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Avapritinib: A Selective Inhibitor of KIT and PDGFRα that Reverses ABCB1 and ABCG2-Mediated Multidrug Resistance in Cancer Cell Lines

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Abstract

The frequent occurrence of multidrug resistance (MDR) conferred by the overexpression of ATP-binding cassette (ABC) transporters ABCB1 and ABCG2 in cancer cells remains a therapeutic obstacle for scientists and clinicians. Consequently, developing or identifying modulators of ABCB1 and ABCG2 that are suitable for clinical practice is of great importance. Therefore, we have explored the drug repositioning approach to identify candidate modulators of ABCB1 and ABCG2 from tyrosine kinase inhibitors with known pharmacological properties and anticancer activities. In this study, we discovered that avapritinib (BLU-285), a potent, selective, and orally bioavailable tyrosine kinase inhibitor against mutant forms of KIT and platelet-derived growth factor receptor alpha (PDGFRA), attenuates the transport function of both ABCB1 and ABCG2. Moreover, avapritinib restores the chemosensitivity of ABCB1- and ABCG2-overexpressing MDR cancer cells at nontoxic concentrations. These findings were further supported by results of apoptosis induction assays, ATP hydrolysis assays, and docking of avapritinib in the drug-binding pockets of ABCB1 and ABCG2. Altogether, our study highlights an additional action of avapritinib on ABC drug transporters, and a combination of avapritinib with conventional chemotherapy should be further investigated in patients with MDR tumors.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

Keywords

Chemoresistance; Combination chemotherapy with BLU-285; Modulators; P-glycoprotein; Breast cancer resistance protein

Introduction

The drug efflux function of the ATP-binding cassette (ABC) proteins ABCB1 (P-glycoprotein, MDR1) and ABCG2 (BCRP, MXR) often contributes to the development of the multidrug resistance (MDR) phenotype in cancer cells, which remains a major therapeutic obstacle[1–3]. Both ABCB1 and ABCG2 are able to utilize energy generated from ATP hydrolysis to actively efflux a broad spectrum of chemotherapeutic agents out of cancer cells and away from their intracellular drug targets[3–5]. Consequently, cancer cells overexpressing ABCB1 or ABCG2 are significantly less sensitive to most conventional anticancer drugs and numerous protein kinase inhibitors[6–9], resulting in cancer recurrence and treatment failure[2, 3]. The results from several studies suggest that patients with solid tumors, such as metastatic breast cancer[10] and advanced non-small cell lung cancer[11], as well as blood cancers such as multiple myeloma (MM)[12–18], chronic lymphocytic leukemia (CLL)[19], chronic myeloid leukemia (CML)[20], acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL)[21–23], are likely to be affected adversely by the drug transport function of ABCB1 and ABCG2. Moreover, in addition to conferring multidrug resistance in cancer cells, ABCB1 and ABCG2 have a crucial role as an endogenous defense mechanism against xenobiotics. Both transporters are highly expressed in cells forming the blood-tissue barrier (BTB) and blood-brain barrier (BBB) sites, influencing the absorption, distribution, metabolism and elimination of a majority of drugs in patients[2, 7, 24]. Consequently, developing modulators that are capable of inhibiting the drug transport function and/or protein expression level of ABCB1 and ABCG2 is of great importance to clinicians and medical researchers.

As currently there are no US Food and Drug Administration (FDA)-approved synthetic chemosensitizing agents for treating MDR cancers, it has become a practical therapeutic strategy against MDR cancers by using either drug repositioning (or drug repurposing) of approved therapeutic agents or agents already in clinical development to resensitize MDR cancer cells to conventional chemotherapeutic agents[25–31]. Here, we identify avapritinib as an effective modulator of ABCB1 and ABCG2. Avapritinib (BLU-285) is a potent, selective, and orally bioavailable inhibitor of mutant forms of KIT and platelet-derived growth factor receptor alpha (PDGFRA), such as D816 V KIT[32] and D842 V PDGFRA[33]. The D816 V KIT mutation is found in most patients with systemic mastocytosis (SM)[34], whereas the D842 V PDGFRA mutation is found in 20% of patients with gastrointestinal stromal tumors (GIST)[35], one of the most common soft tissue sarcomas of the digestive tract[36]. Most GISTs are driven by activating mutations in KIT or PDGFRA, resulting in constitutive activation of kinase activity and downstream kinase effectors in a ligand-independent manner[37]. Although tyrosine kinase inhibitors (TKI), such as imatinib and sunitinib, have been used routinely to treat GIST patients, patients with advanced GIST harboring D842V PDGFRA mutation and a secondary mutation in the activation loop of KIT are not responsive to TKI therapy.

Avapritinib is currently under investigation in patients with GIST and other relapsed and refractory solid tumors (ClinicalTrials.gov: NCT02508532), in patients with locally advanced unresectable or metastatic GIST (NCT03465722), and in patients with advanced systemic mastocytosis (AdvSM) and relapsed or refractory myeloid malignancies (NCT03580655 and NCT02561988).

In the present work, we discovered that avapritinib is capable of reversing ABCB1- and ABCG2-mediated MDR in human cancer cell lines, most likely by attenuating the drug transport function of both ABCB1 and ABCG2. Furthermore, we found that drug-resistant cells overexpressing ABCB1 or ABCG2 were equally sensitive to avapritinib as their drug-sensitive parental cells, suggesting that avapritinib is likely a high-affinity substrate of ABCB1 and ABCG2, and a combination therapy of conventional anticancer drugs and avapritinib could potentially be beneficial for patients with multidrug-resistant cancers.

Experimental Section

Chemicals.

Phosphate-buffered saline (PBS), fetal calf serum (FCS), trypsin-EDTA, RPMI-1640 medium, Iscove's modified Dulbecco's medium (IMDM), Dulbecco's modified eagle's medium (DMEM), penicillin, and streptomycin were obtained from Gibco, Invitrogen (Carlsbad, CA, USA). Annexin V: an FITC Apoptosis Detection Kit was purchased from BD Pharmingen (San Diego, CA, USA); Tools Cell Counting (CCK-8) Kit was purchased from Biotools Co., Ltd (Taipei, Taiwan). Verapamil, Ko143 and all other chemicals were obtained from Sigma (St. Louis, MO, USA), unless stated otherwise. Avapritinib (BLU-285) was purchased from Selleckchem (Houston, TX, USA).

Cell Lines.

HEK293 human embryonic kidney cell line and HEK293 stably transfected with human ABCB1 (MDR19-HEK293) and wild-type human ABCG2 (R482-HEK293) were cultured in DMEM containing 2 mg/mL of G418[38]. The drug-sensitive parental human epidermal cancer cell line KB-3–1 and its drug-resistant ABCB1-overexpressing subline KB-V1 were maintained in DMEM, and 1 mg/mL of vinblastine (KB-V-1)[39]. The drug-sensitive human ovarian cancer cell line OVCAR-8 and drug-resistant ABCB1-overexpressing subline NCI-ADR-RES, drug-sensitive human nonsmall cell lung cancer cell line H460 and drug-resistant ABCG2-overexpressing subline H460-MX20, drug-sensitive human colon cancer cell line S1 and drug-resistant ABCG2-overexpressing subline S1-M1–80 were maintained in RPMI-1640, and 20 nM mitoxantrone (H460-MX20)[40] or 80 nM mitoxantrone (S1-M1–80)[41]. Cells were cultured in medium supplemented with 10% FCS, 2 mM L-glutamine and 100 units/mL of penicillin/streptomycin at 37 °C in 5% CO_2 humidified air and maintained in drug-free medium for 7 days prior to assay.

Flow Cytometry.

Calcein and pheophorbide A (PhA), respectively known as a fluorescent substrate of ABCB1 and ABCG2, were used to monitor intracellular substrate accumulation in cells overexpressing ABCB1 or ABCG2, as described previously[42, 43]. Briefly, cells were

harvested (3×10^5) and incubated in 4 mL of IMDM supplemented with 5% FCS with 0.5 μM calcein-AM or 1 μM PhA in the presence or absence of 20 μM avapritinib or 20 μM verapamil (ABCB1 reference inhibitor) or 3 μM Ko143 (ABCG2 reference inhibitor) at 37 °C in 5% CO_2 humidified air. After being washed, cells were resuspended in ice-cold PBS, and the intracellular accumulation of fluorescent substrate calcein or PhA was analyzed with a FACScan flow cytometer with CellQuest software (BD Biosciences, San Jose, CA, USA) and FlowJo software (Tree Star, Inc., Ashland, OR, USA) according to the method described by Gribar *et al*[44].

Cell Viability Assay.

Cells were first seeded into each well of the 96-well flat bottom plates and allowed to attach overnight at 37 °C in 5% CO_2 humidified air. The specified regimen at various concentrations was then added to each well and incubated at 37 °C in 5% CO_2 humidified air for an additional 72 h. Cell Counting Kit-8 (CCK-8) reagent was used to determine the cytotoxicity of drugs in HEK293 cells and HEK293 cells stably transfected with human ABCB1 or ABCG2, whereas MTT reagent was used to determine the cytotoxicity of drugs in attached human cancer cells according to the method described by Ishiyama *et al.*[45]. Concentration-response curves obtained from at least three independent experiments were fitted, and the IC_{50} values were subsequently calculated. A nontoxic concentration of avapritinib or a reference inhibitor was added to the cytotoxicity assays in drug resistance reversal experiments, and the fold-reversal (FR) values, representing the extent of reversal, were calculated as described previously[38, 46].

Western Blot.

A Western blot immunoassay was performed as described previously[38]. Briefly, cancer cells overexpressing ABCB1 or ABCG2 were treated with avapritinib at different concentrations for 72 h before being harvested and subjected to SDS-polyacrylamide electrophoresis. Blots were probed with the following antibodies: C219 (1: 3000 dilution) for the detection of ABCB1, BXP-21 (1:15000 dilution) for the detection of ABCG2 or α-tubulin (1:100000 dilution) for the detection of tubulin, a positive loading control. Goat anti-mouse IgG secondary antibody (1:100000 dilution) and the enhanced chemiluminescence (ECL) kit (Merck Millipore, Billerica, MA, USA) were used for signal detection and visualization[38].

Apoptosis Assay.

The conventional Annexin V–FITC and propidium iodide (PI) staining method[31] was performed to evaluate the apoptotic cell population induced by avapritinib alone, colchicine alone, topotecan alone or in combinations. Briefly, cells were first treated with each regimen as indicated for 48 h before being labeled with 1.25 μg/mL annexin V–FITC and 0.1 mg/mL PI (PharMingen) for 15 min at room temperature. The labeled cells were analyzed by FACScan equipped with CellQuest software after each regimen, as described previously[31]. The early apoptotic cell population (phosphatidylserine (PS)-positive and PI-negative) consisted of cells with intact plasma membranes, whereas necrotic or late apoptotic cell population (PS-positive and PI-positive) consisted of cells with leaky membranes[47].

ATPase Assay.

Total membrane vesicles of high-five insect cells (Invitrogen, Carlsbad, CA, USA) infected with recombinant baculovirus carrying the MDR1 gene or ABCG2 gene with a hexahistidine or decahistidine tag at the C-terminal end respectively, were prepared by hypotonic lysis and differential centrifugation as described previously[48, 49]. The ATPase assay was performed as follows. Membranes (100 μg /mL protein) were incubated in buffer containing 50 mM MES-Tris pH 6.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM EGTA, 1 mM ouabain and 2 mM DTT in the presence or absence of 0.3 mM sodium orthovanadate. Basal ATPase activity was measured in the presence of 1% v/v DMSO, whereas the effect of avapritinib on ATPase hydrolysis was measured in the presence of increasing concentrations of avapritinib at 37°C. The addition of 5 mM ATP started the reaction and the reaction was ended by the addition of 2.5% SDS. The amount of inorganic phosphate (P_i) released over a period of 20 min was quantified and the Vi-sensitive activity was determined using a colorimetric method, as described previously[50]. The respective IC_{50} values were calculated from fitted concentration-response curves acquired from three independent experiments using Graphpad Prism (La Jolla, CA, USA) software v.7.

Docking of Avapritinib with Modeled Structure of ABCB1 and ABCG2.

The mouse P-glycoprotein crystal structure (PDB:5KPI)[51] was used as a template to generate the homology model of human ABCB1 and the human ABCB1 sequence (UniprotKB: P08183) using SWISS-MODEL[52], whereas the cryo-electron microscopy structure of ABCG2 (PDB: 5NJ3) was used for ABCG2. Ligand and receptor structures were prepared for docking using the MGLtools software package[53]. AutoDock Vina was used for extensive docking studies. A total of 27 and 32 residues on ABCB1 and ABCG2 were set as flexible side chains, respectively. The ABCB1 residues were: L65, M68, M69, F72, Q195, F303, I306, Y307, Y310, F314, F336, L339, I340, F343, Q347, N721, Q725, F728, F732, F759, F770, F938, F942, M949, Y953, F957, L975, F978, V982, M986, Q990, S993 and F994 and ABCG2 residues (on both chains) were: Q393, V401, L405, I409, Y413, Q424, F431, F432, T435, N436, F439, S440, V442, S443, T538, L539, T542, I543, V546, F547, M549, I550, L554, and L555. The receptor grid was centered at x = 20, y = 60 and z = 5 Å for ABCB1 and at x = 125, y = 125 and z = 130 Å for ABCG2, whereas the inner box dimensions were 50 × 50 × 50 Å for ABCB1 and 34 × 30 × 50 Å for ABCG2. The exhaustiveness was set at 100 for both proteins.

Quantification and Statistical Analysis.

Unless stated otherwise, the experimental data and IC_{50} values are presented as the mean ± standard deviation (SD) calculated from at least three independent experiments. GraphPad Prism (La Jolla, CA, USA) software was used for curve plotting and KaleidaGraph (Reading, PA, USA) software was used for statistical analysis. A two-sided Student's t-test was carried out for each experiment, and asterisks indicate the "statistically significant" difference between mean values or improvement in fit, if the probability, *P*, was less than 0.05.

Results

Avapritinib attenuates the drug transport function of ABCB1 and ABCG2

To evaluate the effect of avapritinib on the drug transport function of ABCB1 and ABCG2, we determined the intracellular accumulation of a known substrate drug of ABCB1 or ABCG2 in the presence or absence of avapritinib in cells expressing ABCB1 or ABCG2 as described in the Experimental Section. First, we found that avapritinib had no detectable effect on the accumulation of calcein or pheophorbide A (PhA) in drug-sensitive OVCAR-8 human ovarian cancer cells, S1 human colon cancer cells, or parental HEK293 cells (Figure 1A–D, left panels). In contrast, 20 μM avapritinib substantially increased the accumulation of calcein, a fluorescent product of a known ABCB1 substrate drug calcein-AM[54] in ABCB1-overexpressing human ovarian cancer cell line NCI-ADR-RES (Figure 1A, right panel) and in MDR19-HEK293 cells (Figure 1B, right panel), which are HEK293 cells transfected with human ABCB1. Similarly, 20 μM avapritinib markedly increased the accumulation of PhA, a fluorescent compound known to be a substrate of ABCG2[42] in the ABCG2-overexpressing human colon cancer cell line S1-M1–80 (Figure 1C, right panel) and in R482-HEK293 cells (Figure 1D, right panel), which are HEK293 cells transfected with human ABCG2. Of note, 20 μM verapamil was used as an inhibitor of ABCB1 (Figure 1A and B) and 1 μM Ko143 was used as a reference inhibitor of ABCG2 (Figure 1C and D).

Avapritinib reverses multidrug resistance mediated by ABCB1 and ABCG2

Knowing that avapritinib inhibits ABCB1-mediated drug transport in ABCB1-overexpressing cells and inhibits ABCG2-mediated drug transport in ABCG2-overexpressing cells (Figure 1), we further examined the effect of avapritinib on MDR mediated by ABCB1 or ABCG2 in MDR cancer cell lines overexpressing ABCB1 or ABCG2. First, we discovered that at nonoxic concentrations (0.1 – 1.0 μM) and without affecting the proliferation of drug-sensitive parental cells (Figure 2, left panels), avapritinib resensitized ABCB1-overexpressing NCI-ADR-RES cancer cells (Figure 2A, right panel) and human KB-V1 epidermal cancer cells (Figure 2B, right panel), as well as in ABCB1-transfected MDR19-HEK293 cells (Figure 2C, right panel) to paclitaxel in a concentration-dependent manner. Next, we found that at the same concentrations, avapritinib reversed ABCG2-mediated mitoxantrone resistance in ABCG2-overexpressing S1-M1–80 cancer cells (Figure 2D, right panel) and human H460-MX20 lung cancer cells (Figure 2E, right panel), as well as in ABCG2-transfected R482-HEK293 cells (Figure 2F, right panel). Subsequently, we discovered that avapritinib restored the chemosensitivity of ABCB1-overexpressing MDR cells to colchicine and doxorubicin (Table 1), as well as ABCG2-overexpressing MDR cells to topotecan and SN-38 (Table 2) in a similar manner. As shown in Tables 1 and 2, the fold-reversal (FR) value represents the extent of reversal of a particular drug-resistant cell line to a particular drug by a modulator such as avapritinib[46], which was calculated as described in Experimental Section. Of note, 5 μM of verapamil and 1 μM of Ko143 are positive controls demonstrating the reversal of MDR mediated by ABCB1 and ABCG2[55]. Our results here revealed that avapritinib, even at low concentrations, was able to reverse ABCB1- and ABCG2-mediated MDR in cancer cells, which are consistent with the results of drug accumulation assays (Figure 1).

Avapritinib has no significant effect on the protein level of ABCB1 or ABCG2 in cancer cells

In addition to direct inhibition of drug transport mediated by ABCB1 or ABCG2, another common mechanism for modulators to resensitize MDR cancer cells is by transiently down-regulating the protein expression of ABCB1 or ABCG2 in cancer cells[56, 57]. To this end, we treated ABCB1-overexpressing NCI-ADR-RES (Figure 3A) and KB-V1 cancer cells (Figure 3B), as well as ABCG2-overexpressing S1-M1–80 (Figure 3C) and H460-MX20 cancer cells (Figure 3D) with increasing concentrations of avapritinib (0 – 1 μM) for 72 h and examined the protein level of ABCB1 and ABCG2 in these cell lines by Western blotting, as described in Experimental Section. Our results showed that avapritinib had no significant effect on the protein expression of ABCB1 or ABCG2 in all the cell lines, suggesting that the down-regulation of ABCB1 or ABCG2 is unlikely to play a major role in the chemosensitization of MDR cancer cells by avapritinib.

Avapritinib increases drug-induced apoptosis in cancer cells overexpressing ABCB1 or ABCG2

Given that a cell proliferation assay cannot distinguish growth retardation from drug-induced cytotoxicity, we decided to examine the effect of avapritinib on apoptosis induced by colchicine and topotecan, which are known inducers of apoptosis and substrate drugs of ABCB1 and ABCG2[58, 59], in human cancer cells overexpressing ABCB1 or ABCG2. In addition to examining avapritinib in 72 h cytotoxicity assays (Tables 1 and 2), the effect of avapritinib on MDR cancer cells was examined after a shorter period of time (48 h). Drug-sensitive KB-3–1 cells and drug-resistant KB-V1 cells were treated with DMSO, 2 μM avapritinib, 0.5 μM colchicine or colchicine and avapritinib in combination for 48 h and processed as described in the Experimental Section. As shown in Figure 4A, treatment with colchicine alone substantially increased the level of apoptosis in KB-3–1 cancer cells, from 5% basal level to approximately 66% of early and late apoptosis. As expected, colchicine had no significant effect on KB-V1 cells (from approximately 9 to 11% total apoptosis). Notably, the level of colchicine-induced apoptosis in KB-V1 cancer cells was enhanced significantly by avapritinib, from approximately 9% basal level to 52% of early and late apoptosis (Figure 4A). Similarly, the drug-sensitive S1 cell line and the drug-resistant S1-M1–80 subline were treated with DMSO, 2 μM avapritinib, 5 μM topotecan or topotecan and avapritinib in combination for 48 h. As shown in Figure 4B, topotecan increased the level of apoptosis considerably in S1 cancer cells from approximately 2% basal level to 31%, but had no effect on S1-M1–80 cancer cells. Avapritinib significantly enhanced topotecan-induced apoptosis in S1-M1–80 cells, from approximately 3% basal level to 18% total apoptosis (Figure 4B). Of note, treatment with 2 μM avapritinib alone had no significant apoptotic effect in either drug-sensitive or drug-resistant cell lines. Our results suggest that by blocking the drug efflux function of both ABCB1 and ABCG2, avapritinib increases drug-induced apoptosis in ABCB1- and ABCG2-overexpressing cancer cells and restores the chemosensitivity of these cells.

Avapritinib stimulates the ATPase activity of ABCB1 and ABCG2

Knowing that ABCB1 and ABCG2-mediated substrate transport is coupled to ATP hydrolysis[60, 61], we examined the effect of avapritinib on the vanadate (Vi)-sensitive ATP hydrolysis of ABCB1 and ABCG2 to gain insight into the manner in which avapritinib interacts with the substrate-binding pockets of ABCB1 and ABCG2. Avapritinib stimulated the ATPase activity of ABCB1 in a concentration-dependent manner to the maximum level of approximately 148% and the half maximal effective concentration (EC_{50}) of approximately 5 μM (basal, 80.9 ± 4.9 nmole P_i/min/mg protein) (Figure 5A). Moreover, avapritinib stimulated the ATPase activity of ABCG2 in a similar manner, with approximately 160% maximal stimulation and an EC_{50} value of approximately 0.2 μM (basal, 53.8 ± 1.0 nmole P_i/min/mg protein) (Figure 5B). Our data suggest that avapritinib interacts at the drug-binding pocket of ABCB1 and ABCG2.

Docking of avapritinib in the drug-binding pocket of ABCB1 and ABCG2

To further understand the binding of avapritinib with ABCB1 and ABCG2, docking of avapritinib was performed using a homology model of ABCB1 (based on the PDB:5KPI mouse Abcb1 structure) as a template, and the cryo-electron microscopy (EM) structure of ABCG2 (PDB: 5NJ3). As shown in Figure 6, analysis of the lowest energy docking poses allows for the identification of potential hydrophobic and aromatic interactions between avapritinib and the hydrophobic and aromatic residues located in the substrate-binding pocket of ABCB1 and ABCG2. Our data indicate that avapritinib can bind to the drug-binding pocket in the transmembrane regions of both ABCB1 and ABCG2.

Overexpression of ABCB1 or ABCG2 does not alter the chemosensitivity of cancer cells to avapritinib

Knowing that the overexpression of ABCB1 and/or ABCG2 in cancer cells is associated with reduced susceptibility to several tyrosine kinase inhibitors that have been used to treat patients with GIST[30, 56, 57], such as imatinib[62–65] and sunitinib[66, 67], we thus examined the sensitivity of drug-sensitive parental cells and MDR cells overexpressing either ABCB1 or ABCG2 to avapritinib. As shown in Table 3, the IC_{50} value of avapritinib in MDR cell lines was divided by the IC_{50} value of avapritinib in respective drug-sensitive parental cell lines to obtain the resistance factor (R.F.) value, representing the extent of chemoresistance caused by the overexpression of ABCB1 or ABCG2. We found that none of the ABCB1-overexpressing cells or ABCG2-overexpressing cells were significantly more resistant to avapritinib when compared to their respective drug-sensitive parental cells.

Discussion

Resensitization of ABCB1- and ABCG2-overexpressing cancer cells to conventional anticancer agents remains an important aspect in overcoming multidrug resistance in cancer[3, 5, 9]. Many attempts of introducing novel synthetic inhibitors of ABCB1 and ABCG2 into clinical practice for the treatment of MDR cancer have failed due to adverse drug-drug interactions and unforeseen side effects and toxicity[2, 25]. Therefore, we and others have been examining the potential chemosensitizing effect of TKIs, with established pharmacological and toxicological properties, on multidrug-resistant cancer cells overexpressing either

ABCB1 or ABCG2[28, 31, 68–72]. Previous studies have reported that the multitargeted TKIs tandutinib (inhibitor of KIT, PDGFR, and FLT3) and vatalanib (inhibitor of KIT, PDGFR and VEGFR) resensitized MDR cancer cells overexpressing ABCB1 or ABCG2 to various chemotherapeutic drugs that are substrate drugs of ABCB1 or ABCG2[73, 74]. These results prompted us to investigate the chemosensitization effect of avapritinib, a well-tolerated agent, against mutant forms of KIT and PDGFRA, on MDR mediated by ABCB1 and ABCG2 in human cancer cells. Avapritinib displays significant antiproliferative activity in human mast cell lines and leukemia cell lines, as well as in a murine model of SM[32, 33] and in patient-derived xenograft (PDX) GIST models[75], and is currently in clinical trials for treating GIST, AdvSM, and other relapsed and refractory solid tumors and myeloid malignancies. However, the potential chemosensitizing effect of avapritinib on multidrug-resistant cancer cells remains elusive.

In the present study, we examined the interactions of avapritinib with two key multidrug transporters ABCB1 and ABCG2. First, by measuring the cytotoxicity of avapritinib in each cell line (Table 3) and treating drug-sensitive parental cells with various chemotherapeutic drugs in combination with increasing concentrations of avapritinib, we found that avapritinib at concentrations below 1 μM were nontoxic. Our results show that the chemosensitivity of ABCB1- and ABCG2-overexpressing cells was restored by avapritinib at low, nontoxic concentrations. Avapritinib attenuated fluorescent substrate drug transport mediated by ABCB1 and ABCG2 without having a significant effect on the protein expression of ABCB1 or ABCG2 in multidrug-resistant cancer cells. Consequently, drug-induced apoptosis and the sensitivity of ABCB1- and ABCG2-overexpressing cancer cells to multiple therapeutic drugs were significantly increased by avapritinib. Although other possible mechanisms cannot be ruled out, our experiments measuring the effect of avapritinib on the ATPase activity of ABCB1 and ABCG2, as well as the results of drug docking analysis suggest that avapritinib is a high-affinity substrate of ABCB1 and ABCG2. Considering that the ATPase activity of ABCB1 and ABCG2 was stimulated by avapritinib, which is associated with the presence of a substrate at the drug-binding site of these transporters[76], and neither ABCB1 or ABCG2 confer resistance to avapritinib, it is likely that avapritinib directly competes with the binding of other substrate drugs at the drug-binding pocket of ABCB1 and ABCG2, resulting in increased drug accumulation in cells due to efflux mediated by ABCB1 and ABCG2. Moreover, we did not find any evidence for direct interaction between avapritinib and anticancer drugs. It is worth noting that although avapritinib reverses MDR mediated by ABCB1 or ABCG2 in a similar manner as tandutinib and vatalanib, the chemosensitizing effect of avapritinib on ABCB1- and ABCG2-overexpressing multidrug-resistant cancer cells is significantly higher than that of tandutinib and vatalanib. Zhao *et al.* demonstrated that tandutinib resensitized ABCG2-transfected HEK293 cells to SN-38 resistance in a concentration-dependent manner, with the greatest reversal chemosensitization effect of 11-fold at 10 μM[73]. Moreover, To *et al.* demonstrated that at the maximum tested concentration of 3 μM, vatalanib reversed ABCB1- and ABCG2-mediated resistance to paclitaxel and SN-38 in HEK293 cells transfected with ABCB1 or ABCG2 by 3-fold and 10-fold, respectively[74]. In comparison, we found that at 1 μM, avapritinib reversed ABCB1-mediated paclitaxel resistance by 28-fold (Table 1) and

ABCG2-mediated SN-38 resistance by 41-fold (Table 2) in HEK293 cells transfected with ABCB1 or ABCG2, respectively.

Encouraging results from a recent phase I study of patients with sarcomas receiving nilotinib as coadjuvant treatment with doxorubicin[77] support the findings from multiple pre-clinical studies of combination therapy consisting of a conventional anticancer drug and another therapeutic agent acting as a modulator of multidrug transporters and should be further investigated in future clinical trials[28, 71, 78, 79]. Nilotinib is known to be a high-affinity substrate of ABCB1 and ABCG2 that reverses MDR mediated by ABCB1 and ABCG2 in human cancer cells and in a tumor xenograft model by blocking the drug efflux function of both transporters[29, 80–82]. Considering that avapritinib is also a tyrosine kinase inhibitor that blocks the drug efflux function of ABCB1 and ABCG2, and reverses ABCB1- and ABCG2-mediated MDR in cancer cells, our results suggest that treating patients with standard chemotherapy in combination with avapritinib may benefit patients with multidrug-resistant cancer associated with the overexpression of ABCB1 or ABCG2. Repositioning of avapritinib into a modulator role may be less complicated than developing new inhibitors, considering that avapritinib is already in clinical trials for cancer treatment. Moreover, the overexpression and genetic polymorphisms of ABCB1 and ABCG2 are associated with poor clinical outcome in GIST patients receiving chemotherapy[83] or imatinib[84, 85], a known substrate of ABCB1 and ABCG2[86]. Our data summarized in Figure 7 show that multidrug-resistant cells overexpressing ABCB1 or ABCG2 are equally as sensitive to avapritinib as their respective drug-sensitive parental cells, suggesting that neither ABCB1 or ABCG2 is likely to play a major role in the development of resistance to avapritinib in cancer patients, which remains to be verified in future studies.

In summary, we have shown that not only is avapritinib a potent and selective inhibitor of mutant forms of KIT and PDGFRA, it is also an effective modulator of ABCB1 and ABCG2 that is capable of resensitizing multidrug-resistant cancer cells to anticancer drugs at non-toxic concentrations. Despite encouraging preclinical results, favorable clinical outcomes are still uncertain, as adverse responses may occur as a result of combination therapy[25, 87, 88]. Nevertheless, our results suggest that the administration of avapritinib along with chemotherapeutic drugs could potentially enhance the therapeutic efficacy of the treatment of MDR tumors and should be further evaluated in clinical practice.

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Abbreviations:

ABC	ATP-binding cassette
MDR	multidrug resistance
SM	systemic mastocytosis

PDGFRA	platelet-derived growth factor receptor alpha
MM	multiple myeloma
GIST	gastrointestinal stromal tumors
TKI	tyrosine kinase inhibitors
Vi	sodium orthovanadate
FR	fold-reversal
RF	resistance factor

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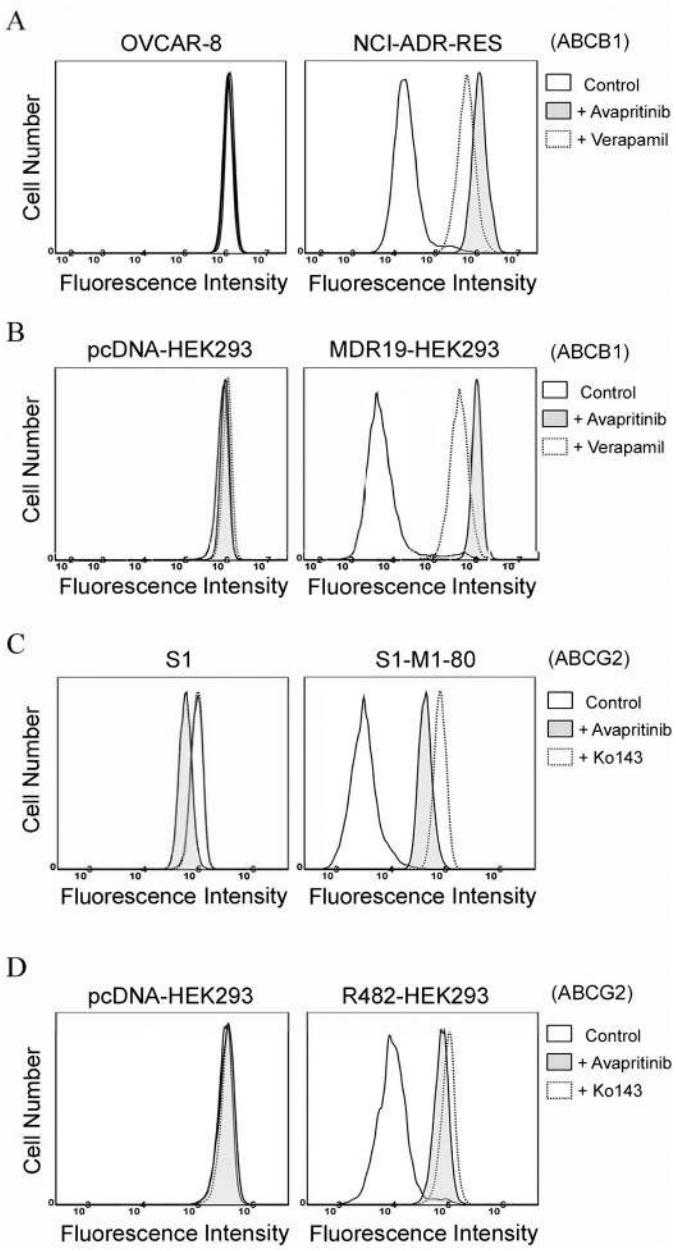

Fig. 1. Avapritinib increases the intracellular drug accumulation in cells overexpressing ABCB1 or ABCG2.

To demonstrate the extent of ABCB1 and ABCG2 inhibition by avapritinib, the drug-sensitive human ovarian cancer cell line OVCAR-8 (A, left panel) and its ABCB1-overexpressing subline NCI-ADR-RES (A, right panel), as well as drug-sensitive parental pcDNA-HEK293 (B, left panels) and human ABCB1-transfected HEK293 cells MDR19-HEK293 cells (B, right panel) were treated with 0.5 μM of a known substrate of ABCB1, calcein-AM, in the presence of DMSO (A, B, solid lines), 20 μM of avapritinib (A, B, filled solid lines) or 20 μM of verapamil, a reference inhibitor of ABCB1 (A, B, dotted lines) as indicated. The drug-sensitive human colon cancer cell line S1 (C, left panel) and its ABCG2-overexpressing subline S1-M1–80 (C, right panel), as well as drug-sensitive

parental pcDNA-HEK293 cells (D, left panels) and human ABCG2-transfected HEK293 cells R482-HEK293 (D, right panel) were treated with 1 μM of a known substrate of ABCG2, fluorescent PhA, in the presence of DMSO (C, D, solid lines) or 20 μM of avapritinib (C, D, filled solid lines) or 1 μM of Ko143, a reference inhibitor of ABCG2 (C, D, dotted lines) as indicated. The intracellular accumulation of fluorescent calcein or PhA in the respective cell line was analyzed by flow cytometry as described previously[38]. Representative histograms of at least three independent experiments are shown.

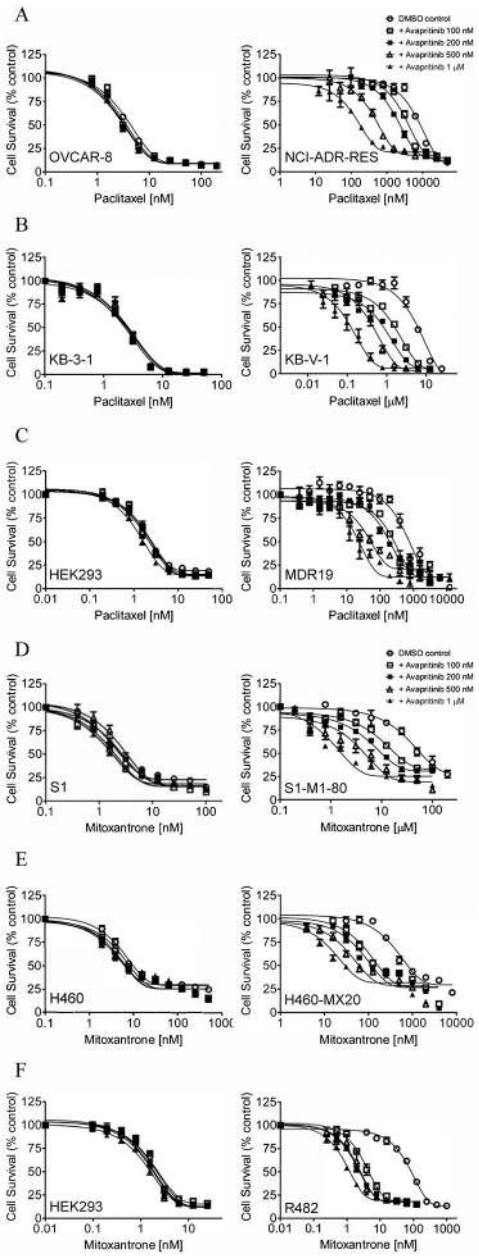

Fig. 2. Avapritinib reverses ABCB1-mediated paclitaxel resistance and ABCG2-mediated mitoxantrone resistance.

The human ovarian cancer cell line OVCAR-8 (A, left panel) and its ABCB1-overexpressing subline NCI-ADR-RES (A, right panel), the human epidermal cancer cell line KB-3–1 (B, left panel) and its ABCB1-overexpressing subline KB-V1 (B, right panel), parental HEK293 cells (C, left panel) and ABCB1-transfected MDR19-HEK293 cells (C, right panel), as well as the human colon cancer cell line S1 (D, left panel) and its ABCG2-overexpressing subline S1-M1–80 (D, right panel), the human lung cancer cell line H460 (E, left panel) and its ABCG2-overexpressing subline H460-MX20 (E, right panel), parental HEK293 cells (E, left panel) and ABCG2-transfected R482-HEK293 cells (E, right panel) were treated with increasing concentrations of paclitaxel (A - C) or mitoxantrone (D - F) in the presence of

DMSO (open circles) or avapritinib at 100 nM (open squares), 200 nM (filled squares), 500 nM (open triangles) or 1.0 μM (filled triangles). Points, mean values from at least three independent experiments; bars; SEM.

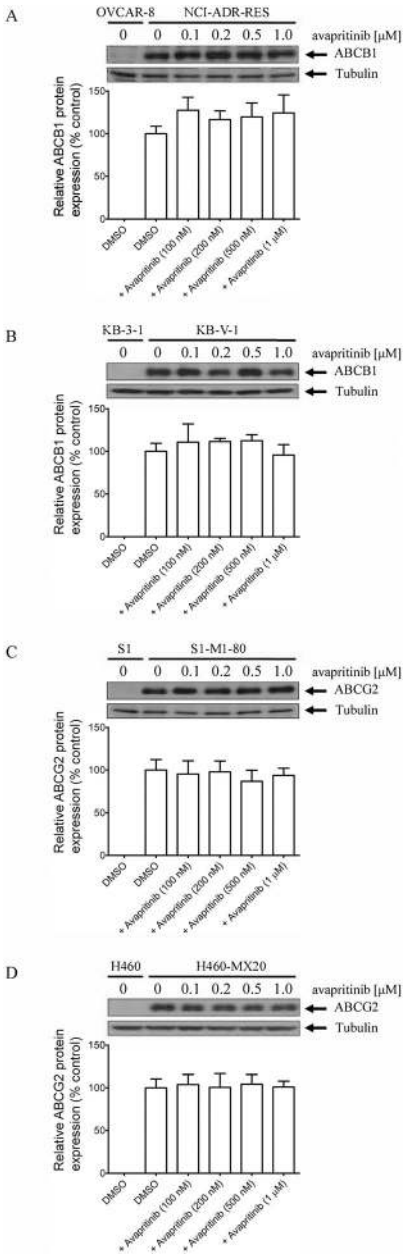

Fig. 3. Avapritinib has no significant effect on the protein expression of ABCB1 or ABCG2 in human cancer cell lines.

Immunoblot detection (upper panels) and quantification (lower panels) of human ABCB1 in ABCB1-overexpressing (A) NCI-ADR-RES and (B) KB-V1 cancer cells or human ABCG2 in ABCG2-overexpressing (C) S1-M1–80 and (D) H460-MX20 cancer cells treated with DMSO (vehicle control) or avapritinib at 0.1 μM, 0.2 μM, 0.5 μM and 1.0 μM as indicated for 72 h before being processed for immunoblotting according to the method described previously[38]. α-Tubulin was used as an internal loading control. Values are presented as mean ± SD calculated from three independent experiments.

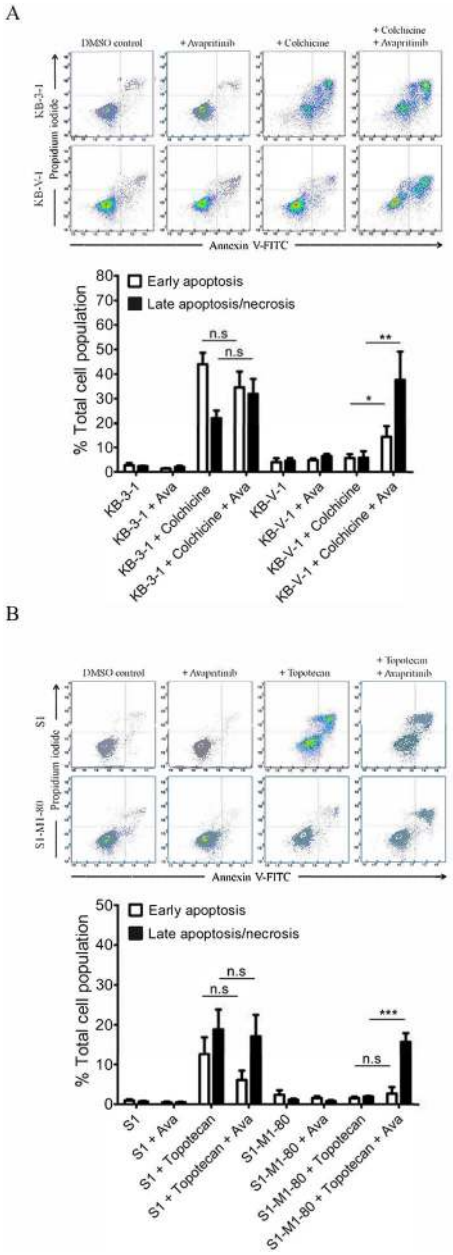

Fig. 4. Avapritinib potentiates drug-induced apoptosis in ABCB1- and ABCG2-overexpressing MDR cancer cells.

Dot plots and quantification of (A) the human epidermal cancer cell line KB-3–1 and its ABCB1-overexpressing subline KB-V1 treated with either DMSO (control), 2 μM of avapritinib (+ avapritinib), 500 nM of colchicine (+ colchicine), or a combination of 500 nM of colchicine and 2 μM of avapritinib (+ colchicine + avapritinib) and (B) the human colon cancer cell line S1 and its ABCG2-overexpressing subline S1-M1–80 treated with either DMSO (control), 2 μM of avapritinib (+ avapritinib), 5 μM of topotecan (+ topotecan), or a combination of 5 μM of topotecan and 2 μM of avapritinib (+ topotecan + avapritinib). Cells were treated with regimens for 48 h, isolated, and analyzed by flow cytometry as described previously[55]. Representative dot plots and quantifications of drug-induced apoptosis in

cancer cell lines presented as the mean ± SD calculated from three independent experiments are shown. $^{**}P < 0.01$; $^{***}P < 0.001$ versus the same treatment in the absence of avapritinib.

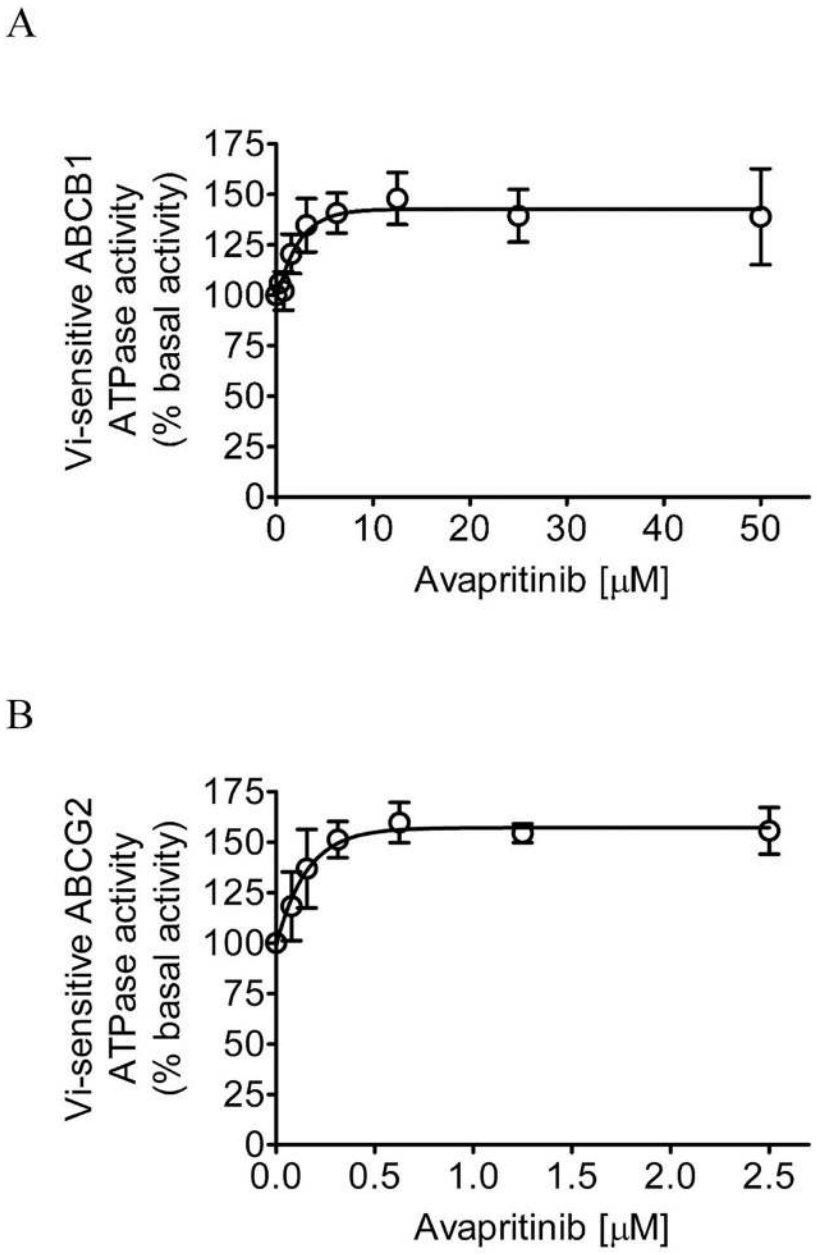

Fig. 5. Avapritinib stimulates ATP hydrolysis of ABCB1 and ABCG2.

An end point P_i assay was used to determine the effect of avapritinib on vanadate-sensitive ATPase activity of (A) ABCB1 and (B) ABCG2, as described previously[50, 89]. Data are presented as the mean ± SEM from at least three independent experiments as a percentage of basal activity.

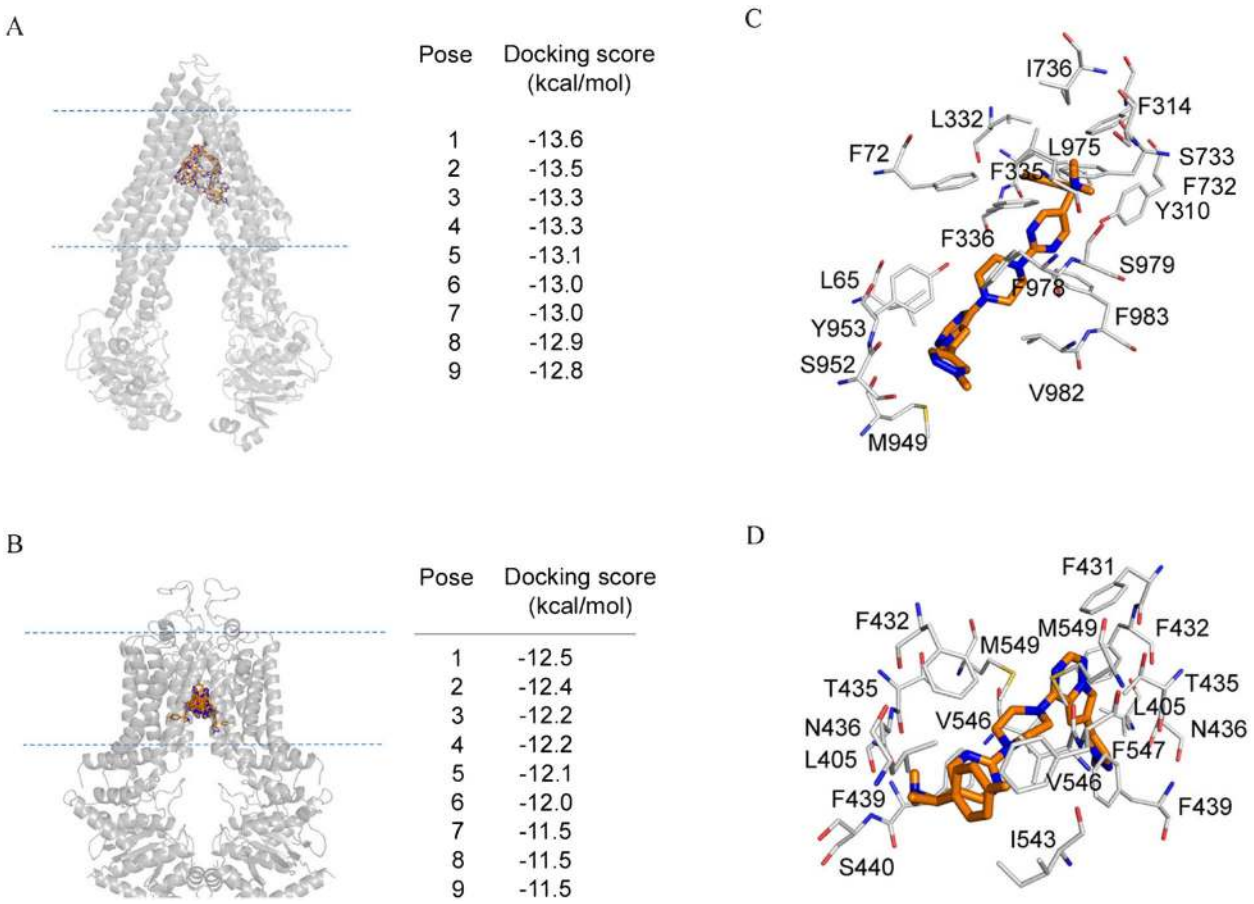

Fig. 6. Docking of avapritinib in the drug-binding pocket of ABCB1 and ABCG2.

Binding modes of avapritinib with (A) a homology model of ABCB1 (based on the PDB: 5KPI mouse P-glycoprotein structure) and (B) cryo-electron microscopy structure of ABCG2 (PDB: 5NJ3) obtained after exhaustive docking using AutoDock Vina software as described in the Experimental Section. The first nine poses with the lowest docking scores (tighter binding) are shown in orange and blue sticks in (A) the homology model of ABCB1 and (B) the ABCG2 structure, accompanied by the pose number and the corresponding docking score. The lowest energy pose (pose 1) for avapritinib in the transmembrane region of ABCB1 (C) and ABCG2 (D) is presented to illustrate the residues that are within 4 Å of the ligand (shown in gray). Figures were prepared using PyMOL software.

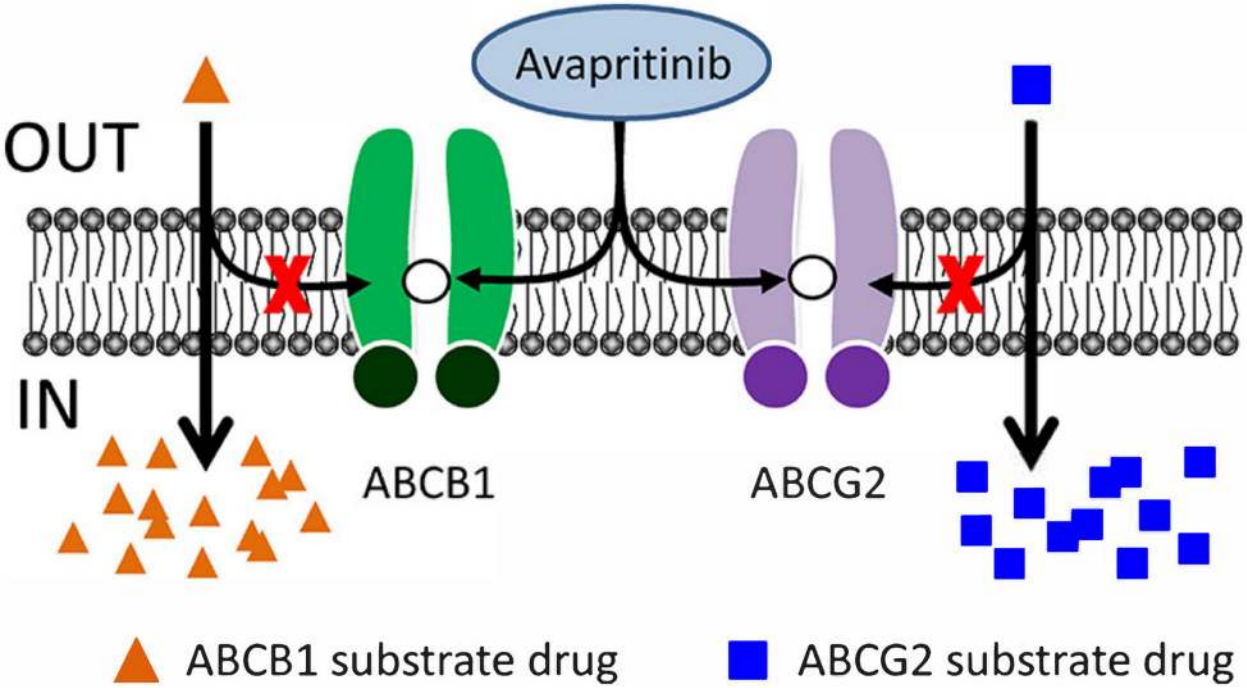

Fig. 7. Schematic diagram illustrating the potential mechanism of avapritinib in reversing drug resistance in cancer cells overexpressing ABCB1 or ABCG2.

Typically, substrate drugs of ABCB1 (orange triangles) and substrate drugs of ABCG2 (blue squares) are actively effluxed out of cancer cells by ABCB1 (green) and ABCG2 (purple), which results in reduced drug accumulation and the emergence of a drug-resistant phenotype. In this model, avapritinib (empty circles) binds to the drug binding pockets of ABCB1 and ABCG2 and blocks the drug transport function of both ABCB1 and ABCG2, consequently elevating intracellular drug accumulation and restoring the chemosensitivity of drug-resistant cancer cells.

Table 1:

Reversal effect of avapritinib on drug resistance mediated by ABCB1

		Mean IC_{50} [†] ± SD and (FR[‡])	
Treatment	**Concentration (μM)**	**OVCAR-8 (parental) [nM]**	**NCI-ADR-RES (resistant) [μM]**
Paclitaxel	-	3.08 ± 0.57 (1.0)	8.03 ± 1.65 (1.0)
+ Avapritinib	0.1	2.36 ± 0.45 (1.3)	3.85 ± 0.74 * (2.1)
+ Avapritinib	0.2	2.17 ± 0.43 (1.4)	2.35 ± 0.28 ** (3.4)
+ Avapritinib	0.5	2.07 ± 0.46 (1.5)	0.65 ± 0.12 ** (12.3)
+ Avapritinib	1.0	2.09 ± 0.38 (1.5)	0.27 ± 0.08 ** (29.7)
+ Verapamil	5.0	1.85 ± 0.40* (1.7)	0.09 ± 0.02 ** (89.2)
		[nM]	**[μM]**
Colchicine	-	24.39 ± 7.67 (1.0)	2.37 ± 0.44 (1.0)
+ Avapritinib	0.1	24.07 ± 8.31 (1.0)	2.46 ± 0.54 (1.0)
+ Avapritinib	0.2	20.71 ± 6.70 (1.2)	1.42 ± 0.32 * (1.7)
+ Avapritinib	0.5	20.02 ± 7.30 (1.2)	0.89 ± 0.24 ** (2.7)
+ Avapritinib	1.0	21.31 ± 7.30 (1.1)	0.55 ± 0.20 ** (4.3)
+ Verapamil	5.0	18.92 ± 6.48 (1.0)	0.97 ± 0.23 ** (1.0)
		[nM]	**[μM]**
Doxorubicin	-	210.77 ± 37.05 (1.0)	7.86 ± 0.67 (1.0)
+ Avapritinib	0.1	210.85 ± 33.53 (1.0)	5.33 ± 0.91 * (1.5)
+ Avapritinib	0.2	167.54 ± 21.59 (1.3)	3.84 ± 0.46 ** (2.0)
+ Avapritinib	0.5	183.78 ± 33.22 (1.1)	2.16 ± 0.32 *** (3.6)
+ Avapritinib	1.0	179.07 ± 24.23 (1.2)	1.82 ± 0.41 *** (4.3)
+ Verapamil	5.0	182.66 ± 36.50 (1.2)	1.36 ± 0.16 *** (5.8)
Treatment	**Concentration (μM)**	**KB-3–1 (parental) [nM]**	**KB-V1(resistant) [μM]**
Paclitaxel	-	1.93 ± 0.56 (1.0)	5.31 ± 1.50 (1.0)
+ Avapritinib	0.1	1.76 ± 0.49 (1.1)	1.52 ± 0.28 * (3.5)
+ Avapritinib	0.2	2.05 ± 0.59 (0.9)	0.79 ± 0.15 ** (6.7)
+ Avapritinib	0.5	1.75 ± 0.49 (1.1)	0.42 ± 0.10 ** (12.6)
+ Avapritinib	1.0	1.99 ± 0.66 (1.0)	0.11 ± 0.01 ** (48.3)
+ Verapamil	5.0	1.39 ± 0.38 (1.4)	0.09 ± 0.01 ** (59.0)
		[nM]	**[nM]**
Colchicine	-	14.89 ± 6.48 (1.0)	1371.11 ± 224.86 (1.0)
+ Avapritinib	0.1	11.49 ± 4.20 (1.3)	1007.30 ± 102.62 (1.4)
+ Avapritinib	0.2	11.89 ± 4.53 (1.3)	486.52 ± 79.69 ** (2.8)
+ Avapritinib	0.5	10.11 ± 3.70 (1.5)	259.41 ± 43.45 ** (5.3)

		Mean IC_{50}[†] ± SD and (FR[‡])	
Treatment	**Concentration (μM)**	**OVCAR-8 (parental) [nM]**	**NCI-ADR-RES (resistant) [μM]**
+ Avapritinib	1.0	9.57 ± 3.47 (1.6)	135.72 ± 59.71*** (10.1)
+ Verapamil	5.0	13.72 ± 2.01 (1.1)	894.39 ± 143.25* (1.5)
		[nM]	**[μM]**
Doxorubicin	-	73.89 ± 11.62 (1.0)	4.36 ± 0.74 (1.0)
+ Avapritinib	0.1	69.41 ± 9.20 (1.1)	1.72 ± 0.25** (2.5)
+ Avapritinib	0.2	98.77 ± 15.74 (0.7)	1.65 ± 0.28** (2.6)
+ Avapritinib	0.5	78.15 ± 15.16 (0.9)	0.68 ± 0.09** (6.4)
+ Avapritinib	1.0	64.45 ± 14.08 (1.1)	0.50 ± 0.06*** (8.7)
+ Verapamil	5.0	54.18 ± 8.45 (1.4)	0.37 ± 0.05*** (11.8)
Treatment	**Concentration (μM)**	**pcDNA-HEK293 (parental) [nM]**	**MDR19-HEK293 (resistant) [nM]**
Paclitaxel	-	2.31 ± 0.47 (1.0)	700.79 ± 108.32 (1.0)
+ Avapritinib	0.1	2.29 ± 0.40 (1.0)	299.54 ± 54.39** (2.3)
+ Avapritinib	0.2	2.48 ± 0.41 (0.9)	229.13 ± 29.16** (3.1)
+ Avapritinib	0.5	2.11 ± 0.35 (1.1)	79.60 ± 17.16*** (8.8)
+ Avapritinib	1.0	1.55 ± 0.30 (1.5)	24.68 ± 4.36*** (28.4)
+ Verapamil	5.0	2.12 ± 0.40 (1.1)	20.36 ± 5.47*** (34.4)
		[nM]	**[nM]**
Colchicine	-	12.35 ± 2.71 (1.0)	155.10 ± 26.87 (1.0)
+ Avapritinib	0.1	11.78 ± 2.69 (1.0)	96.61 ± 17.32* (1.6)
+ Avapritinib	0.2	10.40 ± 2.37 (1.2)	74.80 ± 19.47* (2.0)
+ Avapritinib	0.5	10.46 ± 2.71 (1.2)	26.80 ± 4.84** (5.8)
+ Avapritinib	1.0	11.60 ± 2.94 (1.1)	22.66 ± 5.64** (6.8)
+ Verapamil	5.0	11.83 ± 3.16 (1.0)	102.28 ± 23.89 (1.5)
		[nM]	**[nM]**
Doxorubicin	-	11.94 ± 1.89 (1.0)	289.46 ± 34.80 (1.0)
+ Avapritinib	0.1	16.16 ± 2.38 (0.7)	259.44 ± 35.38 (1.1)
+ Avapritinib	0.2	10.31 ± 1.59 (1.2)	130.95 ± 22.65** (2.2)
+ Avapritinib	0.5	10.63 ± 1.67 (1.1)	67.48 ± 15.08*** (4.3)
+ Avapritinib	1.0	16.17 ± 2.48 (0.7)	31.96 ± 8.76*** (9.1)
+ Verapamil	5.0	5.83 ± 1.11** (2.0)	35.82 ± 3.74*** (8.1)

Abbreviation: FR, fold-reversal.

[†]IC_{50} values are mean ± SD calculated from dose-response curves obtained from at least three independent experiments using cytotoxicity assay as described in *Materials and methods*.

[‡]FR values were calculated by dividing IC_{50} values of cells treated with a particular therapeutic drug in the absence of avapritinib or verapamil by IC_{50} values of cells treated with the same therapeutic drug in the presence of avapritinib or verapamil.

$^{*}P < 0.05$;

$^{**}P < 0.01$;

$^{***}P < 0.001$.

Table 2:

Chemosensitizing effect of avapritinib on drug resistance mediated by ABCG2

		Mean IC_{50}[†] ± SD and (FR[‡])	
Treatment	**Concentration (μM)**	**H460 (parental) [nM]**	**H460-MX20 (resistant) [nM]**
Mitoxantrone	-	19.09 ± 8.36 (1.0)	1024.90 ± 227.94 (1.0)
+ Avapritinib	0.1	16.83 ± 6.24 (1.1)	292.89 ± 86.58 ** (3.5)
+ Avapritinib	0.2	13.63 ± 5.55 (1.4)	271.93 ± 94.05 ** (3.8)
+ Avapritinib	0.5	16.12 ± 6.62 (1.2)	148.73 ± 47.32 ** (6.9)
+ Avapritinib	1.0	17.38 ± 8.21 (1.1)	176.71± 72.45 ** (5.8)
+ Ko143	1.0	15.21 ± 5.95 (1.3)	171.56 ± 55.15 ** (6.0)
Topotecan	-	4.95 ± 0.76 (1.0)	3144.33 ± 997.43 (1.0)
+ Avapritinib	0.1	2.76 ± 0.55 (1.8)	550.16 ± 214.33 * (5.7)
+ Avapritinib	0.2	2.53 ± 0.54 (2.0)	343.39 ± 131.50 ** (9.2)
+ Avapritinib	0.5	2.52 ± 0.56 (2.0)	279.29 ± 107.40 ** (11.3)
+ Avapritinib	1.0	3.07 ± 1.02 (1.6)	270.46 ± 113.44 ** (11.6)
+ Ko143	1.0	3.00 ± 0.74 (1.7)	158.72 ± 59.79 ** (19.8)
SN-38	-	6.85 ± 1.76 (1.0)	1126.10 ± 428.61 (1.0)
+ Avapritinib	0.1	4.84 ± 1.41 (1.4)	354.00 ± 131.06 * (3.2)
+ Avapritinib	0.2	4.55 ± 1.57 (1.5)	390.23 ± 197.02 (2.9)
+ Avapritinib	0.5	3.85 ± 1.36 (1.8)	248.76 ± 107.47 * (4.5)
+ Avapritinib	1.0	2.80 ± 1.10* (2.4)	196.61 ± 93.27 * (5.7)
+ Ko143	1.0	4.58 ± 1.50 (1.5)	38.60 ± 11.19 * (29.2)
Treatment	**Concentration (μM)**	**S1 (parental) [nM]**	**S1-M1–80 (resistant) [μM]**
Mitoxantrone	-	3.11 ± 0.91 (1.0)	65.32 ± 5.31 (1.0)
+ Avapritinib	0.1	2.14 ± 0.42 (1.0)	27.97 ± 5.33 ** (2.3)
+ Avapritinib	0.2	3.06 ± 0.59 (1.0)	17.49 ± 4.36 *** (3.7)
+ Avapritinib	0.5	3.18 ± 0.59 (1.0)	6.48 ± 1.57 *** (10.1)
+ Avapritinib	1.0	2.09 ± 0.54 (1.0)	3.23 ± 1.16 *** (20.2)
+ Ko143	1.0	4.03 ± 1.15 (1.0)	0.93 ± 0.13 *** (70.2)
Topotecan	-	86.40 ± 31.08 (1.0)	17.06 ± 2.71 (1.0)
+ Avapritinib	0.1	90.93 ± 30.56 (1.0)	6.04 ± 1.16 ** (2.8)
+ Avapritinib	0.2	81.34 ± 27.92 (1.1)	2.34 ± 0.61 *** (7.3)
+ Avapritinib	0.5	110.33 ± 42.48 (0.8)	1.20 ± 0.33 *** (14.2)
+ Avapritinib	1.0	96.81 ± 45.24 (0.9)	1.19 ± 0.34 *** (14.3)
+ Ko143	1.0	65.77 ± 26.22 (1.3)	0.51 ± 0.13 *** (33.5)
SN-38	-	3.26 ± 0.46 (1.0)	4.37 ± 1.01 (1.0)

		Mean IC_{50} † ± SD and (FR‡)	
Treatment	**Concentration (μM)**	**H460 (parental) [nM]**	**H460-MX20 (resistant) [nM]**
+ Avapritinib	0.1	3.14 ± 0.45 (1.0)	1.65 ± 0.58 * (2.6)
+ Avapritinib	0.2	2.98 ± 0.46 (1.1)	0.79 ± 0.21 ** (5.5)
+ Avapritinib	0.5	3.54 ± 0.69 (0.9)	0.35 ± 0.09 ** (12.5)
+ Avapritinib	1.0	3.48 ± 0.83 (0.9)	0.29 ± 0.06 ** (15.1)
+ Ko143	1.0	2.93 ± 0.45 (1.1)	0.07 ± 0.01 ** (62.4)
Treatment	**Concentration (μM)**	**pcDNA-HEK293 (parental) [nM]**	**R482-HEK293 (resistant) [nM]**
Mitoxantrone	-	2.02 ± 0.38 (1.0)	87.73 ± 9.89 (1.0)
+ Avapritinib	0.1	2.22 ± 0.40 (0.9)	4.87 ± 0.89 *** (18.0)
+ Avapritinib	0.2	1.76 ± 0.36 (1.1)	2.76 ± 0.65 *** (31.8)
+ Avapritinib	0.5	1.69 ± 0.36 (1.2)	2.75 ± 0.57 *** (31.9)
+ Avapritinib	1.0	1.55 ± 0.22 (1.3)	1.50 ± 0.39 *** (58.5)
+ Ko143	1.0	1.77 ± 0.33 (1.1)	3.58 ± 0.57 *** (24.5)
Topotecan	-	47.33± 9.28 (1.0)	352.32 ± 55.25 (1.0)
+ Avapritinib	0.1	43.97± 6.89 (1.1)	73.17 ± 21.22 ** (4.8)
+ Avapritinib	0.2	45.68 ± 6.56 (1.0)	45.57 ± 12.10 *** (7.7)
+ Avapritinib	0.5	45.76 ± 9.14 (1.0)	38.13 ± 10.77 *** (9.2)
+ Avapritinib	1.0	42.47 ± 5.80 (1.1)	34.19 ± 8.44 *** (10.3)
+ Ko143	1.0	43.69 ± 6.63 (1.1)	29.16 ± 4.88 *** (12.1)
SN-38	-	3.62 ± 0.90 (1.0)	220.49 ± 44.06 (1.0)
+ Avapritinib	0.1	3.52 ± 0.81 (1.1)	29.01 ± 6.19 ** (7.6)
+ Avapritinib	0.2	3.08 ± 0.61 (1.2)	15.91 ± 3.77 ** (13.9)
+ Avapritinib	0.5	2.88 ± 0.62 (1.3)	8.82 ± 1.69 ** (25.0)
+ Avapritinib	1.0	2.57 ± 0.55 (1.4)	5.34 ± 1.05 ** (41.3)
+ Ko143	1.0	3.26 ± 0.70 (1.1)	3.98 ± 0.85 ** (55.4)

Abbreviation: FR, fold-reversal.

† IC_{50} values are mean ± SD calculated from dose-response curves obtained from at least three independent experiments using cytotoxicity assay as described in *Materials and methods*.

‡ FR values were calculated by dividing IC_{50} values of cells treated with a particular therapeutic drug in the absence of avapritinib or Ko143 by IC_{50} values of cells treated with the same therapeutic drug in the presence of avapritinib or Ko143.

* $P < 0.05$;

** $P < 0.01$;

*** $P < 0.001$.

Table 3:

Cytotoxicity of avapritinib in human cell lines overexpressing ABCB1 or ABCG2

Cell line	Type	Transporter expressed	IC_{50} (μM)[†]	RF[‡]
KB-3–1	epidermal	-	34.34 ± 6.90	1.0
KB-V-1	epidermal	ABCB1	26.15 ± 6.89	0.8
OVCAR-8	ovarian	-	15.37 ± 4.16	1.0
NCI-ADR-RES	ovarian	ABCB1	13.10 ± 3.44	0.9
H460	lung	-	12.96 ± 4.37	1.0
H460-MX20	lung	ABCG2	17.16 ± 6.94	1.3
S1	colon	-	7.58 ± 1.28	1.0
S1-M1–80	colon	ABCG2	10.67 ± 3.59	1.4
pcDNA-HEK293	-	-	20.30 ± 4.30	1.0
MDR19-HEK293	-	ABCG2	12.42 ± 3.07	0.6
R482-HEK293	-	ABCG2	15.05 ± 2.90	0.7

Abbreviation: RF, resistance factor.

[†] IC_{50} values are the mean ± SD calculated from dose-response curves obtained from at least three independent experiments using the cytotoxicity assay as described in Experimental Section.

[‡] RF values were obtained by dividing the IC_{50} value of avapritinib in ABCB1- or ABCG2- overexpressing MDR cell lines by the IC_{50} value of avapritinib in respective drug-sensitive parental cell lines.

[*] $P < 0.05$;

[**] $P < 0.01$;

[***] $P < 0.001$.