# Discovery and development of a novel CNS-penetrating ATR inhibitor

## *Dual inhibition of ATR and mTOR in PTEN-deficient tumors*

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## Introduction

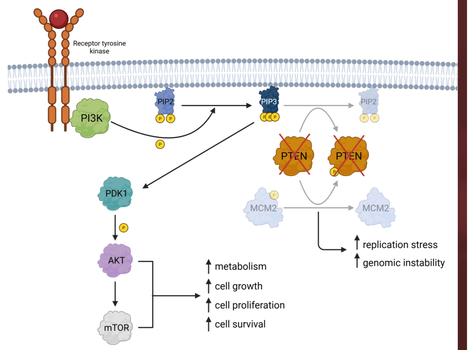
Brain tumors, including primary brain tumors and central nervous system (CNS) metastases, remain among the most challenging malignancies to treat, with therapeutic options often limited by the inability of drugs to penetrate the blood-brain barrier[1,2]. Ataxia telangiectasia and Rad3-related protein serine/threonine kinase (ATR) plays a crucial role in regulating DNA damage repair[3,4]. ATR inhibitors have shown promise against solid tumors as monotherapies and also in combination with chemotherapy, radiotherapy and immunotherapy[5,6,7]. ATR inhibitors currently in clinical development have limited CNS-penetration and are therefore sub-optimal for the treatment of brain tumors and brain metastases. A CNS penetrant ATR inhibitor could be used as a monotherapy or used to potentiate the cytotoxicity of radiotherapy, providing a new therapeutic option for patients with brain tumors[8]. In particular, studies have shown cancer cells with loss of function of phosphate and tensin homolog (PTEN), a negative regulator of PI3K signaling, are vulnerable to ATR inhibition[9,10,11]. PTEN loss has been identified as a biomarker in a variety of cancers, including cancers with high propensity for brain metastases, such as lung cancer[9], breast cancer[10] and melanoma[12]. PTEN deficiency results in activation of mTOR through the PIKT/AKT pathway, as well as PIKT/AKT-independent genomic instability and replication stress[13]. Targeting both ATR and mTOR simultaneously could result in enhanced efficacy of inhibitors against PTEN-deficient disease.

Here, we describe the use of the Enki™ platform, a generative AI approach, to discover novel CNS-penetrating dual ATR and mTOR inhibitors and our progress in the development of these inhibitors to treat PTEN-deficient cancers.

## PTEN-Deficiency in Cancer

PTEN is deficient in a wide variety of cancer indications, including cancers with high rates of brain metastasis. PTEN deficiency could be a useful biomarker for treatment with different inhibitors.

| Indication | Patients with PTEN deficiency (%) | References |
|---|---|---|
| Glioma | 5-40 | Koschmann et al. (2017) NPJ Precis Oncol.<br>Han et al. (22016) 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<br>Cabrita et al. (2020) Cancers |
| Renal Cell Carcinoma | 3-60 | Lee et al. (2014) Journal of Urology<br>Shin Lee et al. (2003) J. Surg. Onc.<br>Cheng et al. (2012) Asian Pacific J Cancer Prev. |
| Colorectal Cancer | 12-40 | Jiang et al. (2013) J. Clin. Onc.<br>Molinari et al. (2014) Front Oncol.<br>Lin et al. (2015) World J. Surg. Onc.<br>Serebriiskii et al. (2023) BPJ Genom. Med. |
| Ovarian Cancer | 32-75 | Martins et al. (2020) Br. J . Cancer<br>Martins et al. (2014) Genome Biol. |
| Prostate Cancer | 2-62 | Jamaspishvili et al. (2018) Nat. Rev. Urol. |




Loss of PTEN results in increased replication stress and genome instability, along with activation of mTOR, leading to increased cell survival and proliferation.

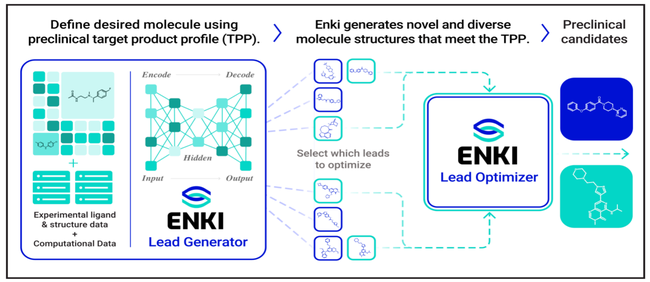
## Drug Discovery Process

**Enki™ Modeling**
138 molecules predicted by Enki™ to satisfy the TPP

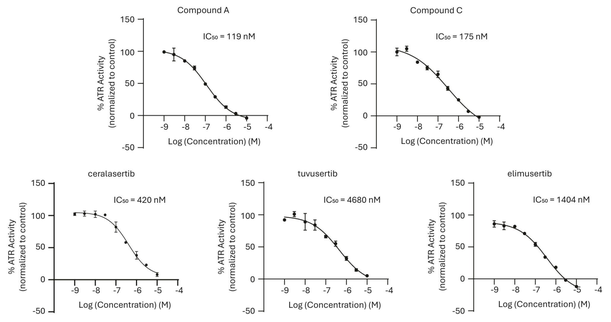
**Chemical Synthesis**
43 most promising compounds synthesized

***In vitro* Validation**
Laboratory testing compounds for activity against the ATR enzyme

**Hit Discovery**
Multiple compounds inhibiting ATR activity by >50% at 1 µM in less than 1 year

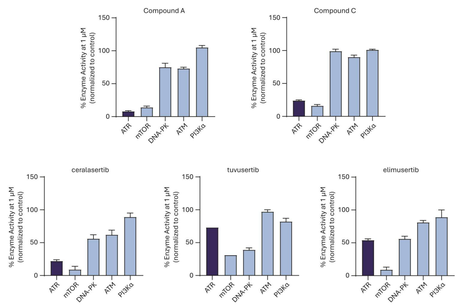



## Inhibition of ATR Enzyme




Compounds are equally or more potent inhibitors of recombinant ATR enzyme compared to reference compounds.

## Selectivity vs. PIKK Kinases




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

## Inhibition of Cell Viability

| Cell viability $EC_{50}$ (µM) | | | | |
|---|---|---|---|---|
| | T47D PTEN WT | 22RV1 PTEN WT | LNCaP PTEN deficient | PC3 PTEN deficient |
| Compound A | 3 | 2.8 | 1.6 | 1.2 |

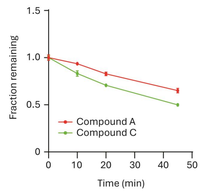
Compound A inhibits cell viability of PTEN-WT and PTEN-deficient cancer cell lines.

| Cell viability $EC_{50}$ (µM) | | | | |
|---|---|---|---|---|
| | Compound A | Compound C | ceralasertib | tuvusertib |
| D283 MED | 0.17 | 2.2 | 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**

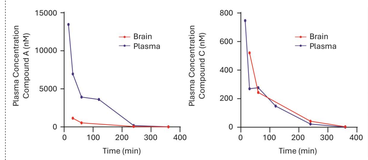



After 45 minutes of incubation with human liver microsomes, compounds are metabolically stable.

***In Vivo* Pharmacokinetic Profiling**

Pharmacokinetic profiling of compounds in mouse plasma and brain after intraperitoneal injection of 5 mg/kg compound. Concentration of drug in plasma and brain tissue was determined using LC/MS.

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




| | Compound A | Compound C |
|---|---|---|
| Dose (mg/kg) | 5 | 5 |
| T½(min) | 32.49 | 33.73 |
| $C_{max}$ (nM) | 13456.14 | 747.11 |
| Plasma AUC (0-∞) (nM*min) | 882610.9 | 45896.86 |
| Brain AUC (0-∞) (nM*min) | 98762.8 | 48039.23 |
| Brain/Plasma (%) | 11.2 | 104.7 |

## References

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## 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.
- PTEN deficiency can serve as a biomarker for inhibition of ATR and mTOR.
- 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.
- Additional *in vitro* and *in vivo* characterization is ongoing.

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