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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Epigenetic Silencing of MTAP in Hodgkin's Lymphoma Renders It Sensitive to a 2 nd Generation PRMT5 Inhibitor

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Background: PRMT5 is type II arginine methyltransferase that catalyses symmetric dimethylation of arginine residues (SDMA) and plays an important role in cancer biology. By methylating a number of substrates, PRMT5 can regulate important processes such as DNA repair, RNA splicing, and cellular proliferation. In addition, PRMT5 is overexpressed in various cancer types and has been identified as a candidate for therapeutic intervention through the development of small molecules that inhibit PRMT5 methyltransferase activity. However, first generation PRMT5 inhibitors have shown limited clinical benefit mainly due to development of on-target toxicity, primarily in the bone marrow. AstraZeneca has developed a second generation PRMT5 inhibitor (AZ-PRMT5i), that selectively inhibits PRMT5 in MTAP deficient tumours while sparing MTAP proficient normal cells. MTAP (methylthioadenosine phosphorylase) is a metabolic enzyme involved in methionine salvage pathway. MTAP deficiency results in accumulation of the metabolite methylothioadenosine (MTA) in tumor cells that induces partial inhibition of PRMT5, rendering these tumors sensitive to PRMT5 inhibition. Homozygous deletion of the *MTAP* gene, that results in the loss of MTAP protein, has been found in approximately 15% of advanced solid tumors. Here, for the first time, we describe epigenetic silencing of the *MTAP* gene in Hodgkin's Lymphoma (HL) cell lines. This silencing results in the loss of MTAP protein expression thus increasing sensitivity to PRMT5 inhibition. Importantly, MTAP protein loss was also observed in primary cHL samples, opening a novel opportunity for the treatment of HL.

Methods: Bioinformatic data mining of the Cancer Cell Line Encyclopedia (CCLE) dataset has been used to overlay levels of expression of *MTAP* mRNA with *MTAP* copy number and *MTAP* promoter DNA methylation. MTAP protein expression and activity of AZ-PRMT5i were assessed in 5 HL cell lines *in vitro*; efficacy of AZ-PRMT5i and levels of target engagement were tested *in vivo* in the L540 xenograft model. MTAP expression levels were determined by IHC analysis using 55 primary samples from cHL patients.

Results: Using an unbiased analysis of the CCLE dataset, we have identified a subset of cell lines that in the absence of *MTAP* genetic loss were showing low levels of MTAP mRNA expression, equivalent to levels of *MTAP* mRNA in MTAP homozygous deleted cell lines. Interestingly, this was predominantly the case in HL cell lines where it was detected in 67% of cases (4 out of 6 cell lines). *MTAP* methylation profile analysis of HL cell lines revealed elevated *MTAP* DNA promoter methylation in cell lines with low *MTAP* mRNA expression. In addition, MTAP protein was not detected by Western Blot. Accordingly, lack of MTAP protein expression resulted in increased sensitivity of these cells to AZ-PRMT5i compared to a HL cell line that expressed MTAP protein. Utilizing RNA-seq for transcriptional profiling of AZ-PRMT5i on a MTAP-deficient HL cell line has identified robust changes in genes that regulate the cell cycle, DNA replication and TP53 pathways thus confirming on target activity. In addition, AZ-PRMT5i demonstrated strong dose dependent efficacy in a *MTAP* silenced L540 xenograft model where greater than 90% of tumour growth inhibition was detected, with no significant body weight loss. Corresponding dose-dependent inhibition of SDMA is observed in the treated tumours.

Finally, IHC analysis of MTAP expression in 55 primary cHL samples has demonstrated that 46 of 55 cHL samples (84%) had absent nuclear MTAP expression.

Conclusion: Here we are presenting a novel finding showing MTAP protein expression loss in the majority of tested primary cHL samples, which could provide a collateral vulnerability and novel therapeutic opportunity to 2 nd generation PRMT5 inhibitors as demonstrated in our pre-clinical models.

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