Preclinical Antitumor Activity of Cabazitaxel, a Semisynthetic Taxane Active in Taxane-Resistant Tumors

Patricia Vrignaud, Dorothée Sémiond, Pascale Lejeune, Hervé Bouchard, Loreley Calvet, Cecile Combeau, Jean-François Riou, Alain Commerçon, François Lavelle, and Marie-Christine Bissery

Abstract

Purpose: Taxanes are important chemotherapeutic agents with proven efficacy in human cancers, but their use is limited by resistance development. We report here the preclinical characteristics of cabazitaxel (XRP6258), a semisynthetic taxane developed to overcome taxane resistance.

Experimental Design: Cabazitaxel effects on purified tubulin and on taxane-sensitive or chemotherapy-resistant tumor cells were evaluated *in vitro*. Antitumor activity and pharmacokinetics of intravenously administered cabazitaxel were assessed in tumor-bearing mice.

Results: *In vitro*, cabazitaxel stabilized microtubules as effectively as docetaxel but was 10-fold more potent than docetaxel in chemotherapy-resistant tumor cells (IC_{50} ranges: cabazitaxel, 0.013–0.414 μmol/L; docetaxel, 0.17–4.01 μmol/L). The active concentrations of cabazitaxel in these cell lines were achieved easily and maintained for up to 96 hours in the tumors of mice bearing MA16/C tumors treated with cabazitaxel at 40 mg/kg. Cabazitaxel exhibited antitumor efficacy in a broad spectrum of murine and human tumors (melanoma B16, colon C51, C38, HCT 116, and HT-29, mammary MA17/A and MA16/C, pancreas P03 and MIA PaCa-2, prostate DU 145, lung A549 and NCI-H460, gastric N87, head and neck SR475, and kidney Caki-1). Of particular note, cabazitaxel was active in tumors poorly sensitive or innately resistant to docetaxel (Lewis lung, pancreas P02, colon HCT-8, gastric GXF-209, mammary UISO BCA-1) or with acquired docetaxel resistance (melanoma B16/TXT).

Conclusions: Cabazitaxel is as active as docetaxel in docetaxel-sensitive tumor models but is more potent than docetaxel in tumor models with innate or acquired resistance to taxanes and other chemotherapies. These studies were the basis for subsequent clinical evaluation. *Clin Cancer Res; 19(11); 2973–83. ©2013 AACR.*

Introduction

Microtubules are highly dynamic cytoskeletal fibers composed of 2 tubulin subunits (α and β). The polymerization and depolymerization of these molecules are crucial processes, not only to mitosis but also to intracellular trafficking. Microtubules are the main target of taxanes, which bind to a specific binding site on the tubulin β-subunit (1, 2). The taxanes paclitaxel and docetaxel suppress microtubule dynamics by promoting tubulin assembly and stabilizing microtubules (3), blocking mitosis at the metaphase/anaphase transition, which results in cell death (ref. 4; Supplementary Fig. S1A and S1B). By stabilizing microtubules, taxanes also impact intracellular trafficking. This was recently reported as one of the main mechanisms of taxane action

in prostate cancer, where taxanes were shown to inhibit nuclear translocation of the androgen receptor, thereby preventing androgen receptor transcriptional activity and leading to prostate cancer cell death (5).

Paclitaxel and docetaxel form the backbone of both first-line and salvage chemotherapy regimens for patients with a wide variety of tumor types. Paclitaxel is indicated for first-line treatment of ovarian, breast, and lung cancer and for second-line treatment of AIDS-related Kaposi's sarcoma (6). Docetaxel is indicated for first-line treatment of breast, head and neck, gastric, lung, and prostate cancer and for second-line treatment of breast cancer (7). However, the use of both paclitaxel and docetaxel is limited by the development of tumor resistance (8–10). During the last 2 decades, considerable efforts have been made to understand, and develop new agents to overcome, taxane resistance.

Cabazitaxel (RPR 116258; XRP6258; TXD258; Jevtana) is a new semisynthetic taxane derived from 10-deacetyl-baccatin III, which is extracted from European yew needles (ref. 11; Supplementary Fig. S1C). Cabazitaxel was identified using a 3-step screening process, assessing activity against microtubule stabilization, *in vitro* activity in resistant cell lines, and *in vivo* activity in a tumor model in which docetaxel resistance had been induced *in vivo*. This article

Authors' Affiliation: Sanofi, Vitry-sur-Seine, France

Translational Relevance

Mechanisms of resistance to taxanes in patients have not been fully elucidated. In cell lines, overexpression of ATP-binding transporters, particularly P-glycoprotein, and alteration of microtubule dynamics are the most common mechanisms of taxane resistance. However, clinical data suggest that other mechanisms, including dysfunctional regulation of apoptotic and intracellular signaling, may operate in tumors escaping taxane therapy. To identify a docetaxel derivative with activity after taxane failure, we developed a clinically relevant docetaxel-resistant tumor model, mimicking tumor resistance development in patients who initially respond to docetaxel, but develop resistance over time. Cabazitaxel was selected from 450 derivatives based on activity in this model. Clinical proof-of-principle was achieved in a phase II study in patients with taxane-resistant metastatic breast cancer and a phase III study in metastatic hormone-refractory prostate cancer post-docetaxel therapy. The current study extends the characterization of cabazitaxel, showing wide ranging *in vitro* and *in vivo* antitumor activity.

describes the development and characterization of the *in vivo*–induced docetaxel-resistant tumor model, the mechanism of action of cabazitaxel on microtubules, and its preclinical evaluation in a wide range of taxane-sensitive and -resistant cell lines, both *in vitro* and *in vivo*.

Materials and Methods

Tubulin polymerization

The effects of cabazitaxel on tubulin polymerization and cold-induced microtubule depolymerization were evaluated using tubulin purified from porcine brain (12, 13). Tubulin was used at a concentration of 6 μmol/L for polymerization assays (at 37°C) and 9 μmol/L for depolymerization assays (at 8°C). Rates of polymerization/depolymerization were measured by optical density (OD) at 350 nm and were expressed in ΔOD/min. Upper and lower limits for drug concentrations reducing polymerization lag time by 50% (LT_{50}) and inhibiting cold-induced disassembly by 50% (dIC_{50}) were determined.

Microtubule and enzymatic parameters in tumors

Microtubule parameters in B16 and B16/TXT tumors were characterized using real-time PCR (RT-PCR) analysis of at least 2 samples per tumor. PCR values in arbitrary units were obtained for the following genes: total α-tubulin (*TUBA*), total β-tubulin (*TUBB*), *TUBB2*, *TUBB3*, *TUBB4A*, *TUBB4B*, and *TUBB7P*.

Glutathione S-transferase (GST) activity was assayed as previously described using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (14). Formation of the CDNB glutathione (GSH) conjugate by cytosols was measured continuously in a spectrophotometer at 340 nm. The results were expressed as the quantity of CDNB conjugated per minute per milligram of cytosolic protein (nmol/min/mg).

Total GSH concentration was determined as the sum of the reduced (GSH) and oxidized (GSSG) forms of GSH (15). In this assay, the sum of the reduced and oxidized forms of GSH is determined using a kinetic assay in which catalytic amounts of GSH or GSSG and GSH reductase bring about the continuous reduction of 5,5′-dithiobis(2-nitrobenzoic acid) by NADPH. The reaction rate is proportional to the concentration of GSH below 2 μmol/L. The formation of 5-thio-2-nitrobenzoate was analyzed using a spectrophotometer at 412 nm. The results were expressed as concentration per milligram of protein (nmol/mg).

Cytochrome P450 3A (CYP3A) levels were determined using the Amersham ELISA system (code RPN 271; Amersham). This assay uses a rabbit primary antibody specific for rat CYP3A, a secondary conjugate of anti-rabbit immunoglobulin (Ig) and horseradish peroxidase antibody, and tetramethylbenzidine substrate. The horseradish peroxidase color that develops is proportional to CYP3A levels. This assay was validated against mouse CYP3A by the manufacturer. Protein concentrations of microsomes, cytosols, and homogenates were determined by the bicinchoninic acid assay (16) using a commercial preparation (Pierce BCA Protein Assay Reagent).

In vitro antiproliferative activity

The HL60/TAX cell line (17) was a kind gift from Dr. K. Bhalla (Medical University of South Carolina, Charleston, SC). Calc18/TXT and P388/TXT were developed internally from Calc18 or P388 parental cell lines. The P388/TXT cell line was selected by mutagenesis with ethyl methane sulfonate and soft agar cloning in the presence of 0.06 μmol/L docetaxel. The Calc18/TXT cell line was established by 6-month exposure to increasing concentrations of docetaxel (up to 0.019 μmol/L). The cross-resistance pattern of these 2 cell lines is shown in Supplementary Table S1. The other tumor cell lines were obtained from the National Cancer Institute (NCI; Bethesda, MD).

Parental and resistant tumor cells were incubated with different drug concentrations for 96 hours at 37°C; cell viability was measured in quadruplicate using neutral red uptake (18). The resistance factor for each drug was calculated by dividing the IC_{50} in resistant cells by the mean IC_{50} in sensitive/parental cells, using data from at least 3 independent experiments. Relative expression of *ABCB1* mRNA was determined by Northern blotting using a human *ABCB1* gene probe.

Antitumor activity in tumor-bearing mice

All experimental procedures were approved by Sanofi, Southern Research Institute (SRI; Birmingham, AL), and Molecular Imaging Research (MIR) Preclinical Services Laboratory Animal Care and Use committees. Protocol design, chemotherapy techniques, and methods of data analysis have been described previously (19–21). Briefly, tumors were implanted subcutaneously and bilaterally on day 0.

Animals were randomly assigned to treatment (T) or control (C) groups. Tumors were measured using a caliper 2 to 5 times weekly (according to tumor growth rate) until the tumor reached 2,000 mm^3. Tumor volumes were estimated from 2-dimensional measurements using the formula: tumor volume (mm^3) = [length (mm) × width^2 (mm^2)]/2. The day of death was recorded, and thoracic and abdominal cavities were examined macroscopically to assess probable cause of death.

Mice. C57BL/6, B6D2F$_1$ and Swiss nude mice were bred at Iffa Credo; C3H/HeN, BALB/c, BALB/c nude, and severe combined immunodeficient (SCID) mice were bred at Charles River; and ICR and NCR nude mice were bred at Taconic. All mice weighed more than 18 g at the start of treatment and had free access to food and water.

Drugs. Cabazitaxel (RPR 116258; XRP6258; TXD258; Jevtana) and docetaxel (RP 56976) were prepared by mixing 1 volume of ethanol stock solution, 1 volume of polysorbate 80, and 18 volumes of 5% glucose in sterile water. Solutions were administered intravenously as a slow bolus (0.4 mL/mouse). Drug doses were adjusted on the basis of body weight at start of treatment. For cytotoxic compounds, such as docetaxel and cabazitaxel, a dose–response evaluation was conducted in each trial to determine the highest nontoxic dose (HNTD), defined as the highest drug dose inducing less than 20% body weight loss with no drug-related deaths. Animal body weights included the tumor weights.

Tumor models. Murine tumors were obtained from Dr. Corbett (Wayne State University, Detroit, MI) and included colon C51 and C38 (19), pancreas P02 and P03 (22), mammary MA17/A and MA16/C (23), Lewis lung (24), and melanoma B16 (20). Tumors were maintained by serial passage in the mouse strain of origin. B16/TXT was isolated by treating C57BL/6 mice bearing docetaxel-sensitive B16 melanoma at the HNTD of docetaxel (60 mg/kg) for 27 passages, until the B16 tumor acquired full resistance to docetaxel. Human tumor cell lines were obtained from American Type Culture Collection and included prostate DU 145 (25), lung NCI-H460 (26) and A549 (27), pancreas MIA PaCa2 (28), and colon HT-29 (29), HCT 116 (30), and HCT-8 (31). Mammary UISO BCA-1 (32) and gastric GXF-209 (33) tumors were obtained from SRI and gastric N87 (34) tumors from MIR Preclinical Services. Murine tumors were grafted into syngenic mice and human tumors were xenografted into immunocompromised mice.

Plasma pharmacokinetics and tumor distribution. Cabazitaxel concentrations in plasma and tumor tissue were evaluated in mice bearing advanced-stage (400 mm^3) murine mammary adenocarcinoma MA16/C after administration of the HNTD of cabazitaxel (40 mg/kg). Mice were treated on day 8 after subcutaneous tumor implantation with a single 45-second intravenous infusion of cabazitaxel in a polysorbate 80/ethanol/5% glucose solution, with a dosing volume of 25 mL/kg and a rate of infusion of 1 mL/min. Blood and tumor samples were collected from 3 animals per sampling time at 2, 5, and 15 minutes and 2, 4, 8, 12, 24, 48, 96, and 168 hours after cabazitaxel treat-

ment. Cabazitaxel concentrations were analyzed by liquid chromatography/tandem mass spectrometry, with limits of quantification of 2.5 ng/mL in plasma and 25 ng/g in tumor tissue. Pharmacokinetic parameters were determined using WinNonLin software, Version 1.0 (Scientific Consulting Inc.), using a noncompartmental infusion model.

Assessments of antitumor activity. Several endpoints were used. Tumor growth delay (T−C) was defined as the difference between tumors in the T and C groups in the median time (days) to reach a predetermined volume (750–1,000 mm^3). Tumor doubling time (T_d) in days was estimated from log linear tumor growth during the exponential phase (range, 100–1,000 mm^3). Log cell kill was calculated using the formula (T−C)/(3.32 × T_d), with antitumor activity defined as a log cell kill value ≥ 0.7 (21). SRI score was used to categorize antitumor activity based on log cell kill values as follows: <0.7 = − (inactive); 0.7–1.2 = +; 1.3–1.9 = ++; 2.0–2.8 = +++; > 2.8 = ++++ (highly active). Complete tumor regression (CR) was defined as tumor regression below the limit of palpation (62 mm^3). Animals without palpable tumors at the end of the study were declared tumor-free survivors (TFS) and were excluded from the T−C value calculation.

Statistical analysis was conducted using either a pairwise Wilcoxon rank-sum test, with *P* value adjustment by the Holm method (N87 study), or by log-rank multiple comparisons test versus control (with Bonferroni–Holm correction for multiplicity) on individual values for time to reach a prespecified tumor size for treated and control groups (UISO BCA-1 study). A *P* value of less than 5% (*P* < 0.05) was considered significant.

Results

Isolation and characterization of B16/TXT, a docetaxel-resistant melanoma

To identify taxane derivatives with activity following taxane failure, a docetaxel-resistant tumor model (B16/TXT) was developed to mimic the gradual development of resistance to docetaxel observed in some patients following an initial tumor response to the agent. Mice bearing the sensitive murine B16 melanoma were treated with docetaxel at the HNTD (60 mg/kg per passage; log cell kill 1.7; Table 1). Resistance occurred very slowly, with 27 passages over 17 months needed to obtain a fully docetaxel-resistant tumor (log cell kill < 0.7). B16/TXT was found to have similar T_d (1.3–2 days) and histologic characteristics to the parental B16 tumor. Cross-resistance (no antitumor activity) was observed to the tubulin-binding drugs paclitaxel, vincristine, and vinblastine, but not to cyclophosphamide (log cell kill 2.9 in B16 vs. 3.0 in B16/TXT), CCNU (log cell kill 3.7 in B16 vs. 4.7 in B16/TXT), and etoposide (log cell kill 1.2 in both). B16/TXT was partially cross-resistant to doxorubicin (log cell kill 2.4 in B16 vs. 0.9 in B16/TXT). There was no difference between the docetaxel-sensitive and -resistant B16 tumors either in factors involved in drug resistance, such as GST activity (B16, 0.42 ± 0.03 μmol/min/mg

Table 1. Dose–response antitumor activity of cabazitaxel and docetaxel in mice bearing murine tumors

Tumor type	Dose, mg/kg per injection (schedule days)	Cabazitaxel					Docetaxel				
		Total HNTD, mg/kg	T–C, days	log cell kill[b]	CR	TFS	Total HNTD, mg/kg	T–C, days	Log cell kill[b]	CR	TFS
B16 melanoma (T_d: 1.2 d)	32.2, 20, 12.4, 7.7 (3, 5, 7)	60	8.5	2.1	N/A	0/5	60	6.6	1.7	N/A	0/5
B16/TXT melanoma (T_d: 1.3 d)	32.2, 20, 12.4, 7.7 (3, 5, 7)	60	5.5	1.3	N/A	0/5	60	2.8	0.6	N/A	0/5
Colon C38 (T_d: 2.8 d)	32.2, 20, 12.4, 7.7 (14[a], 17, 20)	60	—	—	5/5	5/5	60	29.1	3.1	0/5	0/5
Colon C51 (T_d: 3 d)	24.2, 15, 9.3, 5.8 (4, 6, 8)	45	25.8	2.6	N/A	0/5	45	31.1	3.1	N/A	0/5
Pancreas P03 (T_d: 3.5 d)	32.2, 20, 12.4, 7.7 (17[a], 19, 21)	60	—	—	5/5	4/5	ND	ND	ND	ND	ND
Pancreas P02 (T_d: 2.5 d)	32.2, 20, 12.4, 7.7 (3, 5, 7)	60	6.6	0.8	N/A	0/5	ND	ND	ND	ND	ND
Mammary MA16/C (T_d: 1.1 d)	64.5, 40, 24.8, 15.4 (8[a])	40	13.4	3.7	4/5	0/5	ND	ND	ND	ND	ND
Mammary MA17/A (T_d: 1.2 d)	19.4, 12, 7.4, 4.6 (3, 5, 7)	36	15.7	3.9	N/A	0/5	ND	ND	ND	ND	ND
Lung 3LL (T_d: 1.2 d)	31.5, 19.5, 12.1, 7.5 (3, 5, 7)	58.5	4.6	1.2	N/A	0/5	ND	ND	ND	ND	ND

NOTE: Murine tumors were grafted in the syngenic strain of mice of origin of the tumor for MA17/A (C3H/HeN) and C51 (BALB/c) and in B6D2F₁ mice for the C57BL/6 syngenic tumors (B16, B16/TXT, C38, P03, P02, and 3LL).

Abbreviations: N/A, not available as treatment conducted on early-stage disease; ND, not determined in the same study.

[a]Median tumor burden at start of therapy: 290, 310, and 400 mm³ for C38, P03, and MA16/C studies, respectively.

[b]Definition of antitumor activity: log cell kill total <0.7 = inactive; >2.8 = highly active.

protein; B16/TXT, 0.39 ± 0.04 μmol/min/mg protein) and GSH content (B16, 21.7 ± 8.1 μmol/mg protein; B16/TXT, 21.2 ± 2.5 μmol/mg protein), or in activity of CYP3A, involved in TXT metabolism (B16, 2.4 ± 0.8 μg/mg protein; B16/TXT, 2.9 ± 0.06 μg/mg protein). Moreover, no overexpression of P-glycoprotein was found in B16/TXT, either by flow cytometry or Western blot analyses (data not shown). Analyses by RT-PCR of microtubule components revealed that B16/TXT expressed 3.13-fold higher levels of *TUBB3* than the docetaxel-sensitive parental B16 tumor, whereas levels of other microtubule parameters were similar (Supplementary Table S2). As noted earlier, this model was pivotal in the selection of cabazitaxel, the characteristics of which are described hereafter.

Microtubule stabilization

Cabazitaxel had similar efficiency compared with docetaxel for reducing the lag time for tubulin assembly ($LT_{50} = 0$–0.1 μmol/L for both) and the rate of cold-induced microtubule depolymerization ($dIC_{50} = 0.1$–0.25 μmol/L for both) *in vitro* (Table 2).

In vitro antiproliferative activity in chemotherapy-sensitive and -resistant cell lines

Cabazitaxel showed similar antiproliferative activity compared with docetaxel in cell lines sensitive to chemotherapy (murine leukemia P388, human tumor HL60 and KB, and breast Calc18), as shown by the similar IC_{50} ranges across different cell types (cabazitaxel, 0.004–0.041 μmol/L; docetaxel, 0.008–0.079 μmol/L; Table 3). In P-glycoprotein–expressing cell lines with *in vitro*–acquired resistance to taxanes (P388/TXT, Calc18/TXT, and HL60/TAX) or to other chemotherapy agents (P388/DOX, P388/VCR, and KBV1), cabazitaxel was found to be more active than docetaxel (IC_{50} ranges: cabazitaxel, 0.013–0.414 μmol/L; docetaxel, 0.17–4.01 μmol/L). Resistance factors (an indication of the difference in drug concentrations needed to inhibit resistant vs. sensitive/parental cell lines) were 2 to 10 for cabazitaxel and 5 to 59 for docetaxel. Cell lines expressing moderate levels of P-glycoprotein (P388/TXT, P388/VCR, HL60/TAX, and Calc18/TXT), which may be more clinically representative, had minimal cross-resistance to cabazitaxel (resistance factors = 2–4).

Plasma pharmacokinetics and drug distribution in tumors

The pharmacokinetic profile of cabazitaxel was evaluated in mice bearing docetaxel-sensitive murine mammary MA16/C adenocarcinoma tumors. Cabazitaxel was highly active in this tumor model, inducing CRs in 80% of mice and having a log cell kill of 3.7 at the HNTD of 40 mg/kg (Table 1). This antitumor activity was consistent with drug uptake into the tumor, which was both rapid (maximum drug concentrations were reached 15 minutes after dosing) and sustained (at 48 hours post-dose, cabazitaxel concentrations were 40-fold higher in the tumor vs. plasma; Fig. 1). Ratios of cabazitaxel exposure in tumors versus plasma were 1.6 from 0 to 48 hours and 2.9 over the entire experimental period.

Table 2. Effects of taxoids on the assembly–disassembly process of pure tubulin

Drug concentration, μmol/L	Rate of cold-induced microtubule disassembly, ΔOD/min			Lag time, min	
	Cabazitaxel	Docetaxel	Ratio	Cabazitaxel	Docetaxel
Control	7.42×10^{-2} ($n = 5$)			40	
0.1	5.36×10^{-2} ($n = 4$)	6×10^{-2} ($n = 3$)	0.89	4.18	9.16
0.25	2.82×10^{-2} ($n = 5$)	3.05×10^{-2} ($n = 5$)	0.92	1.57	2.61
0.5	1.8×10^{-2} ($n = 6$)	1.95×10^{-2} ($n = 5$)	0.92	1.0	1.04
1	1.2×10^{-2} ($n = 5$)	1.25×10^{-2} ($n = 4$)	0.96	0.5	0.65
2.5	0.61×10^{-2} ($n = 4$)	0.5×10^{-2} ($n = 6$)	1.22		
5	0.56×10^{-2} ($n = 3$)	0.38×10^{-2} ($n = 3$)	1.47		
	dIC$_{50}$: 0.1–0.25 μmol/L	dIC$_{50}$: 0.1–0.25 μmol/L	Mean: 1.06	LT$_{50}$: 0–0.1 μmol/L	LT$_{50}$: 0–0.1 μmol/L

NOTE: Tubulin was used at a concentration of 6 μmol/L for polymerization (at 37°C) and 9 μmol/L for depolymerization (at 8°C). OD was measured at 350 nm. Rates of depolymerization were expressed in ΔOD/min. Ratios between depolymerization rates were calculated for each drug concentration. Boundaries of drug concentrations for dIC$_{50}$ and LT$_{50}$ are given.

Cabazitaxel concentrations were maintained above the range of cellular antiproliferative IC$_{50}$ values [0.004–0.041 μmol/L (see Table 3), corresponding to 3–29 ng/mL, 4-day exposure] for 24 hours in plasma and 96 hours in the tumor.

Schedule of administration

The optimal schedule of cabazitaxel administration *in vivo* was initially determined by assessing the total dose that could be injected without undue toxicity for different sche-dules in nontumor-bearing B6D2F$_1$ female mice (Supplementary Table S3). Three schedules of intravenous cabazi-taxel were administered: intermittent [days 1 and 5 (A$_1$)], daily [days 1–5 (A$_2$)] and split-dose [days 1–5, 3 times daily (A$_3$)]. HNTDs were 58 mg/kg (A$_1$), 29 mg/kg (A$_2$), and 12 mg/kg (A$_3$), suggesting a trend for schedule dependency. These results indicate that, compared with intermittent treatment (A$_1$) of the same duration, the daily (A$_2$) and split-dose (A$_3$) schedules require 2-fold and 4.8-fold dose

Table 3. *In vitro* antiproliferative effects of cabazitaxel and docetaxel against sensitive and P-glycoprotein–expressing resistant cell lines

Cell line	Mean IC$_{50}$, μmol/L, $\pm$ SD		Resistance factor[a]		*ABCB1* mRNA level[b]
	Docetaxel	Cabazitaxel	Docetaxel	Cabazitaxel	
P388 murine leukemia	0.079 ± 0.004	0.041 ± 0.017	—	—	—
P388/DOX	4.01 ± 0.28	0.414 ± 0.036	51	10	+++
P388 murine leukemia	0.039 ± 0.012	0.013 ± 0.005	—	—	—
P388/TXT	0.188 ± 0.022	0.024 ± 0.015	5	2	++
P388 murine leukemia	0.039 ± 0.012	0.013 ± 0.005	—	—	—
P388/VCR	0.227 ± 0.038	0.024 ± 0.003	6	2	++
HL60 human leukemia	0.031 ± 0.004	0.022 ± 0.010	—	—	—
HL60/TAX	0.25 ± 0.11	0.060 ± 0.029	8	3	++
Calc18 human breast adenocarcinoma	0.008 ± 0.002	0.004 ± 0.002	—	—	—
Calc18/TXT	0.17 ± 0.04	0.016 ± 0.004	21	4	++
KB human epidermoid carcinoma	0.042 ± 0.0212	0.035 ± 0.026	—	—	—
KB V1	2.48 ± 0.12	0.27 ± 0.013	59	8	++++

NOTE: Cells were incubated for 96 hours at 37°C in liquid medium with drugs at different concentrations. Viability was assessed by neutral red, with the mean of at least 3 results obtained.

Abbreviations: *ABCB1*, ATP-binding cassette, sub-family B, member 1; Calc18/TXT, Calc18 human breast adenocarcinoma resistant to docetaxel; HL60/TAX, HL60 human leukemia resistant to paclitaxel; KB V1, KB human epidermoid carcinoma resistant to vinblastine; P388/DOX, P388 murine leukemia resistant to doxorubicin; P388/TXT, P388 murine leukemia resistant to docetaxel; P388/VCR, P388 murine leukemia resistant to vincristine.

[a]Resistance factor $=$ IC$_{50}$ (resistant)/IC$_{50}$ (parental) from the same experiment.

[b]Relative expression obtained from Northern blot experiments using the human *ABCB1* gene as probe.

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