Comparison of Thio-deoxycytidine (TdCyd) and Aza-Thio-deoxycytidine (Aza-TdCyd) in solid and liquid tumor cell lines and PPTC pediatric xenografts

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1. Introduction

The mechanism(s) of action of cytostatic agents is incompletely understood, and at least one enigmas remains to be explained. We investigated the anti-clonogenic activity of the two related DNMT1 inhibitors TdCyd and Aza-TdCyd in the NCI60 panel of cell lines which offers a single single-platform in the discovery stage. Here, we analyze the solid and liquid tumor cell lines, and pediatric tumor xenografts in a comprehensive manner. Cytostatic activity was assessed after 2, 3, and 7 days exposure to IC50 concentrations of solid and liquid tumor lines. TdCyd was administered (100 µM, 3 days) to 40 solid tumor cell lines and 9 liquid tumor lines. TdCyd was administered (100 µM, 3 days) to 30 liquid tumor cell lines with different dose-escalation protocols. In the NCI60 panel, we observed an IC50 of 1.2 µM, 15.9 µM, and 4.9 µM for the Sarcoma, Leukemia, and Ovarian lines, respectively. The activity of both compounds was greatest in sarcoma tumors and modest in the leukemias. Aza-TdCyd showed modest activity in solid and liquid tumor lines.

2. Methods

2.1 Cell-Based Experiments

Cells were cultured as complex suspensions in 384-well ultra low attachment (ULA) plates. Complex suspensions consisted of 62% tumor cells, 2% endothelial cells (HUVEC), and 36% Mesenchymal Stem Cells (MSCs). 15% of the cell suspension was used in each well. Cells are cultured in a humidified incubator at 37°C and 5% CO2. Medium is changed every 3-4 days. The doubling time of each cell line is determined as shown in supplemental Figure 1. For cytotoxicity assays, 10,000 cells were plated in 384-well plates and exposed to IC50 concentrations of TdCyd and Aza-TdCyd for 2 days. At the conclusion of the incubations, cytotoxicity was determined using the CellTiter-Blue assay (Promega). Results are presented as IC50's over a time course for Thio-deoxyCytidine & Aza-Thio-deoxyCytidine in 9 human tumor cell lines.

3. Results

3.1 Anticancer activity of 4-thio-2-deoxycytidine and 5-aza-4-thio-2-deoxycytidine in pediatric ALL PDX models.

Table 1: Anticancer activity of 4-thio-2-deoxycytidine and 5-aza-4-thio-2-deoxycytidine in pediatric ALL PDX models.

4. Conclusions

1. The related DNMT1 inhibitors TdCyd and Aza-TdCyd are cytostatic breast human tumor cell lines with IC50’s >10 µM. Aza-TdCyd exhibits major activity in the ALL-31 ALL PDX xenografts at doses which are tolerable. TdCyd showed little activity in these models.

2. Aza-TdCyd is highly active in pediatric ALL PDX xenografts at doses which are tolerable. TdCyd showed little activity in these models.

3. Aza-TdCyd was moderately active in selected pediatric sarcoma and solid xenografts. The KT-1557 cell line exhibits moderate response to Aza-TdCyd and OTC-25 solid xenografts responded to treatment with Aza-TdCyd, while TdCyd had little effect on sarcoma growth.

4. Neither Aza-TdCyd nor TdCyd was effective in slowing the growth of orthotopically implanted pediatric ovarian RPA.

5. There was a moderate correlation between the expression of DNMT1 and the anticancer activity of Aza-TdCyd and TdCyd in ALL PDX models.

6. Both TdCyd and Aza-TdCyd are in clinical trial.

4. References and Acknowledgments

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