ARTS-023: A Potent and Selective CDK4 Inhibitor Demonstrating Anti-Tumor Activity in Preclinical ER+ Breast Cancer Models

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Abstract # 3026

Background

- Cyclin-dependent kinases (CDKs), particularly CDK4 and CDK6, regulate the G1-to-S phase transition, a key checkpoint in cell cycle progression. Dysregulation of the CDK4/6–cyclin D–RB–E2F pathway drives uncontrolled proliferation, making it a prime target in cancer therapy
- Although CDK4/6 inhibitors are approved for HR+/HER2- breast cancers, relapse and toxicities, particularly neutropenia, remain major clinical challenges
- Data suggests that hematopoietic and lymphoid cells depend on CDK6, while breast cancer cells rely largely on CDK4. This supports the use of CDK4-selective inhibitors to reduce hematological toxicity, enhance CDK4 inhibition, and improve HR+/HER2- breast cancer outcomes
- We present here preclinical data supporting the development of a novel CDK4-selective inhibitor, ARTS-023, for patients with ER+ breast cancer

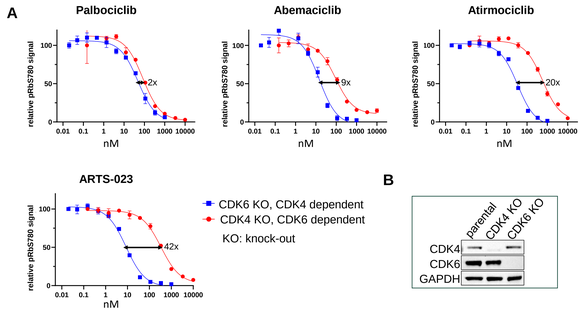
Results

Table 1. ARTS-023 Is a Potent and Selective CDK4 Inhibitor, with Higher CDK4/CDK6 Selectivity Than Atirmociclib*

Enzymatic Assay IC_{50} (nM)[a]						
Compound	**CDK4**	**CDK6**	**CDK1**	**CDK2**	**CDK7**	**CDK9**
Atirmociclib	**5.26**	154 **(29x)**	755	414	1,431	164
ARTS-023	**0.68**	40 **(59x)**	248	127	1,087	89
NanoBret Assay IC_{50} (nM)[b]						
Compound	**CDK4**	**CDK6**	**CDK1**	**CDK2**	**CDK7**	**CDK9**
Atirmociclib	**3.833**	527 **(137x)**	6,014	5,290	>10,000	>10,000
ARTS-023	**0.066**	70 **(1068x)**	442	1,310	>10,000	6,740
Cellular Activity IC_{50} (nM)[c]						
Compound	**pRb (S780) (CDK4 Cell)**	**pRb (S780) (CDK6 Cell)**	**pRb (S780) (CDK2 Cell)**	**pNPM (T199) (CDK1 Cell)**	**pRNAP2 (S2) (CDK9 Cell)**	
Atirmociclib	**30.4**	590 **(20x)**	4,427	>10,000	>10,000	
ARTS-023	**6.9**	288 **(42x)**	>10,000	5,704	>10,000	

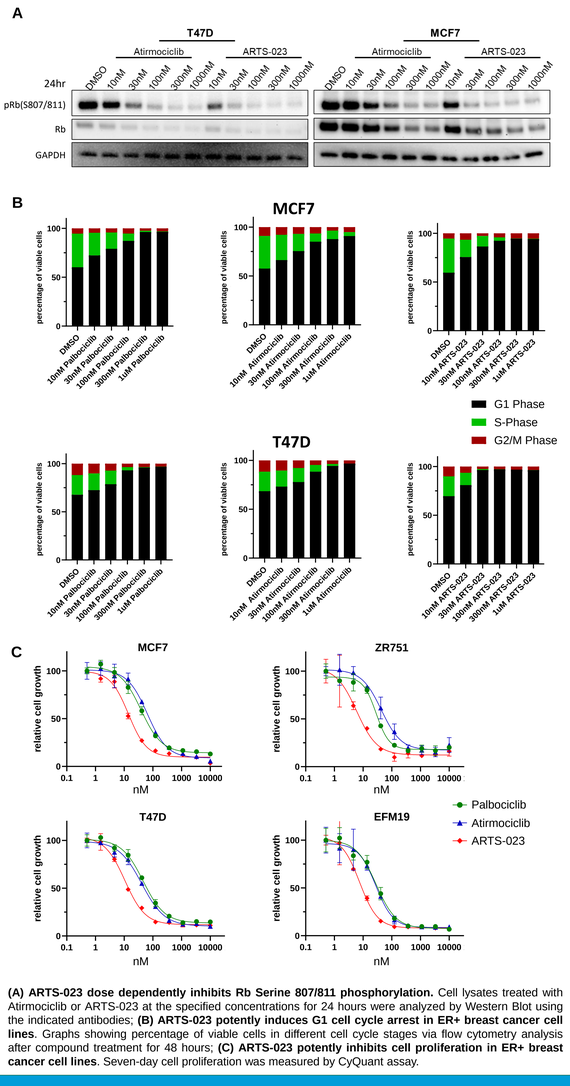
a: Caliper Assay; ATP concentration used at Km of each enzyme; CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 are in complex with Cyclin B1, Cyclin E1, Cyclin D1, Cyclin D3, Cyclin H/MAT1and Cyclin T1, respectively; number in parentheses is fold of activity compared to CDK4; b: HEK-293T cells were transfected with canonical CDK/cyclin pairs as in the enzyme assay and treated with compound and a tracer for 1 hour before measurements were taken; Atirmociclib and ARTS-023 were tested in separate experiment for CDK1/2/7/9; c: Cellular Assay: pRb serine 780 was assessed in isogenic CDK6 or CDK4 knock-out cell lines, or KLE cells for CDK4, CDK6 and CDK2 cellular activity, respectively; p-NPM (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. *: Atirmociclib is a clinical-stage CDK4-selective inhibitor developed by Pfizer (*Palmer etal., Cancer Cell, 2025*).

Figure 1. ARTS-023 Exhibits Strong CDK4 Selectivity Over CDK6



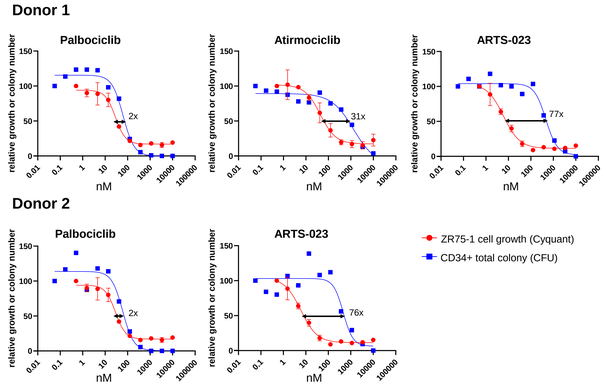
(A) Cellular CDK4 and CDK6 dependent pRb Serine 780 inhibition. Isogenic CDK4 or CDK6 knock-out cell lines were treated with indicated compound for 24 hours and cellular pRbS780 was measured by HTRF; **(B) CDK4 and CDK6 expression in isogenic CDK4 or CDK6 knock-out (KO) cell lines**. KO cell lines were generated by CRISPR technology and lysates were blotted with either CDK4 or CDK6 to confirm knock-out status.

Figure 2. ARTS-023 Potently Inhibits Rb Phosphorylation, Induces G1 Cell Arrest and Inhibits Cell Proliferation in ER+ Breast Cancer Cell Lines



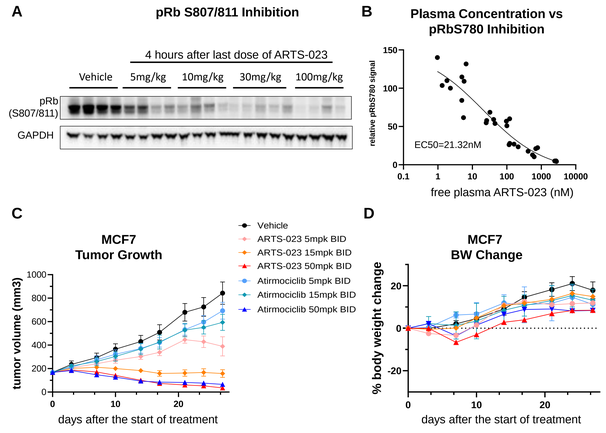
(A) ARTS-023 dose dependently inhibits Rb Serine 807/811 phosphorylation. Cell lysates treated with Atirmociclib or ARTS-023 at the specified concentrations for 24 hours were analyzed by Western Blot using the indicated antibodies; **(B) ARTS-023 potently induces G1 cell cycle arrest in ER+ breast cancer cell lines**. Graphs showing percentage of viable cells in different cell cycle stages via flow cytometry analysis after compound treatment for 48 hours; **(C) ARTS-023 potently inhibits cell proliferation in ER+ breast cancer cell lines**. Seven-day cell proliferation was measured by CyQuant assay.

Figure 3. Reduced Cytotoxicity of ARTS-023 in Human Hematopoietic Stem Cells



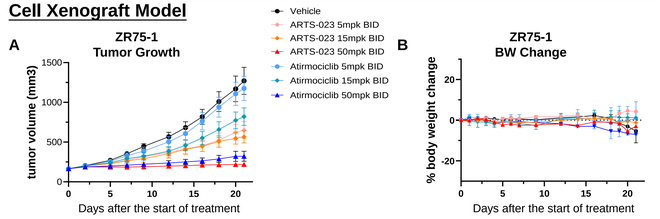
Comparative analysis of ARTS-023 growth inhibition in ER+ breast cancer cell line ZR75-1 and human CD34+ hematopoietic stem cells. ZR75-1 cell growth was assessed using a CyQuant assay, while the proliferative and differentiative capacity of human hematological CD34+ stem cells was evaluated by total colony formation in a CFU (colony-forming unit) assay. Mobilized CD34+ cells from two different donors were tested.

Figure 4. ARTS-023 Induces Dose Dependent pRB Inhibition and Inhibits Tumor Growth in ER+ MCF7 Breast Cancer Cell Xenograft Model



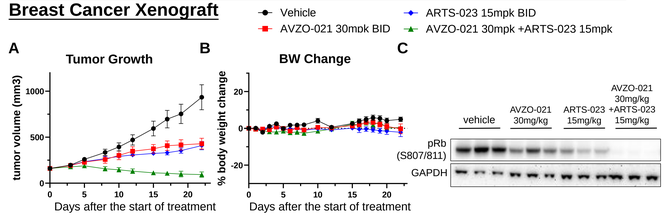
(A) pRb S807/811 inhibition. Tumor lysates collected 4 hours after treatment with four consecutive doses of ARTS-023 at the specified concentrations were analyzed for pRb S807/811 phosphorylation; **(B) Plasma exposure of ARTS-023 and pharmacodynamic modulation.** Unbound ARTS-023 plasma concentration was used in the figure; **(C) Anti-tumor activity in MCF7 xenograft; (D) Body weight (BW) change in MCF7 xenograft.** mpk: mg/kg; BID: twice daily; 6 animals in each group.

Figure 5. ARTS-023 Dose Dependently Inhibits Tumor Growth of ER+ ZR75-1 Cell Xenograft Model



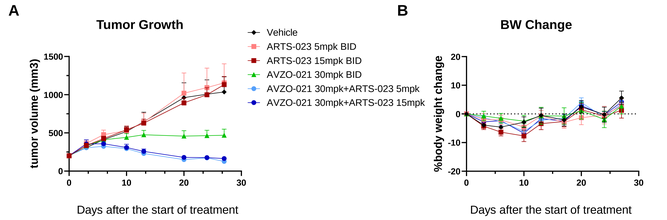
(A) Anti-tumor activity in ZR75-1 xenograft; (D) Body weight (BW) change in ZR75-1 xenograft. mpk: mg/kg; BID: twice daily; 6 animals in each group.

Figure 6. CDK2 Inhibitor AVZO-021* Enhances ARTS-023 Activity in T47D Breast Cancer Xenograft



(A) Anti-tumor activity in T47D xenograft; (B) Body weight (BW) change in T47D xenografts. mpk: mg/kg; BID: twice daily; 6 animals in each group; **(C)pRb S807/811 inhibition.** Tumor lysates collected 4 hours after treatment with four consecutive doses were analyzed for pRb S807/811 phosphorylation. *: Avenzo Therapeutics holds global rights to AVZO-021 outside of Greater China.

Figure 7. ARTS-023 in Combination with AVZO-021 Leads to Tumor Regression in a CDK4/6 Inhibitor-Resistant Breast Cancer PDX Model



(A) Anti-tumor activity in PDX model ST941PBR. ST941PBR is a patient-derived HR+ breast cancer xenograft mouse model resistant to palbociclib; **(B) Body weight (BW) change in ST941PBR.** mpk: mg/kg; BID: twice daily; 8 animals in each group.

Summary

- ARTS-023 is a potent CDK4 inhibitor with high selectivity over CDK6
- ARTS-023 effectively inhibits Rb phosphorylation and blocks the G1/S transition, leading to growth arrest in ER+ breast cancer cells
- ARTS-023 demonstrates reduced cytotoxicity in hematopoietic stem cells, suggesting it may cause fewer hematological side effects compared to currently approved CDK4/6 inhibitors
- ARTS-023 demonstrates strong anti-tumor activity *in vivo* and synergizes with the CDK2-selective inhibitor AVZO-021 in both palbociclib sensitive and resistant xenograft models
- These findings suggest that ARTS-023 is a promising next-generation CDK4-selective inhibitor, offering improved efficacy and a favorable toxicity profile for ER+ breast cancer treatment
- The first-in-human clinical study for ARTS-023 is on track to be initiated in mid-2025

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