Highly specific ATR inhibitor: targeted therapy for a broad spectrum of cancers

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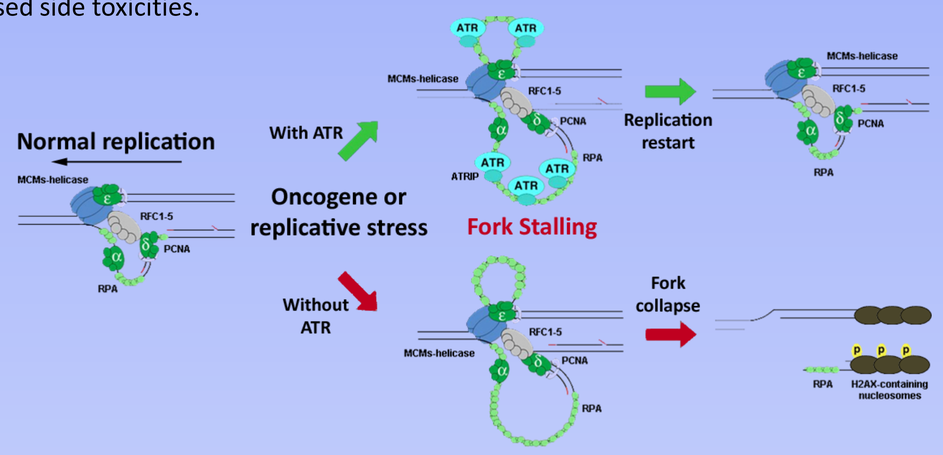
Abstract

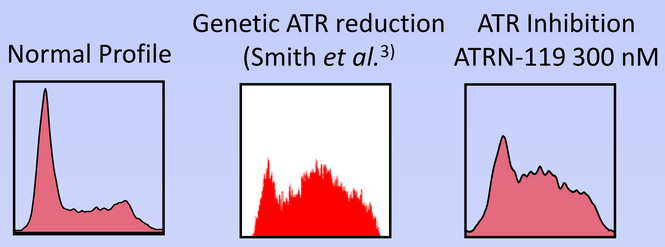
Ataxia Telangiectasia and Rad3-related (ATR) and Checkpoint Kinase 1 (CHK1) stabilize stalled replication forks and prevent their collapse into DNA double strand breaks (DSBs). Combining ATR suppression with over-expression of $HRAS^{G12V}$, $KRAS^{G12D}$ or MYC synergistically increases the formation of DSBs and causes synthetic lethality[1,2]. These findings define a genetic context and level of ATR pathway inhibition in which a broad variety of cancers displaying oncogenic stress can be targeted with minimal impact on normal tissue homeostasis.

We have recently developed and tested a novel series of small molecules that inhibit ATR kinase activity in the low nanomolar range in cell culture. This series exhibits exquisite selectivity for ATR with 800-fold lower inhibitory activity towards other DNA-damage activated PIK-related kinases. ATR inhibition by these compounds causes DSB formation during S-phase and suppresses aphidicolin-induced CHK1 S345 phosphorylation to basal levels at concentrations that are approximately 50-fold lower than other clinically applied ATR inhibitors. ADME profiling indicates that these compounds are stable *in vivo*. Preliminary data suggest these compounds are able to selectively promote lethality in cells expressing $HRAS^{G12V}$, while leaving wild-type unstressed cells relatively unaffected. In addition, single-dose examination of one ATR inhibitor (ATRN-119) indicates that the growth of many NCI 60 cell lines is substantially limited by ATR inhibition.

Many standard-of-care chemotherapies operate through abrogating DNA synthesis. However, these strategies, while effective, have undesirable side effects due to their ability to affect the proliferation of normal cells as much as cancer cells. The specificity of ATR pathway inhibition enables directed killing of replicating cells experiencing oncogenic stress. This targeted approach suggests that Atrin's highly specific ATR inhibitors will efficiently limit the progression of a wide variety of cancers with decreased side toxicities.

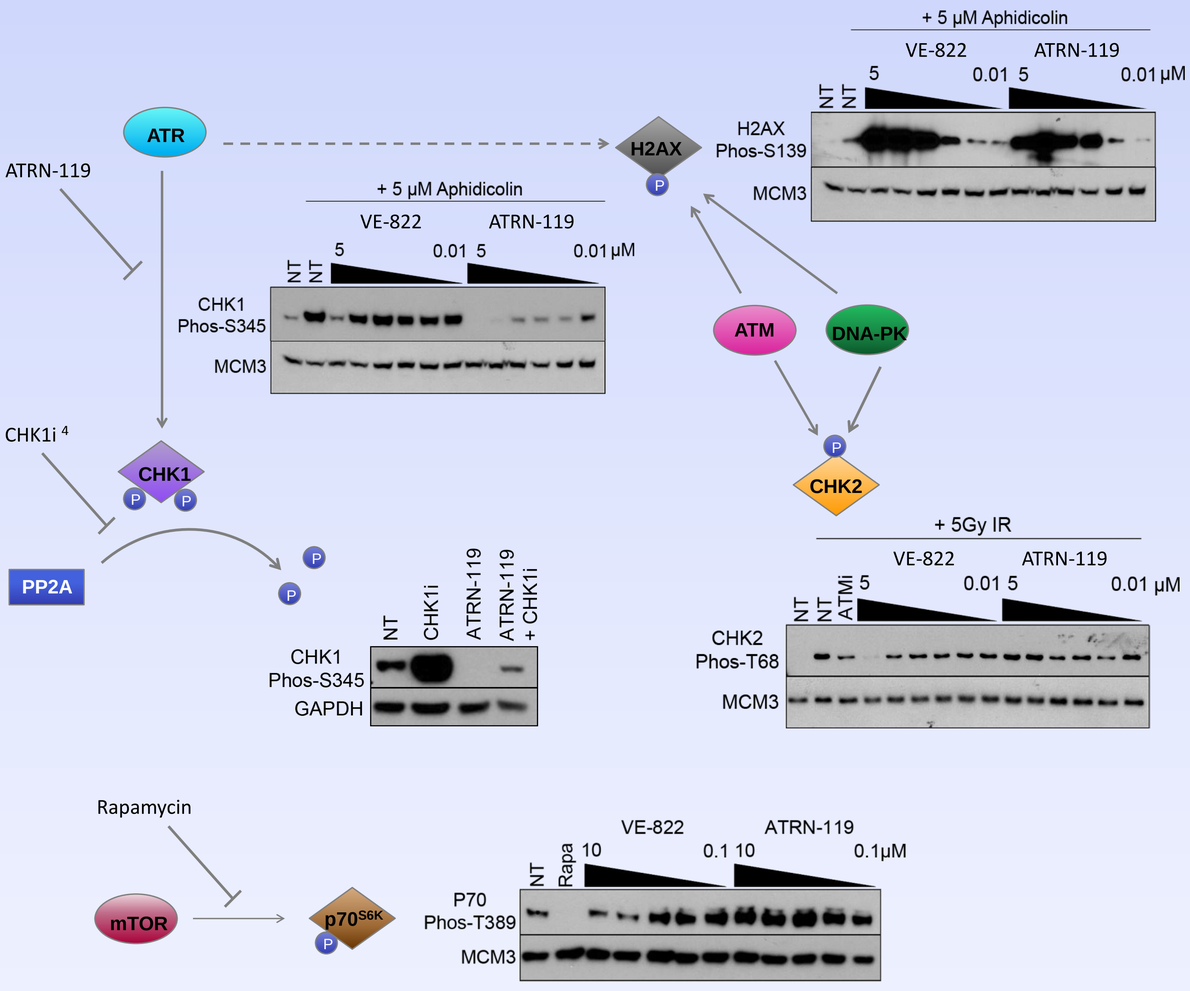


1. ATRN-119 specifically inhibits ATR with high efficacy



ATRN-119 causes stalling of cells in S-phase.

ATRN-119 inhibits ATR much more potently than VE-822, without inhibiting CHK1, or the related PIKKS ATM, DNA-PKcs or mTOR.



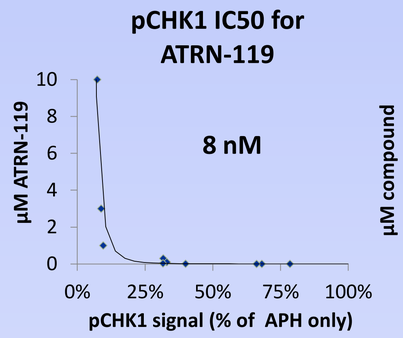
2. ATRN-119 chemical and activity profile

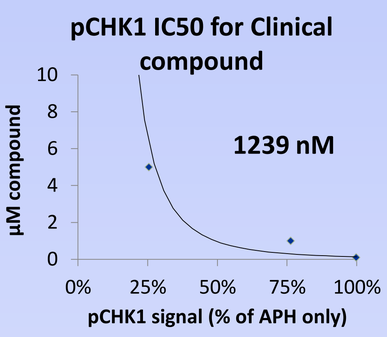
Chemical Details:
Purity Evaluation: 1H NMR, LC/MS, TLC (>97% purity)
CLogP: **1.9** $cLogD_{pH7.4}$: **0.40**

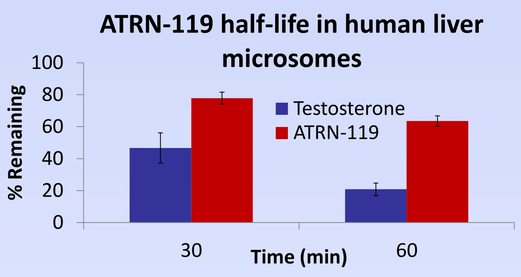
Cellular potency:
ATR IC_{50} (pCHK1) (h) = **8 nM**
Phenotypic MEC (h) = **100 nM**

Cellular kinase selectivity:
ATM;DNAPK IC_{50} (pCHK2) (h)= **> 5,000 nM**
ATM;DNAPK/ATR (pCHK2/pCHK1): **> 833**

Pharmaceutical properties:
Solubility pH 2.0: **>1 mg/mL***
Solubility in deionized water: **>1 mg/mL* (*solid)**
Microsomal Met Stab (t½, min): **>60 (m), >60 (h)**
Plasma Met Stab (% at 60 min): **100%±4 (m), 107%±8 (h)**
CYP Inh (IC_{50}): 3A4: **9 μM**



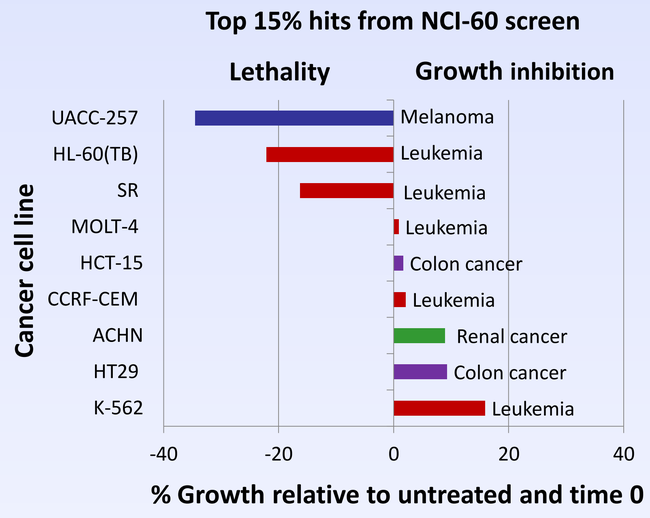




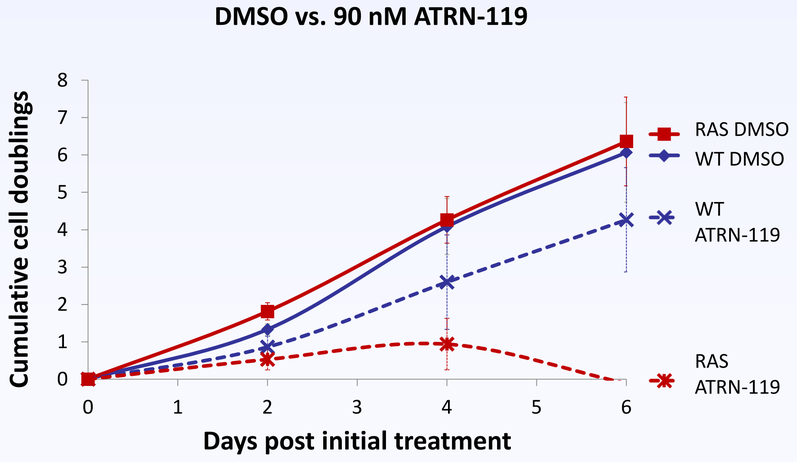
3. Effective single-dose monotherapy in NCI-60 cell lines

This initial screen of the NCI-60 cell lines suggests that ATRN-119 may be promising as a monotherapy for the treatment of a broad spectrum of cancers.

Follow-up studies with five doses are being performed with the NCI-60 panel to give a measure of the drug's LC50.



4. ATRN-119 selectively kills $HRAS^{G12V}$ expressing cells



ATRN-119 is able to inhibit proliferation and decrease survival of cells undergoing oncogenic stress induced by the $HRAS^{G12V}$ mutation.

Conclusions and future directions

- ATRN-119 is able to inhibit ATR with higher specificity and efficacy than a current clinical ATR inhibitor.
- ATRN-119 has favorable ADME , toxicity and pharmaceutical properties.
- ATRN-119 shows promise as a monotherapy in range of cancer cell lines, displaying strong antiproliferative and lethal activity in multiple lines.
- The synthetic lethal interaction between ATRN-119 and human cell lines harboring alterations in other oncogenes or genes involved in homologous recombination will be explored
- The antiproliferative effect of ATRN-119 on cell lines identified from the NCI-60 screen will be examined further
- *In vivo* studies are planned to assess the efficacy of ATRN-119 as a monotherapy in mouse AML

References

1. Schoppy DW *et al.* (2012). Oncogenic stress sensitizes murine cancers to hypomorphic suppression of ATR. *Journal of Clinical Investigation* **122**(1): 241-252.

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3. Smith KD *et al.* (2009). Tim-Tipin dysfunction creates an indispensible reliance on the ATR-Chk1 pathway for continued DNA synthesis. *The Journal of Cell Biology* **187**(1): 15-23.

4. Leung-Pineda V *et al.* (2006). Phosphorylation of Chk1 by ATR is antagonized by a Chk1-regulated protein phosphatase 2A circuit. *Molecular cell biology* **26**(20): 7529-7538.

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