Novel ATR inhibitors with CNS penetrance developed by artificial intelligence

Sarah Truong[1,2,3], Beibei Zhai[1,2,3], Louise Ramos[1,2,3], Mona Marzban[1,2,3], Fariba Ghaidi[1,2,3] , Marshall Drew-Brook[4], Pete Guzzo[4], Ahmad Issa[4], Mehran Khodabandeh[4], Sara Omar[4], Jason Rolfe[4], Seyed Ali Saberali[4], Kally Singh[2], John Langlands[2], Dennis Brown[2], Jeffrey Bacha[2] & **Mads Daugaard**[1,2,3,5]

[1]*Vancouver Prostate Centre, Vancouver, BC, Canada*
[2]*Rakovina Therapeutics, Vancouver, BC, Canada*
[3]*M. H. Mohseni Institute of Urologic Sciences, Vancouver, BC, Canada*
[4]*Variational AI, Vancouver, BC, Canada*
[5]*Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada*

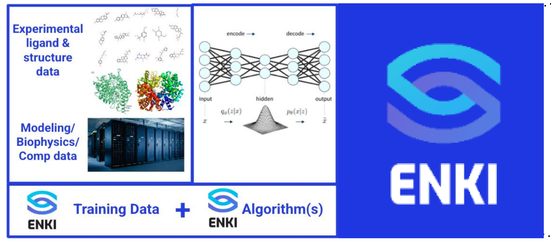
Introduction

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]. Traditional drug discovery methods are time-consuming and costly, necessitating innovative approaches. Harnessing the power of artificial intelligence (AI) for drug discovery and development can accelerate the process significantly.

Here, we describe the application of the Enki™ platform, a generative AI approach, to discover novel ATR inhibitors for the treatment of brain tumors.

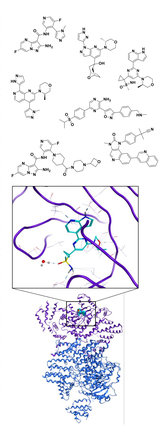
Enki™ Overview

Enki™ has been trained to "learn" what properties and characteristics make a suitable compound to target ATR and possess brain-penetrant properties. It has extrapolated in chemical space to discover a series of novel lead molecules.



Define desired molecule using preclinical target product profile (TPP).

Enki™ generates novel and diverse molecule structures that satisfy the TPP.



Pre-trained foundation model for small molecule discovery
Proprietary algorithm trained on 100's of millions of experimental and computational samples for 700+ drug targets.

Training → Lead Generation & Optimization

Drug Discovery Process

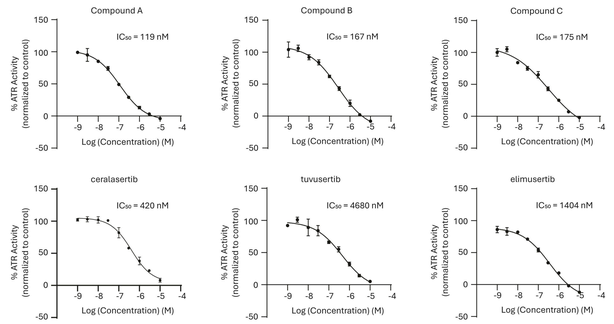
Enki™ Modeling
138 molecules predicted by Enki™ to satisfy the TPP

Chemical Synthesis
35 most promising compounds synthesized

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

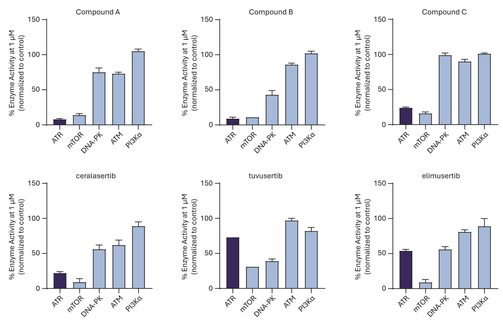
Hit Discovery
Multiple compounds inhibiting ATR activity by >50% at 1 µM in less than 1 year

Compound Potency



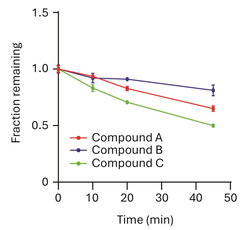
Compounds are equally or more potent inhibitors of recombinant ATR enzyme compared to reference compounds.

Selectivity vs. PIKK family kinases



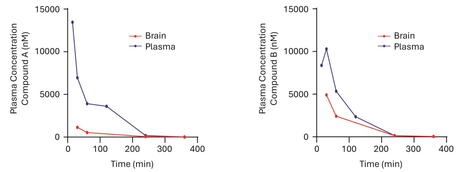
Compounds are equally selective against PIKK family enzymes as reference compounds, ceralasertib, tuvusertib and elimusertib.

Metabolic Stability



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

Mouse Pharmacokinetic Profiling



	Compound A	Compound B
Dose (mg/kg)	5	5
T½(min)	32.49	40.14
C_{max} (nM)	13456.14	10291.95
Plasma AUC (0-∞) (nM*min)	882610.9	832029.1
Brain AUC (0-∞) (nM*min)	98762.8	428018.8
Brain/Plasma (%)	11.2	51.4

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.

Compounds show varying levels of CNS penetrance.

References

1. *Upton DH, et al. Challenges and opportunities to penetrate the blood-brain barrier for brain cancer therapy. Theranostics. 2022 Jun 6;12(10):4734-4752. doi: 10.7150/thno.69682*
2. *Bhowmik A, et al. Blood brain barrier: a challenge for effectual therapy of brain tumors. Biomed Res Int. 2015;2015:320941. doi: 10.1155/2015/320941*
3. *Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. Nat Rev Mol Cell Biol. 2008 Aug;9(8):616-27. doi: 10.1038/nrm2450*
4. *Maréchal A, Zou L. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb Perspect Biol. 2013 Sep 1;5(9):a012716. doi: 10.1101/cshperspect.a012716*
5. *Yap TA, Tolcher AW, Plummer R, Mukker JK, Enderlin M, Hicking C, Grombacher T, Locatelli G, Szucs Z, Gounaris I, de Bono JS. First-in-Human Study of the Ataxia Telangiectasia and Rad3-Related (ATR) Inhibitor Tuvusertib (M1774) as Monotherapy in Patients with Solid Tumors. Clin Cancer Res. 2024 May 15;30(10):2057-2067. doi: 10.1158/1078-0432*
6. *Wang M, Ran X, Leung W, Kawale A, Saxena S, Ouyang J, Patel PS, Dong Y, Yin T, Shu J, Manguso RT, Lan L, Wang XF, Lawrence MS, Zou L. ATR inhibition induces synthetic lethality in mismatch repair-deficient cells and augments immunotherapy. Genes Dev. 2023 Oct 1;37(19-20):929-943. doi: 10.1101/gad.351084.123*
7. *Ngoi NYL, Pilié PG, McGrail DJ, Zimmermann M, Schlacher K, Yap TA. Targeting ATR in patients with cancer. Nat Rev Clin Oncol. 2024 Apr;21(4):278-293. doi: 10.1038/s41571-024-00863-5*
8. *Bright SJ, Manandhar M, Flint DB, Kolachina R, Ben Kacem M, Martinus DK, Turner BX, Qureshi I, McFadden CH, Marinello PC, Shaitelman SF, Sawakuchi GO. ATR inhibition radiosensitizes cells through augmented DNA damage and G2 cell cycle arrest abrogation. JCI Insight. 2024 Oct 8;9(19):e179599. doi: 10.1172/jci.insight.179599*

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 a curated list of small-molecule drug candidates meeting a specific target-product-profile *in silico*, specifically, novel ATR-specific inhibitors which are brain penetrating.
- The most promising candidates were synthesized for evaluation in the wet lab setting and the Enki™ platform predicted several compounds with proven potency against ATR.
- *In vitro* and *in vivo* characterization of efficacy is on going and Enki™ will be used for further lead optimization.

RAKOVINA THERAPEUTICS INC.
VARIATIONAL AI
THE UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER PROSTATE CENTRE
A UBC & VGH Centre of Excellence
M.H. Mohseni Institute of Urologic Sciences