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 a preclinical study

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Introduction

We 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[1-2]. Our collaborators at the NCI developed two novel DNMT1 inhibitors: TdCyd and aza-TdCyd, both of which 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

DNMT1 inhibitors: Two novel DNMT1 inhibitors: 4'-Thio-2'-Deoxycytidine (TdCyd) and 5'-aza-4'-Thio-2'-Deoxycytidine (aza-TdCyd) were obtained from our collaborators at the National Cancer Institute (NCI).

***Men1* KO mice**: We developed a *Men1* conditional KO mouse model that develop islet hyperplasia at 6 months and insulinomas at 12 months.

DNMT1 inhibitors to treat MEN1 tumors: 15 *Men1* KO mice at 12 months of age with insulinomas and hyperinsulinemia were divided into three groups and were ip injected with TdCyd (2mg/kg), aza-TdCyd (1 mg/kg), or PBS (100 µl) as a control, respectively.

DNMT1 inhibitors to prevent MEN1 tumorigenesis: 15 younger *Men1* KO mice at 3 months of age were divided into three groups and ip injected 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. Agents were then administered for 5 days during week 2. No agents were administered during week 3.

DNMT1 inhibitors treat MEN1 tumors

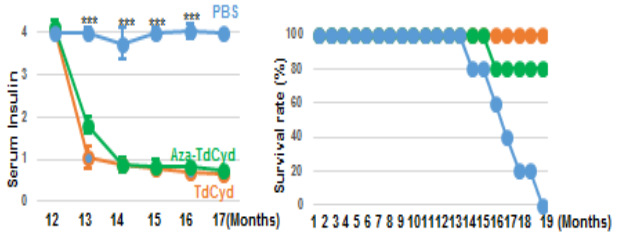

Figure 1. Serum insulin was significantly decreased and the survival rate was improved in the *Men1* KO mice with insulinomas (12 months of age) treated with either DNMT1 inhibitors (at 3 cycles) compared to the PBS control.

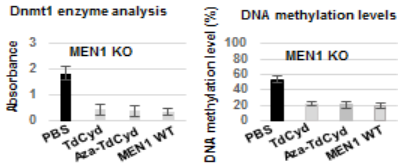

Figure 2. DNMT1 inhibitors can inhibit DNMT1 enzyme activity and decrease global DNA methylation levels in *Men1* KO mice with insulinomas (12 months of age) treated with DNMT1 inhibitors when compared to the PBS control.

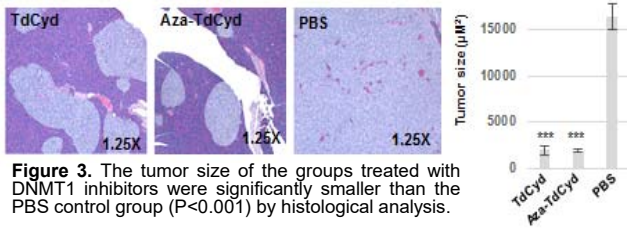

Figure 3. The tumor size of the groups treated with DNMT1 inhibitors were significantly smaller than the PBS control group (P<0.001) by histological analysis.

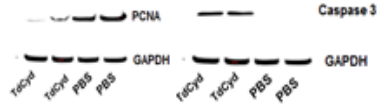

Figure 4. DNMT1 inhibitors can inhibit tumor cell proliferation and induce cell apoptosis in the *Men1* KO mice (12 months of age) with insulinomas by western blot analysis.

DNMT1 inhibitors prevent MEN1 tumorigenesis

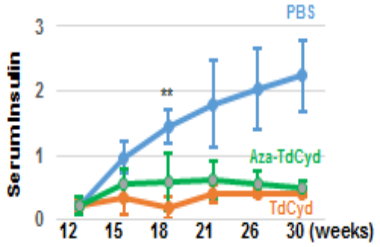

Figure 5. Serum insulin levels showed significant decrease in young *Men1* KO mice (3 months of age) treated with the DNMT1 inhibitors over the three cycle period and remained significantly decreased over 30 weeks compared to the PBS control (P<0.001).

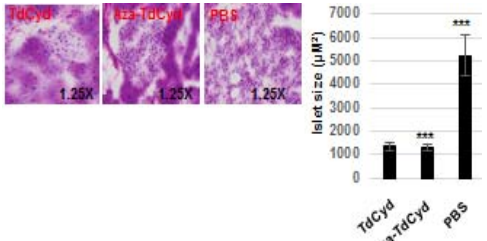

Figure 6. Islet size in the DNMT1 inhibitor-treated mice was significantly smaller (P<0.001) compared to the PBS control in young Men1 KO mice (3 months of age).

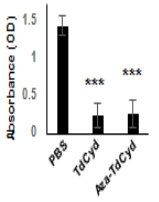

Figure 7. DNMT1 inhibitors can inhibit DNMT1 enzyme activity in young *Men1* KO mice (3 months of age).

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.

Acknowledgements

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References

1. Yuan Z, et al., Loss of MEN1 activates DNMT1 implicating DNA hypermethylation as a driver of MEN1 tumorigenesis. Oncotarget. 2016;7:12633–50.

2. Yuan Z, et al., Tissue-specific induced DNA methyltransferase 1 (Dnmt1) in endocrine pancreas by RCAS-TVA-based somatic gene transfer system promotes β-cell proliferation. Cancer Gene Therapy. 2019; 26:94–102.