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Original Article

Overexpression of ABCB1 confers resistance to FLT3 inhibitor FN-1501 in cancer cells: in vitro and in vivo characterization

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Abstract: FN-1501 is a potent FLT3 inhibitor with antitumor activity. A phase 1 trial of FN-1501 monotherapy in patients with advanced solid tumors and R/R AML is in progress. Since one of the primary causes of multidrug resistance (MDR) is the overexpression of ATP-binding cassette superfamily B member 1 (ABCB1), the objective of this study was to investigate the potential relationship between FN-1501 and the ABCB1 transporter. We found ABCB1 overexpressing-cancer cells conferred FN-1501 resistance, which could be reversed by an ABCB1 inhibitor. Molecular docking study revealed that FN-1501 docked the ligand binding site with an affinity score of -9.77 kcal/mol, denoting a strong interaction between FN-1501 and ABCB1. Additionally, the ABCB1 ATPase assay indicated that FN-1501 could significantly stimulate ABCB1 ATPase activity. Furthermore, we observed a similar trend of ABCB1-facilated FN-1501 resistance in tumor-bearing mice model. In sum, we demonstrate that FN-1501 is a substrate of ABCB1 transporter from both in vivo and in vitro studies. Therefore, our findings provide new insight on the mechanism of chemoresistance due to ABCB1 overexpression.

Keywords: FN-1501, FLT3 inhibitor, multidrug resistance, ABC transporter, ABCB1

Introduction

Multidrug resistance (MDR), one of the leading causes of chemotherapy failure [1], refers to the resistance of cancer cells to drugs through various structures and mechanisms. One of the main factors mediating MDR is an elevated expression level of ATP-binding cassette (ABC) transporters [2]. It has been documented that the primary MDR-associated ABC transporters are ATP-binding cassette superfamily B member 1 (ABCB1), also known as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance protein 1 (ABCC1) [3-5]. ABC transporters utilize the energy derived from ATP hydrolysis to transport the substrate drug out of the cell, thereby reducing the intracellular drug concentration, attenuating the killing effect of drugs, and ultimately leading to the drug resistance of cancer cells [6, 7]. ABCB1 was discovered in drug-resistant Chinese hamster ovarian cells and was identified as the first ABC transporter [8-11]. Further, overexpression of ABCB1 is found in a wide variety of hematologic and solid tumors, including oral epithelial squamous carcinoma [12], breast cancer [13], and ovarian cancer [14]. Chemotherapeutic drugs such as paclitaxel and

doxorubicin are exported by ABCB1 transporters, which reduces their intracellular concentration and decreases their therapeutic effects [15-17]. Notably, ABCB1 is resistant to certain inhibitors of the FMS-like tyrosine kinase-3 (FLT3), such as crenolanib [18, 19].

FLT3 belongs to the class III receptor tyrosine kinase family [18] and is predominantly expressed in normal hematopoietic stem cells and hematopoietic progenitor cells [20]. Importantly, FLT3 is essential for the maintenance, growth, and differentiation of hematopoietic stem cells. FLT3 mutations usually lead to its abnormal activation and autophosphorylation, which activates downstream signaling pathways, leading to the aberrant proliferation of hematopoietic cells and lymphocytes as well as carcinogenesis. FLT3 mutations are especially closely related to the occurrence and progression of acute myeloid leukemia (AML), with about 70% of AML patients overexpressing FLT3 [21-24]. Studies have also demonstrated that the overexpression of FLT3 is associated with a poor prognosis [25]. Hence, the development of small molecule anti-AML drugs targeting FLT3 has emerged as a focus of research. Interestingly, Midostaurin, an inhibitor of FLT3, is not only a drug that targets AML but also an ABCB1 reversal agent [26].

FN-1501, a potent FLT3 inhibitor, has been reported to possess potent antitumor activity [27]. FN-1501 is currently being evaluated as a monotherapy for advanced solid tumors and R/R AML in a phase 1 clinical trial (NCT03690154). Recent studies have demonstrated the effectiveness of FN-1501 in patients with advanced solid tumors [28]. Here, we confirmed that overexpression of ABCB1 confers FN-1501 resistance. Furthermore, we demonstrated that ABCB1 reversal agent can restore the sensitivity of FN-1501 in ABCB1 overexpressing cells, which was supported by in vivo experiments. Therefore, our findings provide the rationale for the development of clinical treatment protocols using FN-1501.

Materials and methods

Chemicals

FN-1501 (HPLC purity: 99.71%), paclitaxel, doxorubicin, verapamil (VPM), SBE-β-CD and polyethylene glycol 300 were obtained from MedChemExpress (Monmouth Junction, NJ, USA). Fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (DMEM) were obtained from Gibco, Invitrogen (CA, USA). The Superoxide Dismutase (SOD) assay kit (WST-1 method) and the Situ TUNEL Apoptosis Detection Kit were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Cell lines and cell culture

The multidrug-resistant ABCB1-overexpressing cell line MCF7/ADR, which was generated by treating MCF7 cells with doxorubicin, was purchased from Guangzhou Xinyuan technology Co., Ltd. SW620/Ad300, a multidrug-resistant cell line overexpressing ABCB1, was generated by treating SW620 cells with doxorubicin. The human embryonic kidney HEK293 were transfected with pcDNA3.1 or ABCB1 to generate pcDNA3.1- or ABCB1-expressing stable cells, HEK293/pcDNA3.1 or HEK293/ABCB1 cells, respectively [29]. All cells were cultured in DMEM supplemented with 10% FBS and maintaimed at 37°C with 5% CO_2.

Cytotoxicity assay

Cytotoxicity assay was conducted as previously described [7]. Briefly, cells were seeded in 96-well plates and cultured overnight. Then, the cells were treated for 68 hours with different concentrations of FN-1501 or alternative chemotherapeutic agents, in the presence or absence of ABCB1 inhibitor verapamil. Cell viability was assessed by incubating the cells in MTT solution for 4 hours, and the absorbance at 570 nm was determined using a microplate reader.

Western blot analysis

Western blot analysis was carried out as described previously [3]. Briefly, cell lysates were collected and quantified. Then, total proteins were separated by SDS-PAGE and transferred onto PVDF membrane. The membrane was incubated with primary antibody overnight at 4°C after blocking, followed by incubation with secondary antibody for 2 hours at room temperature and signal development. Image J software was used to quantitatively analyze the signals. The primary antibodies used were mouse monoclonal anti-ABCB1 and anti-GAP-

DH (1:1,000 dilution, Sigma Chemical Co. (St. Louis, MO, USA)).

ATPase assay

The ATPase activity of the ABCB1 transporter was determined using an SB-MDR1-Hi5-PREDEASY-ATPase kit (Sigma Chemical Co., St. Louis, MO, USA). Briefly, membrane vesicles were incubated in the ATPase assay buffer, with or without the presence of sodium orthovanadate (Na_3VO_4) and Mg-ATP at 37°C. The concentration of inorganic phosphates was measured by colorimetric analysis [30].

[3H]-substrate accumulation assay

Briefly, cells in the logarithmic growth cycle were seeded in a 24-well plate and cultured overnight. Then, the cells were treated with FN-1501 (0, 0.3, and 3 μM) or ABCB1 specific reversal agent (VPM) for 2 hours, followed by treatment with 10 μM tritium-labeled paclitaxel for another 2 hours. The tritium-labeled paclitaxel was washed away with PBS, and the cells were collected and transferred to a liquid scintillation counting bottle. The radioactivity was detected using the liquid scintillation counting machine.

Molecular docking analysis

Molecular docking analysis of FN-1501 with the human ABCB1 model was performed as previously described [31]. The human ABCB1 protein model (7A69) was obtained from RCSB protein data bank, and the molecular structure of FN-1501 was obtained from PubChem. Docking calculation was performed in Maestro v11.1 (Schrödinger, LLC, MA, USA). After the receptor/ligand was prepared, Glide XP docking was performed, and induced-fit docking was conducted with the default protocol.

Tumor xenografts

Male BALB/C nude mice (4-6 weeks) were purchased from the Guangdong Provincial Medical Laboratory Animal Center (permit SCXK (Guangdong) 2018-0002) and housed in the SPF class animal facility of Southern Medical University. The mice were handled according to the Southern Medical University Laboratory Animal Centre's laboratory animal ethics Committee (Ethical approval number: No. 2023-0070). For xenograft tumor model, $1*10^7$ SW620 cells were injected at the left armpit, while $1*10^7$ SW620/AD300 cells were injected at the right armpit. The mice were then randomly divided into the following four treatment groups: a. vehicle control group (10% N-methylpyrrolidinone + 90% polyethylene glycol 300, 10 mL/kg/day orally (p.o.); b. VPM (4 mg/kg, p.o.); c. FN-1501 (15 mg/kg, i.p.); and d. VPM (4 mg/kg, p.o.) plus FN-1501 (15 mg/kg, i.p.). The treatments were given every three days for 15 days. The mouse weight and tumor growth were measured every 3 days. The tumor volume was estimated using the formula (V): $V = (\text{length} \times \text{width}^2) \times 0.5$. When the average diameter of tumors reached 50 mm^3, the experiment was terminated, and blood was extracted from the mice's eyeballs. Mice were sacrificed by euthanasia, and the tumor tissues were excised, weighed, and fixed in 4% formalin for further analysis.

Hematoxylin-eosin staining (HE)

Standard HE staining protocol was used. Briefly, tumor sections were dewaxed and stained in hematoxylin for 10 minutes, followed by eosin staining for 3 minutes. After dehydration with a gradient ethanol concentration, the tissue sections were soaked in xylene and sealed with neutral balsam.

TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay

TUNEL staining was performed to examine the apoptosis of cells according to the manufacturer's instruction (ServiceBiotech). Briefly, tumor sections were treated with protease K at 37°C for 22 minutes. After rinsing, the sections were incubated in dUTP buffer containing TDT enzyme for 2 hours and then stained with DAPI. The staining was examined and quantified under fluorescence microscope.

Serum biochemical assay

Serum was separated by centrifugation (12000 × g, 1 min) and examined with an automated biochemical analyzer (Chemray 800, Raydo, Shenzhen, China) for the levels of alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CREA), creatine kinase isoenzyme (CK-MB),

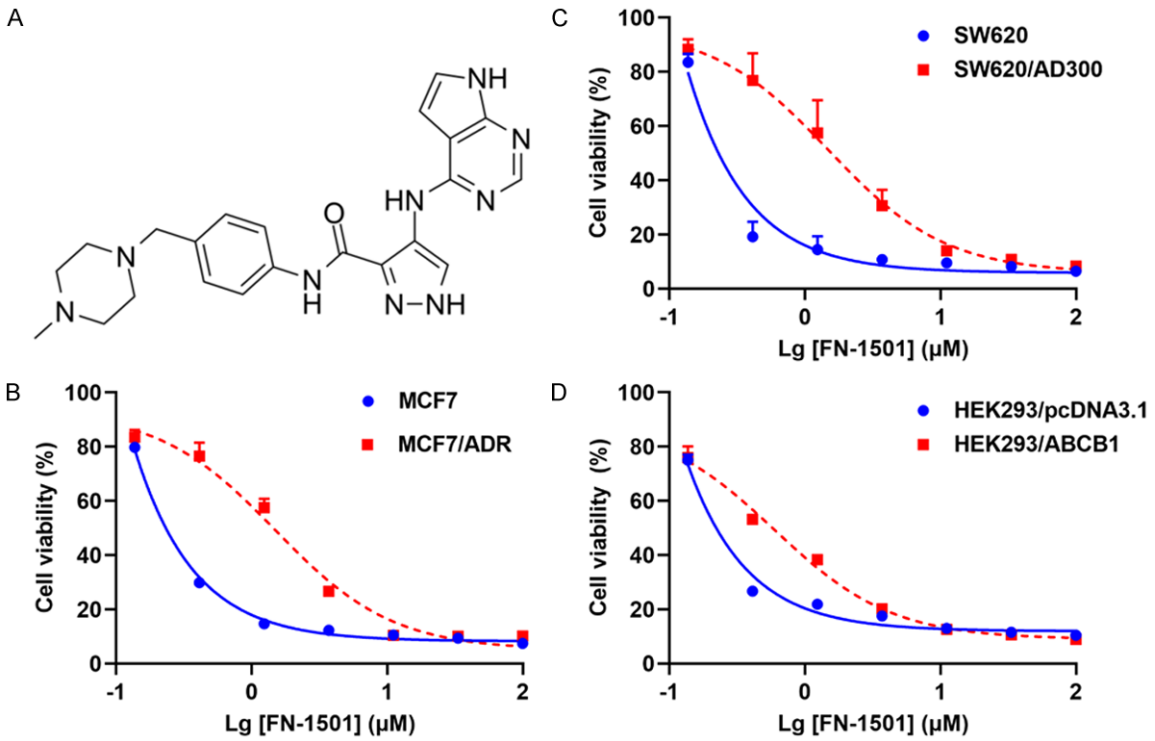

Figure 1. The eytotoxicity of FN-1501 in parental and drug-resistant cells. (A) Chemical structure of FN-1501. Cell vibility curves for (B, C) MCF7, MCF7/ADR, SW620, and SW620/AD300 cells. (D) HEK293/pcDNA3.1, HEK293/ABCB1. For three independent assays, data are presented as mean ± SD.

lactate dehydrogenase (LDH), and superoxide dismutase (SOD).

Data analysis

All assays were performed at least three times. GraphPad Prism 9.0 software and One-way ANOVA were used to analyze the data, and all data were presented as mean ± standard deviations. $*P < 0.05$ was deemed statistically significant.

Results

Cytotoxicity of FN-1501 in parental and ABCB1-overexpressing cells

CCK8 assay was used to determine the cytotoxicity of FN-1501 in parental MCF7 and SW620 cells as well as in ABCB1-overexpressing MCF7/ADR and SW620/Ad300 cells. **Figure 1A** illustrates the chemical structure of FN-1501. As depicted in **Figure 1B**, **1C**, the IC_{50} of FN-1501 in ABCB1-overexpressing MCF7/ADR and SW620/Ad300 cells was 1.739 μmol/L and 1.541 μmol/L, respectively, which was higher than the IC_{50} in their parental cells. To confirm the effect of ABCB1 overexpression on the IC_{50} of FN-1501, we transfected control vector or ABCB1 in HEK293 cells and obtained similar results (**Figure 1D**). The IC_{50} in HEK293/ABCB1 cells was 1.319 μmol/L, higher than that in vector transfected HEK293 cells. In line with this observation, when ABCB1-overexpressing cells were incubated with both FN-1501 and VPM, an effective reversal agent of ABCB1 [32], the reversal fold (RF) decreased significantly. As shown in **Table 1**, the RF value was respectively decreased from 15.389-fold to 1.009-fold, from 6.204 to 1.121, and from 5.004 to 0.774-fold in MCF7/ADR, SW620/Ad300, and HEK293/ABCB1 cells, suggesting that ABCB1 inhibitor can restore the sensitivity of drug-resistant cells to FN-1501 and that FN-1501 may be a potent ABCB1 substrate.

FN-1501 upregulates ABCB1 protein expression levels

We further evaluated the effect of FN-1501 on the expression of ABCB1 protein by western blot analysis. As shown in **Figure 2**, ABCB1 protein levels were upregulated in ABCB1-overexpressing SW620/Ad300 cells by a low

Table 1. The cytotoxicity of FN-1501 in parental and ABCB1-overexpressing cells

Cell lines	IC_{50} ± SD[a] (RF[b]) (μM)					
	Doxorubicin	Doxorubicin+ VPM 3 μM	Paclitaxel	Paclitaxel+ VPM 3 μM	FN-1501	FN-1501+ VPM 3 μM
MCF7	0.313±0.018 (1.000)	0.300±0.020 (0.961)	0.028±0.005 (1.000)	0.026±0.004 (0.929)	0.113±0.016 (1.000)	0.140±0.019 (1.236)
MCF7/ADR	3.207±0.272 (10.258)	0.298±0.019 (0.952)*	1.480±0.240 (52.235)	0.024±0.003 (0.835)*	1.739±0.185 (15.389)	0.114±0.005 (1.009)*
SW620	0.061±0.015 (1.000)	0.064±0.016 (1.043)	0.030±0.005 (1.000)	0.029±0.007 (0.956)	0.248±0.077 (1.000)	0.276±0.020 (1.111)
SW620/AD300	2.122±0.412 (34.598)	0.061±0.024 (0.995)*	1.819±0.258 (59.978)	0.040±0.002 (1.330)*	1.541±0.121 (6.204)	0.278±0.035 (1.121)*
HEK293/pcDNA3.1	0.034±0.004 (1.000)	0.028±0.008 (0.824)	0.028±0.002 (1.000)	0.033±0.008 (1.176)	0.264±0.031 (1.000)	0.306±0.028 (1.161)
HEK293/ABCB1	0.762±0.095 (22.422)	0.028±0.007 (0.833)*	2.784±0.222 (98.259)	0.037±0.004 (1.306)*	1.319±0.124 (5.004)	0.204±0.032 (0.774)*

[a]Values for the IC_{50} are shown as mean ± SD of at least three independent tests. [b]By dividing the IC_{50} values of substrates with or without of inhibitor by the IC_{50} of parental cells without inhibitor, the resistance fold was computed. *$P < 0.05$ vs. control treatment.

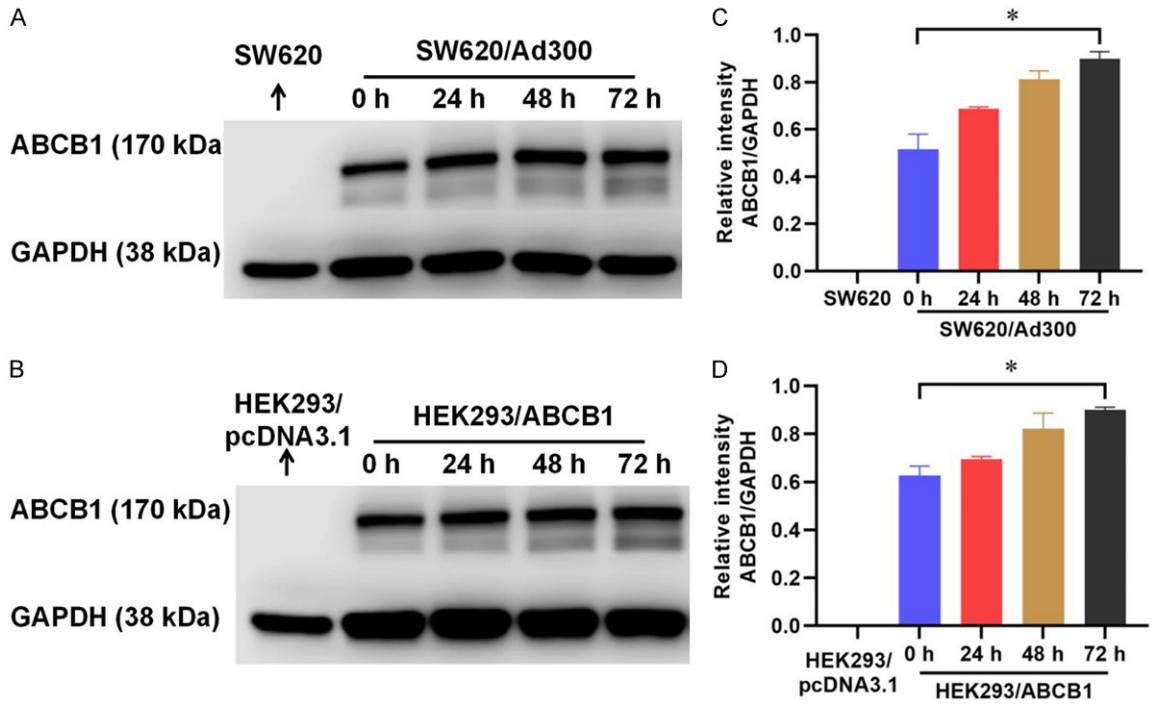

Figure 2. FN-1501 up-regulated the protein expression of ABCB1. A, B. FN-1501 up-regulated the protein expression of ABCB1. C, D. Quantitative analysis of protein expression. Results are mean ± SD, representative of three independent assays. *represents $P < 0.05$.

concentration of FN-1501 treatment in a time-dependent manner.

FN-1501 stimulated the ATPase activity of ABCB1

The activity of ATPase is one of the most crucial elements affecting the ABC transporter's function, as energy is required to pump the substrate drug of ABC transporters out of cells against the concentration gradient, which is supplied by the hydrolysis of ATP. Thus, we investigated the impact of FN-1501 on the ATPase basal activity of ABCB1 transporters by incubating ABCB1 transporters-enriched membrane with various concentrations of FN-1501 (0-40 μM). The results showed that FN-1501 increased the ATPase activity of ABCB1 transporters in a dose-dependent manner with the maximum stimulation of up to 268% of basal activity (**Figure 3**), while the half-maximal effective concentration (EC50) was 6.8 μM.

FN-1501 enhanced the accumulation of [3H]-paclitaxel

We further explored whether FN-1501 affected the function of the ABCB1 transporter by conducting the accumulation assay and found that 3 μM of FN-1501 treatment significantly elevated the accumulation of tritium-labeled paclitaxel in SW620/Ad300 cells (**Figure 4**). This effect was similar with the same concentration of positive control VPM; however, neither FN-1501 nor the positive control VPM significantly altered the accumulation of tritium-labeled paclitaxel in parent SW620 cells, suggesting that FN-1501 might compete with paclitaxel to bind to the ABCB1 transporter as an ABCB1 transporter-binding substance.

Molecular docking study of FN-1501 with ABCB1 protein model

To determine if there is an interaction between FN-1501 and ABCB1 as a ligand and receptor binding, we applied docking simulation in the ligand binding site of the ABCB1 protein (7A69). The results showed that FN-1501 docked into the ligand binding site with an affinity score of -9.77 kcal/mol (**Figure 5**). Specifically, hydrophobic interaction was the primary factor contributing to the binding of FN-1501 to the ABCB1 protein, as FN-1501 was positioned and stabilized in the hydrophobic cavity formed by Tyr310, Tyr307, Ile306, Leu305, Phe303,

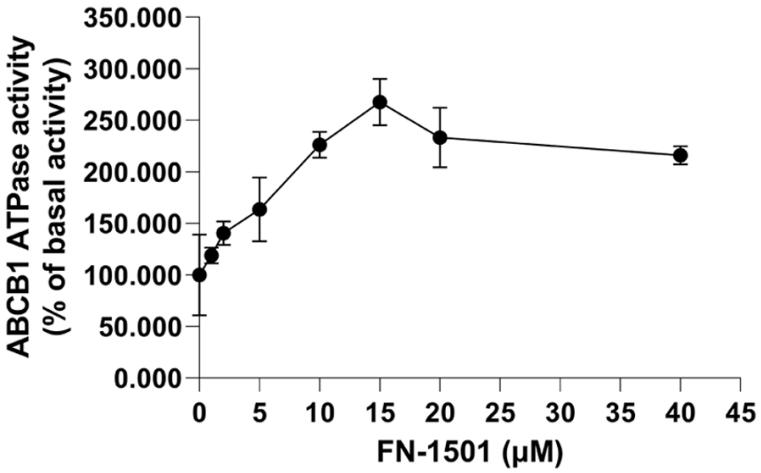

Figure 3. FN-1501 stimulates the activity of ABCB1 ATPase. FN-1501 (0-160 μM) stimulates ABCB1 ATPase activity. Data are mean ± SD of three independent assays.

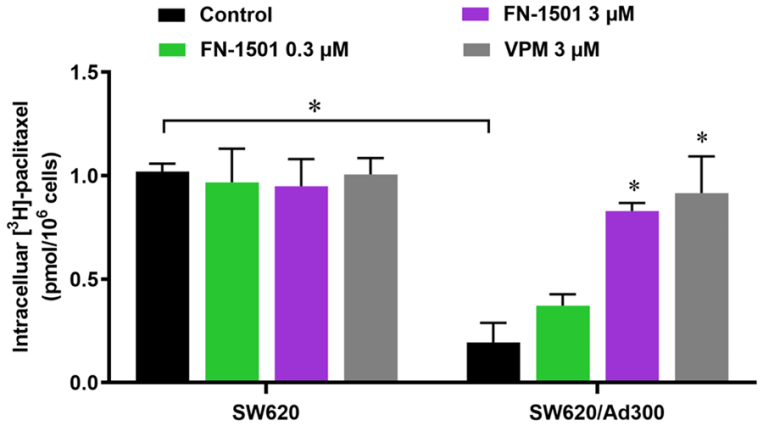

Figure 4. Intracellular [3H]-paclitaxel accumulation was altered by FN-1501 in ABCB1-overexpressing cells. ABCB1-overexpressing cells following co-incubated with FN-1501 at different concentrations (0.3, 3 μM). Results are mean ± SD, three independent experiments were performed. *represents $P < 0.05$.

Ala302, Trp232, Ala229, Ser228, Leu225, Leu339, Phe336, Gln990, Ala987, Met986, and Phe983. Additionally, FN-1501 was further stabilized by pi-pi stacking interactions and cation-pi interaction formed by Phe303 and Trp232 as well as by hydrogen bonds formed by Gln725, Gln990, and Tyr307.

Effect of FN-1501 on cell-derived xenograft (CDX) models

To evaluate the synergistic activity of FN-1501 and VPM in vivo, we used an animal xenograft model and found the growth of SW620 cell-derived tumors was significantly inhibited by FN-1501 treatment. In contrast, FN-1501 alone did not perturb the growth of the SW620/Ad300 cell-derived tumor; however, the growth of SW-620/Ad300 tumor was significantly attenuated when treated by the combination of FN-1501 and VPM (**Figure 6A-D**). HE and TUNEL staining of the tumor samples revealed severe necrosis of tumor cells, nuclear pyknosis, nuclear fragmentation, karyolysis, apoptosis, and vacuolization in the combined treatment group (**Figure 6E**).

To further assess the safety of FN-1501, blood biochemical tests were conducted, and the results indicated that ALT, AST, BUN, CREA, CKMB, LDH, and SOD were all within the normal range without significant changes (**Table 2**).

Discussion

ABCB1 overexpression is closely associated with malignancy and chemotherapy resistance, as it enhances MDR, leading to chemotherapy failure [33]. ABCB1 expression has been shown to positively correlate with tumor progression following chemotherapy in colon cancer, kidney cancer, leukemia, breast cancer, and other malignant tumors [34]. About 50% of AML patients have ABCB1-expressing blast cells [35], which is considered to be a poor prognostic indicator for patients receiving intensive chemotherapy [36]. Overexpression of ABCB1 is also linked to lower complete remission rates and higher recurrence rates in patients receiving standard chemotherapy [37-39].

FLT is one of the most important targets for the treatment of AML. Small molecule drugs targeting FLT3 have been developed, among which

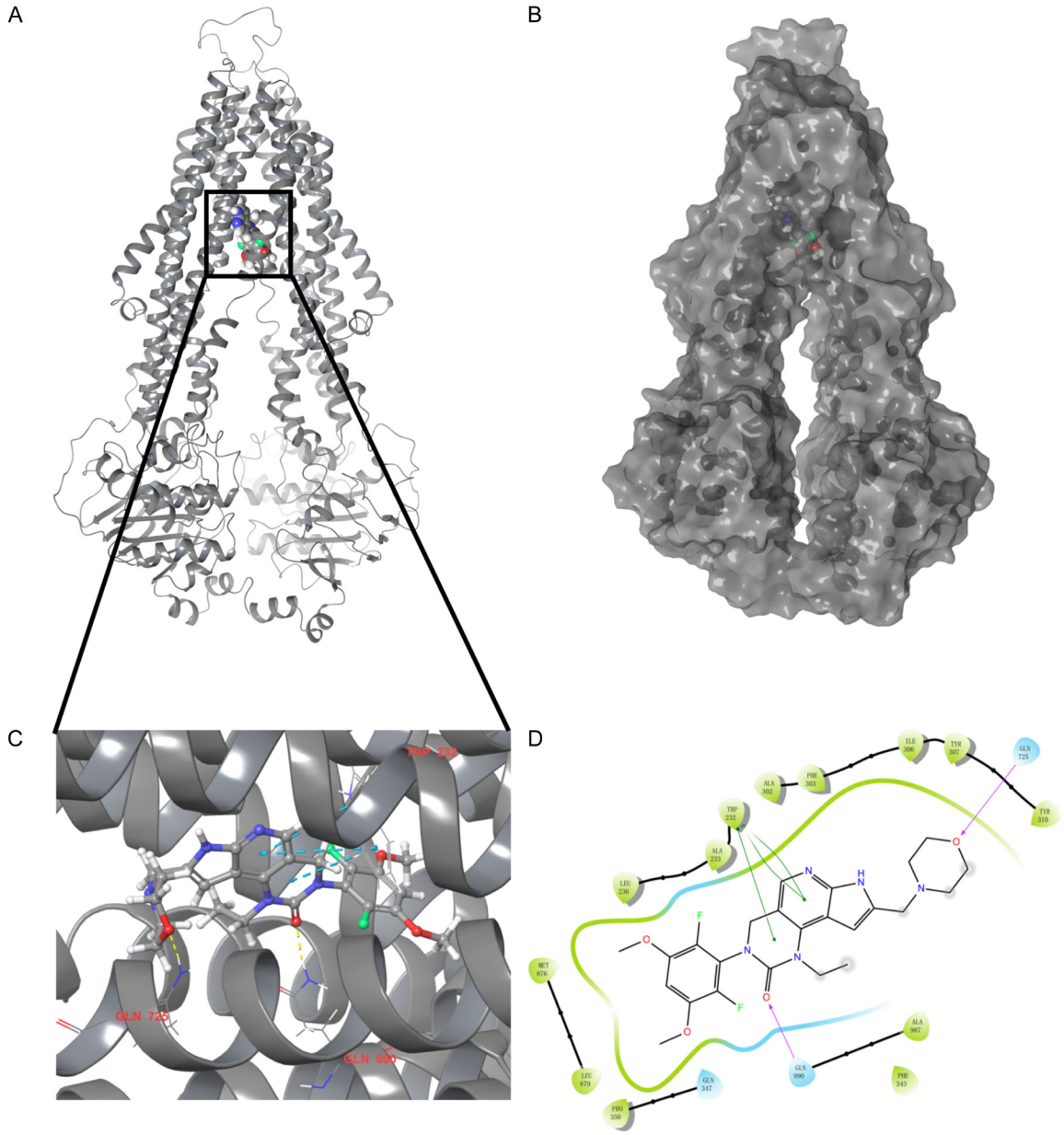

Figure 5. Interaction between FN-1501 and human ABCB1 protein. A. Overview of the best-scoring pose of FN-1501 in the drug binding pocket of ABCB1 protein. ABCB1 was displayed as gray ribbons. FN-1501 was displayed as colored spheres. B. Docked complex displayed with protein surface and ligand surface. FN-1501 was displayed as colored spheres. C. Details of interactions between FN-1501 and ABCB1 binding pocket. ABCB1 was displayed as gray ribbons. Important residues were displayed as colored sticks (gray: carbon; blue: nitrogen; red: oxygen). FN-1501 was displayed as colored sticks (purple: carbon; blue: nitrogen; red: oxygen). p-p stackings were displayed as blue dash lines. Cation-pi interactions were displayed as green dash lines. Hydrogen bonds were displayed as yellow dash lines. D. 2D FN-1501-ABCB1 interaction. Amino acids with 3.0 Å were displayed as color bubbles, cyan indicates polar residues, and green indicates hydrophobic residues. p-p stacking interactions are indicated with green lines. Cation-pi interactions are indicated with red lines. Hydrogen bonds are indicated with purple lines.

FN-1501 is a highly effective FLT3 inhibitor and is undergoing phase I clinical trial (NCT03-690154) in the United States and China. Interestingly, ABCB1-overexpressing cells are resistant to several FLT3 inhibitors, such as crenolanib [18, 19]. However, the effect of FN-1501 on ABCB1-mediated MDR has not been investigated. Here, we studied the possi-

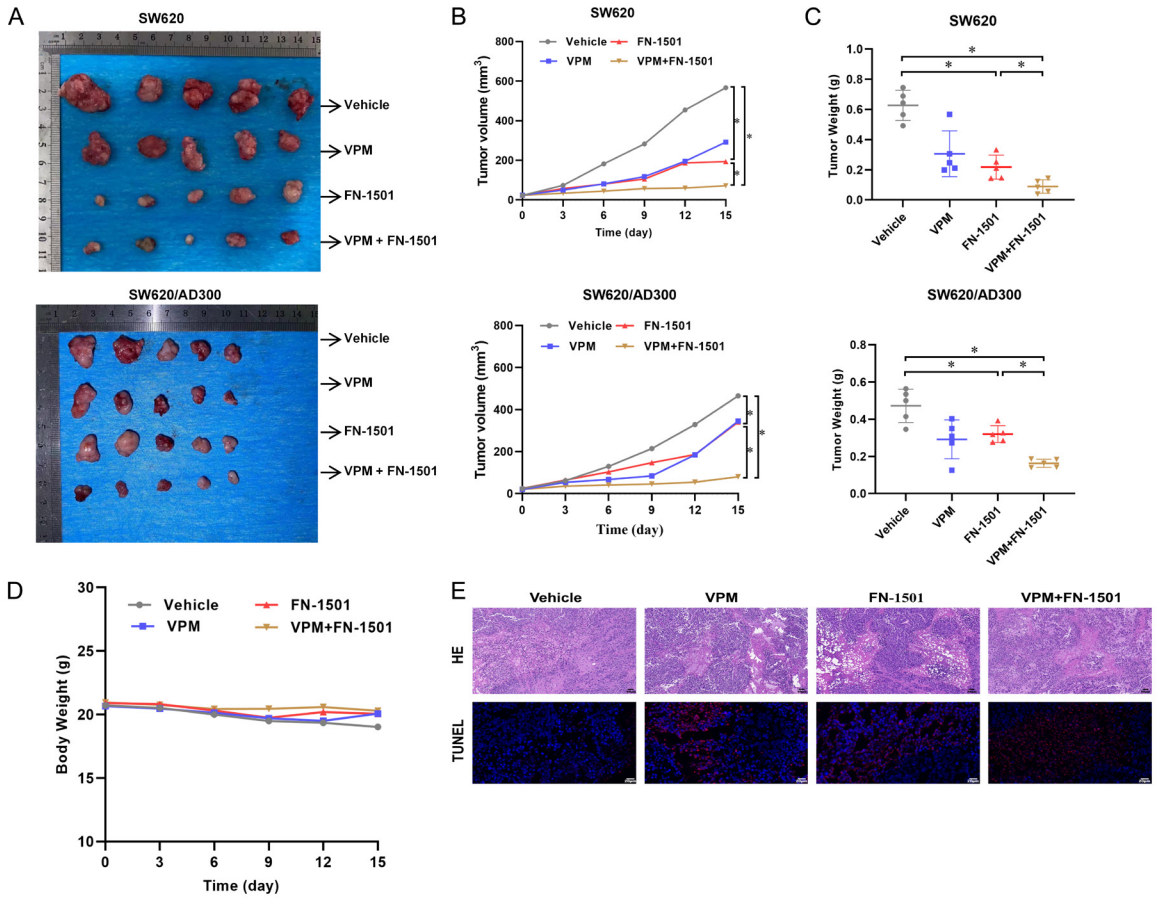

Figure 6. FN-1501's antitumor efficacy in tumor xenograft models. A. Picture of the removed tumors at the conclusion of the trial. B. Tumor volume throughout treatment. C. The weight of the tumor tissues at the conclusion of treatment. D. Changes in mouse body weight throughout therapy. E. HE and TUNEL analysis of tumors. The data are presented as mean ± SD. *$P < 0.05$ vs. the corresponding control group. HE staining scale was 50 μm. The TUNEL scale is 20 μm.

ble interaction between ABCB1 and FN-1501 and observed that ABCB1-overexpressing cells (MCF7/ADR, SW620/AD300) were more resistant to FN-1501 treatment than their parental cells, as the cytotoxicity of FN-1501 in these cells was obviously weakened, suggesting that the upregulation of ABCB1 transporter might cause FN-1501 resistance. Since drug-induced MDR may be the results of multiple mechanisms, we also used ABCB1-transfected HEK293 cells to demonstrate the effect of ABCB1 overexpression. The results showed that FN-1501 had a stronger inhibitory effect on the proliferation of vector-transfected control HEK293/pcDNA3.1 cells than on HEK293/ABCB1 cells. Additionally, the combination of FN-1501 and VPM could restore the sensitivity of FN-1501 in ABCB1-overexpressing cells, indicating that the resistance of FN-1501 was related to the overexpression of the ABCB1 transporter. Together, these findings suggest that ABCB1 is a crucial protein mediating FN-1501 resistance. To further elucidate the mechanism of FN-1501's resistance, we assessed the impact of FN-1501 on the expression of the ABCB1 transporter and found that FN-1501 could significantly upregulate the expression of ABCB1 in SW620/Ad300 cells, suggesting that FN-1501-induced ABCB1 expression leads to FN-1501 resistance. Nonetheless, the precise mechanism of FN-1501-mediated upregulation of ABCB1 transporter expression is unclear and requires further investigation.

ABCB1-mediated MDR is characterized by several features, the most notable being the altered accumulation of antitumor drugs [40].

Table 2. Serum biochemical analysis

Item	Value[a]	
	FN-1501	VPM+FN-1501
ALT, U/L	84.489±14.453	85.807±7.782
AST, U/L	204.609±9.394	192.271±13.703
BUN, mg/dL	30.77±4.837	34.382±5.925
CREA, umol/L	19.656±2.559	19.281±1.152
CKMB, U/L	856.541±66.691	835.240±104.890
LDH, U/L	734.895±114.187	810.898±66.449
SOD, U/mL	259.73±22.452	242.692±14.342

[a]Date are presented as mean ± SD of at least three independent tests.

Overexpressed ABC transporters remove more antineoplastic drugs, resulting in reduced intracellular accumulation, which can lead to the failure of chemotherapy. Paclitaxel is a substrate of ABCB1 and can be transported out of cells through the efflux activity of ABCB1. Therefore, we used tritium-labeled paclitaxel to explore the effect of FN-1501 on paclitaxel accumulation in ABCB1-overexpressing cells and then to indirectly evaluate the interaction between ABCB1 transporter with FN-1501. We observed that 3 μM FN-1501 significantly increased the intracellular accumulation of tritium-labeled paclitaxel in ABCB1-overexpressing SW620/Ad300 cells, indicating that FN-1501 could specifically bind to ABCB1 at the substrate binding site to compete with paclitaxel binding and thereby increasing the intracellular accumulation of tritium-labeled paclitaxel, suggesting a potential mechanism by which ABCB1 mediated FN-1501 resistance.

Since the nucleoside-binding domain of ABC transporters possesses ATPase activity, energy can be obtained by hydrolyzing ATP high-energy phosphoric acid bonds to further facilitate their transporting ability. Therefore, we hypothesized that FN-1501 might influence the ATPase activity of ABCB1. Indeed, our ATPase activity assay showed that FN-1501 significantly stimulated the activity of ABCB1-related ATPase. Furthermore, the computational docking analysis demonstrated the ligand-receptor interaction between the substrate and ABCB1. We simulated the docking at the ligand binding site (7A69) of the ABCB1 protein and found that FN-1501 interacted with ABCB1 at the substrate binding site. Collectively, these data suggest that FN-1501 is a specific substrate of ABCB1 in vitro.

To validate the findings from in vitro study, we subsequently established a mouse xenograft tumor model and proved that FN-1501 significantly inhibited the growth of SW620 cell-derived tumors. In contrast, treatment with FN-1501 alone did not significantly inhibit the growth of SW620/Ad300 cell-derived tumor, while the combination of FN-1501 and VPM did significantly. These results suggest that ABCB1-mediated drug resistance affects the efficacy of FN-1501. HE and TUNEL assays confirmed severe nuclear necrosis and apoptosis in the tumor tissues of the combined treatment group. Importantly, biochemical blood tests showed normal physiological indexes in the combination treatment group, indicating the safety of this treatment regimen.

In conclusion, we have identified and elucidated for the first time the role and molecular mechanism of ABCB1-mediated FN-1501 drug resistance in tumor cells, which provide important information for the further clinical application of FN-1501. Further clinical trials are required to determine whether the overexpression of ABCB1 will affect the prognosis of patients receiving FN-1501.

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Disclosure of conflict of interest

None.

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