The Anti-tumor Activity of CDK2 Inhibition Alone or in Combination with Other Anti-Cancer Agents in Human Cancers

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Background

- Amplification of *CCNE1* gene is prevalent in multiple tumor types, particularly in high-grade ovarian, uterine and gastric cancer and CDK2 activity is important for survival of CCNE1 aberrant cancer cells
- CDK2 activity is also associated with potential resistance to targeted therapies such as CDK4/6 inhibitors or KRAS G12C inhibitors
- 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)
- Here we investigate the efficacy of ARTS-021 as a single agent in CCNE1 aberrant tumor models and in combination with other targeted therapies in ER$^+$/HER2$^-$ breast and KRAS G12C mutant lung tumor models

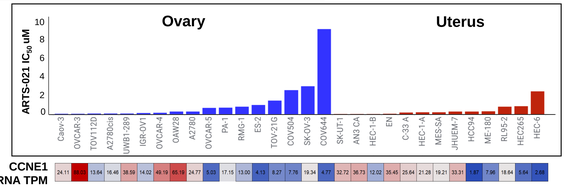
Results

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

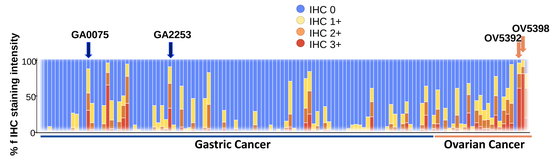
a: Enzymatic assay: Caliper Assay; ATP concentration used at 1mM; CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 are in complex with cyclin B1, Cyclin E1, Cyclin D1, Cyclin D3, Cyclin H/MAT1and Cyclin T1 respectively. Kinome S(10): fraction of kinases with <10 percentage of control at 1uM compound among 403 non-mutant kinases tested, Eurofins Discovery KinomeScan; b: HEK-293T cells were transfected with canonical CDK/cyclin pairs as in the enzyme assay and treated with compound and a tracer for 1 hour 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. ARTS-021 Cell Growth Inhibition Activity in a Panel of 30 Ovarian and Uterine Cancer Cell Lines



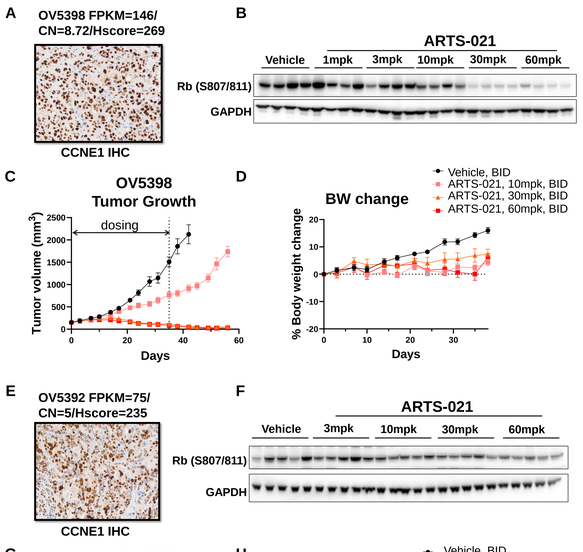
Cell growth was assessed by Cyquant after 7 days of drug treatment in 30 ovarian or uterine cancer cell lines. CCNE1 expression level is expressed as transcript per million (TPM) and colored from low to high:

Figure 2. Tumor CCNE1 Expression Assessed by IHC in Ovarian and Gastric Cancer PDX TMA



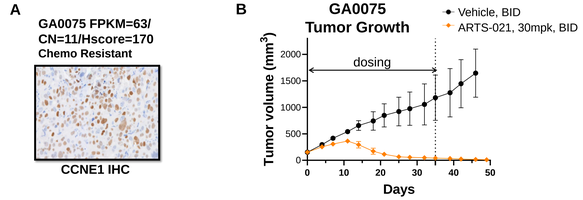
CCNE1 immunohistochemistry (IHC) staining intensity in ovarian and gastric PDX tissue micro array (TMA) containing 93 different gastric and ovarian cancer tissues. TMAs were purchased from CrownBio. Arrow heads represent Gastric and Ovarian PDX models selected for efficacy studies. All models display high percentage of CCNE1 IHC 2+ and 3+ staining.

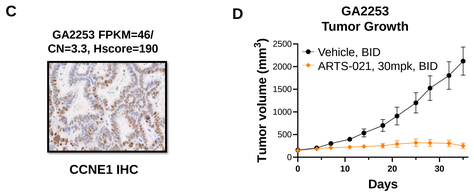
Figure 3. ARTS-021 Potently Inhibits Tumor Growth in Two CCNE1-amplified Ovarian Cancer PDX Models



(A)-(D) model OV5398; (E)-(H) model OV5392; (A and E) CCNE1 IHC of OV5398 and OV5392; (B and F) Dose dependent inhibition of Rb 807/811 phosphorylation. Lysates from tumors treated with single dose ARTS-021 for 4 hours at indicated doses were assessed for Rb S807/811 phosphorylation; **(C and G) Antitumor activity in OV5398 and OV5392 PDX models. (D and H) Relative body weight change in OV5398 and OV5392 xenografts.**

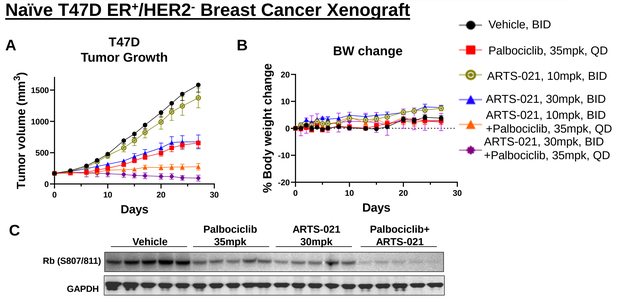
Figure 4. ARTS-021 Potently Inhibits Tumor Growth in Two Gastric Cancer PDX Models with High Cyclin E1 Protein Expression





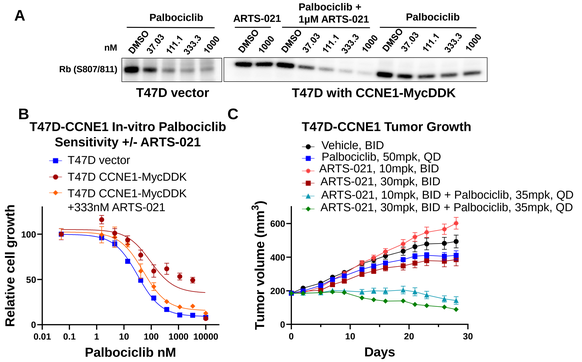
(A, C) CCNE1 IHC of GA0075 and GA2253; (B, D) Anti-tumor activity in GA0075 and GA2253 PDX models. (E) Dose dependent inhibition of Rb 807/811 phosphorylation in GA2253. Lysates from tumor treated with single dose ARTS-021 for 4 hours at indicated doses were assessed for Rb S807/811 phosphorylation.

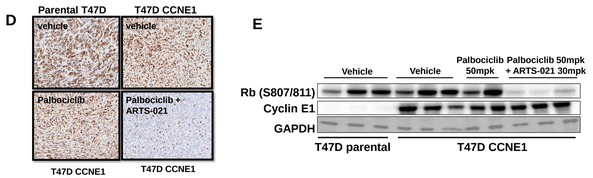
Figure 5. ARTS-021 Enhances the Activity of CDK4/6 Inhibitors in Naïve T47D ER$^+$/HER2$^-$ Breast Cancer Xenograft



(A) Anti-tumor activity in T47D xenograft. Mice inoculated SC with T47D (1x10^7), that were passed in vivo twice; **(B) Relative body weight change in T47D xenografts; (C) Inhibition of Rb S807/811 phosphorylation**. Lysates from tumors treated with either Palbociclib, ARTS-021 or ARTS-021+Palbociclib for 4 hours at indicated doses were assessed for Rb S807/811 phosphorylation.

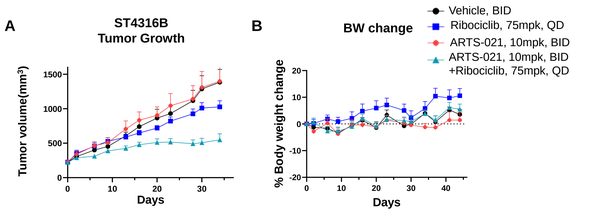
Figure 6. ARTS-021 Re-sensitizes Palbociclib in ER$^+$HER2$^-$ Breast Cancer T47D Over-expressing CCNE1





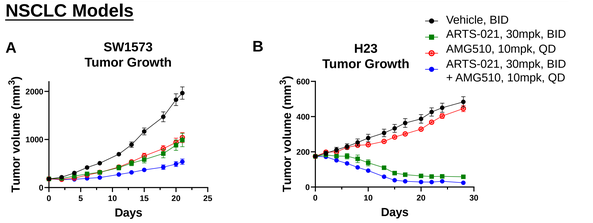
(A) pRb modulation in T47D cells carrying either vector or Myc-CCNE1. Cells were treated with indicated compounds for 24 hours before lysates were collected; **(B) Dose dependent cell growth inhibition by Palbociclib in either parental or CCNE1 over-expressing T47D in the presence or absence of ARTS-021.** Cell growth was assessed by 7-day Cyquant assay; **(C) Anti-tumor activity in CCNE1 over-expressing T47D xenografts.** Mice inoculated SC with T47D-CCNE1 cells (1x10^7); **(D) Rb 807/811 IHC of tumors collected from PD study described in (E); (E) In vivo Rb 807/811 inhibition.** Lysates from vehicle treated parental T47D tumor or T47D-CCNE1 tumors treated with either vehicle, Palbociclib or ARTS-021+Palbociclib for 4 hours at indicated doses were assessed for Rb S807/811 phosphorylation.

Figure 7. ARTS-021 Re-sensitizes Ribociclib in ST4316B, a CDK4/6 Inhibitor Resistant ER$^+$/HER2$^-$ Breast Cancer PDX Model



(A) Antitumor activity in PDX model ST4316B. ST4316B was derived from a patient that progressed on 1L Palbociclib+ Fulvestrant and 2L Abemaciclib+Fulvestrant; **(B) Relative body weight change in ST4316B.**

Figure 8. In-vivo Anti-tumor Activity of ARTS-021 in KRAS G12C NSCLC Models



(A) Anti-tumor activity of ARTS-021 in SW1573 lung xenografts containing the KRAS G12C mutation. Mice inoculated SC with SW1573 cells (1x10^7); **(B) Anti-tumor activity of ARTS-021 in H23 lung xenografts containing the KRAS G12C mutation.** Mice inoculated SC with H23 cells (1x10^7).

Summary

- ARTS-021 is a potent CDK2 inhibitor that selectively inhibited growth of cells with high CCNE1 expression in a collection of ovarian and uterine cancer cell lines
- ATRS-021 displayed strong single agent anti-tumor activity in patient derived ovarian and gastric xenograft models with high CCNE1 protein expression
- ARTS-021 not only enhanced the anti-tumor activity of CDK4/6 inhibitors in naïve and CCNE1 over-expressing T47D models, but also in ST4316B, a patient derived CDK4/6 inhibitor-resistant xenograft model
- ARTS-021 exhibited activity as a single agent and augmented the efficacy of AMG510 in the KRAS G12C mutant NSCLC cell line xenograft models
- These findings suggest that ARTS-021 may have broader applications beyond CCNE1-amplified cancers and can be explored in combination with various treatment modalities