Volasertib (BI 6727), a novel polo-like kinase inhibitor, reverses ABCB1 and ABCG2-mediated multidrug resistance in cancer cells

Kenneth K.W. To^{1*}, Daniel C. Poon¹, X.G. Chen² and L.W. Fu²

*Correspondence: kennethto@cuhk.edu.hk

¹School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China.

²State Key Laboratory of Oncology in South China, Cancer Center, Sun Yat-Sen University, Guangzhou 510060, China.

Abstract

Background: Multidrug resistance (MDR), most often mediated by overexpression of ABC efflux transporters, is severely limiting the usefulness of chemotherapy. Intense research effort has been made to search for inhibitors of these MDR transporters to circumvent resistance, yet with no success in the last decade. The recent discovery of potent and specific inhibition of various MDR transporters by the molecular targeted tyrosine kinase inhibitors has refueled the interest in developing drug transporter inhibitors for MDR circumvention. We investigated the circumvention of MDR by a novel Polo-like kinase (PLK) inhibitor (volasertib) and studied the underlying mechanisms.

Methods: The potential MDR reversal effect of volasertib was evaluated in resistant cancer cell lines with defined overexpression of the three major MDR transporters. Sulforhodamine dye-based cytotoxicity and annexin V apoptosis assay were used to evaluate the combination anticancer activity. Flow cytometric drug efflux assay was used to study the inhibition of transporter activity. ATPase assay was employed to investigate the drug-transporter interaction. Propidium iodide staining was used to study the cell cycle effect of drug combinations.

Results: Volasertib was found to reverse ABCB1- and ABCG2-mediated MDR by inhibiting transport activity of both transporters. The resulting synergistic cytotoxicity in combinations of volasertib with paclitaxel (ABCB1 substrate) or mitoxantrone (ABCG2 substrate) was remarkably higher in drug-resistant cells with transporter overexpression. Transporter inhibition was associated with inhibition of ATPase activity, but not a change in mRNA or protein expression. G2/M arrest mediated by PLK inhibition by volasertib also play an important role in the sensitization of resistant cells.

Conclusions: Our results demonstrated that volasertib reverse ABCB1- and ABCG2-mediated MDR by inhibiting both transporters and promoting more severe G2/M arrest in resistant cancer cells. Further mechanistic studies and animal work are warranted to fully understand and optimize the use of volasertib to sensitize resistant cancer cells to chemotherapy.

Keywords: ABCB1, ABCG2, efflux transporter, multidrug resistance, polo-like kinase inhibitor, volasertib

Introduction

Volasertib (BI 6727) is a highly potent molecular targeted anticancer agent that induces mitotic arrest and apoptosis by selectively targeting the Polo-like kinase (PLK) family of proteins [1]. It is currently in early clinical development against various human tumors. PLK1 is the best characterized member of the PLK family and is recognized to play essential roles in the regulation of mitotic progression [2]. Overexpression of PLK1 is common in various cancers and is associated with poor prognosis [3]. Therefore, PLK1 represents an attractive target for anticancer drug development, particularly related to the antimitotic approach [4,5].

Multidrug resistance (MDR) is a long-standing and unresolved problem hindering successful cancer chemotherapy. Several cellular mechanisms are known to contribute to MDR, such as reduced apoptosis, enhanced DNA damage repair mechanisms or altered drug metabolism.

However, the most common mechanism of resistance is the active efflux of drugs by ATP-binding cassette (ABC) transporters including P-glycoprotein (ABCB1/P-gp), ABCC1/ MRP1 and ABCG2 [6]. These transporters play a key role in the energy-dependent cellular efflux of toxic agents. They are capable of recognizing and extruding a broad range of functionally and structurally unrelated compounds, thereby causing the MDR phenotype in various cancer types.

An obvious strategy to restore drug sensitivity in MDR cancer cells caused by ABC drug transporters is to block transporter-mediated drug efflux. Over the past decade, tremendous efforts have been made to discover and synthesize such inhibitors/modulators. Numerous clinical trials have been performed to evaluate the combination of ABCB1/P-gp modulators with standard chemotherapy regimens in enhancing anticancer efficacy [7]. However, none of them has been successfully put into clinical use, partly because of their low potency and lack of specificity

in inhibiting the MDR transporters.

Tyrosine kinase inhibitors (TKIs) are an important new class of targeted chemotherapeutic agents that specifically inhibit several oncogenic tyrosine kinases, thereby regulating cancer proliferation, invasion, metastasis and angiogenesis. Interestingly, the recent discovery of potent and specific inhibition of various MDR transporters by TKIs has renewed the research interest in developing drug transporter inhibitors for the circumvention of MDR. In this regard, we have previously demonstrated that apatinib [8], axitinib [9], crizotinib [10], and vandetanib [11] reversed MDR by inhibiting various ABC transporters in leukemia and solid tumors. However, the molecular mechanism underlying this circumvention of MDR is still not very clear. In-depth understanding about modulation of the transporters by TKIs is critical for their optimal use in reversing MDR.

To this end, volasertib has been recently shown to exhibit promising anticancer activity against cancer cells resistant to taxanes or Vinca alkaloids, and in a melanoma cell line ectopically expressing the *MDR* gene [1]. Moreover, volasertib was also shown to retain its high anticancer activity in a taxane-resistant colorectal cancer xenograft model in nude mice [1]. Although these data are encouraging, detailed mechanistic understanding is still lacking. Interestingly, a closer look at the chemical structure of volasertib reveals that it possess a quinazolin-4-amine-like functional group, a common structural feature shared by other TKIs (e.g. erlotinib, gefitinib and lapatinib, etc) previously reported to reverse ABCB1- or ABCG2-mediated MDR. We therefore hypothesized that volasertib may also circumvent MDR by inhibiting these transporter(s). In this study, we systematically evaluated the potential reversal of MDR by volasertib and investigated the underlying mechanisms.

Materials and Methods

Chemicals and reagents

Mitoxantrone, paclitaxel and rhodamine were obtained from Sigma Chemical (St Louis, MO). Doxorubicin HCl was purchased from Yick-Vic Chemicals & Pharmaceuticals (Hong Kong). Fumitremorgin C (FTC), Ko143, Pheophorbide A (PhA), PSC833 and tariquidar were kind gifts provided by Dr. Susan Bates (National Cancer Institute, NIH, Bethesda, MD). Phycoerythrin-conjugated anti-ABCB1 antibody UIC2, anti-ABCG2 antibody 5D3 and mouse IgG2b negative control antibody were purchased from eBioscience (San Diego, CA).

Cell culture

All cell lines are generous gift from Dr Susan Bates (National Cancer Institute, Bethesda, MD, USA). Pairs of parental and drug resistant sublines with overexpression of the three major MDR transporters were used in our study, which include human colon cancer SW620/its ABCB1-overexpressing SW620 Ad300 subline [12], human colon cancer S1/its ABCG2-overexpressing S1M1 80 subline [13], and MCF-7/its MRP1-overexpressing MCF-7 VP-16 subline [14].

The resistant sublines were developed from their respective parental cancer cell lines by stepwise selection in increasing concentrations of selecting agents and were maintained in 300 nM doxorubicin, 80 μ M mitoxantrone, and 10 μ M etoposide, respectively. They have been fully characterized and proven to be appropriate models for studying multidrug resistant transporters-mediated resistance and their reversal. The resistant cells were allowed to grow in drug-free culture medium for more than 2 weeks before experiments. The resistance phenotype was stable for at least 3 months in drug-free medium. The human primary embryonic kidney cell line HEK293 and its stably pcDNA3-, ABCB1- or ABCG2-transfected cell lines were also used to demonstrate the specific effect of volasertib on the respective transporters. The transfected cells were cultured in complete culture medium supplemented with 2 mg/mL G418 [15]. The other cell lines were maintained in MEM (S1 and its resistant subline) or RPMI-1640 medium (SW620 and its resistant subline) supplemented with 10% fetal bovine serum, 100 units/mL streptomycin sulfate, and 100 units/mL penicillin G sulfate, and incubated at 37°C in 5% CO₂.

Growth inhibition assay and determination of combination index

The growth inhibitory effect of individual anticancer drugs was evaluated by the sulforhodamine B assay as described previously [16]. The IC₅₀ values of each agent were subsequently determined by Prism 4.0 (Graphpad Software), from which concentrations were established for use in combination experiments. Drugs were given simultaneously for 72 h for the evaluation of combination cytotoxic effects. The synergistic effect of drug combinations was assessed using CompuSyn (CompuSyn Inc., Paramus, NJ), a software program based on the calculations for synergism developed by Chou *et al.*, [17,18]. Combinations with a combination index (CI) < 1 were considered synergistic and with CI < 0.5 highly synergistic.

Flow cytometric analysis of transporter activity using fluorescent probe substrates

A flow cytometry-based assay was employed to study the inhibition of ABCB1 and ABCG2 transport activity by volasertib as described previously with minor modification. Briefly, ABCB1- or ABCG2-overexpressing cells were incubated for 30 min in phenol red-free complete medium with the transporter-specific fluorescent probe substrate (0.5 μ g/mL rhodamine 123 (Rh123) or 1 μ M pheophorbide A (PhA)) in the presence or absence of different concentration of volasertib. Then, the cells were washed twice with ice-cold PBS and incubated in substrate-free medium at 37°C for 1 h continuing with volasertib to generate the inhibitor/efflux histogram, or without volasertib to generate the efflux histogram. Cells were finally washed with ice-cold PBS and placed on ice in the dark until analysis by flow cytometry. Inhibitors specific for ABCB1 (PSC833) and ABCG2 (FTC) were

used as control for comparison. The inhibited efflux was determined as the difference in mean fluorescence intensity (Δ MFI) between the inhibitor/efflux and efflux histograms. To determine significant difference between intracellular fluorescence values, the Students *t*-test was performed with $p < 0.05$ being considered statistically significant. Samples were analyzed on a LSRFortessa Cell Analyzer (BD Biosciences, San Jose, CA). Rh123 was detected with a 488-nm argon laser and a 530-nm bandpass filter whereas PhA fluorescence was detected with a 488-nm argon laser and a 670-nm bandpass filter. 10,000 events were collected for the flow cytometry studies. Cell debris was eliminated by gating on forward versus side scatter and dead cells were excluded by propidium iodide staining. All assays were performed in at least three independent experiments.

Cellular accumulation of ABCB1- or ABCG2- substrate anticancer drugs

The effect of volasertib on the accumulation of doxorubicin (ABCB1 substrate) or mitoxantrone (ABCG2 substrate) in SW620/SW620 Ad300 and S1/S1M1 80, respectively, were determined by flow cytometry. The cells were incubated for 30 min at 37°C with vehicle or various concentrations of volasertib. Then, 10 μ M of doxorubicin or mitoxantrone was added and incubation was continued for another 1 h. The cells were then collected, washed three times with ice-cold PBS, and analyzed by flow cytometry as above. Doxorubicin fluorescence was detected with a 488-nm argon laser and a 670-nm bandpass filter whereas mitoxantrone fluorescence was captured with a 635-red diode laser and a 670-nm bandpass filter.

UIC2 and 5D3 shift assay for assessing interaction between volasertib and transporters

The binding of two conformational sensitive antibodies (UIC2 & 5D3) to ABCB1 and ABCG2, respectively, in intact cells (ABCB1-overexpressing SW620 Ad300 or ABCG2-overexpressing S1M1 80) in the presence or absence of volasertib was measured by flow cytometer as described previously [19,20]. Briefly, cells were preincubated with the tested compounds in 0.5% bovine serum albumin/Dulbecco's PBS for 15 min at 37°C before labeling with 0.5 μ g/mL of either phycoerythrin (PE)-conjugated anti-ABCB1 antibody UIC2 (eBioscience), PE-conjugated anti-ABCG2 antibody 5D3 (eBioscience) or PE-conjugated mouse IgG2b negative control antibody (eBioscience) for another 45 min at 37°C. The tested compounds were present during the antibody labeling. As positive control for maximum labeling, UIC2 and 5D3 binding were determined in the presence of 1 μ M PSC833 or 200 nM Ko143, which are specific ABCB1 and ABCG2 inhibitors respectively.

Transporter ATPase assay

The vanadate-sensitive ATPase activity of ABCB1 or ABCG2 in cell membrane prepared from High-Five insect cells

was measured by using the BD Gentest ATPase assay kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, the ATPase reaction was initiated by the addition of 12 mmol/L Mg-ATP into a total reaction mixture of 60 μ L. After an incubation at 37°C for 20 min (for ABCB1) or 10 min (for ABCG2), the reactions were terminated by the addition of 30 μ L of 10% sodium dodecyl sulfate solution. The liberation of inorganic phosphate was detected by its absorbance at 800 nm and quantified by comparing the absorbance to a phosphate standard curve.

Apoptosis assay

SW620 or SW620 Ad300 cells were grown on 60-mm tissue culture dish at a density of about 2.0×10^5 cells/well. After treatment with 50 nM paclitaxel in the presence or absence of 20 nM volasertib for 48 h, both floating and attached cells were collected and washed twice with ice-cold PBS. The proportion of apoptotic cells was determined by using the APC Annexin V Apoptosis Kit (BD Bioscience) according to the manufacturer's instruction.

Cell cycle analysis by flow cytometry

Cell cycle analysis was performed using the standard propidium iodide staining method. Briefly, cells after the designated treatment were harvested in PBS and fixed in 70% ethanol overnight. Fixed cells were washed once in PBS and then treated with 10 μ g/mL RNase A at 37°C for 30 min. Afterwards, 50 μ g/mL propidium iodide was added to the cells and allowed to incubate at room temperature in the dark for at least 30 min, before analysis for DNA profile on a LSRFortessa Cell Analyzer (BD Biosciences). Cell cycle profiles were then analyzed by fitting the data with the ModFit LT software (Verity Software House, Topsham, ME).

Reverse transcription and quantitative real-time PCR

Total RNA was isolated using the TRI Reagent (Molecular Research Center, Cincinnati, OH). RNA (1 μ g) was reverse transcribed using the PrimeScript First Strand cDNA Synthesis Kit (TaKaRa-Bio, Dalian, China). Quantitative real-time PCR was performed to determine the relative expression level of ABCB1 or ABCG2 transcripts using the KAPA SYBR FAST qPCR Kit (KapaBiosystems, Woburn, MA) in a LightCycler 480 Instrument I (Roche Applied Science, Indianapolis, IN). The human GAPDH transcript level was also measured in parallel for normalization. The specific primers used are as follows: ABCB1 (forward) 5'-CCCATCATTGCAATAGCAGG-3' (reverse) 5'-GTTCAAACCTTCTGCTCCTGA-3'; ABCG2 (forward) 5'-TTTCCAAGCGTTTCATTCAAAA-3' (reverse) 5'-TACGACTGTGACAATGATCTGAGC-3'; and GAPDH (forward) 5'-AGCCACATCGCTCAGACAC-3' (reverse) 5'-GTTCAAACCTTCTGCTCCTGA-3'. PCRs were performed at 95°C for 5 min, followed by 45 cycles of 95°C for 10s and 60°C for 10s. Fluorescence signal was acquired at the end of the elongation step of every PCR cycle (72°C for 10s) to monitor the progress of PCR amplification. Δ Ct was

Table 1. Cytotoxicity of individual anticancer drugs in different cell lines.

Cell lines	IC ₅₀ (μM)				
	Volasertib	Paclitaxel ^a	Doxorubicin ^b	Mitoxantrone ^c	Cisplatin ^d
SW620	1.38 ± 0.19	0.032 ± 0.009	ND	ND	10.2 ± 1.3
SW620 Ad300	1.54 ± 0.22	38.2 ± 4.2	ND	ND	11.6 ± 1.1
MCF-7	0.008 ± 0.003	ND	0.31 ± 0.02	ND	9.85 ± 0.76
MCF-7 VP	0.011 ± 0.007	ND	7.73 ± 0.82	ND	9.16 ± 0.84
S1	2.42 ± 0.23	ND	ND	0.083 ± 0.012	2.45 ± 0.41
S1M1 80	2.15 ± 0.36	ND	ND	115.2 ± 12.5	3.16 ± 0.29
HEK293 -	-	-	-	-	-
pcDNA	6.52 ± 0.41	0.041 ± 0.012	0.022 ± 0.008	0.61 ± 0.25	2.10 ± 0.28
ABCB1	5.93 ± 0.26	0.926 ± 0.121	ND	ND	2.31 ± 0.35
ABCC1	6.26 ± 0.39	ND	0.32 ± 0.05	ND	2.49 ± 0.31
ABCG2	6.25 ± 0.34	ND	ND	34.2 ± 4.1	2.56 ± 0.29

^aPaclitaxel is a probe substrate anticancer drug for ABCB1 (Pgp)

^bDoxorubicin is a probe substrate anticancer drug for ABCC1 (MRP1)

^cMitoxantrone is a probe substrate anticancer drug for ABCG2

^dCisplatin is known to be a non-substrate for ABCB1, ABCC1 and ABCG2

ND = not determined.

calculated by subtracting the Ct of GAPDH from the Ct of ABCB1 or ABCG2. $\Delta\Delta\text{Ct}$ was then calculated by subtracting the ΔCt of the untreated cells (or parental cells) from the ΔCt of the treated cells (or resistant cells). Fold difference of gene expression was calculated by the equation $2^{-\Delta\Delta\text{Ct}}$.

Western blot analysis

ABCB1-overexpressing SW620 Ad300 or ABCG2-overexpressing S1M1 80 cells were treated with a range of different concentrations of volasertib for 24 or 48 h. The cells were then harvested for Western blot analysis. Primary antibody incubation was carried out at 4°C overnight with a mouse monoclonal anti-ABCB1 antibody (C219, Covance, Madison, WI) or mouse monoclonal anti-ABCG2 antibody (BXP-21, Kamiya Biomedical, Seattle, WA) diluted at 1:500 in 5% non-fat milk in PBS-T. Afterwards, the membranes were incubated with HRP-conjugated donkey anti mouse secondary antibody at room temperature for 1 h, and developed using the WesternBright Quantum chemiluminescence detection system (Advansta Corporation, Menlo Park, CA). Anti-GAPDH antibody was used as the loading control (Santa Cruz Biotech, Santa Cruz, CA). Digital chemiluminescence images were captured and analyzed by using the FluorChem Q Imaging System (Alpha Innotech Corporation, Santa Clara, CA).

Results

Volasertib is synergistic with paclitaxel and mitoxantrone in cell lines with overexpression of ABCB1/P-gp and ABCG2, respectively

The potential reversal of multidrug resistance by volasertib was evaluated in three pairs of parental and drug-resistant cancer cell lines with defined overexpression of the major MDR transporters (ABCB1, ABCC1 & ABCG2). The resistant cancer cell lines are remarkably resistant to the corresponding transporter substrate anticancer drugs (Table 1) (i.e. ABCB1-overexpressing SW620 Ad300: 1200-fold resistant to

paclitaxel; ABCC1-overexpressing MCF-7 VP: 25-fold resistant to doxorubicin; ABCG2-overexpressing S1M1 80: 1400-fold resistant to mitoxantrone). A panel of ABCB1-, ABCC1-, or ABCG2-stably transfected HEK293 human embryonic kidney cell lines was also tested, which demonstrated different extent of resistance to the transporter substrate anticancer drugs. Of note, volasertib was found to exhibit similar anticancer activity in pairs of parental and resistant cancer cell lines and within the panel of transfected HEK293 cells (Table 1). Subsequently, combinations of volasertib and various transporter substrate anticancer drugs for ABCB1, ABCC1 or ABCG2 were evaluated in different ratios according to their relative anticancer activity in the cells.

The combination cytotoxic effect of volasertib with various anticancer drugs was evaluated by the CompuSyn software. The combination index values computed at 50% and 90% cell kill, which show similar trend, are summarized in Table 2. Synergistic cytotoxic effect was noted for combinations of volasertib with paclitaxel or mitoxantrone in the two pairs of SW620/SW620 Ad300 and S1/S1M1 80 cells, respectively (i.e. $\text{CI} < 1$). It is noteworthy that the synergistic effect is more pronounced in the resistant SW620 Ad300 and S1M1 80 ($\text{CI} < 0.5$) than in the corresponding parental SW620 and S1 cells. In particular, combination of volasertib and the ABCB1-substrate paclitaxel is remarkably synergistic in the ABCB1-overexpressing SW620 Ad300 cells, with a CI value close to 0.2. In the pair of MCF-7 and ABCC1-overexpressing MCF-7 VP cells, combination of volasertib and the ABCC1-substrate doxorubicin was only found to be additive ($\text{CI} \sim 1$).

To evaluate whether the synergistic effect is related to the overexpression of the multidrug resistance transporters, the drug combinations were also tested in a panel of ABCB1, ABCC1, or ABCG2-stably transfected cells (Table 2). Similar to the findings observed in the drug-selected resistance cell line models above, combination of volasertib with paclitaxel was found to be remarkably synergistic in ABCB1-

Table 2. Combination index (CI) values for the combinations of volasertib with paclitaxel, mitoxantrone, doxorubicin, or cisplatin. Significant synergistic combinations with CI < 0.5 are highlighted in bold.

Cell Line	Drug Combination	CI at ED ₅₀ ± SD	CI at ED ₉₀ ± SD	n
SW620	Paclitaxel ^a + Volasertib	0.79 ± 0.13	0.71 ± 0.11	4
	Cisplatin ^d + Volasertib	1.11 ± 0.16	0.92 ± 0.12	3
SW620 Ad300	Paclitaxel ^a + Volasertib	0.22 ± 0.04	0.16 ± 0.02	4
	Cisplatin ^d + Volasertib	0.98 ± 0.13	0.90 ± 0.12	3
MCF-7	Doxorubicin ^b + Volasertib	1.05 ± 0.11	1.01 ± 0.08	4
	Cisplatin ^d + Volasertib	1.12 ± 0.14	0.98 ± 0.11	3
MCF-7 VP	Doxorubicin ^b + Volasertib	0.93 ± 0.14	0.96 ± 0.09	4
	Cisplatin ^d + Volasertib	1.35 ± 0.13	1.10 ± 0.15	3
S1	Mitoxantrone ^c + Volasertib	0.88 ± 0.16	0.82 ± 0.13	4
	Cisplatin ^d + Volasertib	1.32 ± 0.16	0.98 ± 0.16	3
S1M1 80	Mitoxantrone ^c + Volasertib	0.48 ± 0.11	0.41 ± 0.13	4
	Cisplatin ^d + Volasertib	1.19 ± 0.16	0.94 ± 0.14	3
HEK293 pcDNA	Paclitaxel ^a + Volasertib	0.92 ± 0.09	0.91 ± 0.12	4
	Doxorubicin ^b + Volasertib	1.03 ± 0.11	1.01 ± 0.07	-
	Mitoxantrone ^c + Volasertib	0.89 ± 0.16	0.88 ± 0.14	4
	Cisplatin ^d + Volasertib	1.21 ± 0.15	1.15 ± 0.11	3
HEK293 ABCB1	Paclitaxel ^a + Volasertib	0.37 ± 0.06	0.34 ± 0.04	4
	Cisplatin ^d + Volasertib	0.92 ± 0.11	0.97 ± 0.14	3
HEK293 ABCC1	Doxorubicin ^b + Volasertib	0.97 ± 0.08	0.94 ± 0.07	4
	Cisplatin ^d + Volasertib	1.01 ± 0.13	0.97 ± 0.13	3
HEK293 ABCG2	Mitoxantrone ^c + Volasertib	0.67 ± 0.09	0.64 ± 0.05	4
	Cisplatin ^d + Volasertib	1.22 ± 0.13	0.97 ± 0.13	3

^aPaclitaxel, a well-characterized ABCB1 substrate anticancer drug, was chosen as the model drug for investigating the effect of ABCB1 inhibition on its cytotoxicity.

^bDoxorubicin, known to be a ABCC1 substrate anticancer drug, was chosen as the model drug for evaluating the effect of ABCC1 inhibition on its cytotoxicity.

^cMitoxantrone, a well-characterized ABCG2 substrate anticancer drug, was chosen as the model drug for evaluating the effect of ABCG2 inhibition on its cytotoxicity.

^dCisplatin is known to be a non-substrate for ABCB1, ABCC1 and ABCG2. It was chosen as the negative control for comparison.

transfected HEK293 cells (CI < 0.4) whereas combination of volasertib with mitoxantrone was found to be moderately synergistic in ABCG2-transfected HEK293 cells (CI ~ 0.6). In ABCC1-transfected HEK293 cells, combination of volasertib and the ABCC1-substrate doxorubicin was only additive (CI ~ 1). In all cell line models tested, combination of volasertib and the non-transporters substrate cisplatin was always additive.

Inhibition of ABCB1- and ABCG2-mediated drug efflux by volasertib

The remarkable synergistic cytotoxic effect from combination of volasertib with ABCB1 or ABCG2-substrate anticancer drugs in the corresponding transporter-overexpressing cells suggests the specific reversal of ABCB1- and ABCG2-mediated multidrug resistance. Therefore, the possible inhibition of ABCB1 and ABCG2 transport activity by volasertib was evaluated. As indicated in **Figure 1**, volasertib was found to inhibit the efflux of Rh123 (a fluorescent ABCB1 probe substrate) in ABCB1-overexpressing SW620 Ad300

Figure 1. Inhibition of (A) ABCB1-mediated rhodamine (Rh123) efflux in ABCB1-overexpressing SW620 Ad300 (left panel) and ABCB1-stably transfected HEK293 MDR1 cells (right panel) or (B) ABCG2-mediated pheophorbide a (PhA) efflux in ABCG2-overexpressing S1M1 80 cells (left panel) and ABCG2-stably transfected HEK293 ABCG2 cells (right panel) by volasertib. Cells were incubated with 0.5 μg/mL Rh123 alone (black) (or 1 μM PhA alone), 0.5 μg/mL Rh123 with 1 μM PSC833 (a ABCB1-specific inhibitor) (red) (or 1 μM PhA with 10 μM FTC (a ABCG2-specific inhibitor), or 0.5 μg/mL Rh123 (or 1 μM PhA) with volasertib at the indicated concentrations (0.25, 0.5, 1, or 2 μM) (in various different colors as indicated) at 37°C for 30 min. Rh123 or PhA fluorescence retention in the cells after 1-h fluorescent substrate-free efflux was measured by flow cytometry. Representative histograms from at least three independent experiments are shown.

and ABCB1-transfected HEK293 cells, and also the efflux of PhA (a fluorescent ABCG2 probe substrate) in ABCG2-overexpressing S1M1 80 and ABCG2-transfected HEK293 cells in a concentration dependent manner.

Moreover, doxorubicin and mitoxantrone (fluorescent ABCB1 and ABCG2 substrate anticancer drugs, respectively) were also used to monitor intracellular drug accumulation. Volasertib was found to increase cellular accumulation of doxorubicin (dramatically) and mitoxantrone (to a lesser extent) concentration dependently in the ABCB1-overexpressing SW620 Ad300 and ABCG2-overexpressing S1M1 80 cells, respectively (**Figure 2**). This correlates well with the synergistic combination data reported in **Table 2**. A similar enhancement of doxorubicin and mitoxantrone accumulation was also observed in the ABCB1- and ABCG2-

Figure 2. Increased cellular accumulation of doxorubicin (upper panel) or mitoxantrone (lower panel) in ABCB1-overexpressing SW620 Ad300 and ABCG2-overexpressing S1M1 80 cells by volasertib, respectively. Cells were incubated for 30 min at 37°C with volasertib at various concentrations (0.25, 0.5, 1, or 2 μM) or vehicle. Then, 10 μM of doxorubicin or mitoxantrone was added and incubation was continued for another 1 h. Finally, the cells were collected, washed three times with ice-cold PBS, and analyzed by flow cytometry. The results are presented as flow change in fluorescence intensity relative to untreated control SW620 Ad300 or S1M1 80 cells. Columns, means of triplicate determinations; bars, SD, * $p < 0.05$, ** $p < 0.01$, versus untreated resistant SW620 Ad300 or S1M1 80 group.

stably transfected HEK293 cells (data not shown).

Increased UIC2 and 5D3 labeling by volasertib suggest its interaction with ABCB1 and ABCG2

UIC2 and 5D3 are conformation sensitive monoclonal antibodies, recognizing an extracellular epitope of the human ABCB1 and ABCG2, respectively. UIC2 or 5D3 binding to an extracellular loop of the corresponding transporter was known to be increased in certain conformations of the transporter protein, upon substrate/inhibitor binding and ATP hydrolysis, which has been reported as UIC2 and 5D3 shift [19,20]. The UIC2 and 5D3 shift assay was therefore performed in SW620 Ad300 and S1M1 80, respectively, to demonstrate the interaction of volasertib with ABCB1

Figure 3. UIC2 and 5D3 labeling in ABCB1-(SW620 Ad300) and ABCG2-overexpressing (S1M1 80) resistant cells, respectively, suggesting interaction between volasertib and the two transporters. (A) A typical UIC2 and 5D3 shift exhibited by ABCB1 (PSC833) and ABCG2 (Ko143) inhibitor, respectively. The solid line represents UIC2/5D3 binding of untreated cells (native staining) and dotted line for cells incubated with 1 μM PSC833 and 200 nM Ko143, respectively. The shaded histogram represents the background fluorescent signal upon staining with a mouse IgG2b (isotype control). (B & C) Comparison of UIC2 (B) or 5D3 (C) shift produced by volasertib and other known ABCB1/ABCG2 inhibitors/substrates, respectively. Known ABCB1 inhibitors: PSC833 (1 μM) and crizotinib (10 μM); known ABCB1 substrate: quinine (50 μM); reported non-ABCB1 substrate: cisplatin (50 μM). Known ABCG2 inhibitors: Ko143 (200 nM) and axitinib (5 μM); known ABCG2 substrate: quercetin (25 μM); reported non-ABCG2 substrate: cisplatin (50 μM). The various tested compounds were present during the 45-min antibody incubation with cells. Fluorescence values are shown as the percentage of maximum labeling obtained in SW620 Ad300 cells incubated with 1 μM PSC833 (UIC2 shift; set as 100%) or 200 nM Ko143 (5D3 shift; set as 100%) and labeled with the respective UIC2 or 5D3 antibody. Mean and SD of the mean channel numbers from histograms obtained from three independent experiments are plotted. * $p < 0.05$, versus the untreated cells (native staining), respectively.

and ABCG2. Using the potent ABCB1 or ABCG2 inhibitor (i.e. PSC833 or Ko143) as the positive control (set as 100% UIC2 or 5D3 labeling for comparison (Figure 3), volasertib was found to notably increase both UIC2 and 5D3 labeling in a concentration dependent manner relative to the untreated control (Figure 3B and 3C). Two other tyrosine

kinase inhibitors, crizotinib and axitinib, previously reported by our group to inhibit ABCB1 and ABCG2 respectively were also shown to enhance the UIC2 and 5D3 labeling (Figure 3B and 3C). On the other hand, quinine and quercetin (known ABCB1 and ABCG2 substrate, respectively) were found to increase the UIC2 and 5D3 shift very modestly (~20% that of PSC833 and Ko143, respectively). Cisplatin, a non-ABCB1 and ABCG2 substrate, did not exhibit any UIC2 or 5D3 shift effect.

Modulation of ATPase activity of ABCB1 and ABCG2 by volasertib

Drug transport activity of ABCB1 and ABCG2 are associated with ATP hydrolysis that is modulated in the presence of its substrates or inhibitors. To understand further the mechanism of ABCB1 and ABCG2 inhibition by volasertib, ABCB1- or ABCG2-mediated ATP hydrolysis in the presence of a range of different concentrations of volasertib was

measured as vanadate-sensitive ATPase activity (*i.e.* the difference between the amount of inorganic phosphate released from ATP in the absence and presence of sodium orthovanadate (ATPase inhibitor)). Interestingly, volasertib behaved quite differently to ABCB1 (Figure 4A) and ABCG2 (Figure 4B). When tested at concentrations above 0.02 μ M, volasertib was found to inhibit ABCB1 ATPase activity in a dose dependent manner (Figure 4A). On the other hand, volasertib was shown to stimulate the ATPase activity of ABCG2 in a concentration-dependent manner (Figure 4B). A maximum ABCG2 ATPase activity of 73.5 $\pm$ 2.4 nmol Pi/min per mg protein was attained in the presence of 1.25 μ M volasertib. At higher concentrations of volasertib, a drop in the stimulated ABCG2 ATPase activity was observed.

Volasertib did not alter the expression of ABCB1 and ABCG2 at both mRNA and protein levels

The reversal of ABCB1- and ABCG2-mediated drug resistance by volasertib may also be associated with alteration of the transporter expression. Therefore, the mRNA and protein expressions of ABCB1 and ABCG2 were examined in SW620 Ad300 and S1M1 80 cells, respectively, after incubating the cells with volasertib at concentrations up to 1 mM for 48 h. Higher concentrations were not tested because more prominent cell death was observed. At the tested concentrations, volasertib did not affect the mRNA and protein expressions of ABCB1 and ABCG2 (Supplementary figure S1).

G2/M arrest by volasertib also contribute to the reversal of ABCB1-mediated paclitaxel resistance

Since the combination of volasertib with paclitaxel is so highly synergistic (Table 2), we asked whether volasertib possesses properties other than ABCB1 inhibition to reverse ABCB1-mediated resistance. First, SW620 and SW620 Ad300 were treated with a combination of paclitaxel (50 nM) and low concentration of volasertib (20 nM) for 48 h, after which the extent of apoptosis was measured. While volasertib only led to mild apoptosis at this low concentration (~8% early apoptosis versus ~1.5% in untreated control cells), its combination with paclitaxel was found to dramatically increase the proportion of both early and late apoptotic cells (~20 & ~30%, respectively) in the resistant SW620 Ad300 cells (Figure 5), presumably contributing to the reversal of resistance. Of note, volasertib, at this low concentration (20 nM), is not expected to exert appreciable ABCB1-inhibitory effect (Figure 1A).

Since it has been recently reported that volasertib also causes G2/M cell cycle arrest [1], we then evaluated whether this is related to our observed synergistic effect between volasertib and paclitaxel. The cell cycle effect of volasertib was first evaluated at a range of different concentrations (10 nM–100 nM) in SW620 and SW620 Ad300 cells (Supplementary figure S2). After 24-h drug exposure, notable G2/M arrest was observed even at the

Figure 5. Low concentration of volasertib was sufficient to sensitize paclitaxel-resistant SW620 Ad300 cells to apoptosis. (A) Parental SW620 and ABCB1-overexpressing resistant SW620 Ad300 cells were allowed to expose to paclitaxel (Taxol, 50 nM) alone, volasertib (20 nM) alone, or their combination (V+T) for 48 h before harvest for apoptosis assay. A representative set of data from three independent experiments is shown. (B) Summary of apoptosis assay data from three independent experiments. Data are presented in histogram as means + SD. *, $p < 0.05$; **, $p < 0.01$, versus no treatment control in parental SW620 cells.

lowest concentration of volasertib tested (*i.e.* 10 nM) (**Supplementary figure S2**). At 100 nM of volasertib, more than 75% cells were arrested at the G2/M phase. 20 nM volasertib was then chosen for subsequent experiments to combine with paclitaxel treatment. Intriguingly, while SW620 Ad300 cells were remarkably resistant to paclitaxel (no cell cycle effect on paclitaxel alone), the combination of 20 nM volasertib with paclitaxel was found to greatly increase the proportion of G2/M arrested cells to > 60% (**Figure 6**).

Discussion

TKIs are an important new class of molecularly-targeted cancer chemotherapeutic agents. Several recent reports about the potent and specific inhibition of MDR transporters by various TKIs have renewed the research interest in developing drug transporter inhibitors for the circumvention of MDR. Most of them work by interfering with the efflux activity of MDR transporters, thereby effectively increasing the accumulation of other concomitantly administered transporter substrate anticancer drugs. In this study, volasertib (BI 6727), a novel PLK inhibitor, was studied.

Volasertib was first screened for possible MDR reversal

Figure 6. Potentiation of paclitaxel-mediated G2/M arrest by volasertib in ABCB1-overexpressing SW620 Ad300 cells. (A) Parental SW620 and ABCB1-overexpressing resistant SW620 Ad300 cells were allowed to expose to paclitaxel (Taxol, 20 nM) alone, volasertib (20 nM) alone, or their combination (V+T) for 24 h before harvest for cell cycle analysis. A representative set of data from three independent experiments is shown. (B) Summary of cell cycle analysis results from three independent experiments. Data are presented in histogram as means + SD. *, $p < 0.05$; **, $p < 0.01$, versus the no treatment control in parental SW620 cells.

in cancer cell line models with defined overexpression of the three major MDR transporters (ABCB1/P-gp, ABCC1, and ABCG2). The resistant cell lines have been extensively characterized and only the designated MDR transporter is overexpressed in each individual cell line. Since volasertib is highly cytotoxic (**Table 1**), we evaluated its potential synergistic cytotoxic effect when used in combination with other transporter substrate anticancer drugs. Our data revealed that volasertib was highly synergistic with paclitaxel (a ABCB1 substrate anticancer drug; $CI \sim 0.2$) and moderately synergistic with mitoxantrone (a ABCG2 substrate anticancer drug; $CI \sim 0.5$) in the MDR cell lines mediated by ABCB1/P-gp (SW620 Ad300) and ABCG2 (S1M1 80), respectively (**Table 2**). This was further confirmed in ABCB1- and ABCG2-stably transfected HEK293 cells (**Table 2**). The observation appears to be specific because no synergistic effect was noted when volasertib was combined with the non-substrate anticancer drug (cisplatin) in the ABCB1- or ABCG2-overexpressing cells (**Table 2**). Combination of volasertib with cisplatin was only additive ($CI \sim 1$). On the other hand, volasertib was found to have no effect on ABCC1-mediated

MDR (**Table 2**).

Consistent with the synergistic cytotoxic effect observed, volasertib was found to inhibit efflux of Rh123 and PhA (ABCB1- and ABCG2-specific fluorescent probe substrate [15,21]) (**Figure 1**) and increased the accumulation of doxorubicin and mitoxantrone (ABCB1 and ABCG2 substrate anticancer drug) in the ABCB1-overexpressing SW620 Ad300 and ABCG2-overexpressing S1M1 80 cells (but not in the respective parental cells) (**Figure 2**). Similar results were also obtained in the ABCB1- or ABCG2-stably transfected HEK293 cells (**Figure 1**).

The interaction between volasertib and ABCB1/ABCG2 was further demonstrated by the UIC2 and 5D3 shift assay, respectively. The assay is based on the phenomenon that the binding of a conformation-sensitive antibody UIC2/5D3 to ABCB1/ABCG2 (respectively) could be increased in the presence of an ABCB1/ABCG2 substrate or inhibitor interacting with the transporter [19,20]. Inhibitors of ABCB1/ABCG2 are expected to cause higher UIC2/5D3 shift effects as compared to those caused by transported substrates [22]. In the ABCB1- and ABCG2-overexpressing cells (SW620 Ad300 and S1M1 80, respectively), volasertib was found to cause a UIC2/5D3 shift in a concentration dependent manner (**Figure 3B** and **3C**), comparable to that mediated by known ABCB1 (crizotinib) and ABCG2 (axitinib) inhibitors. A much smaller UIC2/5D3 shift was observed for the ABCB1- and ABCG2- substrates (quinine and quercetin, respectively). As control for comparison, the non-substrate drug (cisplatin) did not appreciably affect the UIC2/5D3 labeling (**Figure 3B** and **3C**). The relatively greater UIC2/5D3 shift observed for volasertib suggested that it may be ABCB1 and ABCG2 inhibitor (but not transported substrate). To this end, volasertib was found to be equally cytotoxic in the parental (SW620 & S1) and the transporters-overexpressing (SW620 Ad300 & S1M1 80) cells, a circumstantial evidence suggesting that volasertib is not likely transported by ABCB1 or ABCG2 (**Table 1**).

ATPase assay is another useful biochemical assay for the study of MDR transporter-drug interactions [23]. Energy is provided from ATP hydrolysis by ATPase for MDR transporters to effectively transport their substrate drugs. Some TKIs (including apatinib [24], erlotinib [25], lapatinib [26]) have been reported to modulate MDR transporters by stimulating the ATPase activity. On the other hand, other TKIs (such as CCT129202 [27] and BIBF 1120 [28]) were found to inhibit the ATPase activity and transport function of the ABCB1 transporter. We found that volasertib inhibited ABCB1 ATPase activity even at very low concentration (in the nM range) (**Figure 4A**). In contrast, volasertib was found to stimulate ABCG2 ATPase activity at low concentration (0.05–1 μ M) (**Figure 4B**). At higher concentration (>1 μ M), the stimulated ABCG2 ATPase activity was found to gradually go down (**Figure 4B**). Since volasertib did not affect ABCB1 and ABCG2 at both mRNA and protein expression (**Figure 5**), results from the ATPase assay suggested that

volasertib may reverse ABCB1-mediated MDR by inhibiting ATP hydrolysis by ABCB1. In the case of ABCG2, stimulation of ATP hydrolysis by volasertib suggests that it may be a substrate for the transporter. It follows that volasertib may work as a competitive inhibitor for ABCG2, though more detailed transporter inhibition kinetic study will be needed to confirm this speculation. Since volasertib is inhibiting ABCB1 ATPase activity at a very low concentration in the nM range (in contrast to stimulation of ABCG2 ATPase activity at a higher concentration), the data may also explain why volasertib is more potent in inhibiting ABCB1 than ABCG2 transport activity (**Figure 1**) and that combination of volasertib with ABCB1 substrate anticancer drug is more synergistic than its combination with ABCG2 substrate anticancer drug (**Table 2**).

The development of PLK inhibitors including volasertib is part of the research effort to explore novel antimitotic agents that avoid the side-effects of the classic tubulin-binding drugs (e.g. taxanes) [29] and overcome taxane resistance [30]. To this end, volasertib is known to cause G2/M arrest and regulate multiple steps in mitosis through inhibition of PLK1 [1]. We investigated whether the reversal of ABCB1- and ABCG2-mediated MDR was related to cell cycle regulation. As revealed in an apoptosis assay, volasertib at a low concentration (20 nM) not inhibitory to ABCB1 transport activity was sufficient to sensitize drug-resistant SW620 Ad300 cells to paclitaxel treatment (**Figure 5**). When used at the same 20 nM, volasertib was found to mediate G2/M arrest (used alone) and, importantly, restore the G2/M arrest in resistant SW620 Ad300 cells after paclitaxel treatment (**Figure 6**). Therefore, besides ABCB1 inhibition, the reversal of ABCB1-mediated resistance by volasertib and the strong synergistic cytotoxicity between volasertib and paclitaxel may also be contributed by the G2/M arrest effect of volasertib. To this end, as mitoxantrone cause predominantly G1 arrest in S1/S1M1 80 cells (**Supplementary figure S3**), its combination with volasertib may not benefit as much in terms of cell cycle regulation. Therefore, besides the weaker ABCG2 inhibition by volasertib (**Figure 1**), the less remarkable synergistic combination effect between volasertib and mitoxantrone (an ABCG2 substrate anticancer drug) may also be caused by the discordant cell cycle effect between the two drugs.

Conclusions

In summary, our results show that volasertib can reverse ABCB1- and ABCG2-mediated MDR by inhibiting the efflux activity of both transporters, thereby leading to synergistic cytotoxicity with ABCB1- and ABCG2- substrate anticancer drugs particularly in the resistant cells. G2/M arrest due to PLK inhibition by volasertib may also contribute to the sensitization of resistant cells to paclitaxel. Further mechanistic investigation and animal studies are therefore warranted to fully understand and optimize the beneficial combination of volasertib with other conventional cancer

chemotherapeutic drugs.

Additional files

[Supplementary figure S1](#)
[Supplementary figure S2](#)
[Supplementary figure S3](#)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Participated in research design: To KK and Fu LW.
Conducted experiments: To KK, Poon DC, and Chen XG.
Performed data analysis: To KK Wrote or contributed
to the writing of manuscript: To KK

Acknowledgement

We would like to thank Dr Susan Bates (National Cancer Institute, NIH) for the MDR transporters-overexpressing cancer cell lines and the various specific transporter inhibitors. The work was supported by a grant from the NSFC/RGC Joint Research Scheme 2010/11 sponsored by the Research Grants Council of Hong Kong and the National Natural Science Foundation of China (Project No. N_CUHK443/10) to Kenneth To.

Publication history

Received: 06-Feb-2013 Revised: 21-Feb-2013
Accepted: 15-Mar-2013 Published: 19-Mar-2013

References

1. Rudolph D, Steegmaier M, Hoffmann M, Grauert M, Baum A, Quant J, Haslinger C, Garin-Chesa P and Adolf GR: **BI 6727, a Polo-like kinase inhibitor with improved pharmacokinetic profile and broad antitumor activity.** *Clin Cancer Res* 2009, **15**:3094-102. | [Article](#) | [PubMed](#)
2. Archambault V and Glover DM: **Polo-like kinases: conservation and divergence in their functions and regulation.** *Nat Rev Mol Cell Biol* 2009, **10**:265-75. | [Article](#) | [PubMed](#)
3. Takai N, Hamanaka R, Yoshimatsu J and Miyakawa I: **Polo-like kinases (Plks) and cancer.** *Oncogene* 2005, **24**:287-91. | [Article](#) | [PubMed](#)
4. Degenhardt Y and Lampkin T: **Targeting Polo-like kinase in cancer therapy.** *Clin Cancer Res* 2010, **16**:384-9. | [Article](#) | [PubMed](#)
5. Strebhardt K: **Multifaceted polo-like kinases: drug targets and antitargets for cancer therapy.** *Nat Rev Drug Discov* 2010, **9**:643-60. | [Article](#) | [PubMed](#)
6. Gottesman MM, Fojo T and Bates SE: **Multidrug resistance in cancer: role of ATP-dependent transporters.** *Nat Rev Cancer* 2002, **2**:48-58. | [Article](#) | [PubMed](#)
7. Sandor V, Fojo T and Bates SE: **Future perspectives for the development of P-glycoprotein modulators.** *Drug Resist Updat* 1998, **1**:190-200. | [Article](#) | [PubMed](#)
8. Tong XZ, Wang F, Liang S, Zhang X, He JH, Chen XG, Liang YJ, Mi YJ, To KK and Fu LW: **Apatinib (YN968D1) enhances the efficacy of conventional chemotherapeutic drugs in side population cells and ABCB1-overexpressing leukemia cells.** *Biochem Pharmacol* 2012, **83**:586-97. | [Article](#) | [PubMed](#)
9. Wang F, Mi YJ, Chen XG, Wu XP, Liu Z, Chen SP, Liang YJ, Cheng C, To KK and Fu LW: **Axitinib targeted cancer stemlike cells to enhance efficacy of chemotherapeutic drugs via inhibiting the drug transport function of ABCG2.** *Mol Med* 2012, **18**:887-98. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
10. Zhou WJ, Zhang X, Cheng C, Wang F, Wang XK, Liang YJ, To KK, Zhou W, Huang HB and Fu LW: **Crizotinib (PF-02341066) reverses multidrug resistance in cancer cells by inhibiting the function of P-glycoprotein.** *Br J Pharmacol* 2012, **166**:1669-83. | [Article](#) | [PubMed](#)
11. Zheng LS, Wang F, Li YH, Zhang X, Chen LM, Liang YJ, Dai CL, Yan YY, Tao LY, Mi YJ, Yang AK, To KK and Fu LW: **Vandetanib (Zactima, ZD6474) antagonizes ABCC1- and ABCG2-mediated multidrug resistance by inhibition of their transport function.** *PLoS One* 2009, **4**:e5172. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
12. Lai GM, Chen YN, Mickley LA, Fojo AT and Bates SE: **P-glycoprotein expression and schedule dependence of adriamycin cytotoxicity in human colon carcinoma cell lines.** *Int J Cancer* 1991, **49**:696-703. | [Article](#) | [PubMed](#)
13. Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, Brangi M, Greenberger L, Dean M, Fojo T and Bates SE: **Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes.** *Cancer Res* 1999, **59**:8-13. | [Article](#) | [PubMed](#)
14. Schneider E, Horton JK, Yang CH, Nakagawa M and Cowan KH: **Multidrug resistance-associated protein gene overexpression and reduced drug sensitivity of topoisomerase II in a human breast carcinoma MCF7 cell line selected for etoposide resistance.** *Cancer Res* 1994, **54**:152-8. | [Article](#) | [PubMed](#)
15. Robey RW, Steadman K, Polgar O, Morisaki K, Blayney M, Mistry P and Bates SE: **Phosphoribide a is a specific probe for ABCG2 function and inhibition.** *Cancer Res* 2004, **64**:1242-6. | [Article](#) | [PubMed](#)
16. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S and Boyd MR: **New colorimetric cytotoxicity assay for anticancer-drug screening.** *J Natl Cancer Inst* 1990, **82**:1107-12. | [Article](#) | [PubMed](#)
17. Chou TC: **Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies.** *Pharmacol Rev* 2006, **58**:621-81. | [Article](#) | [PubMed](#)
18. Chou TC, Martin N: **CompuSyn for Drug Combinations.** *ComboSyn, Parmaus, NJ* 2004, USA.
19. Park SW, Lomri N, Simeoni LA, Fruehauf JP and Mechetner E: **Analysis of P-glycoprotein-mediated membrane transport in human peripheral blood lymphocytes using the UIC2 shift assay.** *Cytometry A* 2003, **53**:67-78. | [Article](#) | [PubMed](#)
20. Ozvegy-Laczka C, Varady G, Koblos G, Ujhelly O, Cervenak J, Schuetz JD, Sorrentino BP, Koomen GJ, Varadi A, Nemet K and Sarkadi B: **Function-dependent conformational changes of the ABCG2 multidrug transporter modify its interaction with a monoclonal antibody on the cell surface.** *J Biol Chem* 2005, **280**:4219-27. | [Article](#) | [PubMed](#)
21. Broxterman HJ, Lankelma J, Pinedo HM, Eekman CA, Wahrer DC, Ossenkoppele GJ and Schuurhuis GJ: **Theoretical and practical considerations for the measurement of P-glycoprotein function in acute myeloid leukemia.** *Leukemia* 1997, **11**:1110-8. | [PubMed](#)
22. Telbisz A, Hegedus C, Ozvegy-Laczka C, Goda K, Varady G, Takats Z, Szabo E, Sorrentino BP, Varadi A and Sarkadi B: **Antibody binding shift assay for rapid screening of drug interactions with the human ABCG2 multidrug transporter.** *Eur J Pharm Sci* 2012, **45**:101-9. | [Article](#) | [PubMed](#)
23. Ambudkar SV: **Drug-stimulatable ATPase activity in crude membranes of human MDR1-transfected mammalian cells.** *Methods Enzymol* 1998, **292**:504-14. | [Article](#) | [PubMed](#)
24. Mi YJ, Liang YJ, Huang HB, Zhao HY, Wu CP, Wang F, Tao LY, Zhang CZ, Dai CL, Tiwari AK, Ma XX, To KK, Ambudkar SV, Chen ZS and Fu LW: **Apatinib (YN968D1) reverses multidrug resistance by inhibiting the efflux function of multiple ATP-binding cassette transporters.** *Cancer Res* 2010, **70**:7981-91. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
25. Shi Z, Peng XX, Kim IW, Shukla S, Si QS, Robey RW, Bates SE, Shen T, Ashby CR, Jr., Fu LW, Ambudkar SV and Chen ZS: **Erlotinib (Tarceva, OSI-774) antagonizes ATP-binding cassette subfamily B member 1 and ATP-binding cassette subfamily G member 2-mediated drug resistance.** *Cancer Res* 2007, **67**:11012-20. | [Article](#) | [PubMed](#)
26. Dai CL, Tiwari AK, Wu CP, Su XD, Wang SR, Liu DG, Ashby CR, Jr., Huang Y, Robey RW, Liang YJ, Chen LM, Shi CJ, Ambudkar SV, Chen ZS and Fu LW: **Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette**

- subfamily B member 1 and G member 2. *Cancer Res* 2008, **68**:7905-14. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
27. Cheng C, Liu ZG, Zhang H, Xie JD, Chen XG, Zhao XQ, Wang F, Liang YJ, Chen LK, Singh S, Chen JJ, Talele TT, Chen ZS, Zhong FT and Fu LW: **Enhancing Chemosensitivity in ABCB1- and ABCG2-Overexpressing Cells and Cancer Stem-like Cells by An Aurora Kinase Inhibitor CCT129202**. *Mol Pharm* 2012, **9**: 1971-1982. | [Article](#) | [PubMed](#)
28. Xiang QF, Wang F, Su XD, Liang YJ, Zheng LS, Mi YJ, Chen WQ and Fu LW: **Effect of BIBF 1120 on reversal of ABCB1-mediated multidrug resistance**. *Cell Oncol (Dordr)* 2011, **34**:33-44. | [Article](#) | [PubMed](#)
29. de Carcer G, Perez de Castro I and Malumbres M: **Targeting cell cycle kinases for cancer therapy**. *Curr Med Chem* 2007, **14**:969-85. | [Article](#) | [PubMed](#)
30. Ismael GF, Rosa DD, Mano MS and Awada A: **Novel cytotoxic drugs: old challenges, new solutions**. *Cancer Treat Rev* 2008, **34**:81-91. | [Article](#) | [PubMed](#)

Citation:

To KKW, Poon DC, Chen XG and Fu LW: **Volasertib (BI 6727), a novel polo-like kinase inhibitor, reverses ABCB1 and ABCG2-mediated multidrug resistance in cancer cells**. *Journal of Cancer Therapeutics and Research* 2013, **2**:13.
<http://dx.doi.org/10.7243/2049-7962-2-13>