior. We determined that both DNMT1 inhibitors were safe in the treatment of NETs in this preclinical setting. We evaluated whether the DNMT1 inhibitors can prevent NET transformation by treating younger MEN1 KO mice at the age of 3 months. We observed a significant decrease in serum insulin, a tumor biomarker, in both DNMT1 inhibitor treated groups at 3 to 8 months post-treatment when compared to the PBS control group ($P < 0.001$). The DNMT1 inhibitor treated mice had a significant decrease in islet size compared with the PBS control mice ($P < 0.001$). Furthermore, we demonstrated that DNMT1 enzyme activity had significantly increased in the islet tissues of PBS control mice compared with the islet tissues of DNMT1 inhibitor treated mice. These results indicate that DNMT1 inhibitors may be useful in the prevention of the formation of NETs in our preclinical Men1 knockout models. **Conclusions:** Our studies indicate that both DNMT1 inhibitors, TdCyd and aza-TdCyd, are safe and effective for the treatment of MEN1 related pancreatic tumors in the pre-clinical setting. Since these agents have been safely administered to patients in phase 1 trials, we believe that our study supports the development of DNMT1 inhibitors as a treatment for patients with MEN1 associated tumors.