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PE-0260, an oral brain-penetrant MTA-cooperative PRMT5 inhibitor, drives tumor regressions in MTAP-deleted solid tumors

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Background

- Protein arginine methyltransferase 5 (PRMT5) has been identified as a synthetic lethal target in cancers with homozygous deletion of the methylthioadenosine phosphorylase (MTAP) gene. PRMT5 is essential for maintaining cellular homeostasis by regulating a variety of processes, including gene transcription, ribosomal biogenesis, mRNA splicing, protein translation, DNA damage response, and immune function through symmetric dimethylation on arginine (SDMA) of its substrate proteins such as histones, TP53, SmD3, EGFR, and RAD9. Dysregulation of PRMT5 has been associated with poor outcomes in several cancers, such as lung cancer and glioblastoma multiforme (GBM), highlighting its potential as a therapeutic target. [1, 2]
- MTAP plays a key role in the methionine salvage pathway, and its loss results in the accumulation of methylthioadenosine (MTA), which partially inhibits PRMT5 activity. In MTAP-deficient tumor cells, PRMT5 expression and activity become essential for cell growth, making PRMT5 an attractive synthetic lethality target for treating these cancers.[3]
- MTAP homozygous deletions occur in 10-15% of human cancers, with higher frequencies observed in various types, including non-small cell lung cancer (NSCLC), mesothelioma, pancreatic cancer, GBM, head and neck cancer, esophageal cancer, and bladder cancer.
- PE-0260 discovered and developed by PharmaEngine Inc. (PEI) is a compound that can utilize the accumulation of MTA by binding to PRMT5 in an MTA-cooperative manner and forming a stable ternary complex, thereby achieving selective PRMT5 inhibition and killing of MTAP-null tumor cells while sparing MTAP-containing normal cells. This selective binding mechanism provides improved therapeutic window compared to unselective first generation PRMT5 inhibitors.

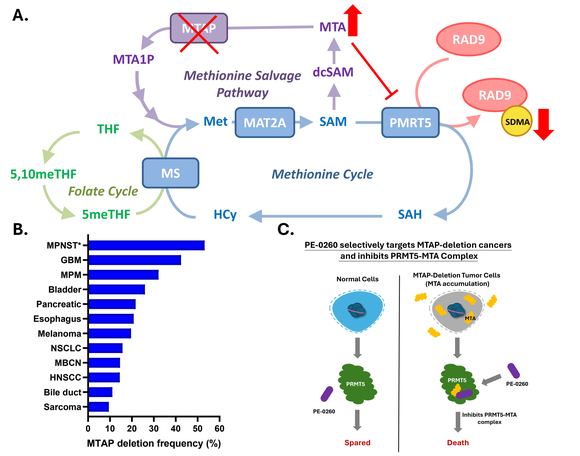


Figure 1: A. Accumulated MTA in MTAP deletion cells reduces PRMT5 activity and decreases SDMA modification in PRMT5 substrates. **B.** *MTAP* deletion frequency in human cancers. (from TCGA Pan-Cancer Atlas, *Cortes-Ciriano et al. Cancer Discov. 2023) **C.** The MTA-cooperative binding mechanism of PEP08 in PRMT5 provides enhanced inhibition selectivity in MTAP deletion cancer cells.

AI/CADD driven drug discovery of structurally distinct series of MTA co-operative PRMT5 inhibitors

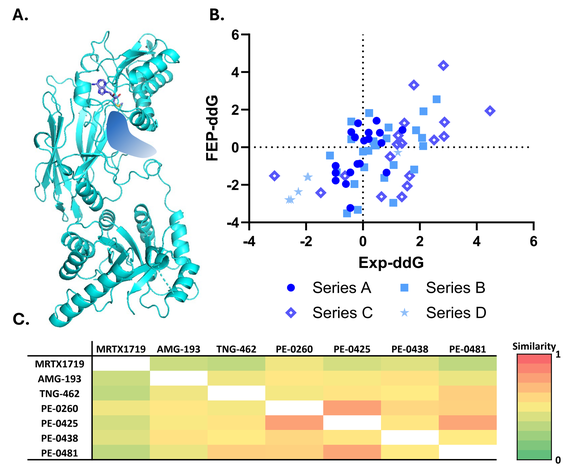


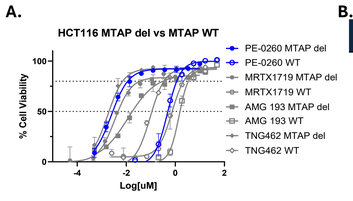
Figure 2: A. CryoEM structure of MTA/PRMT5 complex with a PEI lead molecule in the binding site. **B.** Comparison of calculated ddG of leads using free energy perturbation (FEP) and experimental ddG (converted from IC_{50} of anti-proliferation cell assay) across four series with different scaffold. **C.** Structure similarity of PEI leads and clinical stage MTA-operative PRMT5 inhibitors with publicly disclosed structures.[4-6]

Summary of drug properties of PEI leads and other MTA-cooperative PRMT5 inhibitors in clinical stage

In vitro ADME	PE-0260	PE-0425	PE-0438	PE-0481	MRTX1719	AMG 193	TNG462
HCT116 MTAP del IC_{50} < 10 (nM)	V	V	V	V	10	16	2
IC_{50} HCT116 MTAP WT/del > 100	V	V	X	V	94	88	48
Human LMS, Cl_{int} < 20 (mL/min/Kg)	V	V	V	V	46	68	N/A
Human HTS Cl_{int} < 5 (mL/min/Kg)	V	V	V	V	5.1	3.9	19.8
CYP inhibition > 50% @10 uM	No	No	No	No	3A4	No	No
Mouse PK							
$T_{1/2}$ > 4 (h)	V	V	V	V	1.3	2.1	4.7
CL < 5 (mL/min/Kg)	V	V	V	V	90	11	78
%F > 60	V	V	V	V	63	98	44
Brain, K_p, mouse	0.40	<0.05	0.31	<0.05	<0.05	0.42	N/A

Table 1: *In vitro* ADME properties and *In vivo* mouse PK parameters comparison of PEI leads and other MTA-cooperative PRMT5 inhibitors in clinical stage. PEI MTA-cooperative PRMT5 inhibitors show desired drug properties and potential for further development.

PE-0260 demonstrates superior potency and selectivity in HCT116 isogenic pair



B.

	MTAP WT IC_{50}/ del IC_{80}
PE-0260	43.2
MRTX1719	20.4
TNG462	15.4
AMG193	5

Figure 3: A. HCT116 MTAP del and MTAP WT 10-day CTG viability assays of PE-0260 and other MTA-cooperative PRMT5 inhibitors in clinical stage. PE-260 demonstrates the greatest selectivity (> 200X) and potency. **B.** PE-260 has largest HCT116 MTAP WT IC_{50} to del IC_{80} ratio indicating a larger *in vivo* therapeutic window.

PE-0260 selectively inhibits proliferation of MTAP del cells across different cancer types

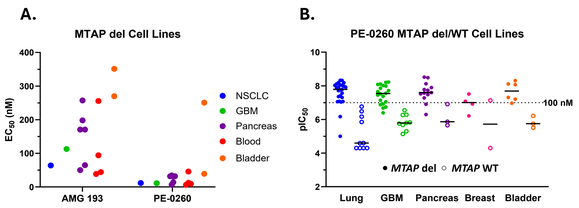


Figure 4: A. Head-to-head comparison of PE-0260 and AMG 193 in 5-day CTG MTAP del cancer cell line viability assay. **B.** PE-0260 screened in a large 10-day CTG viability assay and demonstrates great potency in MTAP del cell lines (solid circle) and desired selectivity to MTAP WT cell lines (empty circle) across different cancer types.

PE-0260 shows dose-dependent antitumor activity and on-target inhibition in Mia Paca-2 PDAC CDX mouse model

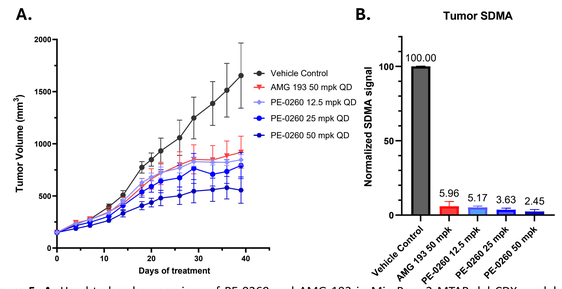


Figure 5: A. Head-to-head comparison of PE-0260 and AMG 193 in Mia Paca-2 MTAP del CDX model. PE-0260 and AMG 193 were dosed as indicated. PE-0260 drives dose-dependent antitumor activity and better tumor growth inhibition comparing to AMG 193 at the same dosage. Average tumor volumes +/- SEM were plotted over time. **B.** Tumor samples collected 4 hours post last dose were analyzed by western blot for SDMA quantification. The result reveals dose-dependent SDMA level reduction and smaller variability in PE-0260 dosing groups.

PE-0260 drives dose-dependent antitumor activity and tumor regressions in LU99 NSCLC CDX mouse model

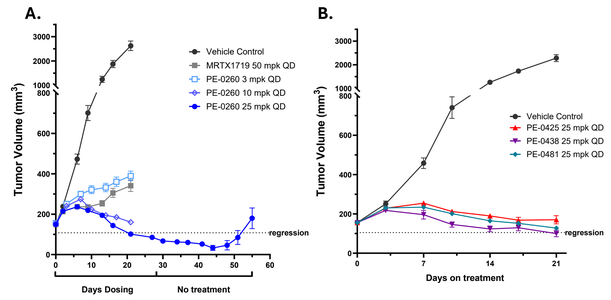


Figure 6: A. PE-0260 and MRTX1719 were dosed as indicated in LU99 CDX mouse model. PE-0260 drives dose-dependent antitumor activity and tumor regressions after 21-day daily dosing above 10 mpk. Tumor size continuously decreased for 14 days after last dosing indicates a long stable PD effect. **B.** Other PEI leads also demonstrate great antitumor activity in the MTAP del LU99 CDX model. Average tumor volumes +/- SEM were plotted over time. Tumor regression is defined as > 30% tumor volume decrease from initial values.

PE-0260 antitumor activity is on-target and with great tumor PK/PD correlations

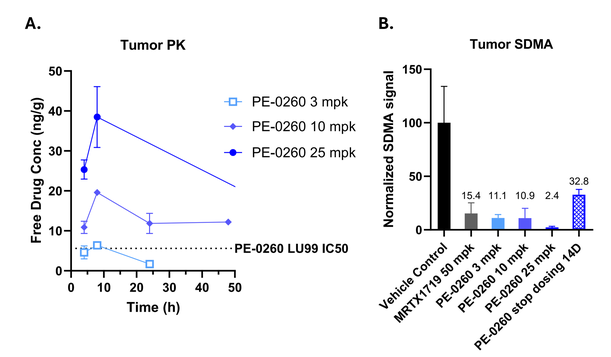


Figure 7: PK/PD analysis of PE-0260 LU99 CDX study (Figure 6A). Tumor and plasma samples were collected at D21 for PK and PD (SDMA) analysis. **A.** Dose-dependent PK profiles in tumor samples are observed. In groups with > 10 mpk QD dosing levels, free drug concentration (converted based on mouse plasma protein binding ratio) was above IC_{50} of PE-0260 obtained from LU99 antiproliferation assay for more than 48 hours. **B.** Tumor samples collected 4 hours post last dose were analyzed by western blot for SDMA quantification. Dose-dependent SDMA level reduction was also observed indicating good PK/PD correlation. The degree of SDMA inhibition is proportional to the tumor growth inhibition suggests PE-0260 antitumor activity is on-target driven. SDMA level in tumor was not restored after 14 dosing holidays which is consistent with the tumor growth curve.

PE-0260 is brain-penetrant and completely inhibited tumor growth in LN-18 GBM orthotopic mouse model

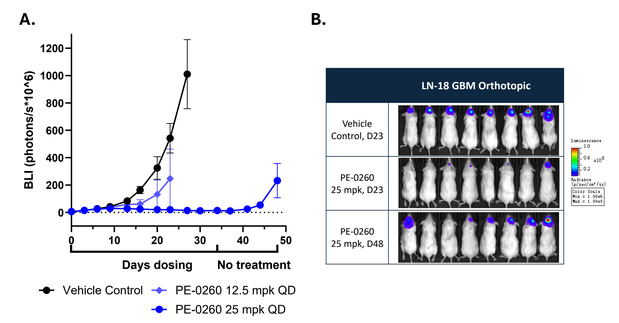


Figure 8: A. PE-0260 was dosed as indicated in LN-18 orthotopic mouse model. PE-0260 drove dose-dependent antitumor activity and effectively inhibited tumor growth comparing to vehicle control. For the 25 mpk QD group, tumor signal remained minimal for 7 days after last dosing indicates a long stable on-site PD effect. **B.** Bio-luminescence intensity (BLI) images of LN-18 GBM orthotopic mouse. BLI decreased significantly in 7/8 animals at day 23 and two complete response (CR) were confirmed in the end of the study.

PE-0260 desired PK profile and enrichment in tumor drives efficacy in LN-18 orthotopic model

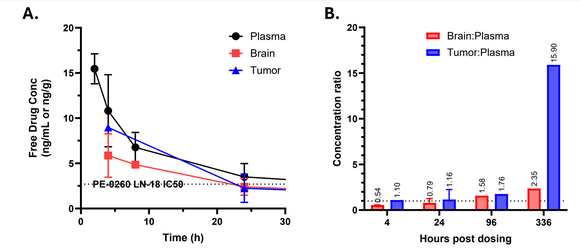


Figure 9: A. PK result of PE-0260 12.5 mpk QD group in LN-18 orthotopic model. The PK profiles in brain and tumor demonstrate the capability of PE-0260 to penetration BBB. **B.** Comparison of concentration ratios, Brain:Plasma and Tumor:plasma at different time points after the last dosing in 12.5 mpk QD group. The concentration ratio of tumor to plasma increases significantly along the time after dosing indicating a favorite drug partition in tumor.

PE-0260 synergize with clinical stage brain-penetrant CHK1 inhibitor PEP07 in CDX mouse models

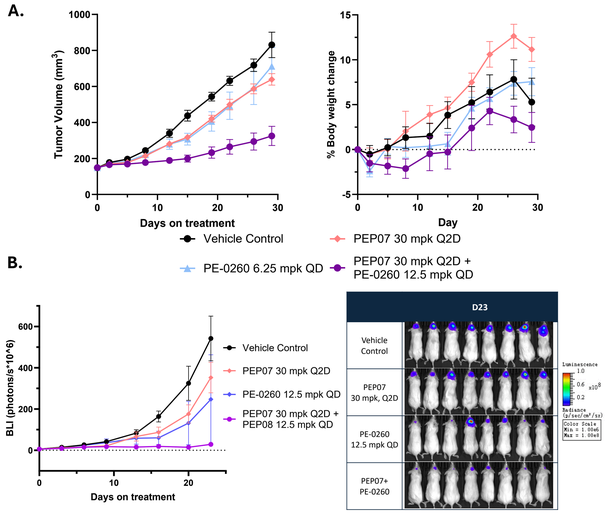


Figure 10: A. PE-0260 and PEP07 were dosed in single or combination use in Mia Paca2 CDX mouse model. The combination group, PEP07 30 mpk Q2D with PE-0260 6.25 mpk QD shows good synergy comparing to single uses while dosing was well tolerated as indicated by animal body weight. **B.** The combination use of PE-0260 and PEP07 in LN-18 orthotopic CDX mouse model. The bio-luminescence signal suggests tumor growth is well controlled with small variability during the 23-day study.

Summary

- Four structurally distinct lead series of MTA-cooperative PRMT5 inhibitors were discovered. Among them, PE-0260 was further developed into IND enabling stage.
- PE-0260 shows good potency of HCT116 MTAP del cell viability (< 5 nM IC_{50}) and > 200X selectivity compared with isogenic MTAP WT cells. In a diverse cell line panel, PE-0260 selectively inhibits MTAP del cell lines over MTAP WT cell lines with median IC_{50} < 30 nM and > 50X selectivity across NSCLC, GBM, pancreatic and breast cancers.
- PE-0260 drives dose-dependent antiproliferation activity in multiple CDX model, including a GBM orthotopic model, demonstrating outstanding efficacy and excellent PK/PD correlation.
- PE-0260 has balanced ADME properties and low risk for drug-drug interaction which enables the potential in combination use with other anticancer agents such as PEP07, a clinical stage CHK1 inhibitor. Synergy and additive effect were observed when dosing low doses of PE-0260 and PEP07 together in multiple CDX models.
- The potential best-in-class MTA-cooperative PRMT5 inhibitor, PE-0260, is IND ready and aiming to enter clinical stage in 2H 2025.

Acknowledgments

The authors would like to acknowledge the following collaborators and CROs for their contributions to this study:
XtalPi for AI/CADD and medicinal chemistry,
Academia Sinica for CryoEM study,
Pharmaron, and Chempartner for *in vitro* and *in vivo* studies.

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