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

Patricia Vrignaud, Dorothée Sémiond, Pascale Lejeune, Hervé Bouchard, Loreley Calvet, Cécile Combeau, Jean-François Rou, 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 (IC50 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.

**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-deacetylbaccatin 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
Glutathione (GSH) conjugate by cytosols was measured (CDNB) as the substrate (14). Formation of the CDNB previously described using 1-chloro-2,4-dinitrobenzene (CDNB) by 50% (dIC50) were determined.

**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% (LT50) and inhibiting cold-induced disassembly by 50% (dLT50) 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 IC50 of resistant cells by the mean IC50 in sensitive/parental cells, using data from at least 3 independent experiments. Relative expression of ABCC1 mRNA was determined by Northern blotting using a human ABCC1 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 mm³. Tumor volumes were estimated from 2-dimensional measurements using the formula: tumor volume (mm³) = [length (mm) × width² (mm²)]/2. The day of death was recorded, and thoracic and abdominal cavities were examined macroscopically to assess probable cause of death.

Mice. C57BL/6, B6D2F1 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 docetaxel. Human tumor cell lines were obtained from American Type Culture Collection and included prostate

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 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 treatment. 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 mm³). Tumor doubling time (Td) in days was estimated from log linear tumor growth during the exponential phase (range, 100–1,000 mm³). Log cell kill was calculated using the formula (T–C)/(3.32 × 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 mm³). 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 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 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

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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 (LT50 = 0–0.1 µmol/L for both) and the rate of cold-induced microtubule depolymerization (dIC50 = 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 IC50 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 (IC50 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.
Preclinical Antitumor Activity of Cabazitaxel

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

<table>
<thead>
<tr>
<th>Drug concentration, μmol/L</th>
<th>Rate of cold-induced microtubule disassembly, ΔOD/min</th>
<th>Lag time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cabazitaxel</td>
<td>Docetaxel</td>
</tr>
<tr>
<td>Control</td>
<td>7.42 x 10^-2 (n = 5)</td>
<td>6 x 10^-2 (n = 3)</td>
</tr>
<tr>
<td>0.1</td>
<td>5.36 x 10^-2 (n = 4)</td>
<td>6 x 10^-2 (n = 3)</td>
</tr>
<tr>
<td>0.5</td>
<td>2.82 x 10^-2 (n = 6)</td>
<td>3.05 x 10^-2 (n = 5)</td>
</tr>
<tr>
<td>1</td>
<td>1.8 x 10^-2 (n = 6)</td>
<td>1.95 x 10^-2 (n = 5)</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>
</tr>
<tr>
<td>5</td>
<td>0.56 x 10^-2 (n = 3)</td>
<td>0.38 x 10^-2 (n = 3)</td>
</tr>
</tbody>
</table>

**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 dIC50 and LT50 are given.

Cabazitaxel concentrations were maintained above the range of cellular antiproliferative IC50 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 schedules in nontumor-bearing B6D2F1 female mice (Supplementary Table S3). Three schedules of intravenous cabazitaxel were administered: intermittent [days 1 and 5 (A1)], daily [days 1–5 (A2)] and split-dose [days 1–5, 3 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

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

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean IC50, μmol/L ± SD</th>
<th>Resistance factor*</th>
<th>ABCB1 mRNA levelb</th>
</tr>
</thead>
<tbody>
<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 adenocarcinoma</td>
<td>0.008 ± 0.002</td>
<td>0.004 ± 0.002</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 carcinoma</td>
<td>0.042 ± 0.0212</td>
<td>0.053 ± 0.026</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>

**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 vincristine; P388/DOX, P388 murine leukemia resistant to doxorubicin; P388/TXT, P388 murine leukemia resistant to docetaxel; P388/VCR, P388 murine leukemia resistant to vincristine.

*Resistance factor = IC50 (resistant)/IC50 (parental) from the same experiment.

*bRelative expression obtained from Northern blot experiments using the human ABCB1 gene as probe.
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 conducted 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, 3 times daily; HNTD 42 mg/kg) compared with an intermittent schedule (days 3 and 7; HNTD 9.3 mg/kg; Supplementary Table S4). Caba-
zitaxel antitumor activity against MA17/A tumors was also lower with the split-dose schedule compared with the inter-
mittent schedule (log cell kill 1.3 vs. 4.6, respectively). These differences between split-dose and intermittent schedules were confirmed in a second tumor model, murine mam-
mary adenocarcinoma MA16/C, which showed a 4.2-fold reduction in HNTD (11 vs. 46 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 inter-
mittent schedule (log cell kill 1.3 vs. 4.6, respectively). These differences between split-dose and intermittent schedules were confirmed in a second tumor model, murine mam-
mary adenocarcinoma MA16/C, which showed a 4.2-fold reduction in HNTD (11 vs. 46 mg/kg, respectively) and decreased antitumor activity (log cell kill 1.6 vs. 5.4, respec-
tively). 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, inter-
mittent 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 per injection on days 3, 5, and 7), murine colon adenocarcinoma C51 (log cell kill 2.6 at HNTD of 9.3 mg/kg per injection on days 4, 6, and 8), and mammary adenocarcinomas MA16/C and MA17/A (reported earlier). Cabazitaxel was highly active, with CRs observed against the advanced-stage murine tumors colon C38 (5 of 5 CR; 5 of 5 TFS) and pancreas P03 (5 of 5 CR; 4 of 5 TFS) as well as human tumor xenografts including: prostate DU145 (6 of 6 CR; 5 of 6 TFS); colon HCT 116 (7 of 7 CR; 2 of 7 TFS) and HT-29 (6 of 6 CR); pancreas MIA PaCa2 (6 of 6 CR; 6 of 6 TFS); breast Cal18 (5 of 8 TFS); lung NCI-H460 (2 of 6 CR) and A549 (2 of 6 CR); head and neck SR475 (6 of 6 CR; 6 of 6 TFS); and kidney Caki-1 (5 of 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 (ref. 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 of 8 TFS; P < 0.0001). The 2 dose levels below the HNTD (15 and 9.3 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 (T1/C0 = 67 days; log cell kill 4.5; 1 of 8 TFS; P < 0.0001), and the activity was observed only at one dose below the HNTD. Thus, cabazitaxel had a greater therapeutic index (3 active dose levels) than doc-
etaxel (2 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 vs. 1.3 in B16/TXT), but no antitumor activity was obtained against the P-glycoprotein–overexpressing tumor Cal18/TXT in which docetaxel resistance was induced in vitro (log cell kill 3.4 in Cal18 vs. 0.5 in Cal18/TXT). Cabazitaxel was also found to be active against 2 aggressive
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, log cell killᵃ</th>
<th>CR</th>
<th>TFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate DU 145 (T₉: 4.5 d)</td>
<td>19.4, 12, 7.4, 4.6 (24, 30, 36, 42)</td>
<td>210</td>
<td>48.0</td>
<td>—</td>
<td>6/6</td>
<td>5/6</td>
</tr>
<tr>
<td>Colon HCT 116 (T₉: 4.3 d)</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>
</tr>
<tr>
<td>Colon HT-29 (T₉: 4 d)</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>
</tr>
<tr>
<td>Colon HCT-8 (T₉: 3.5 d)</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>
</tr>
<tr>
<td>Pancreas MIA PaCa-2 (T₉: 3 d)</td>
<td>19.4, 12, 7.5, 4.6 (15, 19, 23, 27)</td>
<td>310</td>
<td>48.0</td>
<td>—</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Breast Calc18 (T₉: 4.5 d)</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>
</tr>
<tr>
<td>Breast Calc18/TXT (T₉: 4 d)</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>
</tr>
<tr>
<td>Breast UISO BCA-1 (T₉: 2.1 d)</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>
</tr>
<tr>
<td>Lung NCI-H460 (T₉: 2 d)</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>
</tr>
<tr>
<td>Lung A549 (T₉: 6.4 d)</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>
</tr>
<tr>
<td>Gastric N87 (T₉: 4.5 d)</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>
</tr>
<tr>
<td>Gastric GXF-209 (T₉: 4 d)</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>
</tr>
<tr>
<td>Head and neck SR475 (T₉: 4.9 d)</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>
</tr>
<tr>
<td>Kidney Caki-1 (T₉: 4.6 d)</td>
<td>31.2, 19.4, 12.7, 7.5 (10, 14)</td>
<td>140</td>
<td>24.0</td>
<td>25.8</td>
<td>1.7</td>
<td>5/6</td>
</tr>
</tbody>
</table>

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

ᵃDefinition of antitumor activity: log cell kill total < 0.7 = inactive; >2.8 = highly active.

ᵇThe dose–response pattern for docetaxel was different from that of cabazitaxel in the following studies: HT-29, A549 and Caki-1 studies: 51.3, 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.

cDocetaxel groups were not treated on day 27.
murine tumors: Lewis lung carcinoma (which has innate resistance to vincristine and 5-fluorouracil, and modest sensitivity to docetaxel; ref. 35), and pancreatic adenocarcinoma P02 (which has innate resistance to a broad spectrum of chemotherapeutic agents; ref. 22; 1.2 and 0.8 log cell kill, respectively). In addition, cabazitaxel was active against 3 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 vs. docetaxel of 1.9 vs. 0.8, 1.4 vs. 0.5, and >6 vs. 0.6, respectively). In UISO BCA-1, docetaxel at the HNTD of 15 mg/kg per injection (i.v. 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).

Across different models, the HNTD of cabazitaxel ranged from 22.2 to 73.2 mg/kg, and was influenced by the mouse strain in which the tumor was grafted and by tumor aggressiveness.

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 in vitro and 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 in vitro, the 2 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 isoforms 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

Figure 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 (HNTD; no symbol) on days 25, 31, and 35 to mice bearing 140 mm\(^3\) tumors implanted subcutaneously monolaterally at start of therapy on day 25. Interquartile ranges for medians and numbers of animals per group are provided in Supplementary Table S7.

B, human breast UISO BCA-1. Drugs were administered intravenously on days 13, 16, and 19 to mice bearing palpable tumors implanted subcutaneously bilaterally at start of therapy on day 13. Doses were 5.8 (asterisk), 9.3 (triangle), and 15.0 mg/kg/inj (HNTD; solid circle) for cabazitaxel and 15.0 mg/kg/inj (HNTD; open circle) for docetaxel. Interquartile ranges for medians and numbers of animals per group are provided in Supplementary Table S8.

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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.

On the basis of 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 vs. 2.1, respectively). In a preliminary target assay, cabazitaxel showed 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 chemotherapeutic-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 other than P-glycoprotein overexpression (as shown by similar cytotoxicity of doxorubicin 1 μmol/L 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 CRRs. 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 vs. 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 intravenous 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: 2 aggressive murine tumors (Lewis lung and pancreas P02); and 3 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 be active not only in patients with acquired resistance to taxanes but also in patients innately resistant (refractory) to taxanes.

Clinical proof-of-concept was first achieved for cabazitaxel in a phase II study that showed responses to cabazitaxel in patients with metastatic breast cancer resistant to previous docetaxel therapy (44, 45). As suggested by the in vitro preclinical studies, the responders included both patients with tumors refractory to taxane therapy and patients with relapse following taxane therapy. This was further validated in the pivotal phase III study in metastatic hormone-refractory prostate cancer (mHRPC) previously treated with docetaxel, in which cabazitaxel in combination with prednisone/prednisolone provided a statistically significant overall survival benefit compared with mitoxantrone plus prednisone/prednisolone (46), leading to regulatory approval in various countries and regions worldwide in this indication.

Overall, the data presented here provide a comprehensive overview of preclinical studies with the new taxane cabazitaxel, supporting its current use in patients with mHRPC experiencing disease progression following docetaxel therapy. These results also offer an insight into a successful development process for a new anticancer agent, highlighting the importance of conducting relevant, rationally designed studies to accelerate progress in drug discovery.

Disclosure of Potential Conflicts of Interest
The authors disclosed no potential conflicts of interest.

Authors’ Contributions
Conception and design: P. Vrignaud, P. Lejeune, H. Bouchard, A. Commençon, M.-C. Bissery
Development of methodology: P. Vrignaud, C. Combeau, J.-F. Riolu, M.-C. Bissery
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P. Vrignaud, P. Lejeune, C. Combeau, J.-F. Riolu, M.-C. Bissery

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Vrignaud, D. Sémiond, P. Lejeune, J.-F. Riou, M.-C. Bissey

Writing, review, and/or revision of the manuscript: P. Vrignaud, D. Sémiond, P. Lejeune, H. Bouchard, I. Calvet, A. Commerçon, M.-C. Bissey

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Vrignaud, M.-C. Bissey

Study supervision: P. Vrignaud, D. Sémiond, P. Lejeune, I. Calvet, F. Lavelle, M.-C. Bissey

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References


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


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