Presentation #: 1743

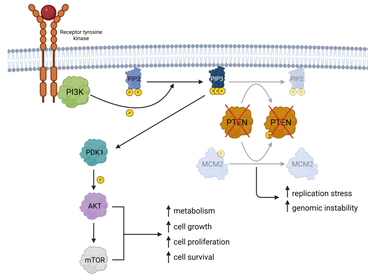
A novel brain-penetrant dual ATR-mTOR inhibitor for PTEN-deficient cancers

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Introduction

Ataxia telangiectasia and Rad3-related protein serine/threonine kinase (ATR), a master regulator of DNA damage repair, maintains genomic stability in cancer cells and allows cancer cells to survive with high replication stress. Inhibition of ATR thus causes genomic instability, leading to mitotic catastrophe and apoptosis[1,2]. ATR inhibitors (ATRi) have been found to be particularly effective against cells harboring PTEN deficiencies due to synthetic lethality[3,4,5]. PTEN regulates the cell cycle and interacts with key proteins like p53 and Chk1, and PTEN deficient cells show increased genomic instability due to compromised DNA repair[6]. Inhibition of ATR in PTEN-deficient cells, therefore, leads to accumulation of DNA damage and cell death. PTEN loss/mutation also leads to activation of mTOR through activation of PDK1 and AKT, contributing to cell survival and growth[6]. Thus, simultaneous inhibition of ATR and mTOR seems rational in the context of PTEN-deficient tumor cells.



PTEN deficiency can be found in up to 40% of gliomas[7] and 63% of breast cancers[8], which often metastasize to the brain. Since combination treatments can be clinically difficult to utilize due to overlapping toxicities and differing pharmacokinetics, we sought to develop a CNS-penetrating dual inhibitor of ATR and mTOR for the treatment of PTEN-deficient cancers. Recent development of a selective, CNS-penetrant mTOR inhibitor for treatment of glioblastoma further supports the development of mTOR-targeting therapeutics[9].

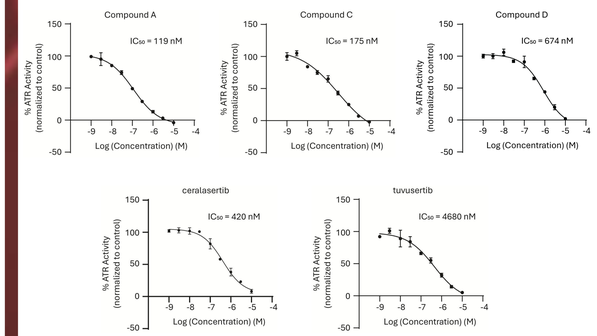
Indication	Patients with PTEN deficiency (%)	References
Glioma	5-40	Koschmann et al. (2017) NPJ Precis Oncol. Han et al. (2016) Onco Targets Ther.
Medulloblastoma	5	Kool et al. (2014) Cancer Cell
Breast Cancer	4-63	Li et al. (2017) Oncotarget
Lung Cancer	15-38	Fischer et al. (2022) Cell BioSci.
Melanoma	5-59	Wu et al. (2003) Oncogene Cabrita et al. (2020) Cancers
Renal Cell Carcinoma	3-60	Lee et al. (2014) Journal of Urology Shin Lee et al. (2003) J. Surg. Onc. Cheng et al., (2012) Asian Pacific J Cancer Prev.
Colorectal Cancer	12-40	Jiang et al. (2013) J. Clin. Onc. Molinari et al. (2014) Front Oncol. Lin et al. (2015) World J. Surg. Onc. Serebriiskii et al. (2023) BPJ Genom. Med.
Ovarian Cancer	32-75	Martins et al. (2020) Br. J . Cancer Martins et al. (2014) Genome Biol.
Prostate Cancer	2-62	Jamaspishvili et al. (2018) Nat. Rev. Urol.

A generative artificial intelligence platform called Enki™ was used to identify de novo molecules with optimized properties for CNS penetrance and specificity for ATR and mTOR. Enki uses a latent diffusion model to optimize many properties simultaneously within the search space, including maximal potency against the primary target, selectivity, ADMET, and physicochemical properties. The most promising molecules were synthesized and tested, and here, we present data on potency, selectivity, ADME and in vivo efficacy.

References

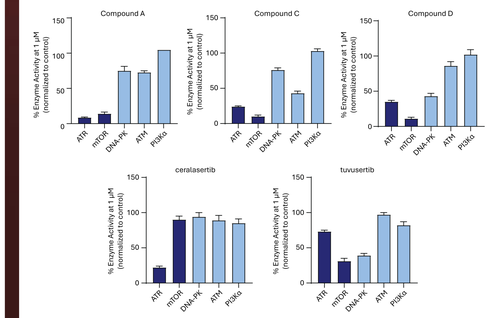
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Inhibition of ATR and mTOR Enzymatic Activity



Compounds are equally or more potent inhibitors of recombinant ATR enzymes compared to reference compounds, ceralasertib and tuvusertib, and are potent inhibitors of recombinant mTOR enzyme.

Selectivity vs. PIKK Kinases



Compounds are equally or more selective against PIKK family enzymes as reference compounds, ceralasertib and tuvusertib.

Inhibition of Cell Viability

Table 1: Cell Viability EC_{50} (µM) PTEN –WT and PTEN Deficient Cells

	T47D PTEN WT	22RV1 PTEN WT	LNCaP PTEN deficient	PC3 PTEN deficient
Compound A	3	2.8	1.6	1.2

Compound A inhibits cell viability of PTEN-WT and PTEN-deficient cancer cell lines.

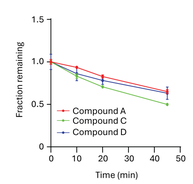
Table 2: Cell Viability EC_{50} (µM) D283 Medulloblastoma Cells

Compound A	Compound C	Compound D	Ceralasertib	Tuvusertib
0.17	2.2	2.0	2.0	0.14

Compounds inhibit cell viability of D283 medulloblastoma cells equally or more than reference compounds, ceralasertib and tuvusertib

ADME Profiling

In Vitro Metabolism



After 45 minutes of incubation with human liver microsomes, compounds are metabolically stable.

In Vivo Pharmacokinetic Profiling

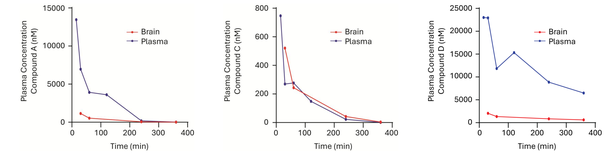


Table 3: Pharmacokinetic parameters

Pharmacokinetic Parameters	Compound A	Compound C	Compound D
Dose (mg/kg; IP)	5	5	5
T½(min)	32.49	33.73	193.40
C_{max} (nM)	13456.14	747.11	23019.27
Plasma AUC (0-∞) (nM*min)	882610.9	45896.86	6034733.41
Brain AUC (0-∞) (nM*min)	98762.8	48039.23	597649.61
Brain/Plasma (%)	11.2	104.7	9.9

Administration of 5 mg/kg compound (ip) to mice. Concentration of drug in plasma and brain tissue was determined using LC/MS.

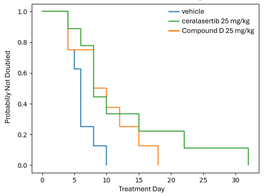
ATR-mTOR compounds are well-tolerated after single dose administration and show varying levels of CNS penetrance.

In vivo Efficacy



Nu/J male mice with subcutaneous LNCaP tumors were treated QD 5 days on, 2 days off with vehicle control, 25 mg/kg ceralasertib, or 25 mg/kg compound D. Caliper measurements were taken every 2-3 days to monitor tumor growth rate.

Kaplan-Meier: Time from Start of Treatment to doubling of tumor volume



KM Log Rank Test: Ceralasertib vs. vehicle p=0.048, Compound D vs. vehicle p=0.027

Table 4: Tumor Doubling Time from start of Treatment and Hazard Ratio Relative to Vehicle

Group	n	Mean	Median	SD	95% CI	HR	HR (95% CI)
Vehicle	8	6.13	6.0	2.03	4.72 - 7.53	-	-
Ceralasertib 25 mg/kg	8	9.88	9.0	4.97	6.43 - 13.32	0.36	0.114 - 1.164
Compound D 25 mg/kg	9	12.56	8.0	9.07	6.63 - 18.48	0.35	0.119 - 1.052

Table 5: Percent Change in Body Weight from Start of Treatment to Last Treatment Day

Group	n	Mean (%)	Median	SD	95% CI (%)
Vehicle	8	-3.83	-4.24	2.91	-5.84 to -1.81
Ceralasertib 25 mg/kg	8	-6.46	-6.12	3.07	-8.59 to -4.33
Compound D 25 mg/kg	9	-5.11	-4.65	1.91	-6.37 to -3.86

ATR-mTOR compound D significantly prolongs tumor doubling time compared to vehicle control and is equally potent as reference compound ceralasertib. Compound D was also well-tolerated with daily dosing, showing less weight loss compared to ceralasertib-treated mice and no clear signs of hematological toxicity at terminal CBC analysis (not shown).

Conclusions and Future Directions

- AI-driven drug discovery has the potential to streamline the identification of new best-in-class drug candidates for the treatment of many diseases, including cancer.
- Using the Enki™ generative AI platform, we have generated and synthesized a curated list of small-molecule drug candidates meeting a specific target-product-profile *in silico*, specifically, novel dual ATR and mTOR inhibitors which are brain penetrating.
- Dual ATR and mTOR inhibitors are effective at inhibiting cell viability of PTEN-deficient cancer cells and are well tolerated after a single dose in mice.
- Candidate inhibitors show varying levels of CNS penetrance, activity, and metabolic stability which is broadly consistent with AI predictions
- Compound D shows equal efficacy *in vivo* compared to ceralasertib with decreased weight loss and no signs of hematological toxicity.
- Further optimization of candidate inhibitors is ongoing.

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