ARTS-021 is a Potent and Selective CDK2 Inhibitor That Demonstrates Anti-cancer Activity in Preclinical Cancer Models With CCNE1 Amplification

Jiaqi Liang [1], Dai Cheng[1], Yali Guo[1], Xiaobin Zhang[2], Nathan Schomer[2], Xipan Liu[1], Jia Fu[1], Lin Xu[1], Cong Zhu[2], Qiang Ding[1], Wen Xiong[1], Tao Ji[2], Fang Li[2], Yaoyu Chen[2], Qing Sheng[2]
[1]Allorion Therapeutics, Guangzhou, China, [2]Allorion Therapeutics, Natick, MA

2568

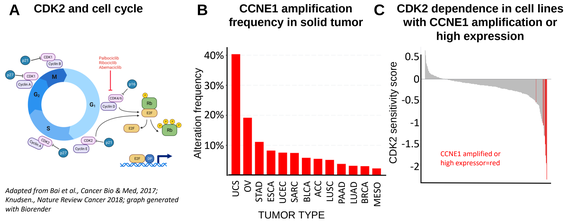
Allorion Therapeutics

Abstract

- Cyclin E1 amplification is prevalent in cancers with high unmet medical need such as high grade serous ovarian cancer, stomach cancer and esophageal cancer.
- Cancer cell lines with high expression level of cyclin E1 gene exhibits profound sensitivity to CDK2 gene depletion, suggesting CDK2 selective inhibitors have the potential to treat patients harboring Cyclin E1 alterations.
- Here we report the development and preclinical characterization of ARTS-021, an orally bioavailable small molecule CDK2 selective inhibitor.
- ARTS-021 demonstrates potent CDK2 inhibition and selectivity against other CDK family members
- ARTS-021 inhibits Rb phosphorylation and blocks G1/S transition, leading to cell growth arrest specifically in CCNE1 amplified cell lines.
- Twice daily ARTS-021 administration leads to tumor stasis in CCNE1 amplified but not in wild type xenograft models.
- ARTS-021 is a promising CDK2 selective inhibitor with strong potential towards the development of effective therapies for CCNE1 altered cancer.

Results

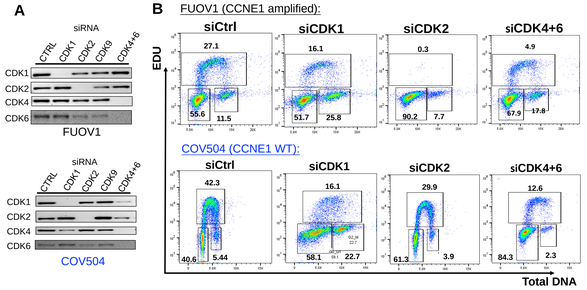
Figure 1. CCNE1 amplification frequently occurs in cancer and renders tumor cells CDK2 dependent



Adapted from Bai et al., Cancer Bio & Med, 2017; Knudsen., Nature Review Cancer 2018; graph generated with Biorender

(A) CDK2 regulates G1/S transition in cell cycle; (B) TCGA CCNE1 amplification frequency in different cancer type, using CBIO-analysis. Cut off>=2%; UCS: Uterine carcinosarcoma; OV: Ovarian serous cystadenocarcinoma; STAD: Stomach adenocarcinoma; ESCA: Esophageal carcinoma; UCEC: Uterine corpus endometrial carcinoma; SARC: Sarcoma; BLCA: Bladder urothelial carcinoma; ACC: Adrenocortical carcinoma; LUSC: Lung squamous cell carcinoma; PAAD: Pancreatic adenocarcinoma; LUAD: Lung adenocarcinoma; BRCA: Breast invasive carcinoma; MESO: Mesothelioma; **(C) CDK2 RNAi sensitivity score replotted from Project DRIVE**. Cell lines with CCNE1 amplification (copy number >4) and high expression (TPM>100) are plotted in red

Figure 2. Loss of CDK2 induces G1 arrest in CCNE1 amplified ovarian cancer cell line



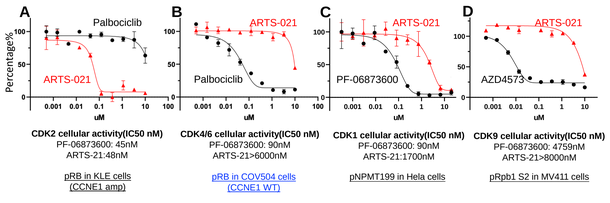
(A) Impact of CDK targeting siRNAs on cell cycle distribution in CCNE1 amplified FUOV1 cells. Western blots indicate successful knock down of different CDKs with corresponding siRNAs. Percentage of cells in different cell cycle stage was assessed by EDU incorporation (EDU-Alexa488) and total DNA content (FxCycle-violet) using flowcytometry 3days after siRNA infection; **(B) Impact of CDK targeting siRNAs on cell cycle distribution in CCNE1 wild type (WT) COV504 cells.**

Table 1. ARTS-021 is a potent and selective CDK2 inhibitor

Compound	Enzyme activity IC_{50} (nM)						Kinome S(10)
	CDK2	CDK1	CDK4	CDK6	CDK7	CDK9	
PF-06873600	1.5	36	13	31	1,823	2,735	NA
ARTS-021	**1.4**	942	477	1,237	2,834	7,440	0.022

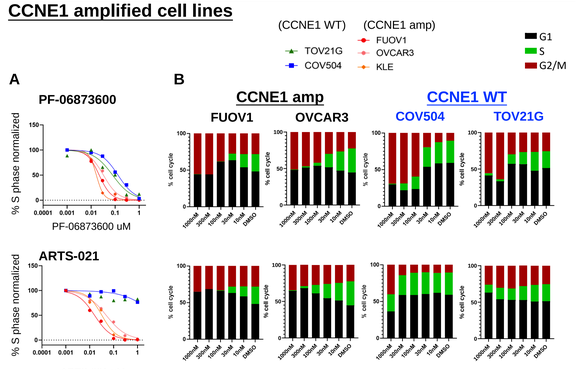
Enzymatic assay: Caliper Assay; ATP concentration used at 1mM; CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 are in complex with cyclin B1, Cyclin E1, Cyclin D1, Cyclin D3, Cyclin H/MAT1and Cyclin T1 respectively. Kinome S(10): fraction of kinases with <10 percentage of control at 1uM ARTS-021 among 403 non-mutant kinases tested, Eruofins Discovery KinomeScan

Figure 3. ARTS-021 potently and selectively inhibits cellular CDK2 activity



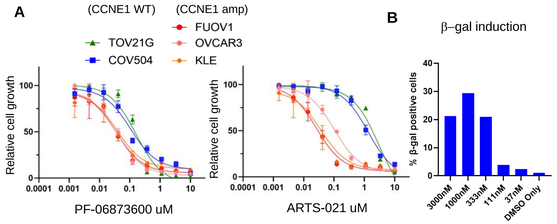
(A),(B) ARTS-021 selectively inhibited Rb780 phosphorylation in CCNE1 amplified KLE cell line (A) but not in CCNE1 WT COV504 cell line (B). Rb S780 phosphorylation was measured by HTRF. CDK4/6 inhibitor Palbociclib was used as a reference compound; **(C) ARTS-021 shows improved CDK1 selectivity over PF06873600, a clinical stage CDK2/4/6 inhibitor.** Cellular CDK1 activity was assessed by the percentage of inhibition of NPM1 T199 phosphorylation in nocodazole arrested Hela cells via high content imaging; **(D) ARTS-021 has minimum cellular CDK9 inhibitory activity.** Cellular CDK9 activity was measured by decrease of Rpb1 Ser2 phosphorylation in MV411 cells using Cisbio CDK9 HTRF assay. AZD4573, a clinical stage CDK9 inhibitor was used as a reference compound.

Figure 4. ARTS-021 blocks S-phase entry and induces G1 arrest in CCNE1 amplified cell lines



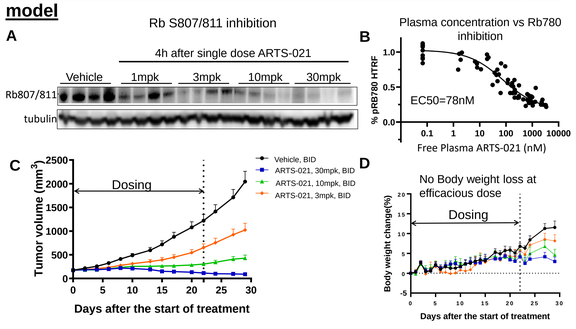
(A) S phase inhibition. %S phase was assessed by EDU incorporation (2h) after 48h drug treatment and normalized to DMSO treated samples; **(B) cell cycle profile.** Percentage of cells in different cell cycle stage was assessed by EDU incorporation and total DNA content (FxCycle) using flowcytometry 48h after drug treatment.

Figure 5. ARTS-021 inhibits cell growth in CCNE1 amplified cells and induces expression of senescence marker beta-galactose



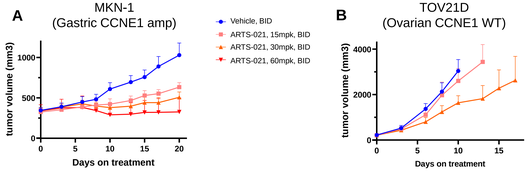
(A) ARTS-021 selectively inhibited cell growth of CCNE11 amplified cells. Cell growth was assessed by Cyquant after 7 days of drug treatment in various celllines; **(B) Induction of senescence associated marker β–gal by ARTS-021 in CCNE1 amplified OVCAR3 cells.** β-galactosidase was detected using the CellEvent® Senescence Green Flow Cytometry Assay Kit by Invitrogen 5 days after cells treated with ARTS-021 at indicated concertation.

Figure 6. ARTS-021 induces dose dependent pRB inhibition and inhibits tumor growth in CCNE1 amplified OVCAR3 cell xenograft model



(A) Rb S807/811 inhibition. Lysates from tumors treated with single dose ARTS-021 for 4hours at indicated doses were assessed for Rb S807/811 phosphorylation; **(B) Plasma exposure of ARTS-021 and PD modulation.** Unbound ARTS-021 plasma concentration was used in the figure; ; **(C) Antitumor activity in OVCAR3 xenograft.** Mice inoculated SC with OVCAR3 ($10x10^6$); **(D) Body weight change in OVCAR3 xenograft.**

Figure 7. ARTS-021 displays anti-tumor activity in a CCNE1 amplified gastric cell xenograft but is inactive in a CCNE1 WT cell line model



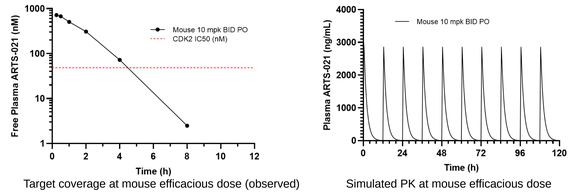
(A) Anti-tumor activity in MKN-1 xenograft. mice inoculated SC with MKN-1 ($10x10^6$); **(B) lack of anti-tumor activity in TOV21D xenograft.** mice inoculated SC with TOV21D ($10x10^6$).

Table 2. The DMPK and safety profiles of ARTS-021

Category	Assay	ARTS-021
in vitro ADME	Caco-2 permeability, $P_{app\ (A-B)}$ (10^{-6} cm/s)/$P_{app\ (B-A)}$ (10^{-6} cm/s)	2.19/31.6
	Solubility pH1.6/7.4 (μM)	122/89.55
	Plasma protein binding (H/M)	93.2/93.2%
	Hepatocyte clearance (H/M) (μL/min/10^6 cells)	1.97/1.39
in vivo PK	Mouse (10 mg/kg) $AUC_{0-\infty}$, C_{max}	8040 h*ng/mL, 1660 ng/mL
Safety	CYP inhibition IC_{50} (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4)	≥ 10 μM
	hERG IC_{50}	> 30 μM

H: human, M: mouse

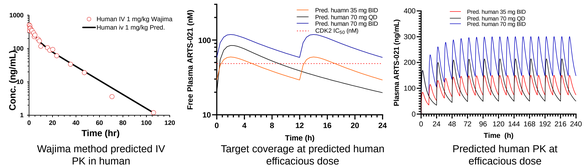
Figure 8. ARTS-021 Mouse PK at efficacious dose of 10 mg/kg BID



Target coverage at mouse efficacious dose (observed) | Simulated PK at mouse efficacious dose

- The target coverage for ARTS-021 is approximately 9 hours daily for 10 mg/kg BID in mouse

Figure 9. Predicted ARTS-021 efficacious dose in human



Wajima method predicted IV PK in human | Target coverage at predicted human efficacious dose | Predicted human PK at efficacious dose

Target coverage at efficacious dose:

- Human PK parameters was predicted by allometric scaling
- Human IV PK profile was projected by wajima method
- Oral bioavailability in human was estimated by average of bioavailability in preclinical species
- The predicted human efficacious dose of 35 mg BID or 70 mg QD meets the target coverage required for efficacy in mouse model, where the steady state free trough concentrations exceed IC_{50} at 70 mg BID

Summary

- ARTS-021 is a potent and selective CDK2 inhibitor that specifically targeting CCNE1-aberrant cancer cells.
- In CCNE1 amplified cells, ATRS-021 induces senescence maker, durably arrests cells at G1/S and inhibits cancer cell proliferation.
- ARTS-021 also induces dose dependent pRB inhibition and inhibits tumor growth in CCNE1 amplified OVCAR3 cell line xenograft model.
- The predicted human efficacious dose of 35 mg BID or 70 mg QD meets the target coverage required for efficacy in mouse model, where the steady state free trough concentrations exceed IC_{50} at 70 mg BID.