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Preclinical and clinical studies of PEP07, a novel brain-penetrant oral CHK1 inhibitor, on solid tumor treatments

Chieh-Fang Cheng[1], Hui-Ling Chen[1], Feng-Yu Lee[1], Cheng-Hao Liu[1], Chien-Fang Li[2], Hong-Ren Wang[1]

1. PharmaEngine, Inc. Taipei, Taiwan 2. Chi Mei Medical Center, Tainan, Taiwan

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Introduction

The DNA damage response is a complex network of cellular pathways that detect and repair DNA damage to prevent mutations, cancer, and other diseases. When DNA damage occurs, the DDR coordinates repair mechanisms and cell cycle checkpoints to prevent the propagation of damaged DNA. The DDR can be triggered by various sources such as UV radiation, chemicals, ionizing radiation, or replication errors.

CHK1 is a serine/threonine kinase that functions as a key regulator in the DNA damage response, especially in response to replication stress or DNA double-strand breaks. CHK1 is primarily activated by the ATR (ataxia telangiectasia and Rad3-related) kinase, which senses DNA damage, and then phosphorylates key substrates (e.g., CDC25, WEE1, etc.) to enforce cell cycle checkpoints and DNA repair mechanisms. Given its central role in regulating the cell cycle and DNA repair, CHK1 has become a target in cancer therapy.

PEP07 is a potent and selective brain-penetrant oral CHK1 inhibitor. The selectivity of PEP07 on CHK1 versus CHK2 is over 1000-fold. PEP07 demonstrated strong activity in repressing cancer cell growth *in vitro* and *in vivo* in different solid cancer models. Currently, PEP07 is being evaluated in clinical studies for both hematologic and solid cancers.

In vitro cell panel of PEP07

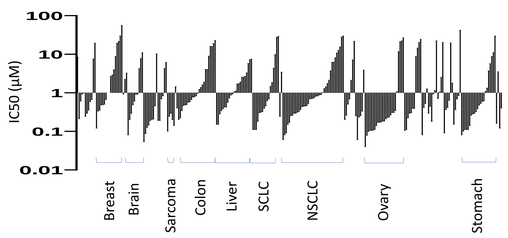


Figure 1. Over 200 cell lines were treated with PEP07 for viability assay. Among them, Over 100 cell lines showed IC50 < 0.5μM toward PEP07. CellTiter-Glo assay was used to examine the effect of PEP07 on cell viability of cancer cells. Cells were treated with PEP07 for 72 hours before conducting the assay. Major cancer types of tested cell lines were summarized.

In vivo sarcoma models

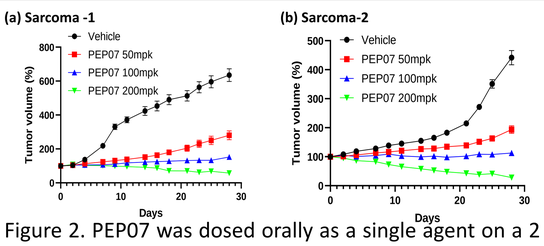


Figure 2. PEP07 was dosed orally as a single agent on a 2 on/5 off dosing schedule in two sarcoma xenograft models. Dose dependent inhibition of tumor growth was observed. Error bar is SEM.

In vivo gastric cancer model

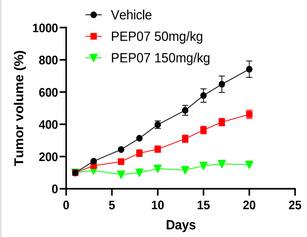


Figure 3. PEP07 was dosed orally as a single agent on a 2 on/5 off dosing schedule in a gastric cancer xenograft model. Dose dependent inhibition of tumor growth was observed. Error bar is SEM.

Combination of PEP07 with ADC

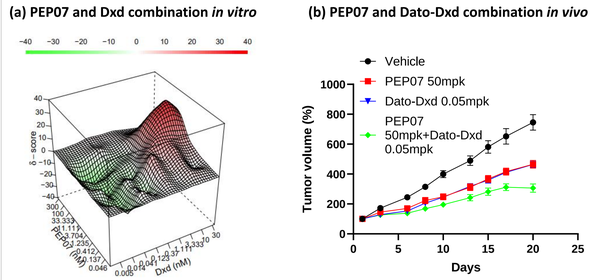


Figure 4. (a) Combination of PEP07 with a topoisomerase I inhibitor deruxtecan (Dxd) was tested in a gastric cancer cell line *in vitro*. Bliss synergy score was shown. (b) Combination of PEP07 with Dato-Dxd was tested in a gastric cancer xenograft model *in vivo*. Error bar is SEM.

Combination of PEP07 with PRMT5i

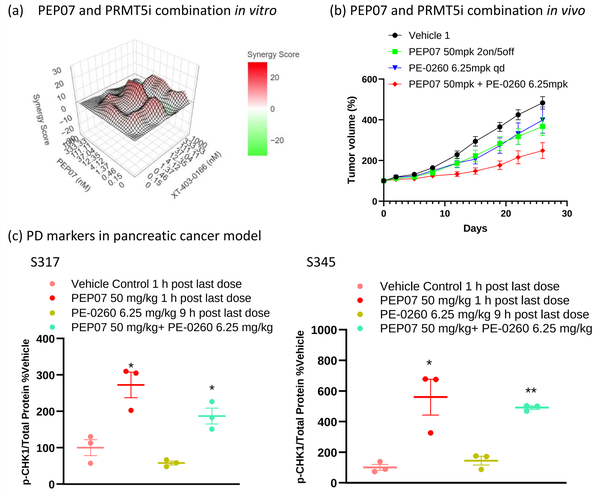


Figure 5. (a) Combination of PEP07 with a PRMT5 inhibitor PE-0260 was tested in a pancreatic cancer cell line *in vitro*. Bliss synergy score was shown[1]. (b) Combination of PEP07 with PE-0260 was tested in a pancreatic cancer xenograft model *in vivo*. (c) Phosphorylations of S317 and S345 on CHK1 were examined as PD markers for CHK1 inhibition. PEP07 treatments activated both sites while PRMT5i had no effect. Error bar is SEM.

PEP07 tumor/brain penetration

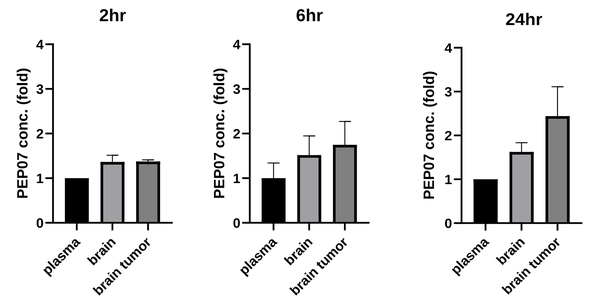


Figure 6. Tumor cell line was implanted in the right brain of mice for 3 weeks before drug delivery for blood, brain, and tumor concentration measurement. Blood, brain, and tumors were collected 2, 6, or 24 hours after treatment. Brain concentration was about 1.2 to 2 folds of blood concentration in the three time points tested. Tumor concentration was 1.3 folds at 2 hours, 1.7 folds at 6 hours, and 2.4 folds at 24 hours of blood concentration post treatment. Error bar is SEM.

PEP07 brain *in vivo* model

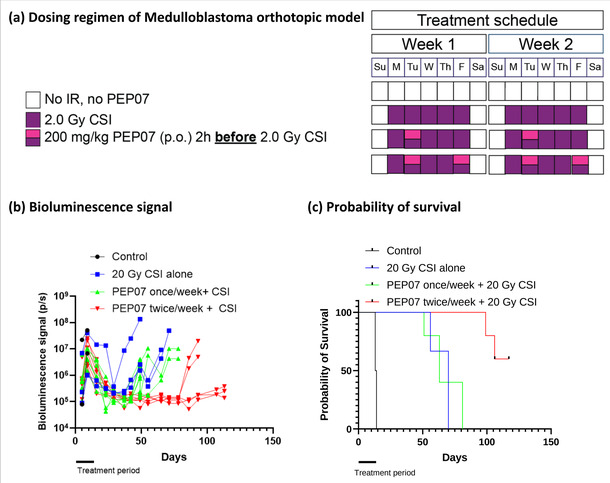


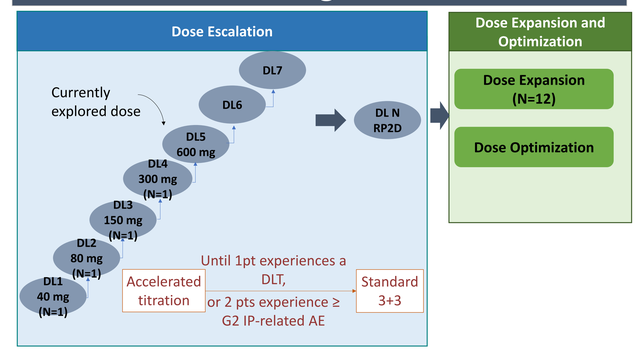
Figure 7. PEP07 was dosed orally for 2 weeks in combination with radiation therapy in an orthotopic medulloblastoma model. (a) Dosing regimen is shown. (b) Medulloblastoma cell line was engineered to express luciferase. Tumor growth was monitored by bioluminescence signal. Bioluminescence signal of each mice was shown. (c) Survival curve of mice in each group was shown. Combination of four times PEP07 treatment with radiation achieved median survival for over 100 days.

NN biomarker prediction and validation

Gastric		Predicted	
		Sensitive	Insensitive
Actual	Sensitive	14 (7)	0 (3)
	Insensitive	0 (0)	16 (10)

Figure 8. Multi-Layer Perceptron (MLP) models to predict PEP07 potency were trained using the z-score normalized RNA data as inputs and experimental PEP07 potency as outputs. Feature reduction was conducted by LASSO regression of DDR-related genes in gastric cancer cell lines and patient-derive cells. The dataset was split into training, validation, and testing sub-datasets. The training and validation sub datasets were used to train MLP models with different architectures. The testing sub-dataset, which was not seen during training, was then used to confirm and select the best performing model from the trained models. Training + validation sub-datasets were shown in green, and testing sub-datasets were shown in red.

Clinical design of PEP07



Conclusions

- PEP07 is a potent and selective brain-penetrant oral CHK1 inhibitor.
- PEP07 showed strong activity in repressing cancer cell proliferation *in vitro* and tumor growth *in vivo* either by monotherapy or combined with standard of care (SoC) treatments.
- *In vitro* cell proliferation and transcriptome data in cancer cell lines were used to generate predictive markers of PEP07, and PDCs were used to validate the predictive markers.
- Brain penetration of PEP07 was demonstrated in tumor bearing mice models.
- Combination of PEP07 with radiation showed strong activity in repressing brain tumor model *in vivo*.

Acknowledgement

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Contact: jack.cheng@pharmaengine.com

Reference:
1. Zheng, S.; Wang, W.; Aldahdooh, J.; Malyutina, A.; Shadbahr, T.; Tanoli Z; Pessia, A.; Tang, J. SynergyFinder Plus: Toward Better Interpretation and Annotation of Drug Combination Screening Datasets. Genomics, Proteomics & Bioinformatics 2022, 20, 587-596 doi:10.1016/j.gpb.2022.01.004