Dual ATR-mTOR inhibitors for PTEN-deficient prostate cancer

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Introduction

Ataxia telangiectasia and Rad3-related (ATR) kinase is a master regulator of the DNA damage response and helps maintain genomic stability. Cancer cells often upregulate ATR to survive replication stress. Inhibiting ATR stalls replication forks, arrests the cell cycle, and causes accumulation of DNA damage[1].

Many tumor types are sensitive to ATR inhibitors (ATRi), but these agents are usually tested clinically in combination with other therapies or in tumors carrying genetic vulnerabilities that increase sensitivity[2]. PTEN loss is one such vulnerability: ATRi are synthetically lethal in PTEN-deficient cells[3–5]. PTEN restrains PI3K–Akt signaling and contributes to cell cycle control and DNA repair, in part through Chk1 — the same checkpoint effector activated by ATR[6]. Cells lacking PTEN, therefore, depend on ATR–Chk1 signaling to manage replication stress, and inhibiting ATR removes that remaining safeguard, leading to genomic instability, DNA damage and cell death.

PTEN is the most commonly lost tumor suppressor gene in primary prostate cancer, usually by genomic deletion[7]. This is also highly correlated with increasing Gleason score and tumor stage, with PTEN loss more commonly found in prostate cancer metastases than primary tumors, nearly 40% in most reports[8,9,10]. The involvement of PTEN in DNA damage repair makes PTEN deficiency a potentially useful biomarker for DNA damage repair inhibitors. Because PTEN loss also hyperactivates PI3K–mTOR signaling, this program initially targeted dual ATR/mTOR inhibition. After evaluation of several compound families, we maintained selective potency against ATR with variable potency against mTOR. Lead optimization converged instead on ATR-selective compounds, avoiding confounding toxicity associated with added mTOR blockade while retaining activity in PTEN-deficient prostate cancer cells. Here we describe ATR-selective inhibitors developed for PTEN-deficient prostate tumors.

Molecules were designed *de novo* using Enki™, a generative AI platform that applies a latent diffusion model to optimize many properties simultaneously — potency against the primary target, selectivity, ADMET and physicochemical properties — against a target product profile requiring CNS penetration. The most promising molecules were synthesized and tested. We present *in vitro* potency and selectivity, cell viability and ADME data for five lead compounds.

Table 1. Summary of percentage of cancer patients with PTEN deficiency in primary and metastatic prostate cancer indications

Indication	Patients with PTEN deficiency (%)	References
Localized prostate cancer	10-20%	Cancer Genome Atlas Research Network (2016) Cell Jamaspishvili et al. (2025) Journal of Clinical Oncology
Metastatic prostate cancer	40-60%	Robinson et al. (2015) Cell Sweeney et al. (2021) The Lancet

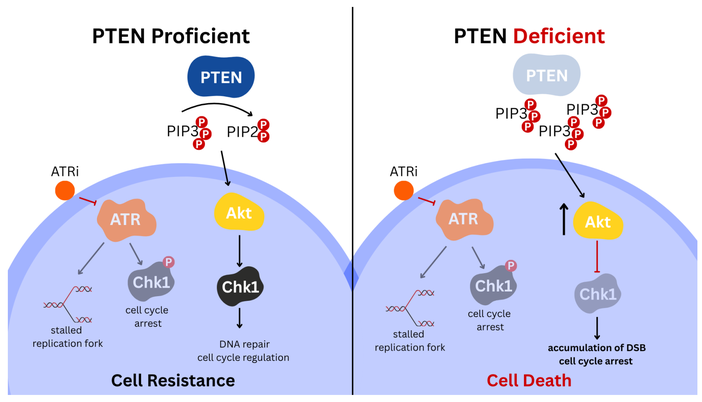

Figure 1. Proposed mechanism of PTEN synthetic lethality with ATR inhibition.

Inhibition of ATR Enzymatic Activity

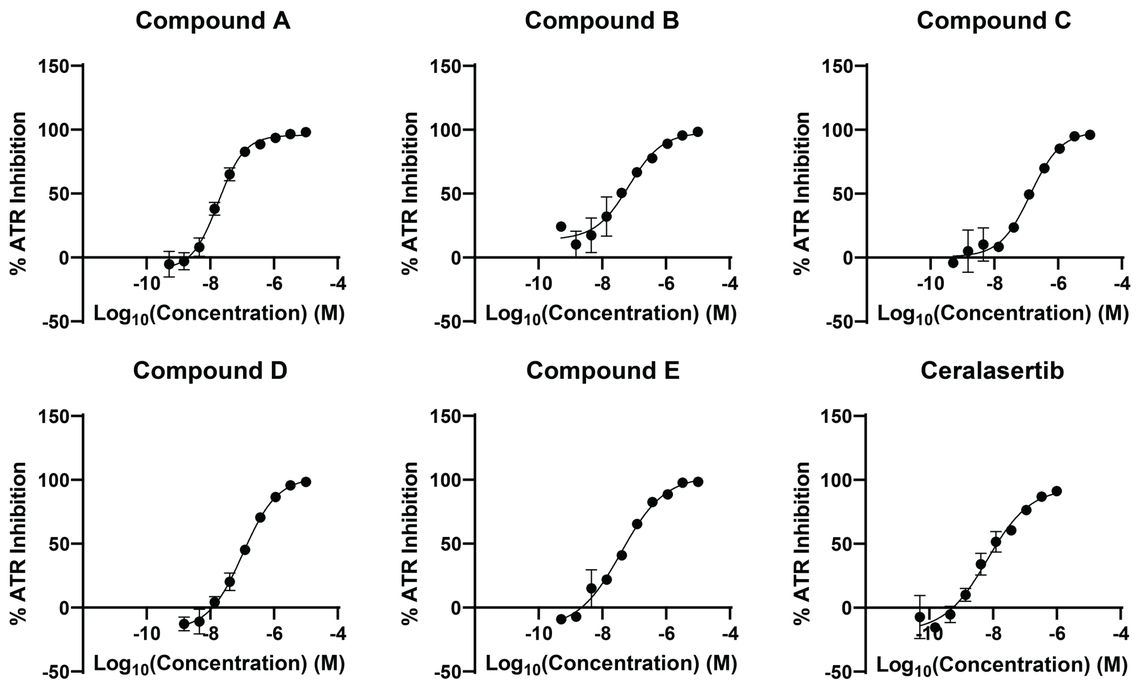

Figure 2. Dose–response inhibition of recombinant ATR/ATRIP kinase activity by lead compounds and ceralasertib. Compounds were tested in a 10-point, half-log dilution series from 10 µM to 1 nM. K_i values were derived from IC_{50} by the Cheng–Prusoff equation (K_m,ATP = 12 nM). Data are from one representative experiment; points show mean ± SD of technical duplicates.

Table 2. ATR inhibitory potency of five lead compounds and the clinical benchmark ceralasertib. K_i values were derived from IC_{50} by the Cheng–Prusoff equation (K_m,ATP = 12 nM). Fold change is calculated relative to ceralasertib (K_i = 0.26 nM).

	ATR K_i (nM)	Compared to ceralasertib
Compound A	0.09	2.9× more potent
Compound B	0.16	1.6× more potent
Compound C	0.54	2.1× less potent
Compound D	0.42	1.6× less potent
Compound E	0.16	1.6× more potent
Ceralasertib	0.26	Reference

66 Enki™-designed compounds were screened against recombinant ATR. The five lead compounds shown are potent, sub-nanomolar ATR inhibitors (Ki 0.09–0.54 nM), comparable to the clinical benchmark ceralasertib (Ki 0.26 nM). Compound A was the most potent (~3-fold more potent than ceralasertib), and all compounds fell within ~3-fold of the reference.

Selectivity vs. PIKK Kinases

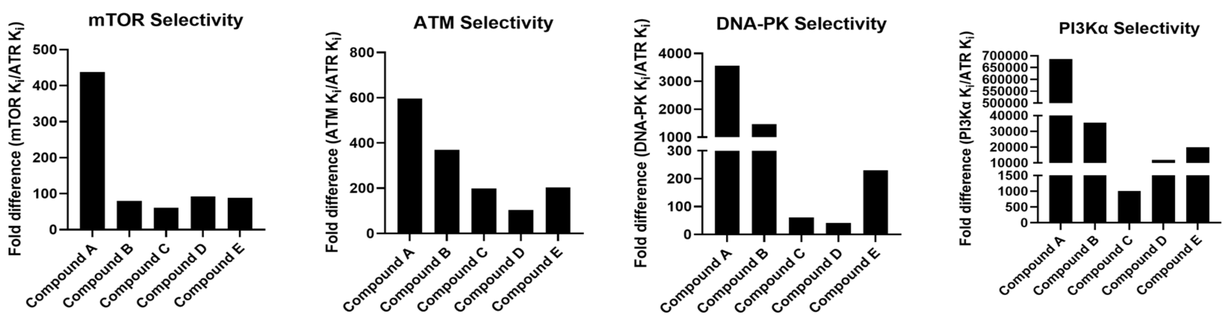

Figure 3. Kinase selectivity of lead compounds, expressed as the fold difference between K_i for each off-target kinase and K_i for ATR. K_i values were derived from IC_{50} by the Cheng–Prusoff equation using K_m,ATP values of 12 nM (ATR), 227 nM (mTOR), 226 nM (ATM), 30 µM (DNA-PK) and 317 µM (PI3Kα). Data are from one representative experiment; bars show mean ± SD of technical duplicates.

All five compounds are highly selective for ATR over the related PIKK family kinases mTOR, ATM and DNA-PK (≥40-fold), and over the lipid kinase PI3Kα (up to ~680,000-fold). Compound A showed the broadest selectivity (>400-fold against all 4 kinases).

Selective ATR inhibition limits the toxicities associated with blocking related kinases: ATM and DNA-PK inhibition sensitizes normal tissue to DNA damage, and PI3K/mTOR inhibition carries metabolic liabilities. A clean profile supports future combination with other standard-of-care therapies.

In vitro Metabolic Stability

Table 3. *In vitro* metabolic stability of lead compounds in mouse and human liver microsomes. Compounds were incubated at 1 µM microsomes (+ NADPH) (0.5 mg/mL); half-life ($T_{1/2}$) and intrinsic clearance (CL_{int}) were determined from the log-lin decline in parent compound over 60 min.

	Mouse liver microsomes		Human liver microsomes	
	$T_{1/2}$ (min)	Cl_{int} (µL/min/mg)	$T_{1/2}$ (min)	Cl_{int} (µL/min/mg)
Compound A	72.6	19.9	336.5	4.1
Compound B	47.1	29.5	63.8	21.8
Compound C	52.7	27.6	103.7	13.6
Compound D	52.5	27.0	67.9	20.6
Compound E	69.5	21.1	365.2	3.8

In liver microsome stability assays, all five compounds showed low to moderate intrinsic clearance in mouse (Clint 19.9–29.5 µL/min/mg; $T_{1/2}$ 47–73 min) and lower clearance in human (Clint 3.8–21.8 µL/min/mg; $T_{1/2}$ 64–365 min). Compounds A and E were the most stable, with human $T_{1/2}$ exceeding 5 hours and Clint below 5 µL/min/mg.

Prostate Cancer Cell Viability

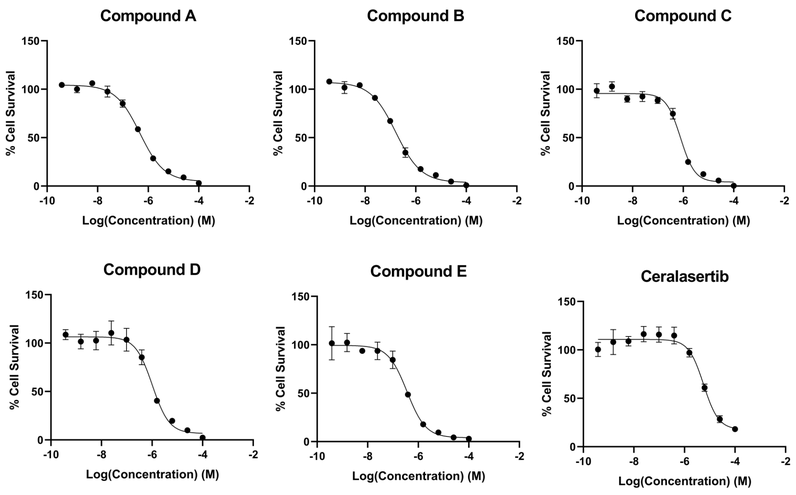

Table 4: Antiproliferative potency in the PTEN-deficient prostate cancer cell line LNCaP. Cells were treated for 72 h and viability measured by GlowQuant™ assay; EC_{50} values were determined from four-parameter logistic fits.

	LNCaP EC_{50} (µM)
Compound A	0.46
Compound B	0.16
Compound C	0.78
Compound D	1.0
Compound E	0.35
Ceralasertib	5.7

Figure 4: Viability of prostate cancer cell line LNCaP after treatment of cells for 72 h; viability by GlowQuant™ cell viability assay. EC_{50} from four-parameter fits, representative data of one experiment, mean ± SD of technical triplicates.

All five compounds inhibited viability of the PTEN-deficient prostate cancer cell line LNCaP after 72 hours, with EC_{50} values of 0.16–1.0 µM. All compounds were more potent than ceralasertib — up to 36-fold — with compound B the most potent.

Conclusions and Future Directions

- Using the Enki™ generative AI platform, we designed and synthesized novel ATR inhibitors against a target product profile combining potency, selectivity and drug-like properties.
- From 89 *in silico* designs, 66 compounds were synthesized and evaluated over a 6-month period
- Lead compounds are sub-nanomolar ATR inhibitors (K_i 0.09–0.54 nM) with ≥40-fold selectivity over ATM, DNA-PK and mTOR, and >1000-fold selectivity over PI3Kα.
- All compounds were more potent than ceralasertib against PTEN-deficient prostate cancer cell line LNCaP — up to 36-fold.
- Compounds showed low intrinsic clearance in human liver microsomes, supporting sustained oral exposure and a practical dosing regimen
- Ongoing work includes *in vivo* plasma exposure and pharmacokinetic profiling, *in vivo* xenograft efficacy, and comparison of PTEN-deficient versus PTEN-proficient models.

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