Original Research
ABC1 limits the cytotoxic activity of TAK-243, an inhibitor of the ubiquitin-activating enzyme UBA1

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Academic Editors: Graham Pawelec
Submitted: 25 September 2021 Revised: 20 November 2021 Accepted: 24 November 2021 Published: 5 January 2022

Abstract

Background: One of the major concerns of cancer therapy is the emergence of multidrug resistance (MDR). The MDR-associated ATP-binding cassette sub-family B member 1 (ABC1) transporter is established to mediate resistance against numerous anticancer drugs. In this study, we demonstrated that the Ubiquitin-like modifier activating enzyme 1 (UBA1) inhibitor TAK-243 is transported by the ABC1.

Methods: MTT assay was performed to evaluate the cytotoxicity of TAK-243. Western blot was carried out to investigate if TAK-243 affect to ABC1 protein expression in cancer cells. High Performance Liquid Chromatography (HPLC) and ATPase assay were carried out to confirm TAK-243 as an ABC1 substrate. [3H]-paclitaxel accumulation assay was used to determine the MDR reversal effect of TAK-243. Computational docking analysis was performed to investigate the drug-transporter binding position. Results: The cytotoxicity profile showed that TAK-243 was less effective in ABC1-overexpressing cells than in the parental cells, but pharmacological inhibition or knockout the gene of ABC1 was able to reverse TAK-243 resistance. Furthermore, TAK-243 potently stimulated ABC1 ATPase activity and the HPLC analysis revealed that TAK-243 accumulation was significantly reduced in ABC1-overexpressing cells. Finally, the computational docking analysis indicates a high binding affinity between TAK-243 and human ABC1 transporter. Conclusions: Our in vitro data characterized TAK-243 as a substrate of ABC1, which may predict limited anticancer effect of this compound in drug resistant tumors.

Keywords: Ubiquitin-activating enzyme; TAK-243; Multidrug resistance; ABC1; In vitro cytotoxicity

1. Introduction

ATP-binding cassette (ABC) transporters are membrane-bound transporters and some of the members of this superfamily play an important role in the energy-dependent efflux of substrate drugs [1]. The well-established ABC transporters responsible for multidrug resistance (MDR) are ABC1/P-gp, ABCG2/BCRP, and ABC1/MRP1 [2], which are capable of protecting cancer cells from a wide range of structurally and mechanistically unrelated anticancer drugs. These ABC transporters may also affect to the pharmacokinetics and toxicities of clinically used anticancer drugs [3,4]. ABC1 is the most studied ABC transporter, which was first discovered in 1976 [5]. ABC1 extrudes neutral or positively charged hydrophobic chemicals and xenobiotics from the cells, serving as a protective mechanism in normal tissues [6]. However, studies on ABC1 have revealed that chemotherapeutic agents such as taxanes, vinca alkaloids and anthracyclines as well as tyrosine kinase inhibitors (TKIs) such as dasatinib [7], ricinolstat [8] and GSK-1070916 are substrates of ABC1 [9]. ABCG2 also has a diverse substrate profile, including mitoxantrone, irinotecan, methotrexate, doxorubicin, and TKIs [10,11].

ABC1 was first identified from a doxorubicin-selected human small cell lung cancer cell line [12]. Compared with ABC1, the substrates of ABC1 are mostly amphipathic organic acids with more diverse structures including vinca alkaloids, anthracyclines and antiandrogens [13,14].

TKIs are small molecular drugs that target specific oncogenic tyrosine kinase to inhibit the proliferation, metastasis, invasion, and angiogenesis of cancer cells [15]. Recent studies have shown that some TKIs can reverse MDR by inhibiting the MDR-related ABC transporters [16–19]. Most of the TKIs reverse MDR by downregulating the expression or blocking the efflux function of ABC transporters, which increase the amount of substrate drugs in cancer cells [20,21]. Therefore, repurposing TKIs as MDR modulators may be a potential strategy to combat MDR. However, it is worth noting that TKIs also are transported by ABC1 or ABCG2, leading to suboptimal anticancer effect in drug resistant tumors [22–25].

TAK-243 is a first-in-class ubiquitin activating enzyme (UA)/UBA1 inhibitor currently under clinical development [26,27]. The anticancer effect of TAK-243 is being evaluated in a clinical trial involving patients with leukemia (NCT03816319). Both UAE and UBA6 are
known as E1 enzymes that regulate ubiquitin conjugation in mammals. UAE, encoded by UBA1 gene, regulates the charging of over 99% of cellular ubiquitin while UBA6 charges less than 1% of cellular ubiquitin [28]. The protein ubiquitylation is triggered when UAE catalyzes ubiquitin-charging for E2 enzymes, which in turns cooperate with cellular E3 ligases [29]. The clinical success of proteasome inhibitors gave rise to the development of small-molecule inhibitors targeting the E1, E2, and E3 units. Several inhibitors are currently in clinical development, including pevonedistat, CGM097, avadomide, and indisulam [30]. Despite that the preclinical studies have established TAK-243 as a potent UBA1 inhibitor, drug resistance remains to be a major concern for targeted therapy. Several TKIs that target the ubiquitin pathway may interact with MDR-related ABC transporters. Besse et al. [31] showed that ABCB1 overexpression contributes to the reduced proteasome-inhibiting effect of carfilzomib but not bortezomib. MLN4924 was identified as an ABCG2 substrate in previous studies [23,32]. Lenalidomide is a weak substrate of ABCB1 but not ABCG2 or ABCC1 [33]. PYR41 was shown to prevent the reduction of ABCB1 protein expression and transport activity levels [34]. Therefore, it is critical to investigate the potential factors that predict TAK-243 sensitivity, which provides direction for the clinical usage. Yasuhisa et al. [35] confirmed that SLFN11 regulates the sensitivity of leukemia cells to TAK-243. In SLFN11-KO cells, TAK-243 produced stronger protein ubiquitylation inhibition than in wide-type cells. Zhuang et al. [36] suggested that TAK-243 is more effective in bortezomib- and carfilzomib-resistant cell models. The significant cytotoxicity was also demonstrated in primary cells that acquired resistance to doxorubicin, melphalan, or dexamethasone.

While TAK-243 was demonstrated as a transported substrate of ABCG2 in previous studies [37,38], it is inconclusive whether ABCB1 or ABCC1 can interact with TAK-243. Our results showed that the cytotoxicity of TAK-243 was attenuated in ABCB1-overexpressing cells but not in ABCC1-overexpressing cells, suggesting that it is a transported substrate of ABCB1.

2. Materials and methods

2.1 Cell lines

The parental cells and drug-resistant sublines overexpressing MDR transporters were used in this study, including human epidermoid carcinoma KB-3-1, its ABCB1-overexpressing KB-C2 subline and ABCC1-overexpressing KB-CV60 subline [39,40], human colon cancer SW620 and its ABCB1-overexpressing SW620/Ad300 subline [41]. HEK293 with stable pcDNA3.1- and ABCB1-transfected cell lines were included. ABCB1 knockout cell lines SW620-ABCB1ko and SW620/Ad300-ABCB1ko were established by CRISPR/CRISPR-associated (Cas) 9 system (unpublished data). All media were supplemented with 10% of fetal bovine serum (FBS) and 1% of penicillin-streptomycin.

2.2 Cellular toxicity assay

MTT assay was conducted in accordance with the previous study [42]. Briefly, cells were harvested, counted, and seeded at 5 x 10^3 cells/well in 96-well plates for 24 h. Then cells were treated with different concentrations of the sample and further incubated for 72 h. After that, MTT solution was added and incubated for 4 h. Lastly, the solution was removed and DMSO was added to dissolve the formazan. OD_{750} value was read on an AccuSkan™ GO UV/Vis Microplate Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.3 Membrane ATPase assay

The effect of TAK-243 on the vanadate-sensitive ATPase activity of ABCB1 in cell membrane prepared from High-Five insect cells was measured using the protocol as previous described [43]. Tepotinib was used as a reference ABCB1 ATPase inhibitor to evaluate the combination effect on ABCB1 ATPase activity [16].

2.4 Determination of ABCB1 protein expression level

ABCB1-overexpressing KB-C2 cells were incubated with TAK-243 for up to 10 days. The total soluble protein lysates were prepared, subjected to SDS-PAGE, and transferred to PVDF membranes [44]. To quantify the expression level of ABCB1, membranes were probed with anti-ABCB1 monoclonal antibody (C219) (MA1-26528, Thermo Fisher Scientific Inc., Waltham, MA, USA). The loading control GAPDH was probed by anti-GAPDH monoclonal antibody (GA1R) (MA5-15738-D680, Thermo Fisher Scientific Inc., Waltham, MA, USA). Mouse IgG HRP conjugates (#7076, Cell Signaling Technology Inc., Danvers, MA, USA) were used as secondary antibody. To visualize the bands, ECL detection reagents were used (Thermo Fisher Scientific Inc., Waltham, MA, USA). The protein band densitometry was measured using ImageJ software (NIH, MD, USA).

2.5 Quantitative measurement of intracellular TAK-243 by HPLC

The sample preparation and HPLC setting follows the previous mentioned protocol with modifications [38]. Cells were harvested, counted, and separated into microcentrifuge tubes (1 x 10^6 cell/tubes) and incubated with 20 μM of TAK-243 with or without verapamil for 2 h. Subsequently, samples were collected in 0.5% SDS with acetoneitrile and centrifuged at 14,000 rpm for 10 min. The supernatants were collected, purified and subjected to HPLC analysis.
2.6 [³H]-paclitaxel accumulation assay

The interaction of TAK-243 and ABCB1 substrate drug paclitaxel was determined by [³H]-paclitaxel accumulation assay [45]. Briefly, cells were seeded into 24-well plates overnight. Then, cells were incubated with plain medium or medium containing TAK-243 or Verapamil. Subsequently, 5 nM of [³H]-paclitaxel was added to each well and incubated for 2 h. At the end, the radioactivity of samples was quantified by a liquid scintillation analyzer (Packard Instrument, Downers Grove, IL, USA).

2.7 Computational drug-protein docking analysis

The three-dimensional structure of TAK-243 was constructed to perform docking simulation with a human ABCB1 model 6QEX (obtained from RCSB Protein Data Bank) as previously described [46,47]. Docking calculations were performed using AutoDock Vina (version 1.1.2, Scripps Research, San Diego, CA, USA) and AutoDockTools (ADT, version 1.5.4, Scripps Research, San Diego, CA, USA) [48]. The top-scoring pose was selected for data analysis and visualization.

2.8 Statistical analysis

Data are collected from three independent experiments and presented as mean ± SD. One-way ANOVA was selected for the experimental analysis. The statistical analysis was performed in GraphPad Prism 8.1 (GraphPad Software, Inc, San Diego, CA, USA). A p value below 0.05 was considered to be statistically significant.

3. Results

3.1 The cytotoxic effect of TAK-243 was attenuated in ABCB1-overexpressing cells but not ABCC1-overexpressing cells

To characterize the interaction between TAK-243 and ABCB1 or ABCC1 transporters, MTT assay was performed to generate the cytotoxicity profiles (presented in Fig. 1) and the calculated IC₅₀ values are summarized in Table 1. Compared to the parental KB-3-1 cells, ABCB1-overexpressing KB-C2 cells demonstrated a 37.45-fold resistance to TAK-243 while ABCC1-overexpressing KB-CV60 cells maintained sensitivity to same level as the parental KB-3-1 cells. We further confirmed these results in additional two pairs of ABCB1-overexpressing cell lines. HEK293/ABCB1 cells showed 10.62-fold resistance to TAK-243 compared to the parental HEK293/pcDNA3.1 cells. Similarly, drug-resistant SW620/Ad300 cells were highly (28.46-fold) resistant to TAK-243 compared to parental SW620 cells. Therefore, our results suggest that overexpression of ABCB1 but not ABCC1 affects the cytotoxic effect of TAK-243 in cancer cells.

3.2 Pharmacological inhibition and knock-out of ABCB1 restore the sensitivity of MDR cells to TAK-243

Since overexpression of ABCB1 resulted in resistance to TAK-243, reversal studies were conducted to evaluate whether inhibiting ABCB1 can restore the cytotoxicity of TAK-243 in MDR cells. Verapamil, a known substrate of ABCB1 when used as a competitive inhibitor, was able to significantly enhances the cytotoxic effect of TAK-243 in ABCB1-overexpressing cells (Fig. 1A,C). When TAK-243 was co-incubated with verapamil, the fold-resistance to TAK-243 significantly decreased from 37.45- to 3.15-fold in KB-C2 cells, and from 10.62- to 1.88-fold in HEK293/ABCB1 cells. Furthermore, the cytotoxicity of TAK-243 was not affected in parental cells when combined with verapamil. The ABCC1 inhibitor MK-571 did not enhance TAK-243 cytotoxicity in parental and drug-resistant cells (Fig. 1B), suggesting TAK-243 is not a substrate of ABCC1. As shown in Fig. 1D, knockout of ABCB1 gene also restored the sensitivity of SW620/Ad300 cell to TAK-243. Furthermore, verapamil did not change the IC₅₀ values of TAK-243 in ABCB1 knockout cells, suggesting that the sensitization effect is due solely to ABCB1 inhibition.

3.3 TAK-243 stimulated the ABCB1 ATPase activity

The interaction between ABCB1 and TAK-243 was evaluated in High-Five insect cell total membrane vesicles containing the ABCB1 transporter. As shown in Fig. 2A, TAK-243 in a concentration-dependent manner increased the ATP hydrolysis mediated by ABCB1. The ABCB1 ATPase activity reached a maximum of 181.3% stimulation when incubated with 20 µM of TAK-243. To further verify that TAK-243 specifically stimulates ABCB1 ATPase activity, tepotinib was included as an ABCB1 ATPase inhibitor. When the membrane vesicles were incubated with both TAK-243 and tepotinib, the stimulatory effect of TAK-243 was significantly diminished (stimulation was decreased from 81.3% to 20.8%). This result shows that TAK-243 can specifically stimulate ABCB1 ATPase activity. The cytotoxicity and ATPase data strongly indicate that it is a substrate of ABCB1 transporter.

3.4 Overexpression of ABCB1 reduced the intracellular accumulation of TAK-243

As an MDR transporter, ABCB1 protects cancer cells by extruding substrate drugs into extracellular matrix. To this end, we measured the intracellular TAK-243 accumulation in empty vector transfectant HEK293/pcDNA3.1 cells and gene transfectant HEK293/ABCB1 and HEK293/ABCC1 cell lines. While drug-selected cancer cells may acquire other drug resistance mechanisms, the gene-transfected cells ensured that overexpression of ABC transporters is the sole contributor to MDR in HEK/ABCB1 and HEK293/ABCC1 cells. The cells were incubated with TAK-243 for 2 h, followed by HPLC analysis. Our results demonstrated that the in-
Fig. 1. The cytotoxic profile of TAK-243 in parental and drug-resistant cell lines. Cell viability curves for (A) KB-3-1 and KB-C2 cells, (B) KB-3-1 and KB-CV60 cells, (C) HEK293/pcDNA3.1, HEK293/ABCB1, and HEK293/ABCC1 cells, and (D) SW620, SW620/Ad300, SW620-ABCB1ko, and SW620/Ad300-ABCC1ko cells. Verapamil and MK571 were used as inhibitors of ABCB1 and ABCC1, respectively. Data are expressed as mean ± SD from three independent experiments.

Fig. 2. TAK-243 stimulated the ABCB1 ATPase activity and was accumulated significantly less in ABCB1-overexpressing cells. (A) The effect of TAK-243 on ABCB1-mediated ATPase activity at concentration range from 0–20 µM. Tepotinib (5 µM) was used as an ABCB1 ATPase inhibitor. (B) The intracellular concentration of TAK-243 in HEK293/pcDNA3.1, HEK293/ABCB1, and HEK293/ABCC1 cells. Verapamil is an ABCB1 inhibitor and MK571 is an ABCC1 inhibitor. Data are expressed as mean ± SD derived from three independent experiments. *p < 0.05 versus the control group. The intracellular concentration of TAK-243 was 3.7-fold lower in HEK293/ABCB1 than in HEK293/pcDNA3.1 cells. In addition, verapamil was able to significantly increase TAK-243 concentration in HEK293/ABCB1 cells but not in the HEK293/pcDNA3.1 cells. In contrast, TAK-243 concentrations were similar between HEK293/pcDNA3.1 and HEK293/ABCC1 cells, and ABCC1 inhibitor MK571 did not affect the drug accumulation. Therefore, it suggests that
ABCB1 is able to pump out TAK-243 from ABCB1 overexpressing cells, and this process can be inhibited by verapamil.

### 3.5 TAK-243 did not upregulate ABCB1 expression level

Some ABCB1 substrates have been shown to upregulate the protein expression level of ABCB1 transporter, therefore Western blot was performed to evaluate ABCB1 expression level after TAK-243 treatment. ABCB1-overexpressing cancer cells KB-C2 were incubated with 1 µM of TAK-243 for 10 consecutive days. As shown in Fig. 3A, the ABCB1 protein level remained constant during the incubation period. Therefore, TAK-243 may not affect the expression of ABCB1 in cancer cells.

### 3.6 TAK-243 did not competitively inhibit the efflux of paclitaxel mediated by ABCB1

To determine whether TAK-243 can competitively inhibit ABCB1 efflux activity, [3H]-paclitaxel accumulation assay was carried out using KB-3-1 and KB-C2 cells. As shown in Fig. 3B, compared to KB-3-1 cells, the accumulation of [3H]-paclitaxel was 10-fold lower in KB-C2 cells, indicating the active efflux function of ABCB1. Verapamil, a substrate of ABCB1 by competing for the binding site, inhibited the efflux and, significantly restored the paclitaxel accumulation in KB-C2 cells to the same level as that in KB-3-1 cells. However, TAK-243 did not demonstrate any effect on the efflux of paclitaxel in KB-C2 cells with concentrations around IC_{50} and IC_{85}. Therefore, the results suggest that TAK-243 may not affect paclitaxel efflux by ABCB1.

### 3.7 Docking of TAK-243 in the drug-binding pocket of human ABCB1

Since we identified TAK-243 as a substrate of ABCB1, protein-ligand docking was carried out to understand the potential binding pattern of TAK-243 with human ABCB1. Our results showed that the TAK-243 bound to ABCB1 with a -8.023 kcal/mol affinity score. The ligand-receptor interactions are highlighted in Fig. 4B,C. TAK-243 was stabilized in the ABCB1 substrate-binding site by both polar and hydrophobic interactions. Specifically, the pyrazolo-pyrimidine group in TAK-243 was stabilized by π–π stacking with Trp232, and the sulfamate group was stabilized by multiple hydrogen bonds formed with Gln195, Gln347 and Glu875, respectively.

### 4. Discussion

In this study, we performed in vitro assays to determine the influence of two MDR-related ABC transporters ABCB1 and ABCC1 on the cytotoxic effect of TAK-243 in cancer cells. TAK-243 is a first-in-class inhibitor of the ubiquitin-activating enzyme UBA1 to induce cancer cell death. TAK-243 was demonstrated to be effective against solid tumors including colon cancer and breast cancer in preclinical study, which distinguished it from proteasome inhibitors such as bortezomib and carfilzomib [26,49]. Many clinically used anticancer drugs were identified as substrates of MDR-related ABC transporters. These ABC transporters are drug efflux transporters that decrease the accumulation of anticancer drugs in cancer cells and therefore attenuate their cytotoxic effect. Clinical data have suggested that expression of ABCB1 and ABCG2 significantly affects the response of chronic myeloid leukemia (CML) patients to nilotinib and imatinib [50–52]. The clinical significance of ABCC1 was also demonstrated in many
Fig. 3. TAK-243 did not affect ABCB1 transporter protein expression level, and did not inhibit paclitaxel efflux. (A) The effect of TAK-243 on ABCB1 expression level in KB-C2 cells after 10 days treatment. (B) The intracellular accumulation of [3H]-paclitaxel in KB-3-1 and KB-C2 cells after co-incubated with 5 or 10 µM of TAK-243. Verapamil was used as an ABCB1 inhibitor. Data are expressed as mean ± SD derived from three independent experiments. *p < 0.05 versus the control group.

studies. High expression of ABCC1 could predict poor response to chemotherapy or induce drug resistance in patients with breast cancer, ovarian cancer, and prostate cancer [53]. Therefore, it is critical to determine the interaction between TAK-243 and the MDR-related ABC transporters, which provides future direction for the development of a drug that can be used in the clinic. Evidence shown that ABCG2 actively extrudes TAK-243 and confers drug resistance in cancer cells [37,38].

The most significant finding of our study was that the cytotoxic effect of TAK-243 was inhibited in ABCB1-but not ABCC1-overexpressing cells. The survival curves and IC$_{50}$ values of TAK-243 were same between ABCC1-overexpressing KB-CV60 cells and the parental cells. In contrast, the IC$_{50}$ values of TAK-243 were significantly higher in ABCB1-overexpressing KB-C2 and SW620/Ad300 cells compared to the parental KB-3-1 and SW620 cells, respectively, suggesting the overexpression of ABCB1 may contribute to TAK-243 resistance. Because the MDR cancer cells may employ multiple drug resistance mechanisms, both ABCB1 gene transfected and ABCB1 gene knockout cell models were used to further validate our finding. In gene transfected HEK293/ABCB1 cells, the cytotoxicity of TAK-243 was decreased as well. Furthermore, inhibition of ABCB1 function by gene knockout or ABCB1 substrate verapamil were able to sensitize MDR cancer cells to TAK-243. While in parental cells, ABCB1 knockout or verapamil did not enhance the cytotoxicity of TAK-243, suggesting that TAK-243 is a transported substrate of ABCB1.

To further characterize the interaction of TAK-243 with ABCB1, the ATPase activity in the presence of TAK-243 was measured. The ABCB1 efflux function is driven by the energy derived from ATP-binding and hydrolysis, which is catalyzed by its nucleotide-binding domains (NBDs). Our results showed that TAK-243 potently stimulated the ATPase activity of ABCB1. Furthermore, the co-incubation with ABCB1 ATPase inhibitor tepotinib decreased the stimulated ATPase activity, suggesting TAK-243 is an ABCB1 substrate. Another important evidence is that the accumulation of TAK-243 was significantly decreased in ABCB1-overexpressing cells. After 2 h incubation with 20 µM of TAK-243, only 30% of the drug remained in the HEK293/ABCB1 cells as compared to the HEK293/pcDNA3.1 cells. In addition, verapamil increased the intracellular level of TAK-243 in HEK293/ABCB1 cells but the effect was limited, which may be due to the insufficient incubation time of verapamil with the drug-resistant cells. Together, our results confirmed that TAK-243 is a transported substrate of ABCB1 and the cytotoxicity may be attenuated because of decreased TAK-243 accumulation in MDR cells.

Subsequently, two potential interactions between substrate and ABCB1 were explored. Some substrate drugs, such as paclitaxel and imatinib, are able to upregulate the expression of ABCB1 [51,54]. Our Western blot results showed that incubating ABCB1-overexpressing cells with TAK-243 for consecutive 10 days did not change the expression of ABCB1, suggesting that TAK-243 is unlikely to induce ABCB1 expression level. However, the effect of higher concentration of TAK-243 or longer incubation of TAK-243 should be further evaluated in parental and drug resistant cells. Another potential interaction is the competitive inhibition of ABCB1 efflux activity. Substrate drugs like gefitinib and nilotinib are able to modulate the efflux function of ABCB1 and reverse ABCB1-mediated drug resistance [55,56]. To this end, [3H]-paclitaxel accumulation assay was performed to determine whether TAK-243 can inhibit drug efflux activity of ABCB1. Direct determination of [3H]-paclitaxel showed that the intracellular pacli-
Fig. 4. Docking of TAK-243 in the drug-binding pocket of human ABCB1 protein model. (A) Overview of the best-scoring pose of TAK-243 in the drug-binding pocket of ABCB1 protein (6QEX). ABCB1 was displayed as colored tubes (helix: red; strand: blue; coil: white). TAK-243 was displayed as colored sticks. Carbon: cyan; oxygen: red; nitrogen: blue; hydrogen: white. (B) Details of the interaction between TAK-243 and ABCB1 binding pocket. ABCB1 helices were displayed as colored tubes (helix: red; strand: blue; coil: white). Important residues were displayed as colored sticks (carbon: red; oxygen: red; nitrogen: blue; hydrogen: white). TAK-243 was displayed as colored sticks (same as in (A). Hydrogen bonds were displayed as yellow dash lines. \(\pi-\pi\) stacking interactions were displayed as green dash lines. (C) 2D diagram of the interaction between TAK-243 and ABCB1 binding pocket. Important amino acids within 3 Å from the ligand were displayed as colored bubbles (green: hydrophobic; blue: polar). Purple solid lines with arrow indicate hydrogen bonds. Green solid lines indicate \(\pi-\pi\) stacking interactions.

Taxel concentration was significantly lower in KB-C2 cells compared to the KB-3-1 cells. While verapamil was able to inhibit paclitaxel efflux and completely restore paclitaxel accumulation in drug-resistant cells, TAK-243 failed to demonstrate any inhibition effect on the accumulation of paclitaxel. Therefore, our data suggests that TAK-243 does not serve as a reversal inhibitor of ABCB1. However, recent study has shown that ABCB1 may have multiple substrate-binding sites in the transmembrane domains that allows the binding of different substrates [57]. It is possible that TAK-243 may limit the efflux of some ABCB1 substrates that have the overlapping binding sites.

Lastly, the docking simulation analysis was carried out to predict the binding mode of TAK-243 with ABCB1. The computational analysis has been applied to the screening of substrate drugs with high accuracy, though the predicted interaction may not represent the actual binding mode [58]. The actual binding position should be further evaluated by cryogenic electron microscopy. The docking analysis showed that TAK-243 can form chemical bonds with Trp232, Gln195, Gln347 and Glu875 in the ABCB1 substrate-binding pocket with a high affinity score.

5. Conclusions

In summary, here we have shown that the ABCB1 actively transported TAK-243 and limited its cytotoxic effect in MDR cancer cells. Using an ABCB1 inhibitor or knock-out of ABCB1 gene was able to significantly abolish the TAK-243 resistance in MDR cancer cells. Thus, further in vivo studies with the animal models are necessary to validate these results. Therefore, if the results can be validated in vivo, it suggests that TAK-243 should be used in combination with an ABCB1 inhibitor in patients with MDR tumors to achieve optimal therapeutic effect.
Author contributions
Conceptualization—ZXW, FFP, ZSC; methodology—ZXW, QYY, JQW, ZNL, QXT, SN; resources—MM, SVA; writing-original draft preparation—ZXW; writing-review and editing—ZXW, FFP, SVA, ZSC; supervision—FFP and ZSC. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
Not applicable.

Acknowledgment
The authors would like to thank Shin-Ichi Akiyama (Kagoshima University, Kagoshima, Japan), and Susan E. Bates, Robert W. Robey (NCI, NIH, Bethesda, 331 Maryland, MD, USA) for kindly providing the cell lines. The authors would also like to thank ChemieTek (Indianapolis, IN) for providing the TAK-243.

Funding
This study was funded by the National Natural Science Foundation of China (81874330); Wuxi Taihu Lake Talent Plan Top Talents Project (BJ 2020001). The work was also partially supported by Department of Pharmaceutical Sciences, St. John’s University. MM and SVA were supported by the Intramural Research Program, National Institutes of Health, National Cancer Institute, Center for Cancer Research.

Conflict of interest
The authors declare no conflict of interest. ZSC is serving as one of the Guest editors of this journal. We declare that ZSC had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GP.

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