CDK2 Inhibition Demonstrates Synthetic Lethality in SCLC through Apoptotic Induction

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Abstract 34905

Background

- Small Cell Lung Cancer (SCLC) is a fast-growing malignant neoplasm representing approximately 15% of lung cancers and typically genetically defined by the absence or severe disruption of both the retinoblastoma gene RB1 and the tumor suppressor TP53.
- SCLC remains one of the deadliest cancers with a 5-year survival rate in the single digit percentages.
- Standard of Care for SCLC remains chemotherapy, which typically works well initially but in nearly all cases leads to resistance and relapse. There are currently no approved targeted therapies for SCLC.
- ARTS-021 is a highly selective and potent CDK2 inhibitor, with favorable drug-like properties
- Phase 1/2 clinical trial ARTS-021-001 is now ongoing in US(NCT05867251)

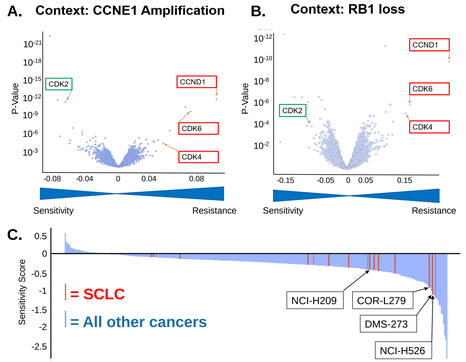
Results

Table 1. ARTS-021 Is a Potent and Selective CDK2 inhibitor

Enzyme activity IC_{50} (nM) [a]						Kinome S(10)
CDK2	CDK1	CDK4	CDK6	CDK7	CDK9	
1.4	942	477	1,237	2,834	7,440	0.022
Nano Bret Assay IC_{50} (nM) [b]						
CDK2	CDK1	CDK4	CDK6	CDK7	CDK9	
0.22*	107.4	788.9	412	5073	>10,000	
Cellular activity IC_{50} (nM) [c]						
pRb (S780) (CDK2 Cell)		pNPM (T199) (CDK1 Cell)		pRb (S780) (CDK4/6 Cell)		RNAP2 S2 (CDK9 Cell)
44.9		1342.8		9241.9		>10,000

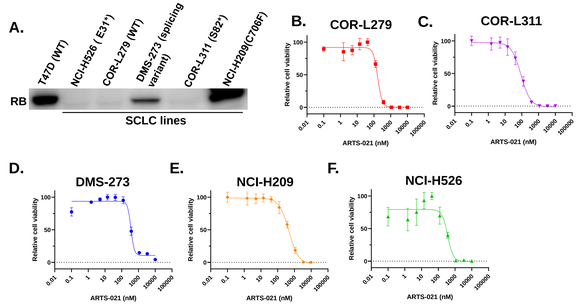
a: Enzymatic assay: Caliper Assay; ATP concentration used at 1mM; CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 are in complex with cyclin B1, Cyclin E1, Cyclin D1, Cyclin D3, Cyclin H/MAT1and Cyclin T1 respectively. Kinome S(10): fraction of kinases with <10 percentage of control at 1uM compound among 403 non-mutant kinases tested, Eruofins Discovery KinomeScan; b: HEK-293T cells were transfected with canonical CDK/cyclin pairs in the enzyme assay and treated with compound and a tracer for 1 hours before measurements were taken; c: Cellular assay: pRb serine 780 was assessed in KLE and COV504 cells for CDK2 and CDK4/6 cellular activity respectively; pNPM (nucleophosmin) threonine 199 was assessed in mitotic arrested Hela cells for cellular CDK1 activity; pRNAP2 (RNA polymerase II) serine 2 was assessed in MV411 cells for CDK9 cellular activity. *: below low limit of quantification (<0.5nM).

Figure 1: *RB1* Loss SCLC Cells are Sensitive to CDK2 Depletion



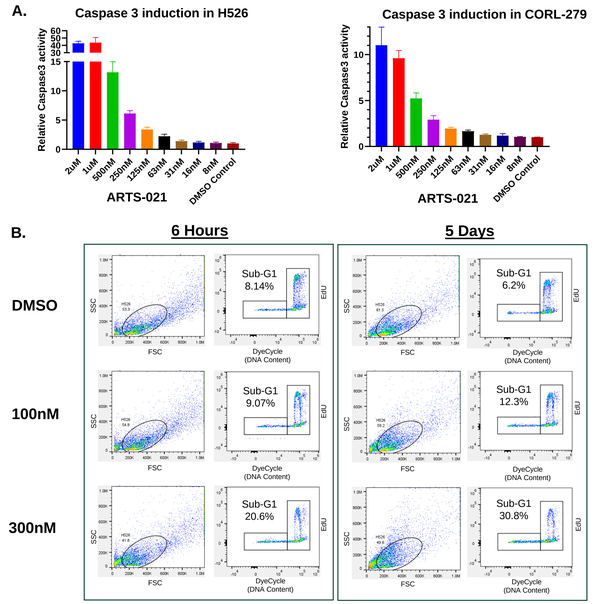
(A) Volcano plot of gene depletion sensitivities in the context of CCNE1 Amplification. (B) Volcano plot of gene depletion sensitivities in the context of *RB1* loss of function mutation. (C) A waterfall plot of cancer cell line susceptibility to CDK2 RNAi. All data from these figures was derived from DepMap.org stored databases (DEMETER2, Achilles+DRIVE+Marcotte).

Figure 2. SCLC Cells with Aberrant RB are Sensitive to ARTS-021



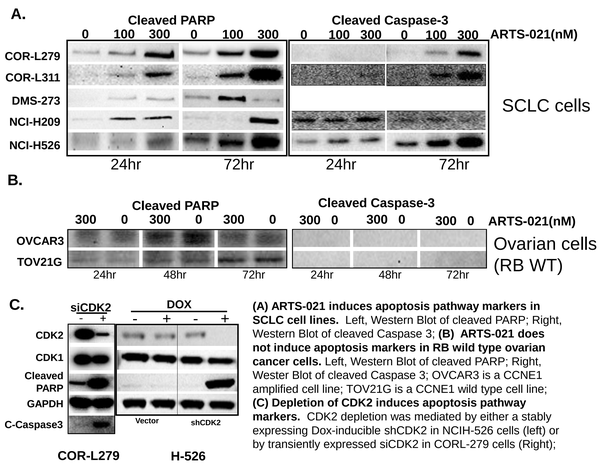
(A) Rb protein level in five SCLC cell lines. Breast Cancer cell line T47D is used as a positive control; **(B-F) Dose response curves of ARTS-021 in five SCLC lines measured by CellTiter-Glo (B)** COR-L279, EC50=166.2nM **(C)** COR-L311, EC50=88.2nM, **(D)** DMS-273, EC50=353.5nM, **(E)** NCI-H209, EC50=464.8nM, and **(F)** NCI-H526, EC50=353.5nM.

Figure 3. ARTS-021 Induces Apoptosis in SCLC Cells



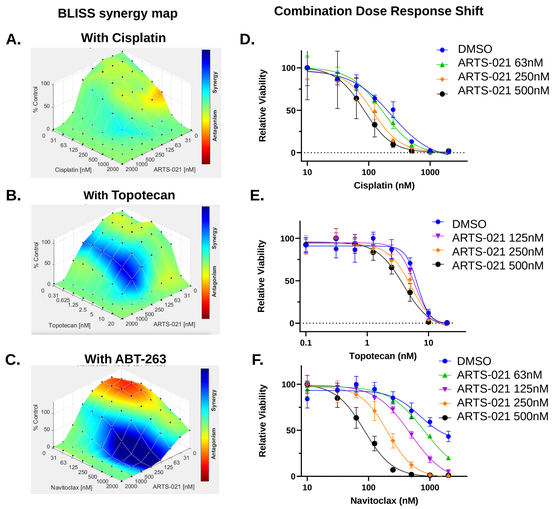
(A) Induction of Caspase-3 activity by ARTS-021. Relative Caspase 3 activity was measured by CaspaseGlo 3/7 and normalized to CTG to correct the cell number variations; **(B) ARTS-021 increases Sub-G1 cell population in NCIH-526 cells.** Cells were treated with indicated amount of ARTS-021 for 6 hours (left panel) or 5 days (right panel) before one-hour EdU labeling, followed by fixation and EdU click-labelling, and staining of total DNA content. Cells were then run on an Attune NXT flow cytometer, and cell cycle analysis performed using FlowJo.

Figure 4. ARTS-021 Induces Apoptosis Pathway Markers in SCLC Cells



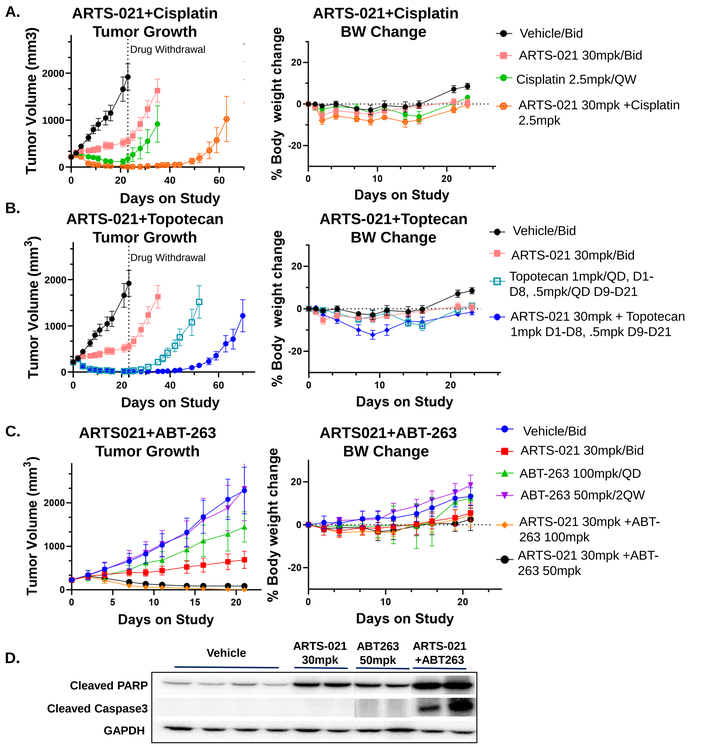
(A) ARTS-021 induces apoptosis pathway markers in SCLC cell lines. Left, Western Blot of cleaved PARP; Right, Western Blot of cleaved Caspase 3; **(B) ARTS-021 does not induce apoptosis markers in RB wild type ovarian cancer cells.** Left, Western Blot of cleaved PARP; Right, Wester Blot of cleaved Caspase 3; OVCAR3 is a CCNE1 amplified cell line; TOV21G is a CCNE1 wild type cell line; **(C) Depletion of CDK2 induces apoptosis pathway markers.** CDK2 depletion was mediated by either a stably expressing Dox-inducible shCDK2 in NCIH-526 cells (left) or by transiently expressed siCDK2 in CORL-279 cells (Right);

Figure 5. ARTS-021 Enhances Chemotherapy Activity and Synergizes with BCL-xL/BCL2 Dual Inhibitor ABT-263 in H526 Cells



(A-C) Contour plots showing combinatorial synergy Calculated by the Combenefit software package using N=3 data from 96-well plates combining the ARTS-021 inhibitor with Cisplatin in (A), Topotecan in (B) and ABT-263(Navitoclax) in (C); **(D-E) Dose response curve of Cisplatin (D), Topotecan (E) and ABT-263(Navitoclax) (F) in the presence or absence of indicated concentration of ARTS-021.**

Figure 6. ARTS-021 Inhibited Tumor Growth as a Single Agent or in Combination with Cisplatin, Topotecan and ABT-263 in H526 Xenograft Tumor Model



(A) In vivo efficacy of ARTS-021+Cisplatin. Nude mice(N=6/cohort) inoculated SC with NCI-H526 cells (1x107); ARTS-021 was dosed orally; Cisplatin was dosed via intraperitoneal injection; **(B) In vivo efficacy of ARTS-021+Topotecan.** Topotecan was dosed via intraperitoneal injection at 1mg/kg daily from Day1-8 and reduced to 0.5mg/kg daily from Day 9-22 due to body weight loss; **(C) In vivo efficacy of ARTS-021+ABT-263(Navitoclax).** ABT-263 was dosed via oral gavage, study on going; **(D) Induction of apoptosis markers in tumor samples.** Lysates from tumors treated with either vehicle, single agent ARTS-021, ABT-263 or ARTS-021+ABT-263 for 48 hours at indicated doses were assessed for cleaved PARP and cleaved Caspase-3.

Summary

- Small cell lung cancer cells with altered RB are sensitive to CDK2 gene depletion.
- ARTS-021 treatment leads to induction of apoptotic markers and apoptosis in SCLC cell lines.
- ARTS-021 combines well with standard of care chemotherapeutics, demonstrating improved survival in a SCLC cell line mouse xenograft model.
- ARTS-021 synergizes with BCL-xL/BCL2 dual inhibitor ABT-263(Navitoclax) both in vitro and in vivo in a SCLC cell line model.
- Taken together, our data suggest ARTS-021 may have the potential to be a combination partner for treating SCLC