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

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

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

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Corresponding author: Patricia Vrignaud, Sanofi Oncology, 13 quai Jules Guesde, Vitry-sur-Seine, 94403 Cedex, France. Phone: +33 1 58 93 36 29; Fax: +33 1 58 93 34 71; Email: patricia.vrignaud@sanofi.com

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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 ten-fold more potent than docetaxel in chemotherapy-resistant tumor cells (IC$_{50}$ ranges: cabazitaxel, 0.013–0.414 µM; docetaxel, 0.17–4.01 µM). 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 DU145, 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 UIISO 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
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resistance to taxanes and other chemotherapies. These studies were the basis for subsequent clinical evaluation.
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, demonstrating wide-ranging in vitro and in vivo antitumor activity.
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Introduction

Microtubules are highly dynamic cytoskeletal fibers composed of two 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 (4) (Supplementary Fig. S1[a] and [b]). 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 utility of both paclitaxel and docetaxel is limited by the development of tumor resistance (8–10). During the last two decades, considerable efforts have been made to understand, and develop new agents to overcome, taxane resistance.

Cabazitaxel (RPR116258; XRP6258; TXD258; Jevtana®) is a new semisynthetic taxane derived from 10-deacetylbaccatin III, which is extracted from European yew needles (11) (Supplementary Fig. S1[c]). Cabazitaxel was identified using a three-step research.
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screening process, assessing activity against microtubule stabilization, \textit{in vitro} activity in resistant cell lines and \textit{in vivo} activity in a tumor model in which docetaxel resistance had been induced \textit{in vivo}. This article describes the development and characterization of the \textit{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 taxane-resistant cell lines, both \textit{in vitro} and \textit{in vivo}. 
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1 Materials and Methods

2 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 µM for polymerization assays (at 37°C) and 9 µM 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% (LT50) and inhibiting cold-induced disassembly by 50% (dIC50) were determined.

3 Microtubule and enzymatic parameters in tumors

Microtubule parameters in B16 and B16/TXT tumors were characterized using rt-PCR analysis of at least two 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 glutathione (15). In this assay, the sum of the reduced and oxidized forms of glutathione is determined using a kinetic assay in which catalytic amounts of GSH or GSSG and glutathione reductase bring about the continuous reduction of 5,5′-dithiobis(2-nitrobenzoic acid) by NADPH. The reaction rate is
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proportional to the concentration of GSH below 2 µM. 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, Les Ulis, France). This assay uses a rabbit primary antibody specific for rat CYP3A, a secondary conjugate of anti-rabbit 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, CS, USA). Calc18/TXT and P388/TXT were developed internally from P388 or Calc18 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 µM docetaxel. The Calc18/TXT cell line was established by 6-month exposure to increasing concentrations of docetaxel (up to 0.019 µM). The cross-resistance pattern of these two cell lines is shown in Supplementary Table S1. The other tumor cell lines were obtained from the National Cancer Institute (NCI).

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 mean half-maximal inhibitory concentration (IC$_{50}$) in resistant cells by the mean IC$_{50}$
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in sensitive/parental cells, using data from at least three independent experiments.

Relative expression of \textit{ABCB1} mRNA was determined by northern blotting using a
human \textit{ABCB1} gene probe.

\textbf{Antitumor activity in tumor-bearing mice}

All experimental procedures were approved by Sanofi, Southern Research Institute
(SRI; Birmingham, AL, USA), and Molecular Imaging Research (MIR) Preclinical
Services (Ann Arbor, MI, USA) 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 (SC) and bilaterally
on Day 0. Animals were randomly assigned to treatment (T) or control (C) groups.

Tumors were measured using a caliper two to five times weekly (according to tumor
growth rate) until the tumor reached 2000 mm$^3$. Tumor volumes were estimated from
two-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.

\textit{Mice:} C57BL/6, B6D2F1, and Swiss nude mice were bred at Iffa Credo (Les Oncins,
France); C3H/HeN, BALB/c, BALB/c nude and SCID mice were bred at Charles River
(Les Oncins, France); and ICR and NCR nude mice were bred at Taconic (Hudson, NY,
USA). All mice weighed > 18 g at the start of treatment and had free access to food and
water.

\textit{Drugs:} Cabazitaxel (RPR116258; XRP6258; TXD258; Jevtana$^{\text{\textregistered}}$) and docetaxel
(RP 56976) were prepared by mixing one volume of ethanol stock solution, one volume
of polysorbate 80, and 18 volumes of 5\% glucose in sterile water. Solutions were
administered intravenously (IV) as a slow bolus (0.4 mL/mouse). Drug doses were
adjusted based on body weight at start of treatment. For cytotoxic compounds, such as

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docetaxel and cabazitaxel, a dose–response evaluation was performed in each trial to
determine the highest non-toxic dose (HNTD), defined as the highest drug dose inducing
< 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, USA) 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 ATCC (Rockville, MD, USA) and
included prostate Du145 (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 mm³)
murine mammary adenocarcinoma MA16/C after administration of the HNTD of
cabazitaxel (40 mg/kg). Mice were treated on Day 8 after SC tumor implantation with a
single 45-second IV 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 three animals per sampling time at 2, 5 and 15
minutes, and 2, 4, 8, 12, 24, 48, 96 and 168 hours after cabazitaxel treatment.
Cabazitaxel concentrations were analyzed by liquid chromatography–tandem mass
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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., Apex, NC, USA), using a non-compartmental
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 (750–1000 mm$^3$). Tumor doubling
time (Td) in days was estimated from log linear tumor growth during the exponential
phase (range 100–1000 mm$^3$). Log cell kill was calculated using the formula (T−C)/(3.32
x Td), 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 performed 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 probability value of less than 5% (p < 0.05) was
considered significant.
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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 Td (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 versus 3.0 in B16/TXT), CCNU (log cell kill 3.7 in B16 versus 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 versus 0.9 in B16/TXT). There was no difference between the docetaxel-sensitive and docetaxel-resistant B16 tumors either in factors involved in drug resistance, such as GST activity (B16: 0.42 ± 0.03 µmol/min/mg protein; B16/TXT: 0.39 ± 0.04 µmol/min/mg protein) and glutathione content (GSH; 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.
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(Supplementary Table S2). As noted above, 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 ($LT_{50} = 0–0.1 \mu M$ for both) and the rate of cold-induced microtubule depolymerization ($dIC_{50} = 0.1–0.25 \mu M$ for both) in vitro (Table 2).

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

Cabazitaxel demonstrated 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 $\mu M$; docetaxel, 0.008–0.079 $\mu M$; 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 $\mu M$; docetaxel, 0.17–4.01 $\mu M$). Resistance factors (an indication of the difference in drug concentrations needed to inhibit resistant versus sensitive/parental cell lines) were 2–10 for cabazitaxel and 5–59 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
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uptake into the tumor, which was both rapid (maximum drug concentrations were reached 15 minutes post-dosing) and sustained (at 48 hours post-dose, cabazitaxel concentrations were 40-fold higher in the tumor versus 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. Cabazitaxel concentrations were maintained above the range of cellular antiproliferative IC_{50} values (0.004–0.041 μM [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 schedules in non-tumor-bearing B6D2F1 female mice (Supplementary Table S3). Three schedules of IV cabazitaxel were administered: intermittent (Days 1 and 5 [A1]), daily (Days 1–5 [A2]) and split dose (Days 1–5, three times daily [A3]). HNTDs were 58 mg/kg (A1), 29 mg/kg (A2) and 12 mg/kg (A3), suggesting a trend for schedule dependency. These results indicate that, compared with intermittent treatment (A1) of the same duration, the daily (A2) and split-dose (A3) schedules require 2-fold and 4.8-fold dose reductions, respectively. In addition, host recovery time (time from last treatment to recovery of initial body weight) was shorter with the intermittent schedule (A1; 8 days) compared with the daily (A2; 12 days) and split-dose (A3; 15 days) schedules. Results of these dose-scheduling studies were consistent with further evaluations performed in C3H/HeN mice bearing mammary adenocarcinoma MA17/A tumors, which showed a 4.5-fold lower HNTD for a split-dose schedule (Days 3–7, three-times daily; HNTD 42 mg/kg) compared with an intermittent schedule (Days 3 and 7; HNTD 9.3 mg/kg) (Supplementary Table S4). Cabazitaxel antitumor activity against MA17/A tumors was also lower with the split-dose schedule compared with the intermittent schedule (log cell
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kill 1.3 versus 4.6, respectively). These differences between split-dose and intermittent
schedules were confirmed in a second tumor model, murine mammary adenocarcinoma
MA16/C, which showed a 4.2-fold reduction in HNTD (11 versus 46 mg/kg, respectively)
and decreased antitumor activity (log cell kill 1.6 versus 5.4, respectively). As the
intermittent schedule allowed the highest drug dose to be administered, with the best
host recovery, and had the greatest antitumor activity of the schedules tested,
intermittent dosing was selected for further evaluation.

Antitumor activity in docetaxel-sensitive tumors

The antitumor properties of cabazitaxel in vivo were evaluated using murine
tumors grafted in syngenic mice and human tumors xenografted in immunocompromised
mice (Tables 1 and 4). Cabazitaxel was found to be very active against murine B16
melanoma (log cell kill 2.1 at HNTD of 20 mg/kg injection on days 3, 5 and 7), murine
colon adenocarcinoma C51 (log cell kill 2.6 at HNTD of 9.3 mg/kg injection on days 4, 6,
and 8), and mammary adenocarcinomas MA16/C and MA17/A (reported above).
Cabazitaxel was highly active, with complete regressions observed against the
advanced-stage murine tumors colon C38 (5/5 CR; 5/5 TFS) and pancreas P03 (5/5 CR;
4/5 TFS) as well as human tumor xenografts including: prostate DU145 (6/6 CR; 5/6
TFS); colon HCT116 (7/7 CR; 2/7 TFS) and HT29 (6/6 CR); pancreas MIAPaCa-2 (6/6
CR; 6/6 TFS); breast Calc18 (5/8 TFS); lung NCI-H460 (2/6 CR) and A549 (2/6 CR);
head and neck SR475 (6/6 CR; 6/6 TFS); and kidney Caki-1 (5/6 CRs). In most of the
above models, cabazitaxel and docetaxel exhibited similar antitumor activity.

Dose–response effects were examined in the advanced human gastric carcinoma
N87 model, a tumor expressing HER2 (34) (Fig. 2a). At the HNTD (24.4 mg/kg on days
27, 31 and 35), cabazitaxel was highly active and delayed tumor growth by 101 days
(log cell kill > 6, 1/8 TFS; p < 0.0001). The two dose levels below the HNTD (15 and 9.3
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mg/kg per injection) also had a high level of antitumor activity (4.5 and 2.5 log cell kill; p < 0.0001 and 0.091, respectively). In comparison, docetaxel was also highly active at the HNTD, but to a lesser extent (T-C = 67 days; log cell kill 4.5; 1/8 TFS; p < 0.0001), and the activity was observed only at one dose below the HNTD. Thus cabazitaxel had a greater therapeutic index (three active dose levels) than docetaxel (two active dose levels) in this gastric tumor model.

**Antitumor activity in tumor models poorly or not sensitive to docetaxel**

Cabazitaxel showed antitumor activity against the fully docetaxel-resistant B16/TXT tumor model (log cell kill 2.1 in B16 versus 1.3 in B16/TXT), but no antitumor activity was obtained against the P-glycoprotein-overexpressing tumor Calc18/TXT in which docetaxel resistance was induced *in vitro* (log cell kill 3.4 in Calc18 versus 0.5 in Calc18/TXT). Cabazitaxel was also found to be active against two aggressive murine tumors: Lewis lung carcinoma (which has innate resistance to vincristine and 5-fluorouracil, and modest sensitivity to docetaxel; 35), and pancreatic adenocarcinoma P02 (which has innate resistance to a broad spectrum of chemotherapeutic agents; 22) (1.2 and 0.8 log cell kill, respectively). In addition, cabazitaxel was active against three human tumors that are poorly or not sensitive to docetaxel, namely colon HCT-8, gastric GXF-209, and breast UISO BCA-1 (log cell kill values for cabazitaxel versus docetaxel of 1.9 versus 0.8, 1.4 versus 0.5, and > 6 versus 0.6, respectively). In UISO BCA-1, docetaxel at the HNTD of 15 mg/kg per injection (IV on days 13, 16 and 19 after tumor implantation) did not delay tumor growth (log cell kill 0.6, p > 0.5), whereas cabazitaxel was highly active, both at the HNTD of 15 mg/kg per injection (log cell kill > 6, p = 0.0016) and also at the dose level below the HNTD (log cell kill 4.4 at 9.3 mg/kg per injection, p = 0.0016) (Fig. 2b).
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Across different models, the HNTD of cabazitaxel ranged from 22.2–73.2 mg/kg, and was influenced by the mouse strain in which the tumor was grafted and by tumor aggressiveness.
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Discussion

During the clinical development of docetaxel, we initiated a program with the objective of selecting a taxane derivative as potent as docetaxel that had activity in tumors unresponsive to docetaxel therapy. Cabazitaxel was selected for further development based on positive results in a number of preclinical studies in which cabazitaxel showed excellent antitumor activity in both docetaxel-sensitive and chemotherapy-resistant tumors \textit{in vitro} and \textit{in vivo}.

The majority of patients receiving docetaxel therapy for advanced prostate cancer eventually experience disease progression due to innate or acquired drug resistance (36). Despite substantial efforts over many years, mechanisms of resistance to taxanes in patients have not been fully elucidated, and resistance appears not to be mediated by a single mechanism. In tumor cell lines in which taxane resistance is induced \textit{in vitro}, the two mechanisms most commonly associated with resistance are overexpression of members of the ATP-binding cassette family of transporters, of which P-glycoprotein is the best known, and mutations in tubulin, the cellular target of taxanes (9, 37, 38).

Clinical data suggest that additional mechanisms may operate in patient tumors, such as altered expression of tubulin isotypes and expression or binding of microtubule-regulatory proteins (39). Regulators of mitotic checkpoints, including Aurora A, BUBR1, MAD2 and synuclein-\(\gamma\), and specific checkpoint proteins, such as BRCA1, have all emerged as potential predictive markers of taxane resistance (40). However, conflicting results with these markers have been reported in patient samples. Loss of functional p53 may facilitate the development of resistance, potentially by providing a clonal advantage (41). Dysfunctional regulation of apoptotic and intracellular signaling (such as by HER2 overexpression) may also contribute to taxane resistance (42). Finally, resistance may be caused by decreased tumor cell permeability, limiting the passive influx of taxanes.
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Based on the multiplicity of factors potentially involved in taxane resistance, it was important when screening taxane derivatives to use a clinically relevant tumor model rather than a cell line in which resistance was induced by continuous exposure to taxanes. For this purpose, we developed a docetaxel-resistant tumor model with behavior similar to that observed in the clinic, in which tumors initially respond to docetaxel before developing resistance over time. The acquisition of full resistance to docetaxel was slow (17 months of repeated docetaxel exposure) when compared with the development of resistance to other chemotherapeutic agents, such as anthracycline, against which resistance took 6 months to emerge under similar in vivo conditions. In addition, the resistance factor for docetaxel was less than 4, which is potentially more clinically relevant. Cross-resistance was mainly observed with tubulin-binding agents.

The B16/TXT tumor did not overexpress P-glycoprotein but changes in β-tubulin isotypes suggested that the resistance may be due to changes in microtubule dynamics. Approximately 450 taxane derivatives were screened in the in vivo B16/TXT model, with cabazitaxel emerging as a promising candidate based on its similar activity against fully docetaxel-resistant B16/TXT and taxane-sensitive B16 tumors (log cell kill 1.3 versus 2.1, respectively). In a preliminary target assay, cabazitaxel demonstrated equivalent potency to docetaxel for stabilizing microtubules, suggesting a cytotoxic mechanism of action similar to that of docetaxel. In addition, antiproliferative IC50 values in vitro were similar for cabazitaxel and docetaxel in a range of chemotherapy-sensitive tumor cell lines. One of the most striking observations was the improved antiproliferative activity of cabazitaxel versus docetaxel in P-glycoprotein-expressing docetaxel-resistant cell lines (18, 43). Resistance ratios were lower for cabazitaxel than for docetaxel in cell lines with acquired P-glycoprotein-mediated resistance to doxorubicin, vincristine, vinblastine and paclitaxel. Furthermore, in murine and human cell lines with resistance mechanisms.
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other than P-glycoprotein overexpression (as shown by similar cytotoxicity of doxorubicin
1 µM in both parental and resistant cell lines), there was no cross-resistance to
cabazitaxel (Supplementary Table S5).

These in vitro data were supported by subsequent studies in in vivo tumors. In
mice bearing very advanced-stage MA16/C tumors, drug concentrations were higher
than the cellular antiproliferative IC₅₀ for up to 24 hours in plasma and up to 96 hours in
the tumor. This rapid and sustained drug uptake into the tumor was consistent with the
high antitumor activity of cabazitaxel in this model, which included induction of CRs. A
trend toward schedule dependency was observed: maximum tolerated doses were
4.8-fold higher with an intermittent schedule than with a split-dose schedule (2 versus
15 administrations over 5 days). Optimum antitumor activity and therapeutic index were
obtained with schedules allowing administration of the highest doses of cabazitaxel.
Using the intermittent IV schedule, cabazitaxel exhibited a broad spectrum of antitumor
activity in murine tumors, including activity against advanced-stage disease. Cabazitaxel
also showed a high level of antitumor activity in human tumor models, including not only
prostate but other tumor types, such as colon, lung, pancreatic, gastric, head and neck,
and renal tumors. Most importantly, cabazitaxel was also found to be active in vivo in
tumor models poorly or not sensitive to docetaxel, not only in a model with acquired
resistance to docetaxel (B16/TXT), but also in models innately resistant to docetaxel:
two aggressive murine tumors (Lewis lung and pancreas P02); and three human tumors
(colon HCT-8, gastric GXF-209 and mammary UISO BCA-1). Although UISO BCA-1 was
obtained from a patient never treated with a taxane, the HNTD of docetaxel was inactive
in this model, whereas cabazitaxel was highly active. UISO BCA-1 does not express P-
glycoprotein but does express HER2 (32), which might explain its docetaxel-refractory
properties (42). Overall, these preclinical data show that cabazitaxel has the potential to
Preclinical antitumor activity of cabazitaxel

1 be active not only in patients with acquired resistance to taxanes but also in patients
2 innately resistant (refractory) to taxanes.
3
4 Clinical proof of concept was first achieved for cabazitaxel in a Phase II study that
5 showed responses to cabazitaxel in patients with metastatic breast cancer resistant to
6 previous docetaxel therapy (44, 45). As suggested by the \textit{in vivo} preclinical studies, the
7 responders included both patients with tumors refractory to taxane therapy and
8 patients with relapse following taxane therapy. This was further validated in the pivotal
9 Phase III study in metastatic hormone-refractory prostate cancer (mHRPC) previously
10 treated with docetaxel, in which cabazitaxel in combination with prednisone/prednisolone
11 provided a statistically significant overall survival benefit compared with mitoxantrone
12 plus prednisone/prednisolone (46), leading to regulatory approval in various countries
13 and regions worldwide in this indication.
14
15 Overall, the data presented here provide a comprehensive overview of preclinical
16 studies with the new taxane cabazitaxel, supporting its current use in patients with
17 mHRPC experiencing disease progression following docetaxel therapy. These results
18 also offer an insight into a successful development process for a new anticancer agent,
19 highlighting the importance of conducting relevant, rationally designed studies to
20 accelerate progress in drug discovery.
Preclinical antitumor activity of cabazitaxel

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Preclinical antitumor activity of cabazitaxel

References


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Table 1. Dose–response antitumor activity of cabazitaxel and docetaxel in mice bearing murine tumors. Murine tumors were grafted in the syngenic strain of mice of origin of the tumor for MA17/A (C3H/HeN) and C51 (BABL/C) and in B6D2F1 mice for the C57BL/6 syngenic tumors (B16, B16/TXT, C38, P03, P02, 3LL).

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Dose, mg/kg per injection (schedule days)</th>
<th>Cabazitaxel</th>
<th></th>
<th>Docetaxel</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total HNTD, mg/kg</td>
<td>T-C, days</td>
<td>Log cell kill&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CR</td>
</tr>
<tr>
<td>B16 melanoma (T&lt;sub&gt;d&lt;/sub&gt;: 1.2 days)</td>
<td>32.2, 20, 12.4, 7.7 (3, 5, 7)</td>
<td>60</td>
<td>8.5</td>
<td>2.1</td>
<td>NA</td>
</tr>
<tr>
<td>B16/TXT melanoma (T&lt;sub&gt;d&lt;/sub&gt;: 1.3 days)</td>
<td>32.2, 20, 12.4, 7.7 (3, 5, 7)</td>
<td>60</td>
<td>5.5</td>
<td>1.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Colon C38 (T&lt;sub&gt;d&lt;/sub&gt;: 2.8 days)</td>
<td>32.2, 20, 12.4, 7.7 (14&lt;sup&gt;a&lt;/sup&gt;, 17, 20)</td>
<td>60</td>
<td>–</td>
<td>–</td>
<td>5/5</td>
</tr>
<tr>
<td>Colon C51 (T&lt;sub&gt;d&lt;/sub&gt;: 3 days)</td>
<td>24.2, 15, 9.3, 5.8 (4, 6, 8)</td>
<td>45</td>
<td>25.8</td>
<td>2.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Pancreas P03 (T&lt;sub&gt;d&lt;/sub&gt;: 3.5 days)</td>
<td>32.2, 20, 12.4, 7.7 (17&lt;sup&gt;a&lt;/sup&gt;, 19, 21)</td>
<td>60</td>
<td>–</td>
<td>–</td>
<td>5/5</td>
</tr>
<tr>
<td>Pancreas P02 (T&lt;sub&gt;d&lt;/sub&gt;: 2.5 days)</td>
<td>32.2, 20, 12.4, 7.7 (3, 5, 7)</td>
<td>60</td>
<td>6.6</td>
<td>0.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Mammary MA16/C (T&lt;sub&gt;d&lt;/sub&gt;: 1.1 days)</td>
<td>64.5, 40, 24.8, 15.4 (8&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>40</td>
<td>13.4</td>
<td>3.7</td>
<td>4/5</td>
</tr>
<tr>
<td>Mammary MA17/A (T&lt;sub&gt;d&lt;/sub&gt;: 1.2 days)</td>
<td>19.4, 12, 7.4, 4.6 (3, 5, 7)</td>
<td>36</td>
<td>15.7</td>
<td>3.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Lung 3LL (T&lt;sub&gt;d&lt;/sub&gt;: 1.2 days)</td>
<td>31.5, 19.5, 12.1, 7.5 (3, 5, 7)</td>
<td>58.5</td>
<td>4.6</td>
<td>1.2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<sup>a</sup> Median tumor burden at start of therapy: 290 mm<sup>3</sup>, 310 mm<sup>3</sup> and 400 mm<sup>3</sup> for C38, P03 and MA16/C studies, respectively.

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

HNTD = highest non-toxic dose; T-C = tumor growth delay; CR = complete regression; TFS = long-term tumor-free survival; N/A = not available as treatment performed on early-stage disease; ND = not determined in the same study.
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Table 2. Effects of taxoids on the assembly–disassembly process of pure tubulin. Tubulin was used at a concentration of 6 µM for polymerization (at 37°C) and 9 µM 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 dIC₅₀ and LT₅₀ are given.

<table>
<thead>
<tr>
<th>Drug concentration, µM</th>
<th>Rate of cold-induced microtubule disassembly, ΔOD/min</th>
<th>Ratio</th>
<th>Lag time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cabazitaxel</td>
<td>Docetaxel</td>
<td>Cabazitaxel</td>
</tr>
<tr>
<td>Control</td>
<td>7.42 x 10⁻² (n=5)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>5.36 x 10⁻² (n=4)</td>
<td>6 x 10⁻³ (n=3)</td>
<td>0.89</td>
</tr>
<tr>
<td>0.25</td>
<td>2.82 x 10⁻² (n=5)</td>
<td>3.05 x 10⁻² (n=5)</td>
<td>0.92</td>
</tr>
<tr>
<td>0.5</td>
<td>1.8 x 10⁻² (n=6)</td>
<td>1.95 x 10⁻² (n=5)</td>
<td>0.92</td>
</tr>
<tr>
<td>1</td>
<td>1.2 x 10⁻² (n=5)</td>
<td>1.25 x 10⁻² (n=4)</td>
<td>0.96</td>
</tr>
<tr>
<td>2.5</td>
<td>0.61 x 10⁻² (n=4)</td>
<td>0.5 x 10⁻² (n=6)</td>
<td>1.22</td>
</tr>
<tr>
<td>5</td>
<td>0.56 x 10⁻² (n=3)</td>
<td>0.38 x 10⁻² (n=3)</td>
<td>1.47</td>
</tr>
</tbody>
</table>

dIC₅₀: concentration of drug that inhibits the cold-induced disassembly by 50%. LT₅₀: concentration of drug that reduces the lag time by 50%.
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Table 3. In vitro antiproliferative effects of cabazitaxel and docetaxel against sensitive and P-glycoprotein-expressing resistant cell lines. Cells were incubated for 96 h at 37°C in liquid medium with drugs at different concentrations. Viability was assessed by neutral red, with the mean of at least three results obtained.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean IC$_{50}$, µM, ± SD</th>
<th>Resistance factor$^a$</th>
<th>ABCB1 mRNA level$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Docetaxel</td>
<td>Cabazitaxel</td>
<td>Docetaxel Cabazitaxel</td>
</tr>
<tr>
<td>P388 murine leukemia</td>
<td>0.079 ± 0.004</td>
<td>0.041 ± 0.017</td>
<td>–</td>
</tr>
<tr>
<td>P388/DOX</td>
<td>4.01 ± 0.28</td>
<td>0.414 ± 0.036</td>
<td>51</td>
</tr>
<tr>
<td>P388 murine leukemia</td>
<td>0.039 ± 0.012</td>
<td>0.013 ± 0.005</td>
<td>–</td>
</tr>
<tr>
<td>P388/TXT</td>
<td>0.188 ± 0.022</td>
<td>0.024 ± 0.015</td>
<td>5</td>
</tr>
<tr>
<td>P388 murine leukemia</td>
<td>0.039 ± 0.012</td>
<td>0.013 ± 0.005</td>
<td>–</td>
</tr>
<tr>
<td>P388/VCR</td>
<td>0.227 ± 0.038</td>
<td>0.024 ± 0.003</td>
<td>6</td>
</tr>
<tr>
<td>HL60 human leukemia</td>
<td>0.031 ± 0.004</td>
<td>0.022 ± 0.010</td>
<td>–</td>
</tr>
<tr>
<td>HL60/TAX</td>
<td>0.25 ± 0.11</td>
<td>0.060 ± 0.029</td>
<td>8</td>
</tr>
<tr>
<td>Calc18 human breast</td>
<td>0.008 ± 0.002</td>
<td>0.004 ± 0.002</td>
<td>–</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calc18/TXT</td>
<td>0.17 ± 0.04</td>
<td>0.016 ± 0.004</td>
<td>21</td>
</tr>
<tr>
<td>KB human epidermoid</td>
<td>0.042 ± 0.0212</td>
<td>0.035 ± 0.026</td>
<td>–</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KB V1</td>
<td>2.48 ± 0.12</td>
<td>0.27 ± 0.013</td>
<td>59</td>
</tr>
</tbody>
</table>

$^a$ 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.

IC$_{50}$ = concentration required to reduce cell survival by 50%; ABCB1 = ATP-binding cassette, sub-family B, member 1; P388/DOX = P388 murine leukemia resistant to doxorubicin; P388/TXT = P388 murine leukemia resistant to docetaxel; P388/VCR = P388 murine leukemia resistant to vincristine; HL60/TAX = HL60 human leukemia resistant to paclitaxel; Calc18/TXT = Calc18 human breast adenocarcinoma resistant to docetaxel; KB V1 = KB human epidermoid carcinoma resistant to vinblastine.
Table 4. Dose–response antitumor activity of cabazitaxel and docetaxel in mice bearing human tumors.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Dose per injection, mg/kg (Schedule in days)</th>
<th>Median tumor burden at start of therapy, mm³</th>
<th>Total HNTD, mg/kg</th>
<th>T-C, days</th>
<th>Log cell killa</th>
<th>CR</th>
<th>TFS</th>
<th>Total HNTD, mg/kg</th>
<th>T-C, days</th>
<th>Log cell killa</th>
<th>CR</th>
<th>TFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate Du145 (Td: 4.5 days)</td>
<td>19.4, 12, 7.4, 4.6 (24, 30, 36, 42)</td>
<td>210</td>
<td>48.0</td>
<td>–</td>
<td>–</td>
<td>6/6</td>
<td>5/6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Colon HCT 116 (Td: 4.3 days)</td>
<td>19.4, 12, 7.5, 4.6 (16, 19, 22)</td>
<td>220</td>
<td>36.0</td>
<td>48.2</td>
<td>3.4</td>
<td>7/7</td>
<td>2/7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Colon HT-29 (Td: 4 days)</td>
<td>19.4, 12, 7.5, 4.6 (8, 12, 16)</td>
<td>140</td>
<td>22.2</td>
<td>27.2</td>
<td>2.0</td>
<td>6/6</td>
<td>0/6</td>
<td>96.6b</td>
<td>45.8</td>
<td>3.4</td>
<td>6/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Colon HCT-8 (Td: 3.5 days)</td>
<td>22.4, 14, 8.6, 5.4 (13, 17)</td>
<td>250</td>
<td>28.0</td>
<td>21.5</td>
<td>1.9</td>
<td>0/5</td>
<td>0/5</td>
<td>50b</td>
<td>8.8</td>
<td>0.8</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Pancreas MIA PaCa-2 (Td: 3 days)</td>
<td>19.4, 12, 7.5, 4.6 (15, 19, 23, 27c)</td>
<td>310</td>
<td>48.0</td>
<td>–</td>
<td>–</td>
<td>6/6</td>
<td>6/6</td>
<td>75b</td>
<td>–</td>
<td>–</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Breast Calc18 (Td: 4.5 days)</td>
<td>33, 20.5, 12.7, 7.9 (5, 7, 9)</td>
<td>N/A</td>
<td>61.5</td>
<td>50.2</td>
<td>3.4</td>
<td>N/A</td>
<td>5/8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Breast Calc18/TXT (Td: 4 days)</td>
<td>33, 20.5, 12.7, 7.9 (5, 7, 9)</td>
<td>N/A</td>
<td>38.1</td>
<td>7.3</td>
<td>0.5</td>
<td>N/A</td>
<td>0/8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Breast UISO BCA-1 (Td: 2.1 days)</td>
<td>24.2, 15, 9.3, 5.8 (13, 16, 19)</td>
<td>70</td>
<td>45.0</td>
<td>75.0</td>
<td>&gt; 6</td>
<td>N/A</td>
<td>0/5</td>
<td>45</td>
<td>4.1</td>
<td>0.6</td>
<td>N/A</td>
<td>0/5</td>
</tr>
<tr>
<td>Lung NCI-H460 (Td: 2 days)</td>
<td>19.4, 12, 7.4, 4.6 (10, 13)</td>
<td>130</td>
<td>24.0</td>
<td>17.8</td>
<td>2.7</td>
<td>2/6</td>
<td>0/6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lung A549 (Td: 6.4 days)</td>
<td>19.4, 12, 7.4, 4.6 (21, 27, 33)</td>
<td>130</td>
<td>36.0</td>
<td>46.0</td>
<td>2.2</td>
<td>2/6</td>
<td>0/6</td>
<td>96.6b</td>
<td>40</td>
<td>1.9</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Gastric N87 (Td: 4.5 days)</td>
<td>39.4, 24.4, 15, 9.3 (27, 31, 35)</td>
<td>140</td>
<td>73.2</td>
<td>100.9</td>
<td>&gt; 6</td>
<td>N/A</td>
<td>1/8</td>
<td>73.2</td>
<td>66.8</td>
<td>4.5</td>
<td>N/A</td>
<td>1/8</td>
</tr>
<tr>
<td>Gastric GFX-209 (Td: 4 days)</td>
<td>32.3, 20, 12.4, 7.7 (14, 17, 20)</td>
<td>130</td>
<td>37.2</td>
<td>18.0</td>
<td>1.4</td>
<td>0/8</td>
<td>0/8</td>
<td>23.1</td>
<td>6.8</td>
<td>0.5</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Head and neck SR475 (Td: 4.9 days)</td>
<td>22.5, 14, 8.6, 5.4 (16, 20, 24)</td>
<td>250</td>
<td>42.0</td>
<td>–</td>
<td>–</td>
<td>6/6</td>
<td>6/6</td>
<td>45b</td>
<td>40.5</td>
<td>2.5</td>
<td>1/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Kidney Caki-1 (Td: 4.6 days)</td>
<td>31.2, 19.4, 12, 7.5 (10, 14)</td>
<td>140</td>
<td>24.0</td>
<td>25.8</td>
<td>1.7</td>
<td>5/6</td>
<td>0/6</td>
<td>64.4b</td>
<td>21.3</td>
<td>1.4</td>
<td>2/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

a Definition of antitumor activity: log cell kill total < 0.7 = inactive; > 2.8 = highly active.
b The dose–response pattern for docetaxel was different from that of cabazitaxel in the following studies: HT-29, A549 and Caki-1 studies: 51.9, 32.2, 20 and 12.4 mg/kg per injection; HCT-8, MIA PaCa-2 and SR475 studies: 41.7, 25 and 15 mg/kg per injection.
c Docetaxel groups were not treated on day 27.
HNTD = highest non-toxic dose; T-C = tumor growth delay; CR = complete regression; TFS = long-term tumor-free survival; N/A = not available as treatment performed on early-stage disease; ND = not determined in the same study.
Preclinical antitumor activity of cabazitaxel

Figure Legends

**Fig. 1.** Pharmacokinetics of cabazitaxel in plasma and tumor tissue in mice.

Cabazitaxel concentrations in plasma and tumor tissue were measured after a single intravenous dose of cabazitaxel at its highest non-toxic dose of 40 mg/kg in C3H/HeN female mice bearing advanced-stage (≥ 400 mm³) mammary adenocarcinoma MA16/C. Mean concentration ± SD from three animals was determined at each sampling time.

**Fig. 2.** Head-to-head comparison of the antitumor activity of cabazitaxel and docetaxel in human tumor xenografts.

Antitumor activity was measured in human tumors xenografted subcutaneously into female nude mice and reported as median tumor volumes ± interquartile ranges. (a) Human gastric N87. Both drugs were administered intravenously at 5.8 (asterisk), 9.3 (triangle), 15.0 (circle) and 24.4 mg/kg/inj (highest non-toxic dose [HNTD]; no symbol) on Days 25, 31 and 35 to mice bearing 140 mm³ tumors implanted SC monolaterally at start of therapy on Day 25. Interquartile ranges for medians and numbers of animals per group are provided in Supplementary Table S6. (b) Human breast UIISO BCA-1. Drugs were administered intravenously on Days 13, 16 and 19 to mice bearing palpable tumors implanted SC bilaterally at start of therapy on Day 13. Doses were 5.8 (triangle), 9.3 (circle) and 15.0 mg/kg/inj (HNTD; no symbol) for cabazitaxel and 15.0 mg/kg/inj (HNTD; cross) for docetaxel. Interquartile ranges for medians and numbers of animals per group are provided in Supplementary Table S7.

IV, intravenously; HNTD, highest non-toxic dose.
Figure 1

Cabazitaxel concentration (ng/mL or ng/g) vs. Time (h)

- Plasma
- Tumor
Figure 2a

Tumor volume (mm$^3$) vs. Days post implantation for various treatments:
- Docetaxel (24.4 mg/kg/inj) (HNTD)
- Docetaxel (15.0 mg/kg/inj)
- Docetaxel (9.3 mg/kg/inj)
- Docetaxel (5.8 mg/kg/inj)
- Cabazitaxel (24.4 mg/kg/inj) (HNTD)
- Cabazitaxel (15.0 mg/kg/inj)
- Cabazitaxel (9.3 mg/kg/inj)
- Cabazitaxel (5.8 mg/kg/inj)
- Control

IV treatment
Figure 2b

Tumor volume (mm$^3$) vs. Days post implantation

- Cabazitaxel (15.0 mg/kg/inj) (HNTD)
- Cabazitaxel (9.3 mg/kg/inj)
- Cabazitaxel (5.8 mg/kg/inj)
- Docetaxel (15.0 mg/kg/inj) (HNTD)
- Control

IV treatments
Preclinical Antitumor Activity of Cabazitaxel, a Semi-Synthetic Taxane Active in Taxane-Resistant Tumors

Patricia Vrignaud, Dorothee Sémiond, Pascale Lejeune, et al.

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