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Original Article

Onvansertib inhibits cell proliferation and increases sensitivity to paclitaxel in uterine serous cancer cells

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Abstract: Uterine serous carcinoma (USC) belongs to the non-endometrioid subtype of endometrial cancer that is known for its highly aggressive behavior and poor prognosis, highlighting the warrant of novel strategies for the treatment of USC. PLK1 is a type of serine/threonine kinase that is crucial for controlling the progression of the cell cycle, DNA damage response, and genome stability. Targeting PLK1 exhibits potent anti-tumorigenic activity in pre-clinical models of multiple cancer types, and several PLK1 inhibitors have shown significant clinical benefit and favorable safety profiles alone or in combination with other chemotherapeutic agents. Onvansertib is an oral, selective PLK1 inhibitor that exhibits anti-proliferative activity in multiple types of cancer cell and animal models and has demonstrated clinical activity and a favorable safety profile in recent clinical trials. Hence, we investigated the anti-tumorigenic effects of onvansertib in USC cell lines. Nanomolar concentrations of onvansertib significantly inhibited cellular proliferation, led to cell cycle G2 arrest, induced cellular stress and apoptosis, caused DNA damage, and reduced cell adhesion and invasion in ARK-1 and SPEC-2 cells. The combination of onvansertib with paclitaxel demonstrated a synergistic effect in cell proliferation inhibition via inducing cell apoptosis and DNA damage. Our results provide preclinical evidence that onvansertib may be an effective strategy to treat USC and deserves further evaluation in animal models and clinical trials.

Keywords: Onvansertib, uterine serous carcinoma, PLK1, apoptosis, paclitaxel, synergy

Introduction

Endometrial cancer (EC) is the fourth most common malignancy in US women with an estimated 67,880 new cases and 13,250 deaths in 2024 [1]. While rates of most other cancers have declined or plateaued, the incidence and mortality of EC continue to rise [1], paralleling the increasing prevalence of obesity [2]. The high-grade histologic subtype uterine serous carcinoma (USC) accounts for about 10% of all endometrial malignancies; however, its behavior is more aggressive, and nearly half of women with USC present with at least stage II disease, compared to 21% of women with endometrioid histology [3]. Prognosis is poor, and USC accounts for nearly 40% of disease-related EC deaths each year [4]. Systemic chemotherapy is the standard adjuvant treatment for high-risk disease [5, 6]; however, treatment options after recurrence of USC are limited. Therefore, novel treatment options are urgently needed.

Dysregulation of the cell cycle is a hallmark of cancer development and progression. Cancer-associated mutations that control and regulate the cell cycle disrupt the normal regulation of cell growth and division, thereby resulting in continuous cancer cell division and proliferation [7, 8]. Since CDK4/6-selective inhibitors have demonstrated notable clinical advantages in some metastatic breast cancers, targeting individual cell-cycle checkpoints could be an

efficient approach in the development of new cancer treatments [9, 10]. PLK1, known as Polo-like Kinase 1, is a type of serine/threonine kinase that is crucial for controlling the progression of the cell cycle and is thought to have significant crosstalk with TP53 and the PI3K/Akt/mTOR pathway, two pathways that are frequently mutated in USC [11-14]. Elevated levels of PLK1 activity promote cell proliferation by overriding mitotic checkpoints, leading to immature cell division, oncogenesis, and tumor growth [15]. Numerous studies have shown that overexpression of PLK1 is found in various types of human cancers and is associated with worse prognosis in many tumor types [15-17]. The Cancer Genome Atlas (TCGA) data revealed that the expression of PLK1 in EC tissues was 21.3 times that of normal endometrium, and the level of PLK protein expression was closely associated with histological grade and invasiveness in EC patients [18, 19]. Inhibition of PLK1 expression by siRNA or small molecule inhibitors is highly effective in controlling cell proliferation, increasing apoptosis, enhancing sensitivity to chemotherapy, and reducing tumor growth in multiple preclinical models of cancer [20, 21]. Several clinical trials have shown that small molecule inhibitors of PLK1, including rigosertib and volasertib, exhibit promising results in improving overall survival in some types of solid tumors while exhibiting an acceptable safety profile [10, 22].

Different from the molecular biological alterations in endometrioid endometrial cancers, several common molecular genetic alterations were identified in USC through comprehensive whole-genome analysis [23]. Most commonly, somatic mutations in the tumor suppressor TP53 are found in more than 85% of USC tumors, but other aberrations include PIK3CA, ERBB2, CCNE1, FBXW7, PPP2R1A and MYC mutations [24, 25]. These changes drive tumorigenesis and progression of USC through alteration of downstream pathway activities and signaling cascades that control cell proliferation, invasion, genomic stability, and survival [4, 25]. More importantly, all these genetic changes, either individually or in combination, are directly or indirectly involved in the positive or negative regulation of the cell cycle by altering cyclin-dependent kinase activity and pRB, p21WAF/CIP, and p27KIP function [26, 27]. Therefore, evaluating the effects of known cell cycle inhibitors on USC cell proliferation and invasion may be a good strategy for developing new treatments for USC. Onvansertib is an orally available, selective PLK1 inhibitor that has demonstrated anti-tumorigenic activity in preclinical models of triple-negative breast, colon, and ovarian cancer and is now being explored in phase 1/2 clinical trials [28-30]. Furthermore, several studies have demonstrated that *TP53*-mutated cell lines are more sensitive to PLK1 inhibition [31, 32]. Thus, in this study, we aimed to investigate the anti-tumorigenic effects of onvansertib in human USC cell lines.

Methods

Cell culture and reagents

The human USC cell lines ARK-1 (*PIK3CA* gene mutation, TP53 wild-type) and SPEC-2 (TP53 deletion, *PTEN* null) were used for all experiments [33]. ARK-1 cells were maintained in RPMI 1640 medium with 10% fetal bovine serum (FBS). SPEC-2 cells were maintained in DMEM/F12 with 10% FBS. All media contained penicillin (100 U/mL) and streptomycin (100 µg/mL). Cells were cultured in a humidified 5% CO_2 incubator at 37°C. Onvansertib was kindly provided by Cardiff Oncology (San Diego, CA). Paclitaxel was purchased from Sigma-Aldrich (St. Louis, MO). Antibodies were purchased from Cell Signaling Technology (Beverly, MA) and Abclonal Science (Woburn, MA, USA). All other reagents and chemicals were purchased from Sigma-Aldrich.

MTT assay

Cell proliferation was assessed by MTT assay in the ARK-1 and SPEC-2 cells. Cells ($4\text{-}6\times10^3$ cells/well) were seeded in 96-well plates and cultured at 37°C in 5% CO_2 for 24 hours. Cells were then treated with varying concentrations of onvansertib (0.1-500 nM) and paclitaxel as single agents or in combination for 72 hours. A volume of 5 µL of MTT (5 mg/mL) was added to each well and the plates were incubated for 1 hour. DMSO (100 µl/well) was added to lyse the formazan crystal and terminate the MTT reaction. Optical density was measured at a wavelength of 562 nm with a Tecan microplate reader (Morrisville, NC). IC_{50} values were calculated using the AAT Bioquest calculator (Sunnyvale, CA). Each experiment was performed in triplicate to ensure consistency of results. The Bliss

Independence model was used to calculate the combination index (CI) to determine whether the combination of onvansertib and paclitaxel is additive (CI=1), synergistic (CI <1), or antagonistic (CI >1) on cell growth inhibition [34].

Colony assay

ARK-1 and SPEC-2 cells were plated in 6-well plates at a concentration of 400 cells/well. After 24 hours, cells were treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 48 hours. After treatment, the media was changed every 3 days for 12-14 days. Cells were then washed with PBS and stained with 0.5% crystal violet for 30 minutes. Colonies were captured and counted by bright-field microscopy. Each experiment was performed in triplicate to ensure consistency of results.

Cell cycle analysis

Cell cycle analysis was performed by Cellometer Vision (Nexcelom Bioscience, Lawrence, MA). ARK-1 and SPEC-2 cells were cultured in 6-well plates at a concentration of 3×10^5 and 4×10^5/well, respectively, for 24 hours. Cells were treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 24 hours and then harvested from plates using 0.25% Trypsin (Sigma-Aldrich). After centrifuging, cell pellets were washed with PBS and then fixed in 90% methanol for 15 minutes. Before analysis, cells were again washed with PBS and centrifuged, and the pellet was resuspended in a solution containing RNase, propidium iodide (PI), and Triton X-100 for 30 minutes. Samples were measured by Cellometer Vision to evaluate cell cycle progression. The cell cycle profile was analyzed by FCS Express (De Novo, Pasadena, CA). Each experiment was performed in triplicate to ensure consistency of results.

Reactive oxygen species (ROS) assay

To measure cellular stress, the production of reactive oxygen species (ROS) was measured by the DCFH-DA assay. ARK-1 (1×10^4 cells/well) and SPEC-2 (2×10^4 cells/well) cells were seeded in 96-well plates for 24 hours and then treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 6 hours. Cells were then incubated at 37°C with phenol-red-free regular media containing DCFH-DA (15 μM) for 30 minutes. Fluorescence intensity was measured using a Tecan microplate reader (Ex/Em = 485/526 nm). Each experiment was performed in triplicate to ensure consistency of results.

JC-1 assay

The mitochondrial membrane potential was measured by the fluorescent cationic dye JC-1 assay (AAT Bioquest). ARK-1 (1×10^4 cells/well) and SPEC-2 (2.5×10^4 cells/well) cells were seeded in 96-well plates overnight. Cells were then treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 6 hours. A volume of 1 μL of JC-1 buffer (200 μM) was added to each well and the plate was incubated at 37°C in the dark for 30 minutes. Cells were then washed with PBS. The levels of JC-1 aggregates (Ex/Em = 535/590 nm) and JC-1 monomers (Ex/Em = 490/530 nm) were measured using a Tecan microplate reader. Each experiment was performed in triplicate to ensure consistency of results.

Cleaved caspase-3 ELISA assay

ARK-1 and SPEC-2 cells were plated in 6-well plates at a concentration of 3×10^5 and 4×10^5/well, respectively, for 24 hours, then treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 12 hours. Media was replaced with 150 μL 1× caspase lysis buffer and cell lysates were collected and incubated with reaction buffer containing 200 μM of caspase-3 substrates (AAT Bioquest) for 30 minutes. Fluorescence intensity was measured using a Tecan microplate reader (Ex/Em = 400/505 nm). Each experiment was performed in triplicate to ensure consistency of results.

Laminin-1 adhesion assay

ARK-1 and SPEC-2 cells were seeded in 96-well plates coated with laminin-1 (Sigma-Aldrich). Cells were then treated with onvansertib and incubated at 37°C for 2 hours. Adherent cells were fixed with 5% glutaraldehyde (100 μL/well) for 30 minutes at room temperature, then washed with PBS and stained with 0.1% crystal violet (100 μL/well) for 15 minutes. Each well was washed twice with PBS and 100 μL of 10% acetic acid was added to each well to solubilize the dye. Absorbance was measured at 575 nm using a Tecan microplate reader. Each experiment was performed in triplicate to ensure consistency of results.

Wound healing assay

The capacity for migration in ARK-1 and SPEC-2 cells was measured with the wound healing assay. ARK-1 and SPEC-2 cells were plated in 6-well plates at a concentration of 6×10^5 cells/well overnight. When cells grew to >80% confluence, each well was scratched with 3 uniform straight lines through the cell monolayer using a 20 µL sterile pipette tip. After washing twice with PBS, cells were treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 48 hours. The wounds in each well were photographed using a microscope after 24 and 48 hours of treatment. The width of wounds was measured and recorded with ImageJ software (National Institutes of Health, Bethesda, MD). Each experiment was performed in triplicate to ensure consistency of results.

Western immunoblotting

The ARK-1 and SPEC-2 cells were plated in 6-well plates and then treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) at different times. Cells were disrupted in RIPA lysis buffer and centrifuged at 12,000 rpm for 15 minutes. Protein concentrations were detected by BCA assay. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane at 4°C. Membranes were blocked in a 5% non-fat milk solution at room temperature for one hour and incubated with the indicated primary antibody overnight at 4°C. Membranes were then washed three times with TBS-T and incubated with the appropriate secondary antibodies for one hour. Target protein bands were visualized using an enhanced chemiluminescence kit (Thermo Fisher Scientific) on the ChemiDoc Image System (Bio-Rad; Hercules, CA). Each experiment was performed in duplicate to ensure consistency of results.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Differences between groups were analyzed by the unpaired Student's *t*-test or one-way analysis of variance (ANOVA) test. GraphPad Prism 10 (La Jolla, CA, USA) was used for comparisons. Any *p*-value <0.05 was considered statistically significant.

Results

Onvansertib inhibits cellular proliferation in USC cells

The anti-proliferative effects of onvansertib were evaluated in ARK-1 and SPEC-2 USC cell lines treated with 0.1-500 nM of onvansertib for 72 hours. Results of the MTT assay demonstrated that cellular proliferation was significantly inhibited in a dose-dependent manner in both cell lines (**Figure 1A**). Mean IC_{50} values were 22.71 nM for ARK-1 and 45.34 nM for SPEC-2. Because the colony count assay is an effective measure of the long-term effects of cytotoxic agents on cellular viability, the ARK-1 and SPEC-2 cells were treated with onvansertib for 48 hours, and the cells continued to be cultured for 12-14 days. Onvansertib demonstrated an inhibitory effect on the clonogenicity of both cell lines in a dose-dependent fashion. Compared to controls, a dose of 25 nM onvansertib significantly decreased ARK-1 colony formation by 96% ($P<0.01$) and a dose of 50 nM significantly decreased SPEC-2 colony formation by 98% ($P<0.01$) (**Figure 1B**). These results confirm that onvansertib inhibits cellular proliferation in USC cells.

Next, the role of onvansertib on PLK1 expression was evaluated by Western blotting assay. After treatment for 24 hours, onvansertib inhibited the expression of phosphorylated PLK1 in both cell lines, indicating that onvansertib effectively inhibits PLK1 activity (**Figure 1C**). In addition, to evaluate the role of onvansertib on the PI3K/Akt/mTOR and MAPK pathways, both cell lines were treated with onvansertib for 24 hours, and the expression of phosphorylated AKT, phosphorylated S6, and phosphorylated p38 were assessed by Western blotting. The results showed that onvansertib decreased the expression of phosphorylated AKT and phosphorylated S6 while increasing the expression of phosphorylated p38 in ARK-1 cells (**Figure 1D**). In SPEC-2 cells, onvansertib treatment decreased the expression of phosphorylated S6 while increasing the expression of phosphorylated AKT and phosphorylated p38 (**Figure 1D**). These results suggest that the effects of onvansertib on phosphorylated AKT depend on different feedback loops of the AKT/mTOR pathway in the ARK1 and SPEC-2 cells and that the inhibitory effects of onvansertib may be mediated through inhibition of the

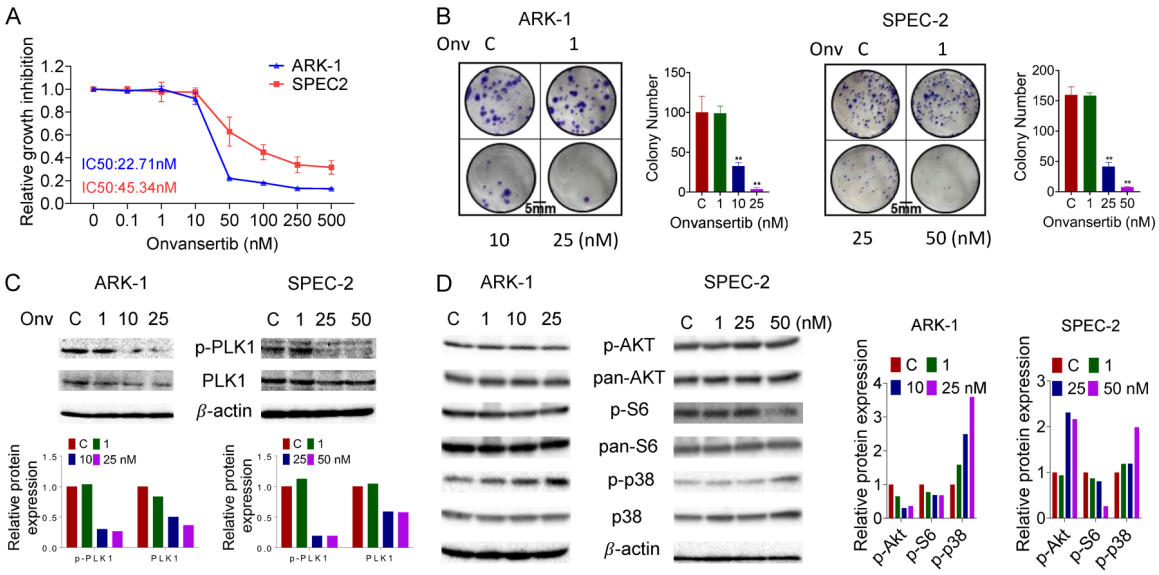

Figure 1. Onvansertib inhibits cellular proliferation in USC cells. ARK-1 and SPEC-2 cells were treated with onvansertib at 0.1-500 nM for 72 hours. Cell proliferation was detected by MTT assay. Onvansertib inhibited the proliferation of these cell lines in a dose-dependent manner (A). Onvansertib inhibited colony formation in the ARK-1 and SPEC-2 cells (B). Effect of onvansertib on the expression of phosphorylated Plk1 and Plk1 in both cells after treatment for 24 hours (C). Western blotting results of phosphorylated AKT, phosphorylated S6, and phosphorylated p38 in both cells after 24 hours of onvansertib treatment (D). *P<0.05, **P<0.01.

PI3K/Akt/mTOR pathway and activation of the MAPK-p38 pathway.

Onvansertib induces G2 cell cycle arrest in USC cells

As an inhibitor targeting PLK1, a key regulator of the G2/M transition, onvansertib can disrupt cell cycle progression and lead to cell death. To better understand the role of onvansertib on the cell cycle process of USC cells, the cell cycle profile was analyzed by Cellometer after treatment of AKR-1 and SPEC-2 cells with onvansertib for 24 hours. Onvansertib decreased the G0/G1 phase and increased the G2 phase in both ARK-1 and SPEC-2 cells in a dose-dependent manner (**Figure 2A**). At a dose of 25 nM onvansertib, the G2 phase increased from 31.75% to 42.50% as compared to the control group in ARK-1 cells (P<0.01), and at a dose of 50 nM, the G2 phase increased from 21.10% to 49.52% as compared to the control group in SPEC-2 cells (P<0.01). Moreover, western blotting results showed that onvansertib decreased protein expression of CDK2, CDK4, cyclin D1, and cyclin E2 in both cell lines after 24 hours of treatment (**Figure 2B**). These results confirm that onvansertib induces G2 arrest in USC cells.

Onvansertib induces cellular stress, apoptosis, and DNA damage in USC cells

To evaluate the role of onvansertib in cellular stress, the ROS assay was used to detect the changes in ROS production after the ARK-1 and SPEC-2 cells were treated with onvansertib for 6 hours. Onvansertib significantly increased ROS production by 17% in ARK-1 cells at 25 nM (P<0.01) and by 13% in SPEC-2 cells at 50 nM (P<0.01) compared to controls (**Figure 3A**). Additionally, the JC-1 assay showed that onvansertib significantly reduced the mitochondrial membrane potential after 6 hours of treatment. In ARK-1 cells, 25 nM onvansertib reduced it by 16%, and in SPEC cells, 50 nM onvansertib reduced it by 17% (**Figure 3B**). Western blotting results demonstrated increased expression of the cellular stress proteins Calnexin, PDI, and BIP after 24 hours of treatment in both cell lines (**Figure 3C**).

Given that increased ROS is a trigger for inducing apoptosis in cancer cells, the levels of cleaved caspase-3 were detected by ELISA in both cell lines. After treatment of both cells with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2) for 12 hours, increased levels of cleaved caspase-3 were found in both

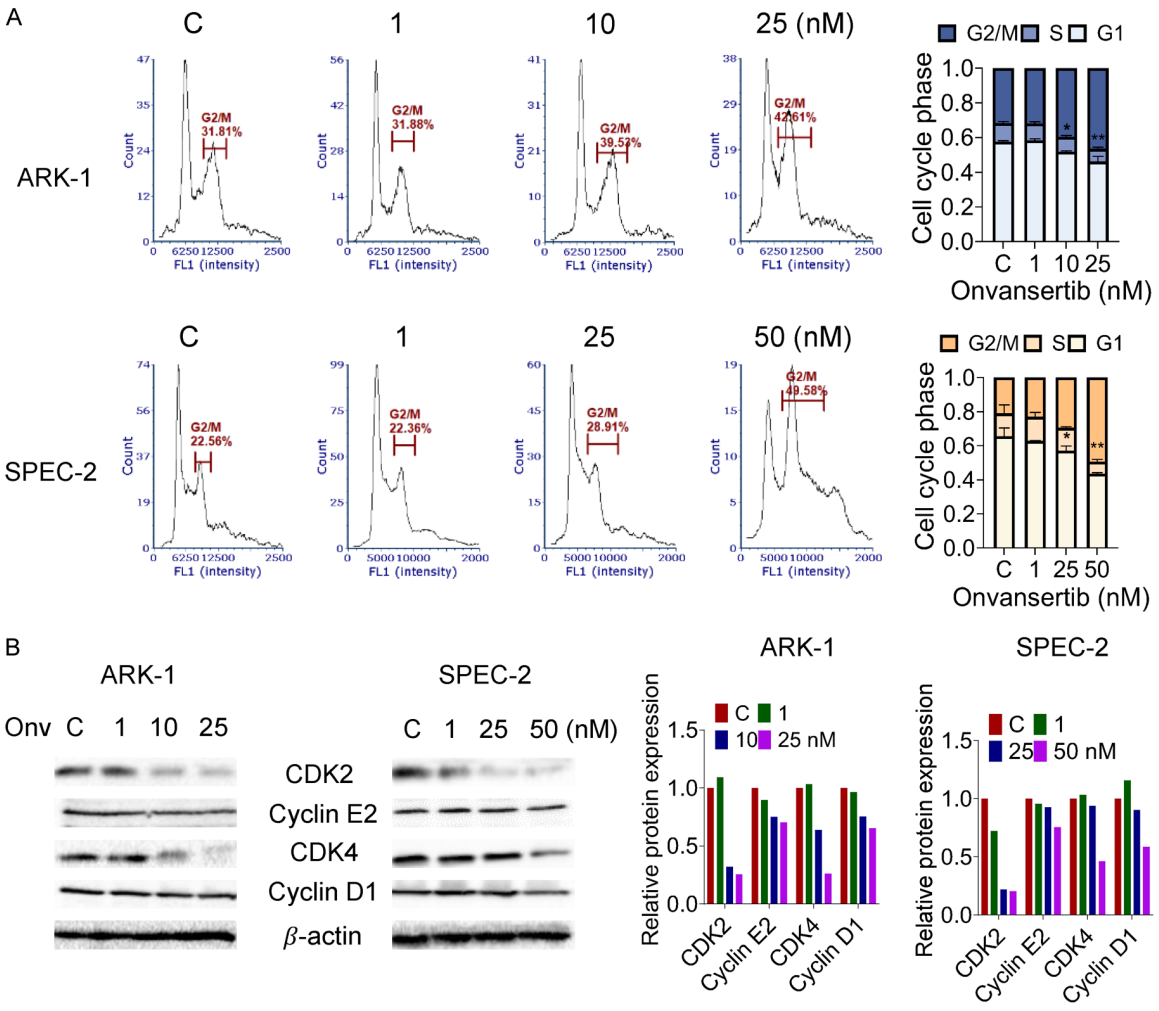

Figure 2. Onvansertib induces G2 cell cycle arrest in USC cells. ARK-1 and SPEC-2 cells were treated with onvansertib for 24 hours. The cell cycle profiles were evaluated by Cellometer. Onvansertib led to cell cycle G2 phase arrest in ARK-1 and SPEC-2 cells in a dose-dependent manner (A). Western blotting results showed that onvansertib inhibited the expression of CDK4, cyclin D1, CDK2, and cyclin E2 after treatment for 24 hours in both cells (B). *P<0.05, **P<0.01.

cell lines. In ARK-1 cells, cleaved caspase-3 activity was increased by 1.38-fold (P<0.05) at 25 nM onvansertib compared to control cells. In SPEC-2 cells, cleaved caspase-3 activity was increased by 2.21-fold (P<0.05) in SPEC-2 cells at 50 nM onvansertib compared to control cells (**Figure 3D**). Additionally, western blotting results demonstrated that treatment of cells with onvansertib for 24 hours decreased the expression of the anti-apoptotic proteins Mcl-1 and Bcl-2 and increased the expression of the pro-apoptotic protein Bax and DNA damage markers Rad51, H2AX, and Geminin in both cell lines (**Figure 3E**).

Onvansertib inhibits cellular adhesion and invasion in USC cells

As the propensity for metastasis is a crucial characteristic of USC, the role of onvansertib in adhesion and migration in ARK-1 and SPEC-2 cells was investigated by the laminin-1 adhesion and wound healing assays, respectively. The adhesion assay demonstrated that onvansertib inhibited cellular adhesion in both ARK-1 and SPEC-2 cells in a dose-dependent fashion. At doses of 10 nM and 25 nM onvansertib, cellular adhesion was decreased by 10.1% (P<0.05) and 21.5% (P<0.01) in ARK-1 cells, respectively, compared to control cells. Similar

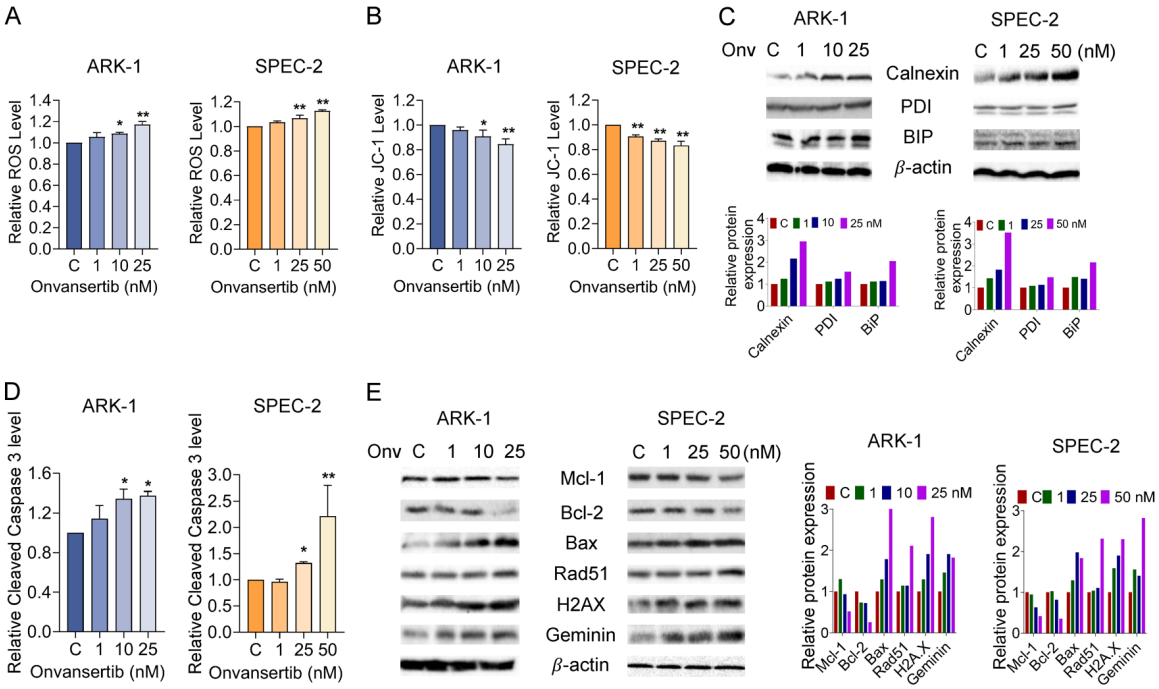

Figure 3. Onvansertib induces cellular stress, apoptosis, and DNA damage in USC cells. ARK-1 and SPEC-2 cells were treated with onvansertib for 6 hours. Onvansertib increased the ROS levels and decreased the JC-1 levels in both cells (A and B). Western blotting results demonstrated that onvansertib increased the expression of Calnexin, PDI, and BiP after 24 hours of treatment in both cell lines (C). Onvansertib increased cell cleaved caspase-3 levels after treatment for 12 hours (D). Western blotting results showed that onvansertib increased the expression of H2AX, Rad51, Geminin, and BAX, and decreased the expression of Mcl-1 and Bcl-2 after 24 hours of treatment in both cell lines (E). *P<0.05, **P<0.01.

results were observed in SPEC-2 cells, as doses of 25 nM and 50 nM onvansertib decreased cell adhesion by 14.7% (P<0.05) and 19.1% (P<0.05), respectively, compared to control cells (**Figure 4A**). The wound healing assay also demonstrated the effect of onvansertib on cell migration inhibition in both cell lines (**Figure 4B**). At a dose of 25 nM, onvansertib significantly increased wound width by 2.17-fold in ARK-1 cells compared to the control group (P<0.01). In SPEC-2 cells at a dose of 50 nM, onvansertib increased wound width by 1.4-fold compared to the control group after 48 hours of treatment (P<0.01). Western blotting showed that treatment of both cells with onvansertib for 24 hours decreased expression of the epithelial-mesenchymal transition (EMT) related proteins N-cadherin and β-catenin (**Figure 4C**). These results indicate that onvansertib has anti-metastatic effects in USC cells.

Onvansertib displays synergy with paclitaxel in USC cells

While prior studies have demonstrated synergy between onvansertib and paclitaxel in pre-clinical models of breast and ovarian cancer [28, 29, 35], this therapeutic combination has not been assessed for EC. Thus, we evaluated the effect of the combination of onvansertib and paclitaxel on cell proliferation in the ARK-1 and SPEC-2 cells. Both cell lines were treated with onvansertib alone (1, 10, and 25 nM for ARK-1 and 1, 25, and 50 nM for SPEC-2), paclitaxel alone (0.1, 1, and 10 nM), and a combination of both drugs for 72 hours. CI calculated at multiple paired concentrations demonstrated synergistic activity at low doses of onvansertib and paclitaxel in ARK-1 cells and at slightly higher doses in SPEC-2 cells (**Figure 5A**). Furthermore, treatment with onvansertib (10 nM) plus paclitaxel (1 nM) resulted in 19.66% cell growth inhibition in ARK-1 cells (P<0.01), whereas onvansertib alone and paclitaxel alone had minimal effect on cell growth at these same doses (**Figure 5B**). A similar result was observed in SPEC-2 cells, as onvansertib (25 nM) plus paclitaxel (1 nM) resulted in 25.28% cell growth inhibition (P<0.01), whereas onvansertib alone and paclitaxel alone led to 13.05% and 2.64% cell growth inhibition, respectively. These results

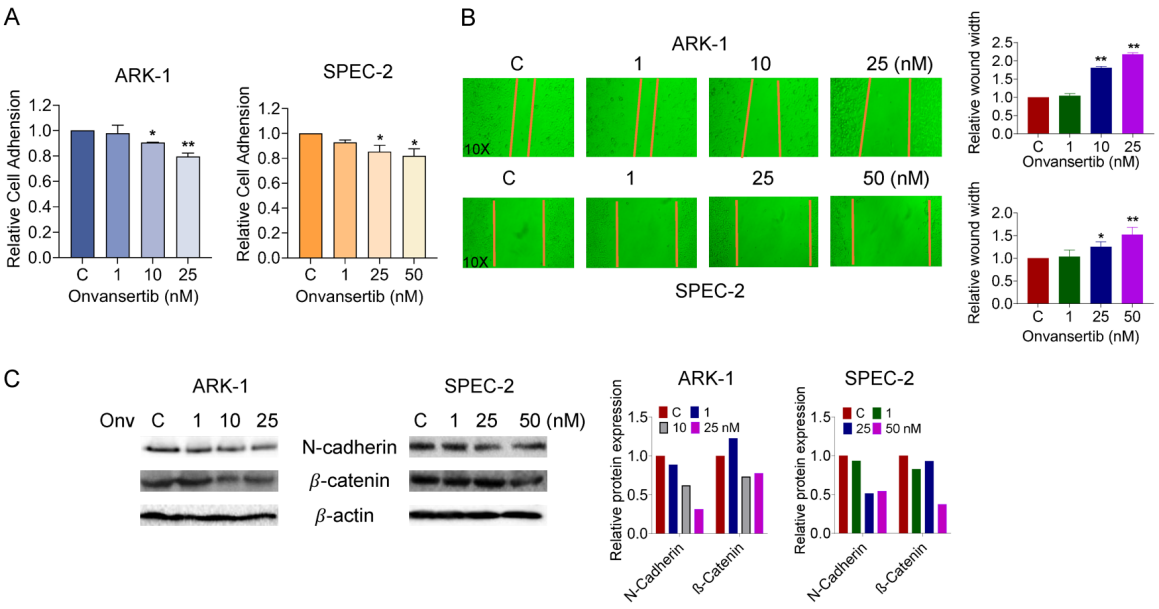

Figure 4. Onvansertib inhibits cellular adhesion and invasion in USC cells. The laminin-1 assay showed that onvansertib significantly inhibited cell adhesion in the ARK-1 cells at 10 and 25 nM, and in the SPEC-2 cells at 25 and 50 nM after 2 hours of treatment (A). The wound healing assay showed that onvansertib inhibited cell migration in the ARK-1 and the SPEC-2 cells after 48 hours of treatment (B). Western blotting results of onvansertib on the expression of β-catenin and N-cadherin after 24 hours of treatment in both cell lines (C). *P<0.05, **P<0.01.

A

ARK-1

CI value of combination

Onv (nM)	Paclitaxel (nM) 0	0.1	1	10
0	-	1	1	1
1	1	0.8334	0.7121	0.9206
10	1	0.8362	0.6285	1.0241
25	1	1.0249	0.9265	1.2778

SPEC-2

CI value of combination

Onv (nM)	Paclitaxel (nM) 0	0.1	1	10
0	-	1	1	1
1	1	0.6344	0.5349	0.8265
25	1	0.9558	0.8409	1.1228
50	1	0.9328	0.8464	1.3745

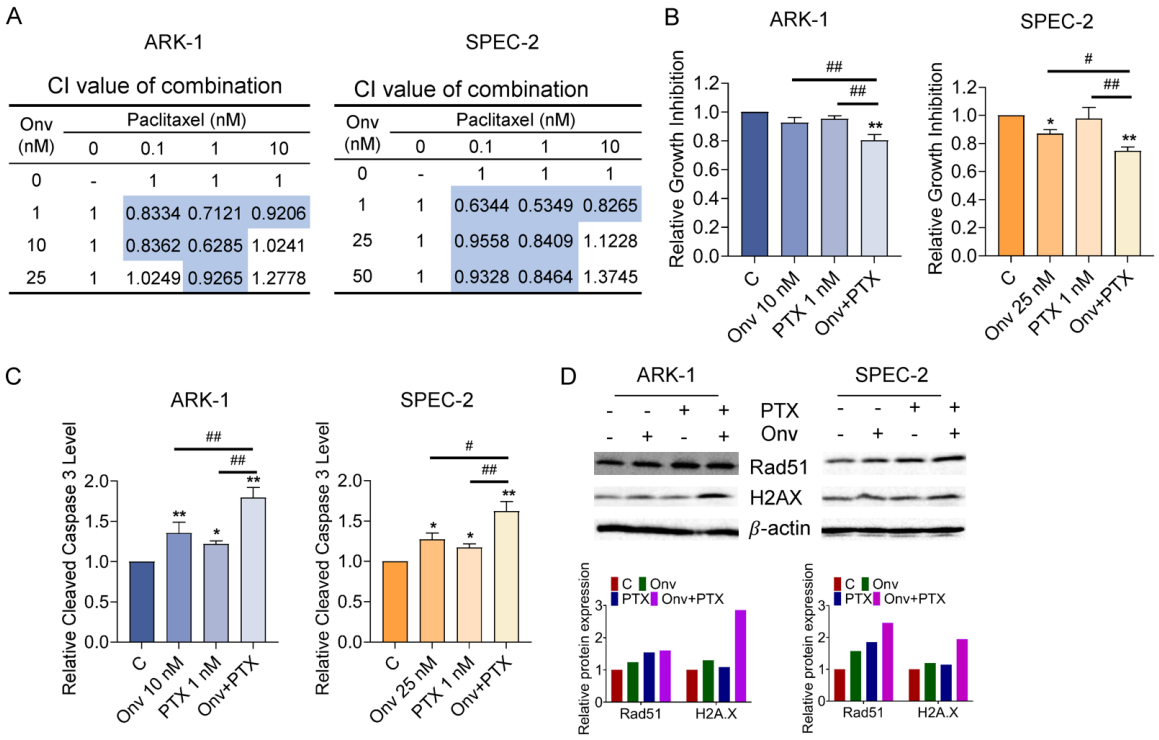

Figure 5. Onvansertib displays synergy with paclitaxel in USC cells. ARK-1 and SPEC-2 cells were each treated with onvansertib (1, 10, 25 nM for ARK-1 and 1, 25, 50 nM for SPEC-2), paclitaxel (0.1, 1, 10 nM), and their combination for 72 hours. The Bliss independence model was used to calculate the combination index (CI) for each combination group (A). The combination of onvansetinib (10 nM for ARK-1 and 25 nM for SPEC-2) and paclitaxel (1 nM) showed a higher inhibitory effect on cell growth in both cell lines than either paclitaxel or onvansetinib alone after 72 hours

of treatment (B). The combination of onvansertib and paclitaxel significantly increased cleaved caspase-3 levels compared with either paclitaxel or onvansertib alone after treatment for 12 hours in both cell lines (C). Western blotting showed that the combination group demonstrated a more potent effect on the expression of H2AX and RAD51 compared with paclitaxel or onvansertib alone (D) *P<0.05, **P<0.01 compared with C. #P<0.05, ##P<0.01 compared with each group.

demonstrate that the combination of onvansertib and paclitaxel may have more potent anti-proliferative activity against USC cells than either of these drugs alone.

To evaluate the synergistic mechanism of onvansertib and paclitaxel in both cells, the effects of onvansertib, paclitaxel, and the combination on cell apoptosis were evaluated by ELISA cleaved caspase-3 assay. The results demonstrated that 10 nM onvansertib plus 1 nM paclitaxel increased cleaved caspase-3 levels 1.79-fold (P<0.01) compared with 1.35-fold and 1.22-fold with onvansertib or paclitaxel alone in ARK-1 cells (**Figure 5C**). Similarly, 25 nM onvansertib plus 1 nM paclitaxel increased cleaved caspase-3 levels 1.62-fold (P<0.01) compared with 1.27-fold and 1.17-fold in onvansertib or paclitaxel alone, respectively, in SPEC-2 cells. The effect of this combination on DNA damage was determined by western blotting assay after the ARK-1 and SPEC-2 cells were treated with indicated doses of onvansertib alone, paclitaxel alone, and the combination for 24 hours. The results showed that combined treatment induced the highest expression of Rad51 and H2AX in both cells compared with onvansertib and paclitaxel alone (**Figure 5D**). These results indicate that apoptosis and DNA damage pathways are involved in the synergistic effects of onvansertib and paclitaxel in USC cell lines.

Discussion

Onvansertib is a second-generation ATP-competitive oral PLK1 inhibitor with a short half-life and controllable side effects, showing antitumor activity and increasing sensitivity to chemotherapy in multiple pre-clinical cancer models [28, 30, 36]. In this study, we evaluated the effects of onvansertib on cellular proliferation, cell cycle progression, cellular stress and apoptosis, adhesion/invasion, and DNA damage, as well as synergy with paclitaxel in USC cell lines. Our findings demonstrated that onvansertib inhibits cellular proliferation, provokes G2/M cell cycle arrest, induces cellular stress and apoptosis, reduces invasion, and decreases AKT/mTOR pathway activity. Additionally, onvansertib combined with paclitaxel produced a synergistic effect in inhibiting cell proliferation and inducing DNA damage and apoptosis in USC cells. These results provide pre-clinical evidence that onvansertib or onvansertib combined with paclitaxel could be an effective strategy for treating USC.

Our data demonstrate that onvansertib inhibited the phosphorylation of S6 and increased the expression of phosphorylated p38 in both cell lines; however, in SPEC-2 cells, onvansertib increased the expression of phosphorylated AKT, which may be related to AKT/mTOR/S6 feedback loops [37]. It has been confirmed that PLK1 physically interacts with PTEN and mTOR, resulting in inactivation of the AKT/mTOR pathway [38, 39]. p38 directly participates in the regulation of cell mitosis by PLK1 during prophase and metaphase and transduces stress signals following PLK1 treatment [40, 41]. Targeting the AKT/mTOR pathway by small molecular inhibitors or in combination with other chemotherapeutic agents has been extensively investigated in multiple cancer types, including USC, but has fallen short of expectations in clinical trials, likely due to lack of target specificity and activation of alternative pathways that compensate for inhibition of the AKT/mTOR pathway [42]. Recent RNAseq data showed that inhibition of the AKT/mTOR pathway by VS-5584 (a specific AKT inhibitor) resulted in a significant reduction of PLK1 expression in differentially expressed genes [43]. The combination of AKT/mTOR inhibitors with PLK1 inhibitors produced a synergetic inhibitory effect on cell proliferation and tumor growth in non-small cell lung cancer cells and mouse models [43, 44]. Thus, considering the results above and the molecular profile of USC, it would be valuable to investigate the potential effects of onvansertib combined with AKT/mTOR inhibitors in our upcoming work.

Oxidative stress has the potential to cause methylation of the PLK1 promoter, thereby resulting in its epigenetic changes and affecting its functions, and these functional changes

may be p53-dependent [45]. PLK1 also regulates induction of apoptosis and inhibition of proliferation caused by cellular stress response through inositol-requiring protein 1a (IRE1a) in cancer cells [46]. Several PLK1 inhibitors cause intracellular ROS accumulation, leading to mitochondrial dysfunction and induction of apoptosis. Inhibition of cellular stress pathways partially alleviates the inhibition of cell proliferation caused by PLK1 inhibitors [41, 47, 48]. Our results similarly show a dose-dependent increase in ROS and cleaved caspase-3 levels in both ARK-1 and SPEC-2 cell lines, as well as a decrease in the anti-apoptotic proteins Mcl-1 and Bcl-2 and an increase in the pro-apoptotic protein Bax, clearly demonstrating the pro-apoptotic effect of onvansertib. More importantly, it has been confirmed that PLK1 physically interacts with p53 in mammalian cultured cells and that the pro-apoptotic effect of p53 was inhibited by increased activity of PLK1. Inhibition of PLK1 significantly increased p53-mediated apoptosis in both triple-negative breast cancer and prostate cancer cell lines [14, 49, 50]. This interaction suggests that inhibiting PLK1 may be particularly effective in tumors with p53 mutations, such as USC, as p53 mutations could potentially be exploited to make PLK1 inhibitors such as onvansertib more effective [51].

Extrauterine metastases are commonly found at the time of initial diagnosis of USC, thereby contributing to the treatment difficulty and complexity of USC [52]. Hence, efficacious management of metastasis of USC is essential for the improvement of the survival of USC patients. PLK1 has been identified as an important driver of EMT, facilitating cancer cell dissemination and metastasis across various malignancies, including prostate cancer, non-small cell lung cancer, and cholangiocarcinoma [53-55]. Elevated PLK1 activity promotes cell proliferative and invasive capacities, and inhibition of PLK1 activity using siRNA or pharmacological inhibitors such as BI-2356 abrogates cancer cell invasion in gastric cancer and esophageal squamous cell carcinoma [56, 57]. Onvansertib significantly inhibited spheroid growth, cell invasion and migration of head and neck squamous cell carcinoma (HNSCC) cells, reduced the expression of angiogenesis markers in a xenograft mouse model, and attenuated local invasion and reduced the size and the number of distant metastases in a zebrafish metastatic model of HNSCC [36]. Consistent with these findings, our results showed that onvansertib significantly inhibited cell adhesion and migration and decreased the expression of EMT markers such as β-catenin and N-cadherin in the ARK-1 and SPEC-2 cells. These results underscore the potential of onvansertib in mitigating the invasive and metastatic propensities of USC cells. Our further work will investigate the anti-metastatic activity of onvansertib in a PDX mouse model of USC.

Normal PLK1 activity has the function of repairing double-strand breaks, and inhibiting PLK1 activity can cause post-mitotic DNA damage and senescence [58]. The PLK1 inhibitor BI2536 has been shown to inhibit cell proliferation, impair DNA damage repair, and increase sensitivity to radiotherapy in medulloblastoma cells and xenograft mice [59]. Therefore, we speculate that the combination of PLK1 inhibitors and DNA damaging agents may have a synergistic effect in inhibiting cell proliferation and tumor growth in certain types of cancer. Onvansertib is highly specific for PLK1 and has a short half-life, allowing for flexible dosing regimens and the potential to mitigate side effects associated with combination therapy [60]. Onvansertib combined with paclitaxel proved to be more effective in reducing tumor weight and improving survival rates in a PDX mouse model of ovarian cancer, as well as increasing the expression of apoptosis and DNA damage markers in tumor tissue, compared to single-drug treatments [28]. Similar results were found in a xenograft model of mucinous ovarian carcinoma where the combination of onvansertib and paclitaxel exhibited a stronger inhibition of tumor growth and longer survival times in mice than single-agent treatments [35]. These results bolster our findings of synergy between onvansertib and paclitaxel in USC cell lines. Our data show that the combination of low doses of onvansertib and paclitaxel produced stronger inhibition of cell proliferation, more caspase-3 cleavage production, and higher expression of H2AX compared with the single agents in both cell lines.

Conclusion

The aggressive nature and poor prognosis of USC necessitate novel therapeutic approach-

es. Our results indicate that onvansertib appears promising, as it exhibits significant anti-tumorigenic activity via inhibiting cell proliferation and invasion, inducing cell cycle arrest, cellular stress, apoptosis, and DNA damage, meanwhile also acts synergistically with paclitaxel in USC cell lines and acts synergistically with paclitaxel in USC cell lines. Onvansertib is already being explored alone and in combination with other chemotherapies in multiple phase I/II clinical trials in solid tumors [61, 62], including in breast, colorectal, and lung cancer. Our data deserves further evaluation of onvansertib as a single or combination of paclitaxel in our PDX mouse models of USC in order to provide a basis for future USC clinical trials.

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Disclosure of conflict of interest

None.

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References

[1] Siegel R, Giaquinto A and Jemal A. Cancer statistics, 2024. Cancer 2024; 74: 12-49.

[2] Smrz SA, Calo C, Fisher JL and Salani R. An ecological evaluation of the increasing incidence of endometrial cancer and the obesity epidemic. Am J Obstet Gynecol 2021; 224: 506.e501-506.e508.

[3] Mhawech-Fauceglia P, Herrmann RF, Kesterson J, Izevbaye I, Lele S and Odunsi K. Prognostic factors in stages II/III/IV and stages III/IV endometrioid and serous adenocarcinoma of the endometrium. Eur J Surg Oncol 2010; 36: 1195-1201.

[4] Ferriss JS, Erickson BK, Shih IM and Fader AN. Uterine serous carcinoma: key advances and novel treatment approaches. Int J Gynecol Cancer 2021; 31: 1165-1174.

[5] de Boer S, Powell MA, Mileshkin L, Katsaros D, Bessette P, Haie-Meder C, Ottevanger P, Ledermann J, Khaw P, Colombo A, Fyles A, Baron M, Kitchener H, Nijman H, Kruitwagen R, Jurgenliemk-Schulz I, Nout R, Smit V, Putter H and Creutzberg C. Final results of the international randomized PORTEC-3 trial of adjuvant chemotherapy and radiation therapy (RT) versus RT alone for women with high-risk endometrial cancer. J Clin Oncol 2017; 2017.

[6] Matei D, Filiaci V, Randall ME, Mutch D, Steinhoff MM, DiSilvestro PA, Moxley KM, Kim YM, Powell MA, O'Malley DM, Spirtos NM, Small W Jr, Tewari KS, Richards WE, Nakayama J, Matulonis UA, Huang HQ and Miller DS. Adjuvant chemotherapy plus radiation for locally advanced endometrial cancer. N Engl J Med 2019; 380: 2317-2326.

[7] Matthews HK, Bertoli C and de Bruin RAM. Cell cycle control in cancer. Nature Reviews Molecular Cell Biology 2022; 23: 74-88.

[8] Jamasbi E, Hamelian M, Hossain MA and Varmira K. The cell cycle, cancer development and therapy. Mol Biol Rep 2022; 49: 10875-10883.

[9] Yuan K, Wang X, Dong H, Min W, Hao H and Yang P. Selective inhibition of CDK4/6: a safe and effective strategy for developing anticancer drugs. Acta Pharm Sin B 2021; 11: 30-54.

[10] Wei X, Song M, Huang C, Yu Q, Jiang G, Jin G, Jia X and Shi Z. Effectiveness, safety and pharmacokinetics of Polo-like kinase 1 inhibitors in tumor therapy: a systematic review and meta-analysis. Front Oncol 2023; 13: 1062885.

[11] Li Z and Zhang X. Kinases involved in both autophagy and mitosis. Int J Mol Sci 2017; 18: 1884.

[12] Leonard MK, Hill NT, Bubulya PA and Kadakia MP. The PTEN-Akt pathway impacts the integrity and composition of mitotic centrosomes. Cell Cycle 2013; 12: 1406-1415.

[13] Ruf S, Heberle AM, Langelaar-Makkinje M, Gelino S, Wilkinson D, Gerbeth C, Schwarz JJ, Holzwarth B, Warscheid B, Meisinger C, van Vugt MA, Baumeister R, Hansen M and Thedieck K. PLK1 (polo like kinase 1) inhibits MTOR complex 1 and promotes autophagy. Autophagy 2017; 13: 486-505.

[14] Ando K, Ozaki T, Yamamoto H, Furuya K, Hosoda M, Hayashi S, Fukuzawa M and Nakagawara A. Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. J Biol Chem 2004; 279: 25549-25561.

[15] Degenhardt Y and Lampkin T. Targeting Polo-like kinase in cancer therapy. Clin Cancer Res 2010; 16: 384-389.

[16] King SI, Purdie CA, Bray SE, Quinlan PR, Jordan LB, Thompson AM and Meek DW. Immunohistochemical detection of Polo-like kinase-1 (PLK1) in primary breast cancer is associated with TP53 mutation and poor clinical outcom. Breast Cancer Res 2012; 14: R40.

[17] Rödel F, Keppner S, Capalbo G, Bashary R, Kaufmann M, Rödel C, Strebhardt K and Spänkuch B. Polo-like kinase 1 as predictive marker and therapeutic target for radiotherapy in rectal cancer. Am J Pathol 2010; 177: 918-929.
[18] Takai N, Miyazaki T, Fujisawa K, Nasu K, Hamanaka R and Miyakawa I. Polo-like kinase (PLK) expression in endometrial carcinoma. Cancer Lett 2001; 169: 41-49.
[19] Liu Z, Sun Q and Wang X. PLK1, a potential target for cancer therapy. Transl Oncol 2017; 10: 22-32.
[20] Shakeel I, Basheer N, Hasan GM, Afzal M and Hassan MI. Polo-like Kinase 1 as an emerging drug target: structure, function and therapeutic implications. J Drug Target 2021; 29: 168-184.
[21] Yu Z, Deng P, Chen Y, Liu S, Chen J, Yang Z, Chen J, Fan X, Wang P, Cai Z, Wang Y, Hu P, Lin D, Xiao R, Zou Y, Huang Y, Yu Q, Lan P, Tan J and Wu X. Inhibition of the PLK1-coupled cell cycle machinery overcomes resistance to oxaliplatin in colorectal cancer. Adv Sci (Weinh) 2021; 8: e2100759.
[22] Otto T and Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nature Reviews Cancer 2017; 17: 93-115.
[23] Bell DW and Ellenson LH. Molecular genetics of endometrial carcinoma. Annu Rev Pathol 2019; 14: 339-367.
[24] Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, Yau C, Laird PW, Ding L, Zhang W, Mills GB, Kucherlapati R, Mardis ER and Levine DA. Integrated genomic characterization of endometrial carcinoma. Nature 2013; 497: 67-73.
[25] Lin DI, Fine A, Danziger NA, Huang RSP, Mata DA, Decker B, Killian JK, Ramkissoon SH, Lechpammer M, Janovitz T, Ross JS, Sokol ES and Elvin JA. Molecular analysis of endometrial serous carcinoma reveals distinct clinicopathologic and genomic subgroups. Gynecol Oncol 2022; 164: 558-565.
[26] Fan J, Bellon M, Ju M, Zhao L, Wei M, Fu L and Nicot C. Clinical significance of FBXW7 loss of function in human cancers. Mol Cancer 2022; 21: 87.
[27] Kurimchak A and Graña X. PP2A counterbalances phosphorylation of pRB and mitotic proteins by multiple CDKs: potential implications for PP2A disruption in cancer. Genes Cancer 2012; 3: 739-748.
[28] Affatato R, Chiappa M, Guffanti F, Ricci F, Formenti L, Fruscio R, Jaconi M, Ridinger M, Erlander M and Damia G. Onvansertib and paclitaxel combined in platinum-resistant ovarian carcinomas. Ther Adv Med Oncol 2022; 14: 17588359221095064.
[29] Giordano A, Liu Y, Armeson K, Park Y, Ridinger M, Erlander M, Reuben J, Britten C, Kappler C, Yeh E and Ethier S. Polo-like kinase 1 (Plk1) inhibition synergizes with taxanes in triple negative breast cancer. PLoS One 2019; 14: e0224420.
[30] Valsasina B, Beria I, Alli C, Alzani R, Avanzi N, Ballinari D, Cappella P, Caruso M, Casolaro A, Ciavolella A, Cucchi U, De Ponti A, Felder E, Fiorentini F, Galvani A, Gianellini LM, Giorgini ML, Isacchi A, Lansen J, Pesenti E, Rizzi S, Rocchetti M, Sola F and Moll J. NMS-P937, an orally available, specific small-molecule polo-like kinase 1 inhibitor with antitumor activity in solid and hematologic malignancies. Mol Cancer Ther 2012; 11: 1006-1016.
[31] Sur S, Pagliarini R, Bunz F, Rago C, Diaz LA Jr, Kinzler KW, Vogelstein B and Papadopoulos N. A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53. Proc Natl Acad Sci U S A 2009; 106: 3964-3969.
[32] Degenhardt Y, Greshock J, Laquerre S, Gilmartin AG, Jing J, Richter M, Zhang X, Bleam M, Halsey W, Hughes A, Moy C, Liu-Sullivan N, Powers S, Bachman K, Jackson J, Weber B and Wooster R. Sensitivity of cancer cells to Plk1 inhibitor GSK461364A is associated with loss of p53 function and chromosome instability. Mol Cancer Ther 2010; 9: 2079-2089.
[33] Van Nyen T, Moiola CP, Colas E, Annibali D and Amant F. Modeling endometrial cancer: past, present, and future. Int J Mol Sci 2018; 19: 2348.
[34] Foucquier J and Guedj M. Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect 2015; 3: e00149.
[35] Affatato R, Carrassa L, Chilà R, Lupi M, Restelli V and Damia G. Identification of PLK1 as a new therapeutic target in mucinous ovarian carcinoma. Cancers (Basel) 2020; 12: 672.
[36] Hagege A, Ambrosetti D, Boyer J, Bozec A, Doyen J, Chamorey E, He X, Bourget I, Rousset J, Saada E, Rastoin O, Parola J, Luciano F, Cao Y, Pagès G and Dufies M. The Polo-like kinase 1 inhibitor onvansertib represents a relevant treatment for head and neck squamous cell carcinoma resistant to cisplatin and radiotherapy. Theranostics 2021; 11: 9571-9586.
[37] Rozengurt E, Soares HP and Sinnet-Smith J. Suppression of feedback loops mediated by PI3K/mTOR induces multiple overactivation of compensatory pathways: an unintended consequence leading to drug resistance. Mol Cancer Ther 2014; 13: 2477-2488.

[38] Cao YY, Qiao Y, Wang ZH, Chen Q, Qi YP, Lu ZM, Wang Z and Lu WH. The Polo-Like Kinase 1-mammalian target of rapamycin axis regulates autophagy to prevent intestinal barrier dysfunction during sepsis. Am J Pathol 2023; 193: 296-312.
[39] Li Z, Li J, Bi P, Lu Y, Burcham G, Elzey BD, Ratliff T, Konieczny SF, Ahmad N, Kuang S and Liu X. Plk1 phosphorylation of PTEN causes a tumor-promoting metabolic state. Mol Cell Biol 2014; 34: 3642-3661.
[40] Tang J, Yang X and Liu X. Phosphorylation of Plk1 at Ser326 regulates its functions during mitotic progression. Oncogene 2008; 27: 6635-6645.
[41] Yang Z, Liang SQ, Saliakoura M, Yang H, Vassella E, Konstantinidou G, Tschan M, Hegedüs B, Zhao L, Gao Y, Xu D, Deng H, Marti TM, Kocher GJ, Wang W, Schmid RA and Peng RW. Synergistic effects of FGFR1 and PLK1 inhibitors target a metabolic liability in KRAS-mutant cancer. EMBO Mol Med 2021; 13: e13193.
[42] Makker V, Green AK, Wenham RM, Mutch D, Davidson B and Miller DS. New therapies for advanced, recurrent, and metastatic endometrial cancers. Gynecol Oncol Res Pract 2017; 4: 19.
[43] Zhao S, Li Y, Li G, Ye J, Wang R, Zhang X, Li F, Gao C, Li J, Jiang J and Mi Y. PI3K/mTOR inhibitor VS-5584 combined with PLK1 inhibitor exhibits synergistic anti-cancer effects on non-small cell lung cancer. Eur J Pharmacol 2023; 957: 176004.
[44] Montaudon E, El Botty R, Vacher S, Déas O, Naguez A, Chateau-Joubert S, Treguer D, de Plater L, Zemoura L, Némati F, Nicolas A, Chapelier A, Livartowski A, Cairo S, Daniel C, Brevet M, Marangoni E, Meseure D, Roman-Roman S, Bieche I, Girard N and Decaudin D. High in vitro and in vivo synergistic activity between mTORC1 and PLK1 inhibition in adenocarcinoma NSCLC. Oncotarget 2021; 12: 859-872.
[45] Ward A and Hudson JW. p53-Dependent and cell specific epigenetic regulation of the polo-like kinases under oxidative stress. PLoS One 2014; 9: e87918.
[46] Li X, Zhu H, Huang H, Jiang R, Zhao W, Liu Y, Zhou J and Guo FJ. Study on the effect of IRE1a on cell growth and apoptosis via modulation PLK1 in ER stress response. Mol Cell Biochem 2012; 365: 99-108.
[47] Feng Y, Li T, Lin Z, Li Y, Han X, Pei X, Fu Z, Wu Q, Shao D and Li C. Inhibition of Polo-like kinase 1 (PLK1) triggers cell apoptosis via ROS-caused mitochondrial dysfunction in colorectal carcinoma. J Cancer Res Clin Oncol 2023; 149: 6883-6899.
[48] Gao J, Huang W, Zhao S, Wang R, Wang Z, Ye J, Lin L, Cai W and Mi Y. Polo-like kinase 1 inhibitor NMS-P937 represses nasopharyngeal carcinoma progression via induction of mitotic abnormalities. J Biochem Mol Toxicol 2024; 38: e23590.
[49] Kudo M, Zalles N, Distefano R, Nigita G, Veneziano D, Gasparini P and Croce CM. Synergistic apoptotic effect of miR-183-5p and Polo-Like kinase 1 inhibitor NMS-P937 in breast cancer cells. Cell Death Differ 2022; 29: 407-419.
[50] Chen L, Ahmad N and Liu X. Combining p53 stabilizers with metformin induces synergistic apoptosis through regulation of energy metabolism in castration-resistant prostate cancer. Cell Cycle 2016; 15: 840-849.
[51] Wang X and Simon R. Identification of potential synthetic lethal genes to p53 using a computational biology approach. BMC Med Genomics 2013; 6: 30.
[52] Alessandrino F, Goncalves N, Metalonis SW, Luna C, Mason MM, Lyu J and Huang M. Uterine serous carcinoma: assessing association between genomics and patterns of metastasis. Front Oncol 2023; 13: 1066427.
[53] Wu J, Ivanov AI, Fisher PB and Fu Z. Polo-like kinase 1 induces epithelial-to-mesenchymal transition and promotes epithelial cell motility by activating CRAF/ERK signaling. Elife 2016; 5: e10734.
[54] Shin SB, Jang HR, Xu R, Won JY and Yim H. Active PLK1-driven metastasis is amplified by TGF-β signaling that forms a positive feedback loop in non-small cell lung cancer. Oncogene 2020; 39: 767-785.
[55] Pan YR, Lai JC, Huang WK, Peng PH, Jung SM, Lin SH, Chen CP, Wu CE, Hung TH, Yu AL, Wu KJ and Yeh CN. PLK1 and its substrate MISP facilitate intrahepatic cholangiocarcinoma progression by promoting lymphatic invasion and impairing E-cadherin adherens junctions. Cancer Gene Ther 2024; 31: 322-333.
[56] Song R, Hou G, Yang J, Yuan J, Wang C, Chai T and Liu Z. Effects of PLK1 on proliferation, invasion and metastasis of gastric cancer cells through epithelial-mesenchymal transition. Oncol Lett 2018; 16: 5739-5744.
[57] Zeng TT, Deng TH, Liu Z, Zhan JR, Ma YZ, Yan YY, Sun X, Zhu YH, Li Y, Guan XY and Li L. HN1L/AP-2γ/PLK1 signaling drives tumor progression and chemotherapy resistance in esophageal squamous cell carcinoma. Cell Death Dis 2022; 13: 1026.
[58] Driscoll DL, Chakravarty A, Bowman D, Shinde V, Lasky K, Shi J, Vos T, Stringer B, Amidon B, D'Amore N and Hyer ML. Plk1 inhibition causes post-mitotic DNA damage and senescence in a

range of human tumor cell lines. PLoS One 2014; 9: e111060.
[59] Wang D, Veo B, Pierce A, Fosmire S, Madhavan K, Balakrishnan I, Donson A, Alimova I, Sullivan KD, Joshi M, Erlander M, Ridinger M, Foreman NK, Venkataraman S and Vibhakar R. A novel PLK1 inhibitor onvansertib effectively sensitizes MYC-driven medulloblastoma to radiotherapy. Neuro Oncol 2022; 24: 414-426.
[60] Su S, Chhabra G, Singh CK, Ndiaye MA and Ahmad N. PLK1 inhibition-based combination therapies for cancer management. Transl Oncol 2022; 16: 101332.
[61] Weiss GJ, Jameson G, Von Hoff DD, Valsasina B, Davite C, Di Giulio C, Fiorentini F, Alzani R, Carpinelli P, Di Sanzo A, Galvani A, Isacchi A and Ramanathan RK. Phase I dose escalation study of NMS-1286937, an orally available Polo-Like Kinase 1 inhibitor, in patients with advanced or metastatic solid tumors. Invest New Drugs 2018; 36: 85-95.
[62] Ahn DH, Barzi A, Ridinger M, Samuëlsz E, Subramanian RA, Croucher PJP, Smeal T, Kabbinavar FF and Lenz HJ. Onvansertib in combination with FOLFIRI and bevacizumab in second-line treatment of KRAS-mutant metastatic colorectal cancer: a phase Ib clinical study. Clin Cancer Res 2024; 30: 2039-47.