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## Review paper

# Docetaxel (Taxotere®): a review of preclinical and clinical experience. Part I: preclinical experience

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**Docetaxel is a taxoid which is currently in phase II/III clinical trials in Europe, the US and Japan. It was found to promote tubulin assembly in microtubules and to inhibit their depolymerization. *In vitro*, the docetaxel concentrations required to reduce murine and human cell survival by 50% ranged from 4 to 35 ng/ml and the cytotoxic effects were greater on proliferating than on non-proliferating cells. It was also found to be cytotoxic on fresh human tumor biopsies. *In vivo*, the drug was found to be schedule independent. A total of 13/14 murine transplantable tumors were found very sensitive to i.v. docetaxel and complete regressions of advanced stage tumors were obtained. Activity was also observed in 15/16 human tumor xenografts in nude mice at an advanced stage. In combination studies, synergism was observed *in vivo* with 5-fluorouracil, cyclophosphamide and etoposide. Pharmacokinetic evaluation revealed linear pharmacokinetics in tumor-bearing mice. There was a good tumor retention with a 22 h elimination half-life. Plasma protein binding ranged from 76 to 89%. Preclinical toxicology evaluation of docetaxel included single-dose toxicity in rats, mice and dogs, 5-day toxicity in mice and dogs, intermittent-dose toxicity in rats, dogs and monkeys, genetic and reproductive toxicity, as well as investigation of the irritation and sensitization potential. The principal toxicities were hematopoietic (all species), gastrointestinal (dog, monkey) and neuromotor (mice). Dogs appeared to be the most sensitive species. The clinical entry dose of 5 mg/m$^2$ was based on one-third of the 'toxic dose low' in dogs (15 mg/m$^2$).**

*Keywords:* Docetaxel, Taxotere®, RP 56976, antitumor efficacy, mechanism of action, resistance, biodynamics, toxicology.

## Introduction

In the late 1960s, a crude alcohol extract from the bark of the Pacific yew, *Taxus brevifolia* L., was revealed to be cytotoxic *in vitro* in the National Cancer Institute screening program.[1,2] That extract's principle, paclitaxel (Taxol®), was isolated and characterized in 1971 by Wani *et al.*[1]

Paclitaxel was found to be a mitotic spindle poison[3] that stabilizes microtubules and inhibits their depolymerization to free tubulin.[4,5] Subsequent phase I clinical development suggested paclitaxel's activity against various tumors.[6] However, paclitaxel's development was impeded by the difficulties in obtaining an adequate drug supply.[6] Once these problems were solved, development progressed rapidly and phase II clinical trials indicated activity in ovarian and breast cancers.[6,7]

In 1981, Rhône-Poulenc and the Institut de Chimie des Substances Naturelles (Gif sur Yvette, France) concluded a cooperative research agreement about natural products extracted from yews and thujas. This collaboration led to the discovery of docetaxel (Taxotere®, [(2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13-ester with 5$\beta$, 20-epoxy-1,2$\alpha$,4,7$\beta$,10$\beta$,13$\alpha$-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate; RP 56976; NSC 628503; (Figure 1)] in 1986.[8] It was obtained by semisynthesis from a non-cytotoxic precursor, 10-deacetyl baccatin III, extracted from a renewable resource, the needles of *Taxus baccata* L. This precursor was then condensed by esterification with the side chain prepared by chemical synthesis.[9,10] Compared with paclitaxel, docetaxel presents two chemical modifications: a hydroxy group replaces an acetyl group on the 10-position of the baccatin III and a $OC(CH_3)_3$ moiety replaces a benzamide phenyl group in the 3′ position on the C-13 side chain. It is a stable crystalline white powder, available as the trihydrate form, with a molecular weight of 861.94.

This compound has widespread *in vivo* and *in vitro* antitumor activity in preclinical models and is currently undergoing phase II and III evaluation. This review will focus on the mechanism of action and cellular pharmacology, the antitumor activity,

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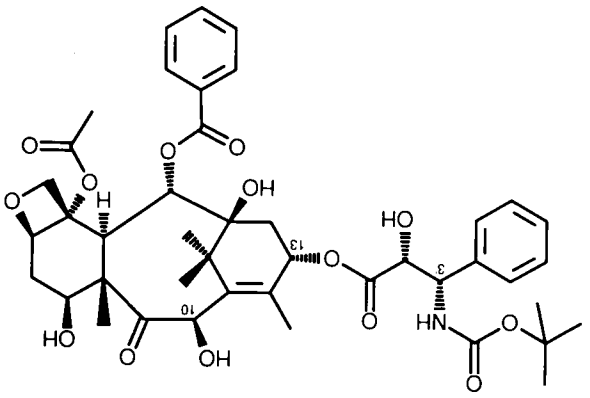

**Figure 1.** Docetaxel, (Taxotere®, RP 56976, NSC 628503); [(2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13-ester with 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate].

the resistance, the drug disposition, and the toxicology of docetaxel. Comparison with paclitaxel will also be discussed where appropriate.

## Preclinical pharmacology

### Mechanism of action and cellular pharmacology

The most studied target of the taxoids is the microtubule. Microtubules are ubiquitous components of eukaryotic cells and have important cellular functions including chromosome movement during mitosis, regulation of cell morphology, hormone secretion, transport of granules, anchorage of receptors in the membrane and cellular motility. The microtubules are assembled from tubulin, a 100 kDa protein which comprises two 50 kDa subunits, α and β. These microtubules are long, hollow cylinders composed of 13 protofilaments aligned longitudinally along the axis of the cylinder and are in a dynamic equilibrium with free tubulin. The shift of the equilibrium toward microtubule assembly or disassembly is controlled by various biochemical entities and proteins including microtubule associated proteins (MAPs), and can be modified by different reagents and drugs.[11]

*Effects on microtubules/tubulin system.* Using purified tubulin from calf and pig, docetaxel has been shown to reduce the lag time for initiation of polymerization and to promote the assembly of tubulin into stable microtubules, i.e. it enhanced both the rate and extent of microtubule assembly. The docetaxel–microtubules formed were found to be stable to depolymerization by the cold. In comparison with paclitaxel, docetaxel has been found slightly more active as a tubulin assembly promoter and as a microtubule stabilizer,[12] and 2-fold more potent as an inhibitor of microtubule depolymerization.[13] This taxoid interaction results in a shift of the tubulin–microtubule equilibrium toward the microtubule. Upon measurement of the critical tubulin concentration (i.e. the minimum concentration of tubulin required for microtubule assembly) in the presence of taxoids, it was found that docetaxel was twice as efficient as paclitaxel in decreasing this critical concentration.[14] Because of this decrease in critical concentration, docetaxel can assemble tubulin under conditions in which polymerization would not normally occur, e.g. the polymerization of tubulin in the absence of guanosine triphosphate[12] or the polymerization of guanosine diphosphate (GDP)–tubulin; similarly, the docetaxel induced assembly of GDP–tubulin required a critical concentration of tubulin 2.1 times lower than that of paclitaxel.[14]

In addition to these quantitative differences between docetaxel and paclitaxel, qualitative differences have been also noted for these two taxoids. First as observed by X-ray scattering and electron microscopy, paclitaxel alters the number of protofilaments per microtubules (i.e. 12 protofilaments) whereas docetaxel does not.[15,16] Docetaxel-bound microtubules have an average of 13.4 protofilaments, similar to control microtubules.[16] Second, at the structural level, it was also found that taxoids have the capacity to induce the formation of abnormal tubulin polymers (sheets or waved microtubules).[17]

Furthermore, differences between docetaxel and paclitaxel efficiencies to disassemble tubulin polymers were more important if the assembly was promoted by the microtubule associated protein Tau than if it was induced by MAP2.[17] This finding suggested that the tubulin polymers generated by paclitaxel differed structurally from those generated by docetaxel.[17]

*Docetaxel interaction site.* It was shown that docetaxel interacted with microtubules but not with dimeric tubulin.[14] Studies were performed to determine if docetaxel and paclitaxel bind to the same site on microtubules. Both taxoids were able to induce the assembly of GDP–tubulin and bind to assembled tubulin with a stoichiometry approaching 1 molecule per αβ tubulin dimer. They both bind to the same site but the affinity of docetaxel is 1.9-fold higher than that of paclitaxel.[14]

This difference may account for the higher efficiency of docetaxel to promote tubulin polymerization, to stabilize microtubules against cold induced disassembly and to decrease the critical concentration of tubulin assembly.

The taxoid site does not overlap those of other molecules such as colchicine, vinblastine and podophyllotoxin.[18,19] Studies are being performed to localize the docetaxel interaction site by photoaffinity.[20,21]

*Cellular effects.* Docetaxel produced changes in cell shape including alteration of the cytoskeleton morphology.[12,22]

It was found that the amount of tubulin measured by flow cytometry was increased in KB 3-1 human epidermoid carcinoma cells treated by both paclitaxel and docetaxel before the accumulation of cells in the $G_2$/M phase. Docetaxel leads to the formation of bundles and asters in KB 3-1 cells and in J82 human bladder carcinoma cells, and accumulates cells in $G_2$/M phase leading to an inability of the cells to divide.[22]

Interestingly, it has been recently demonstrated in HeLa synchronized cells that docetaxel and paclitaxel are active during different phases on the cell cycle: docetaxel seems to be mainly active during S phase, whereas paclitaxel is preferentially active during late $G_2$/M phase.[23]

Finally, docetaxel did not inhibit synthesis of DNA, RNA or protein using P388 leukemia cells.[26]

*Cellular uptake and efflux.* Uptake and efflux studies were performed on P388 leukemia cells *in vitro* with radiolabeled docetaxel and paclitaxel. Uptake experiments revealed that a 3-fold higher intracellular concentration of docetaxel was obtained as compared with paclitaxel, for the same initial extracellular concentration (0.1 μM).[24] Efflux studies revealed that the half-time of efflux of docetaxel from P388 cells was at least three times slower than that of paclitaxel (150 versus 45 min, respectively).

Thus the higher potency of docetaxel observed *in vitro* may be explained by the combination of its higher affinity for microtubules, its higher achievable intracellular concentration and the slower cellular efflux.

## *In vitro* antitumor activity

The antiproliferative and cytotoxic properties of docetaxel have been evaluated in several murine and human cell lines under various experimental conditions (liquid or semi-solid medium; continuous or short term exposure).

*Cytotoxicity in long-term tumor cell cultures.* Docetaxel is a potent inhibitor of cell replication *in vitro* and was found to be cytotoxic against both murine (P388 leukemia and SV1 Ras4 fibrosarcoma) and human tumor cell lines (Calc18 breast adenocarcinoma, KB epidermoid carcinoma, HCT-116 colon adenocarcinoma and T24 bladder carcinoma), the latter being the most drug sensitive (Figure 2).[25,26] $IC_{50}$ values (concentration required to reduce cell survival by 50%) of docetaxel ranged from 4 to 35 ng/ml and the cytotoxic effects were greater on proliferating cells than on quiescent cells.[26] These effects were found to be both time- and concentration-dependent on proliferating cells[26,27] Interestingly, pharmacokinetic-efficacy evaluation in mice bearing colon adenocarcinoma 38 revealed that at optimal dosage, the area under the plasma and tumor concentration versus time curves (AUC) were much higher than the AUC required to kill the most sensitive human cell lines. Indeed if the AUC needed to kill 50% of the cells *in vitro* [AUCs = $IC_{50}$ value × (4 × 24 h exposures)] are calculated, the values obtained (range: 0.4–3.4 μg h/ml) are much lower than the pharmacologically achievable AUCs in mice at non-toxic dosage in plasma (17 μg h/ml) and in tumor (44 μg h/g).[28] Compared with paclitaxel, docetaxel is generally more active *in vitro* (1.3 to 12-fold),[26,27,29,30] a result that could be explained by its higher achievable intracellular concentration, its higher affinity for microtubules which is two times higher than that of paclitaxel and its slower cellular efflux.[12,13,24] Docetaxel was also evaluated in the National Cancer Institute disease oriented primary antitumor screen. Using the COMPARE computer program, it was concluded that the response profile on 50 human tumor cell lines correlated with the data pattern of test agents which act on the tubulin/microtubule system, the closest compound being paclitaxel (NCI, unpublished results).

*Cytoxicity in freshly explanted cells.* The antiproliferative action of docetaxel was also studied and compared with that of paclitaxel in a variety of freshly explanted human tumor cells at clinically relevant concentrations using an *in vitro* soft agar colony formation assay.[31,32] Cytotoxicity of docetaxel was observed against tumor colony-forming units from breast, lung, ovarian, colorectal cancer and melanoma[31,32] at concentrations that are achievable in human plasma (3.7 μg ml, following a dose of 100 mg $m^2$, administered as a 1–2 h infusion).[33] In a direct comparison of 78 spe-

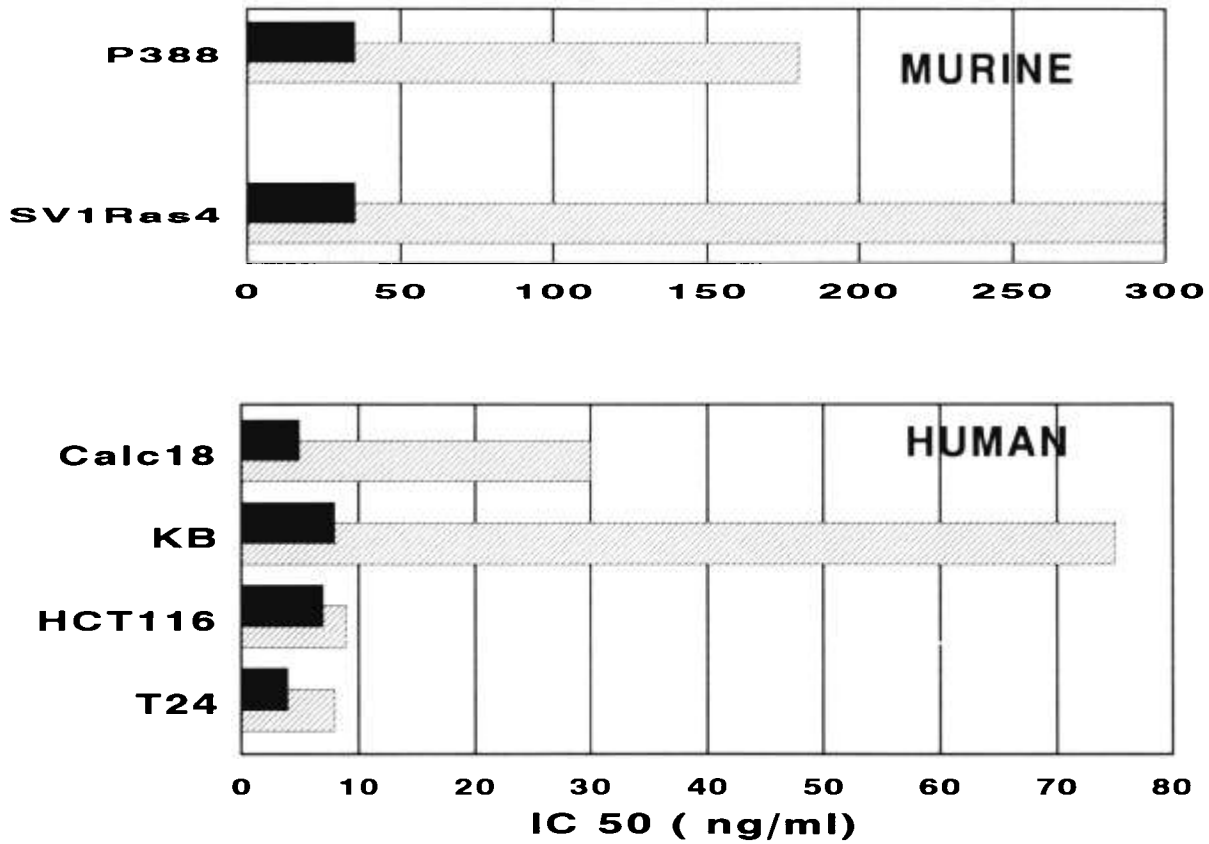


**Figure 2.** Cytotoxicity profiles of docetaxel (filled boxes) and paclitaxel (hatched boxes) in murine and human cell lines. Cells were incubated in liquid medium with different concentrations of drug for 4 days (5 days for Calc18) at 37°C. $IC_{50}$ values were determined from semi-logarithmic plots of the mean values.

cimens, 29 tumor specimens were more sensitive to docetaxel than to paclitaxel, whereas only 13 were more sensitive to paclitaxel. These data indicate that cross-resistance between these two agents was incomplete with freshly implanted human tumors.[31]

### *In vivo* antitumor activity

*In vivo*, docetaxel was evaluated for its antitumor activity against 30 tumor models including a variety of transplantable tumors of mice and human tumor xenografted in nude mice, representing a variety of tissue types and chemosensitivity patterns. The tumors were grafted in distal sites [generally subcutaneously (s.c.)] and several tumors were treated at advanced and metastatic stages. Dose response was evaluated in all trials and evaluation is reported at the maximum tolerated dose.

*Formulation evaluation*. Docetaxel was formulated in a 1:1 ethanol/polysorbate 80 solution and administered after a 1:10 dilution in glucose 5% in water.[34] The only exceptions to this procedure were the evaluation of a formulation with Chremophor® and a formulation in polysorbate 80 alone. In the former, docetaxel was formulated in a 1:1 ethanol/Chremophor® solution and administered after a 1:10 dilution in NaCl 0.9%. In the latter case, docetaxel was formulated in polysorbate 80 and was administered after a 1:20 dilution in glucose 5% in water. Most of the trials were performed using the i.v. route.

All the formulations were evaluated using B16 melanoma *in vivo*, docetaxel being administered i.v. on days 3, 5 and 7. The comparison of the ethanol/polysorbate 80/glucose 5% in water (EPW), with the ethanol/Chremophor®/NaCl 0.9% formulations (ECN), revealed that a dosage of 17.4 mg/kg/injection yielded similar antitumor efficacy, EPW: 2.5 log tumor cell kill, ECN: 2.4 log tumor cell kill, the log cell kill representing the tumor growth delay in days divided by 3.32 × the tumor doubling time in days.

A polysorbate 80 formulation was developed for clinical use and compared with the ethanol containing formulation. The two formulations were also

found equally active with 2.5 log tumor cell kill for the formulation with ethanol and 2.7 log tumor cell kill for the formulation without ethanol at the optimal dosage.

*Efficacy in murine tumor models.* Clearly docetaxel had a good spectrum of efficacy against syngeneic transplantable tumors of mice.[25,34,35] Upon i.v. administration, 13/14 tumor models tested responded to docetaxel (Table 1).[35,36] Nine were found active at the DN-2 level, the level used by the NCI to justify further development.

The s.c. B16 melanoma was found highly sensitive to docetaxel, with a tumor growth inhibition (T/C, with T and C, median tumor weight of the treated and control groups, respectively) of 0% and a 3.0 log tumor cell kill at the maximum tolerated dose. In the same trial, at optimal dosages docetaxel demonstrated clear superiority to paclitaxel, with the former having a 2.7 times greater log cell kill than the latter; paclitaxel producing only a 1.1 log tumor cell kill at the maximum tolerated dose (Figure 3). The maximum tolerated dose of docetaxel was found to be 0.6 times the maximum tolerated dose of paclitaxel. Docetaxel cured s.c. early-stage pancreatic ductal adenocarcinoma 03 (PO3, 6/6 cures) and s.c. colon adenocarcinoma 38 (C38, 7/7 cures). For both tumors, complete regressions of advanced-stage disease were noted in greater than 80% of cases. Docetaxel was active against early and advanced stage s.c. colon adenocarcinoma 51 (C51), with 2.3 and 1.7 log cell kill, respectively. Of particular note was the efficacy of docetaxel against 3/4 mammary tumors evaluated (MA13/C, MA16/C and MA44) with complete regressions of advanced stage tumors in the case of MA16/C and MA13/C.[36] Five other tumors responded to a lesser extent to docetaxel: s.c. Lewis lung (5.5% T/C), s.c. Glasgow osteogenic sarcoma (GOS, 27.2% T/C), pancreatic ductal adenocarcinoma 02 (39% T/C), s.c. colon carcinoma 26 (33% T/C), and i.p. L1210 and P388 leukemias (70 and 54% increase in life span, respectively). Docetaxel was found inactive against M5076 histiocytosarcoma. Evaluation of docetaxel against tumors with acquired resistance revealed that P388/Dox, P388/Vcr,

**Table 1.** *In vivo* antitumor activity of docetaxel against murine tumors

| Tumor | Highest non-toxic i.v. dosage (mg/kg/dose) | Schedule days | Total dose (mg/kg) | T/C[a] (%) | T–C[b] (days) | Total log cell kill[c] | Activity rating[d] |
|---|---|---|---|---|---|---|---|
| Solid tumors s.c. | | | | | | | |
| melanoma B16 early | 24 | 3,5,7,9 | 96 | 0 | 18.8 | 3.8 | ++++ |
| pancreas PO2 | 32.2 | 3,5,7 | 96.6 | 39 | – | – | +/– |
| PO3 early | 20.5 | 3,5,7,9 | 82 | 0 | – | 6/6 cures | ++++ |
| PO3 advanced | 18.0 | 22,24,26,28 | 72 | – | 21.4 | 1.8 | 5/6 CR[e] |
| mammary MA16/C early | 15 | 3,5,7 | 45 | 0 | 13 | 2.4 | +++ |
| MA16/C advanced | 10.8 | 7,9,11 | 32.4 | – | 14.3 | 2.9 | 5/5 CR |
| MA13/C early | 14.2 | 3,5,7 | 42.6 | 0 | 36 | 4.3 | ++++ |
| MA13/C advanced | 15 | 24,27,30 | 45 | – | 23.9 | 2.5 | 3/5 CR |
| MA44 early | 22 | 3,5,7 | 66 | 39 | – | – | +/– |
| colon C26 early | 5 | 1–4 | 20 | 33 | – | – | + |
| C38 early | 23.5 | 3,5,7 | 70.5 | 0 | – | 7/7 cures | ++++ |
| C38 advanced | 26.8 | 14,16,18 | 80.4 | – | 16.5 | 1.7 | 5/5 CR |
| C51 early | 12.7 | 3,5,7 | 38.1 | 2.4 | – | 2.3 | +++ |
| C51 advanced | 15.2 | 10,12,14 | 45.6 | – | 17.2 | 1.7 | ++ |
| lewis lung early | 23.2 | 3–7 | 116 | 5.5 | 6.1 | 1.2 | + |
| osteosarcoma GOS early | 18.6 | 3–7 | 93 | 27.2 | – | – | + |
| hystiocytosarcoma M5076 early | 8.6 | 3–7 | 43 | 51 | – | – | – |
| Leukemias i.p. | | | | | | | |
| P388 $10^6$ cells | 23.2 | 1–4 | 92.8 | 154 | – | – | + |
| L1210 $10^5$ cells | 21.7 | 1–4 | 86.8 | 170 | – | – | ++ |

[a] T/C % = for solid tumors: 100 × median tumor weight of the treated/median tumor weight of the controls, or for leukemias: median survival time of treated animals/median survival time for control animals × 100.
[b] T–C in days = median time in days required for the treatment group T and the control group C tumors to reach a predetermined size.
[c] Log cell kill = T–C in days/3.32 × tumor doubling time of control mice.
[d] Activity rating: ++++ = highly active (log cell kill >2.8), +++ = highly active (log cell kill = 2.0–2.8), ++ = active (log cell kill = 1.3–1.9; T/C ≥ 150% for L1210), + = active (log cell kill = 0.7–1.2 for s.c. tumors, T/C = 125–174% for P388), – = inactive.
[e] CR = complete regressions.

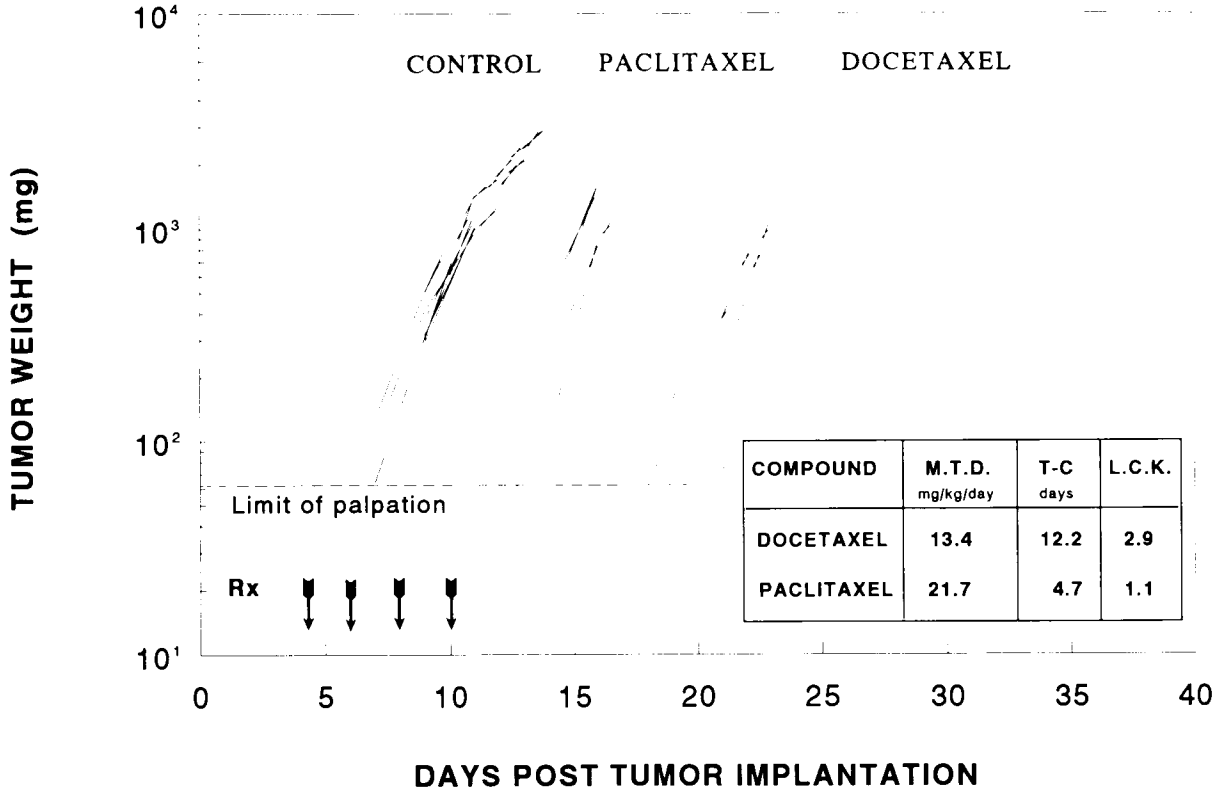


**Figure 3.** *In vivo* comparison of i.v. docetaxel and paclitaxel against SC B16 melanoma. $B6D2F_1$ mice were implanted with 30–60 mg tumor fragments on day 0. The tumor weights were plotted for each mouse for the control group and for the highest non-toxic dose of each drug. Arrows indicate the days when treatment was administered.

L1210/BCNU and L1210/cisplatin were cross-resistant to docetaxel.[35]

A review of all trials performed showed that there was a clear dose–response relationship. The mean optimal total dose of docetaxel (80 mg/kg) produced only body weight loss (13% average at nadir) and no delayed toxicity. The mean host recovery time occurred 8 days post nadir.[35]

Docetaxel was found active i.p. and i.v. against s.c. implanted tumors, indicating that it crosses physiological barriers well. However, it was found inactive by the oral route. This was not unexpected as the three esters and the oxetane ring system on the molecule could be cleaved by low pH in the stomach.

Docetaxel was tested using three different schedules comparing the effect of two, three and 10 administrations over the same duration of treatment. Overall the administration schedule did not influence markedly the total dosage that can be administered and thus the compound was considered schedule-independent for the MTD. A schedule-dependent agent (e.g. 1β-D-arabinofuranosylcytosine) would have at least a 10-fold lower dosage on the split dose schedule compared with the intermittent schedule. Docetaxel antitumor activity correlated with the total dosage administered and dose-splitting did not appear to alter efficacy.[35,37] The best host recovery occurred with the most spaced dosage schedule. Interestingly, this type of study has also been performed with paclitaxel and seems to indicate some schedule-dependency (a better efficacy was obtained with a every day × 7 than with a every 6 days × 3).[38]

*Efficacy in human tumor xenografts.* A broad spectrum of activity was also obtained against human tumor xenografts in nude mice with 15/16 tumor models being responsive to docetaxel.[39–41] At non-toxic drug levels (22–33 mg/kg/injection for three administrations every 4 days), impressive delays in tumor growth were obtained in advanced tumors (200–400 mg at start of therapy) including a mammary adenocarcinoma Calc18, a lung carcinoma LX-1 and two colon carcinomas, KM 20L2 and CX-1 (Table 2). With this last tumor, regressions were observed but with no cures. Complete regressions and cures were seen against advanced MX-1 (a mammary tumor xenograft) (Figure 4) and advanced SK-MEL-2, a melanoma.[39] Cures were also

**Table 2.** Docetaxel evaluation against six human tumor xenografts in mice

| Tumor (site) | Histology | Dose i.v. (mg/kg/injection) | Schedule (days) | Total dose (mg/kg) | T–C[d] (days) | Tumor-free survivors | Comments |
|---|---|---|---|---|---|---|---|
| CALC18[a] (s.c.) | mammary adenocarcinoma | 32.2 | 11,15,19 | 99 | 41 | – | highly active |
| MX-1[b] (s.c.) | mammary adenocarcinoma | 22 | 11,15,19 | 66 | NA | 10/10 | curative |
| LX-1[b] (s.c.) | lung carcinoma | 22 | 9,13,17 | 66 | 19.0 | 0/10 | active |
| SK-MEL-2[b] (s.c.) | melanoma | 33 | 27,31,35 | 99 | NA | 10/10 | curative |
| OVCAR-3[b] (i.p.) | ovarian carcinoma | 33 | 3,7,11 | 99 | NA | 4/10 | highly active |
| OVCAR-3[b] (i.p.) | ovarian carcinoma | 33[c] | 3,10,17 | 99 | NA | 6/10 | curative |
| CX-1[b] (s.c.) | colon carcinoma | 15 | 12,16,20 | 45 | 42.1 | 0/10 | active |
| KM20L2[b] (s.c.) | colon carcinoma | 33 | 14,18,22 | 99 | 19.3 | 0/10 | modest activity |

[a] Bissery *et al.*[36]
[b] Dykes *et al.*[37]
[c] In this experiment, the treatment was administered i.p.
[d] End point defined as in Table 1.

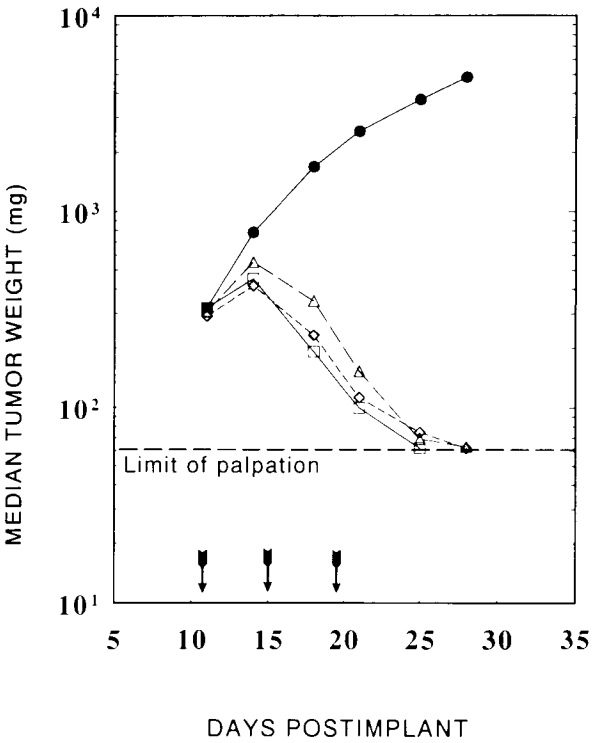


**Figure 4.** *In vivo* evaluation of i.v. docetaxel against s.c. MX-1 human mammary tumor. Athymic NCr-nu mice were implanted with 30–60 mg tumor fragments on day 0. The median tumor weights were plotted for the control group (closed circle) and for the three dose levels tested; open square, 33 mg/kg/injection; closed diamond, 22 mg/kg/injection; open triangle, 15 mg/kg/injection. Arrows indicate the days when treatment was administered.

obtained with early stage OVCAR-3.

Five additional human ovarian cancer lines grafted s.c. were evaluated (Ov.Pe, Ov.Sh, FMa, FKo, MRI-H-207) (Table 3).[40] At the maximum tolerated dose of 15–20 mg/kg administered as two i.v. injections, 1 week apart, docetaxel was found active in 4/5 of these human ovarian cancer lines. Docetaxel was found active against the cisplatin insensitive Ov.Pe tumor, more effective than cisplatin, cyclophosphamide and doxorubicin in FMa, and active in MRI-H-207 and Ov.Sh but less than cisplatin, cyclophosphamide and doxorubicin. It was found inactive against FKo as were the other agents.[40] Docetaxel also had activity against three other ovarian carcinoma xenografts: HOC8, HOC18 and HOC22.[41]

Finally docetaxel was evaluated against head and neck squamous cell carcinoma xenografts HNX-14C and HNX-22B, and was found more active than cisplatin against these two tumors.[42]

## Drug combinations

### *In vitro* combination therapy

In studies of SKBR-3 human breast cells, synergistic effects were noted after cell pretreatment with edatrexate followed by docetaxel; however, the reverse schedule demonstrated antagonism.[43]

Docetaxel may also have a radiation sensitizing

**Table 3.** *In vivo* antitumor activity of docetaxel in combination with anticancer drugs

| Drug i.v. | Tumor site | Schedule[a] | % HNTD of single agents[b] | Therapeutic response[c] |
|---|---|---|---|---|
| Docetaxel + | C51 | simultaneous | 40 | < |
| cisplatin | s.c. | 3 h apart | 40 | < |
| | | 24 h apart | 40 | < |
| Docetaxel + | PO3 s.c. | simultaneous | 60 | < |
| doxorubicin | MA13/C s.c. | simultaneous | 60 | < |
| Docetaxel + | MA13/C | simultaneous | 50 | = |
| mitomycin C | s.c. | | | |
| Docetaxel + | P388 | simultaneous | 80–100 | = |
| vincristine | i.p. | 4 h apart | 80–100 | = |
| | | 24 h apart | 65 | = |
| Docetaxel + | MA13/C | simultaneous | 90 | < |
| vinblastine | s.c. | | | |
| Docetaxel + | MA13/C s.c. | simultaneous | 80 | = |
| navelbine | MA16/C s.c. | simultaneous | 100 | > |
| | | 24 h apart | 80 | > |
| Docetaxel + | B16 | simultaneous | 60 | > |
| etoposide | s.c. | | | |
| Docetaxel + | MA13/C | simultaneous | 60 | > |
| cyclophosphamide | s.c. | | | |
| Docetaxel + | C38 | simultaneous | 70 | > |
| 5-fluorouracil | s.c. | | | |
| Docetaxel + | P388 | simultaneous | 65 | > |
| methotrexate | i.p. | | | |

[a] When drugs were administered *X* h apart, the two sequences (docetaxel first and docetaxel second) were studied.
[b] This represents the fraction of the highest non-toxic dose (HNTD) of each single agents that can be administered in the highest non-toxic combination.
[c] The therapeutic response represents the efficacy of the highest non-toxic combination compared to the efficacy of the best single agent; > greater efficacy than docetaxel, = identical efficacy, < lower efficacy than docetaxel.

effect.[44] This agent blocks cells in the $G_2$ and M phases of the cell cycle, the most radiosensitive phases. Choy *et al.* demonstrated the radiation-sensitizing effects of docetaxel using the human leukemia HL-60.[44] However, Hennequin *et al.* could not show this radiosensitizing effect using HeLa cells.[45]

### *In vivo* combination therapy

Ten two-drug combinations were evaluated in mice bearing s.c. transplantable tumors (Table 3).[36,46,47] The effects of the optimal docetaxel based combination were found greater than the effect of the best single agent in the case of docetaxel–vinorelbine (against MA16/C), docetaxel–etoposide (against B16 melanoma), docetaxel–cyclophosphamide (against MA13/C), docetaxel–5–fluorouracil (against colon 38) and docetaxel–methotrexate (against P388 leukemia). A similar level of efficacy was obtained in the case of docetaxel–vincristine (against P388) and docetaxel–mitomycin C (against MA13/C), compared with the activity of the best single agent. Good activity was obtained with the docetaxel–vinblastine and docetaxel–doxorubicin combinations, both tested against MA13/C. However, their activity was lower than that of docetaxel alone.

In terms of toxicity, the combination toxicity index (CTI, i.e. sum of the fractions of the $LD_{10}$ of each agent used in the combination) ranged from 0.75 for the most toxic combination (docetaxel–cisplatin), indicating complete overlap in dose limiting toxic effects, to 2 for the least toxic combination docetaxel–vinca alkaloids, indicating that the maximum tolerated dose of each agent could be administered without additional toxicity. All the other combinations had a CTI index around 1.2 indicating that approximately 60% of the full dose of each agent can be used in combination without an increase of the overall toxicity.[36,46,47]

Although most of the combinations were evaluated using simultaneous administration, a few studies were performed using different schedules. This factor was found important in the case of the

**Table 4.** *In vivo* evaluation of docetaxel with and without dexamethasone against B16 melanoma

| Dosages (mg/kg/dose) | | Schedule | T–C (days) | LCK |
|---|---|---|---|---|
| Docetaxel | Dexamethasone | | | |
| 32.2 | — | 5,7,9 | toxic | — |
| 20 | — | | 13.50 | 2.0 |
| 12.4 | — | | 9.08 | 1.4 |
| 7.7 | — | | 4.53 | 0.7 |
| — | 0.5 | 5,7,9 | 0.36 | 0.05 |
| 32.2 | 0.5 | 5,7,9 | toxic | — |
| 20 | 0.5 | | 13.45 | 2.0 |
| 12.4 | 0.5 | | 10.49 | 1.6 |
| 7.7 | 0.5 | | 5.52 | 0.8 |

Docetaxel was administered i.v., dexamethasone was administered s.c. See Table 1 for abbreviation identification and for rating basis.

docetaxel–vincristine combination, where administering the two drugs 24 h apart lead to a greater level of host toxicity.[46]

Combination chemotherapy studies have also been performed with paclitaxel using the M109 tumor model.[38] The combined agents included cisplatin, etoposide, doxorubicine, cyclophosphamide, methotrexate, pentamethylmelamine and bleomycin. Taxol–cisplatin and Taxol–bleomycin were the two combinations reported as showing hints of therapeutic synergy.[38]

### Effect of premedication

Dexamethasone was administered s.c. simultaneously with docetaxel i.v. every 2 days for three injections in mice bearing s.c. B16 melanoma. Premedication did not influence the maximum tolerated dosage (MTD = 20 × 3 mg/kg) and the efficacy was similar at all the dose levels tested (Table 4).

## Resistance

Three types of studies have been performed covering the acquired and the innate resistance to docetaxel and the cross-resistance toward clinically used anticancer agents.

### Acquired resistance

The emergence of drug resistant clones of tumor cells has often been the cause of treatment failure.

In terms of mechanisms of acquired taxoids resistance, two mechanisms have been described *in vitro*. One, characterized by the multidrug resistance phenotype,[48–50] related to overproduction of P-glycoprotein, and one, characterized by the presence of altered tubulins.[51]

Two cell lines resistant to docetaxel have been developed *in vitro* to characterize the mechanism of resistance to docetaxel. Using a human breast adenocarcinoma Calc18/docetaxel cell with a 15-fold resistance factor to docetaxel, it was found highly cross-resistant to vinblastine, modestely cross-resistant (i.e. resistance factor $\leq 3$) to doxorubicin and cisplatin, but not to camptothecin and 5-fluorouracil. This cell line was found to overexpress the *mdr*-1 gene and to have a decrease in $\beta$-tubulin mRNA levels compared with the parental cells.[50] Using docetaxel resistant CHO cells (CHO/Doce-R) with a 6-fold resistance factor, low levels of cross-resistance were found with vincristine and VP-16 and no cross-resistance was found with cisplatin.[27]

A mouse macrophage cell line resistant to paclitaxel, J774.2/paclitaxel, was also used to evaluate the cross-resistance to docetaxel.[12] This cell line displays the multidrug resistance MDR phenotype with the amplification of P-glycoprotein. Using this cell line, docetaxel was at least 5-fold more potent than paclitaxel in inhibiting the replication of the J774.2/paclitaxel cell line.[12]

A docetaxel resistant B16 melanoma (B16/TXT) was recently established *in vivo*.[52] Resistance to docetaxel was slow to develop since the s.c. tumor became fully resistant only after 17 months of repeated i.v. docetaxel exposure. Murine *mdr* gene expression was detected in the resistant line, whereas it was absent in the parental line. B16/TXT was found cross-resistant to vincristine and vinblastine, and partially cross-resistant to doxorubicine. No cross-resistance was observed with cyclophosphamide and etoposide.[52]

### Innate resistance

This type of resistance is also called intrinsic insensitivity. It relates to these tumors that have never been exposed to docetaxel and that are not sensitive to docetaxel.

Although this field has barely been touched, it has recently been shown that other mechanisms could be involved such as at the level of MAPs. Indeed it was recently presented that in murine mammary tumors (sensitive MA16 C, MA13 C and poorly sensitive MA44 to docetaxel *in vivo*), differences in

MAPs could account for differences in docetaxel tumor sensitivity and docetaxel tumor intrinsic insensitivity.[53]

### Cross-resistance of docetaxel with anticancer agents

In terms of patterns of cross-resistance with other antitumor agents, cross-resistance to docetaxel has been observed in multidrug-resistant sublines such as P388/DOX,[26] CEM/VLB 1000, MCF-7/VCR6E and in the CHO/CHRC5.[27] However, absence of cross-resistance to docetaxel was observed in cells that expressed low levels of vincristine or etoposide resistance but were P-glycoprotein positive such as CHO/AUX-10E, CHO/DXR-101, Susa/VPC3 and Susa/VPC4.[27] This suggests that cross-resistance to docetaxel was not automatically observed in sublines expressing the MDR phenotype. In addition, in certain cell lines, a lack of cross-resistance was noted to cell lines with acquired resistance to 5-fluorouracil (colon COLO/5-FU-R and LOVO/5-FU-R)[27] or to cisplatin (ovarian 41McisR, CH1cisR and OVCAR-3carboR).[27,29]

### Reversal of resistance

Reversal of resistance to docetaxel was examined in human myeloma cell lines. In that study the effects of eight chemosensitizers on P170-associated docetaxel resistance were evaluated in clonogenic assays. The results suggest that oral quinidine could prove useful for clinical reversal of P170-associated resistance to docetaxel.[54]

Finally, another agent, dexniguldipine–HCl (a dihydro pyridine pure enantiomer), that was found to reverse multidrug resistance, was shown to enhance the sensitivity to docetaxel of 2780 AD, an adriamycin resistant variant of the ovarian carcinoma cell line A2780 in a MTT assay.[55]

## Drug disposition

Drug disposition of docetaxel has been studied following i.v. administration to mice, rats and dogs. Both unlabeled and $^{14}$C-labeled docetaxel have been used. Docetaxel was administered in polysorbate 80/ethanol (50/50 v/v) or polysorbate 80 alone, diluted with 5% glucose or 0.9% sodium chloride.

### Pharmacokinetics

In pharmacokinetic studies, docetaxel has been determined in plasma, urine and tissues by HPLC with UV detection at 225 nm after solid–liquid extraction, similar to the method described by Vergniol *et al.*[56] The lowest limit of quantitation of this method in plasma and urine was 10 ng/ml.

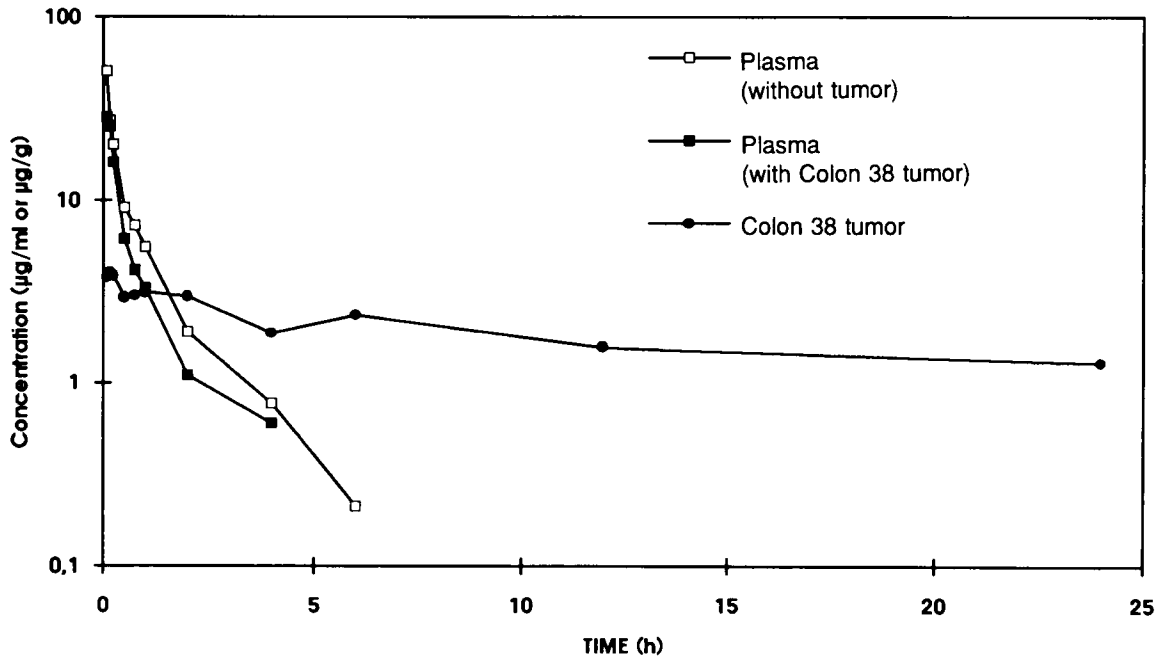


**Figure 5.** Docetaxel kinetics in plasma of normal B6D2F$_1$ mice and in plasma and tumor of colon adenocarcinoma 38 tumor-bearing mice following a 111 mg/m$^2$ i.v. bolus dose.

**Table 5.** Pharmacokinetic parameters of docetaxel in mice, rats and dogs (single dose, unlabeled product)

| Species | Normal mouse | Tumor bearing mouse (C38) | Rat | Dog |
|---|---|---|---|---|
| i.v. dose ($mg/m^2$) | 111 | 111 | 30 | 30 |
| $C_0$ (μg/ml) | 54[a] | 51[a] | 4.1[b] | 3.5[b] |
| $AUC_{0-\infty}$ (μg h/ml) | 24.4 | 17.1 | 0.91 | 1.7 |
| $t_{1/2\alpha}$ (h) | 0.15 | 0.12 | 0.02 | 0.07 |
| $t_{1/2\beta}$ (h) | 1.12 | 1.18 | 0.78 | 6.6 |
| $V_{d_{ss}}$ (l/kg) | 1.6 | 2.2 | 4.0 | 9.1 |
| $Cl_t$ (l/h/kg) | 1.5 | 2.2 | 5.5 | 0.9 |

[a] Extrapolated value (bolus administration).
[b] Concentration at the first sampling time, i.e. 2 min post end of infusion (10 min duration).

After i.v. administration of a highly active 111 mg/$m^2$ dose, the plasma pharmacokinetics in normal $B6D2F_1$ mice and in colon adenocarcinoma 38 tumor-bearing mice were similar (Figure 5).[28] Plasma curves showed a biphasic profile, with a very short distribution phase ($t_{1/2}$ = 7 min) and an elimination half-life of about 1.1 h. Total clearance was 1.6 l/h/kg, the steady-state distribution volume was 1.5 l/kg and the plasma AUC was 17 μg h/ml (Table 5).

In mice, docetaxel showed a good proportionality of plasma $C_{max}$, plasma AUC and tumor AUC values with i.v. administered doses of 39 to 186 mg/$m^2$ (Figure 6). Maximum levels in tumor tissue were attained within minutes following the end of injection, but were considerably lower than corresponding plasma levels (4 against 50 μg/ml). Despite this fact, exposure of tumors was remarkably high, with AUCs in the range of 17 to 71 μg h/g, which is several times higher than the docetaxel AUC in plasma (Figure 6). As shown in Figure 5, this high AUC is caused by the very slow elimination of the drug from tumor tissue; the elimination half-life from tumor tissue was more than 20 h, compared with a plasma elimination half-life of 1.1 or 2–4 h in other tissues such as liver, kidney, spleen or muscle.[28] Twenty-four hours after administration, tumor levels of docetaxel were still 1.29 μg/g for a 111 mg/$m^2$ dose. This high affinity of docetaxel for tumor tissue *in vivo* is consistent with that for tumor cells *in vitro* and the observation of a slow efflux.[24]

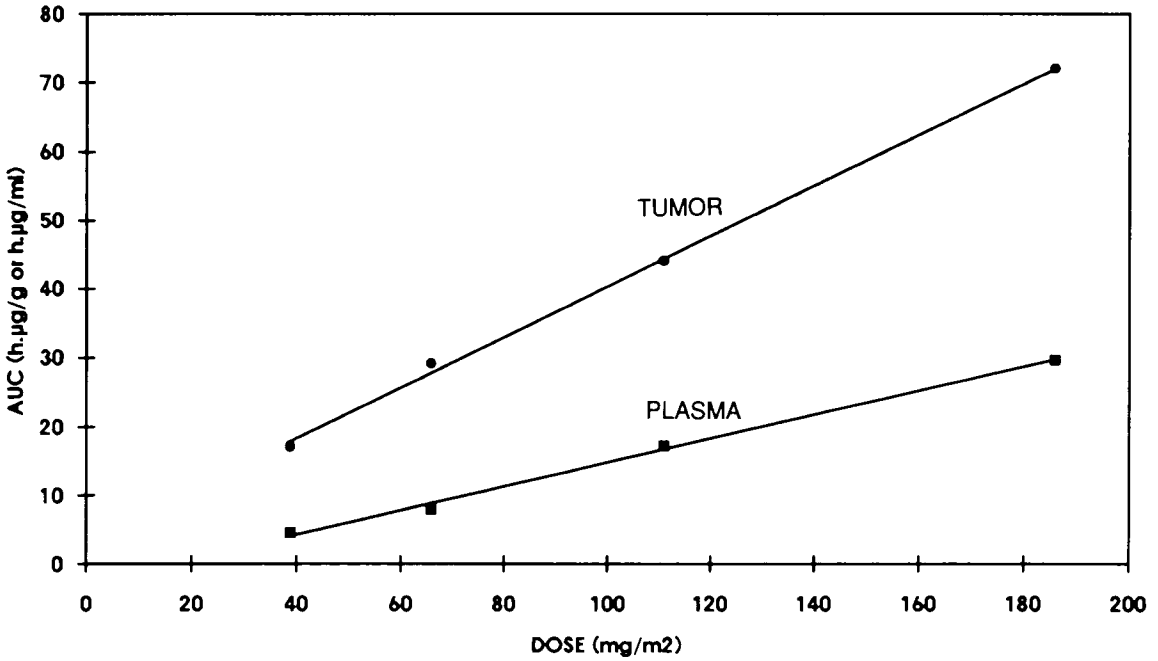


**Figure 6.** Dose proportionality and comparison of exposure in plasma and tumor of colon adenocarcinoma 38 tumor-bearing mice following i.v. bolus administration (39, 66, 111 and 186 mg/$m^2$/injection).

It is of interest that, at all doses, tumor levels were considerably higher than the $IC_{50}$ values of cytotoxicity in tumor cell cultures (see above), up to 24 h after administration. This long exposure of tumor tissue may be an essential factor for docetaxel activity in human patients, where plasma exposure at therapeutic doses is in the same range as in the mouse (4.7 μg h/ml at 100 mg/m$^2$).[33]

Upon repeated administration to female mice (three injections once every 2 days) the tumor AUC on day 5 was about 40% lower than after a single equivalent dose. This lower AUC did not appear to be caused by metabolic enzyme induction; docetaxel at a dose of 30 mg/m$^2$/day for 5 days did not increase inducible cytochrome P450-dependent enzyme activities or its own biotransformation rate in liver microsomes in mice and rats (Sanderink, unpublished observation).

In the rat, plasma docetaxel half-lives were comparable to those in the mouse: 0.014–0.21 h for the first phase and 0.78–1.66 h for the second phase (Table 5). For doses from 15 to 60 mg/m$^2$, the AUC increased proportionally from 0.6 to 2.5 μg h/ml. However, at 120 mg/m$^2$ the increase was more than proportional (9.4 μg h/ml). Plasma clearance was 4–5.5 l/h/kg for the lower doses, but decreased to 2.1 l/h/kg at 120 mg/m$^2$ (Renard, unpublished results). These findings are consistent with results obtained in the isolated perfused rat liver. In this model non-linearity of the AUC was observed when increasing docetaxel concentration in the perfusate from 5 μM (corresponding to the $C_{max}$ at 30 mg/m$^2$) to 50 μM. This was probably caused by cholestasis and/or saturation of metabolic enzymes.[57] This conclusion is further supported by the low $K_m$ of docetaxel metabolism (about 6 μM) in rat liver microsomes.[58]

Docetaxel pharmacokinetics in the dog were characterized by half-lives of 4 min and 6.6 h and a plasma clearance of 0.93 ± 0.28 l/h/kg. The main difference in comparison to the mouse was a larger volume of distribution (9 l/kg). The AUC at 30 mg/m$^2$ was 1.7 μg h/ml (Table 5).

## Distribution

Docetaxel (parent compound) was detected in most tissues of mice, including tumor tissue, but not in the central nervous system. Distribution studies with radiolabeled compound in mice and rats showed rapid tissue uptake, especially into liver, bile, intestine and gastric contents, and also into spleen, myocardium, bone marrow, pancreas and salivary glands. Radioactivity was further detected in virtually all tissues, including fetal tissues and milk but autoradiography did not show any compound in the central nervous system. Levels in reproductive organs were higher in females than in males.[59]

*In vivo* plasma protein binding of the radiolabeled compound was high in mice and rats (from 84.1 to 89.1% at 0.25 h). Binding to plasma proteins *in vitro* was also high in mouse (89–95%), rat (70–76%), dog (83–89%) and man (79–83%). Among human plasma proteins, docetaxel was mainly bound to albumin and $\alpha_1$-acid glycoprotein, the association with the latter might be concentration dependent (Paccaly, personal communication). In humans, more than 90% of plasma radioactivity is protein bound.[60]

## Excretion

Radiolabeled docetaxel was largely excreted by the fecal route in mice, rats and dogs.[59] Excretion in humans has also been shown to be in large majority in the feces.[60] Excretion was virtually complete 96 h after administration. Urinary excretion of parent drug or radioactivity was always low (<10% of the dose). Recovery of radioactivity after 7 days was >95% in mice, >85% in rats and >90% in dogs.

Studies in the bile duct cannulated rat *in vivo* and in the isolated perfused rat liver confirmed the predominance of biliary excretion of radiolabeled docetaxel-derived compounds. In the rat, enterohepatic cycling of radioactivity was not important, with only 13% of the radioactivity excreted in bile being reabsorbed.[57,61]

## Metabolism

Several studies have shown that hepatic metabolism and biliary excretion is the major pathway of docetaxel elimination in all species. Only a minor fraction of the dose is excreted in the form of parent drug.

The structures of docetaxel metabolites have been elucidated by the groups of Monsarrat and Wright at the CNRS in Toulouse and of Gaillard and Vuilhorgne at Rhône-Poulenc Rorer.[61–63]

The main pathway of docetaxel metabolism consists of successive oxidations of the tert-butyl ester group on the side chain, with spontaneous cyclization occurring for the putative aldehyde and acid derivatives. Two metabolites from this pathway, the

alcohol and the cyclized acid have been synthesized and were found inactive against murine leukemia P388 cells *in vitro* and inactive in B16 melanoma-bearing mice *in vivo*.[64]

A minor pathway is caused by 7-epimerization, a reaction previously observed for paclitaxel.[65] Both the diastereoisomer of docetaxel, RP 70617, a known chemical degradation product, and the diastereoisomers of the alcohol metabolite and of the cyclized aldehydes have been found in low quantities in feces. RP 70617 has been shown to have moderate activity *in vitro* and *in vivo* compared with docetaxel. No other metabolites resulting from modification of the taxane ring have been detected for docetaxel. This in contrast to paclitaxel, which is mainly hydroxylated at the 6-position of the main ring by human liver microsomes.[66]

Hydroxylation on the phenyl group of the side chain, which is a major pathway for paclitaxel in the rat,[67] is only a very minor reaction for docetaxel in this species.[61]

*In vivo* metabolism studies in the mouse, the rat, the rabbit and the dog did not indicate any major species or gender differences in the meta-bolic pathway. In all four species the metabolites from the tert-butyl oxidation pathway represented the large majority of fecal metabolites, with the alcohol derivative being the most abundant one.[59,61,62]

Only in the mouse significant levels of docetaxel metabolites were detectable in plasma. These metabolites resulted from the first two steps of tert-butyl oxidation.[59]

*In vitro* metabolism studies in mouse, rat, dog and human liver microsomes showed a good correlation with *in vivo* data. Biotransformation was more rapid in mouse liver microsomes than in other species.[58] Oxidation of the tert-butyl group of docetaxel represented the main biotransformation pathway in all four species, the rest resulting from the same reaction on the 7-epimer, RP 70617, or from epimerization of the metabolites.[33]

The main enzymes involved in docetaxel metabolism are monooxygenases (phase I enzymes). Conjugation of parent drug and metabolites appeared to be of minor importance. In human liver microsomes, cytochrome P-450 isoenzymes of the CYP3A subfamily were mainly responsible for docetaxel biotransformation.[58,68] Studies in rat liver microsomes and in the isolated perfused rat liver also indicated a major role of CYP3A in this species, but other (inducible) enzymes might also be involved.[57,58]

Possible metabolic drug–drug interactions were indicated by these findings. These are most likely to occur with potent CYP3A inhibitors such as ketoconazole or macrolide drugs.

Overall, docetaxel pharmacokinetics in animals have been extensively studied. The drug is eliminated very rapidly although at a slower rate from tumor tissue than from normal tissue and excreted mostly in the feces. Docetaxel metabolism is similar across species *in vivo* and in *in vitro* models. In humans, tert-butyl oxidation is also the main pathway, although the cyclized acid is more abundant in human feces than in other species.[60,62,64] Thus the studied animal species are very likely to be good models for human metabolism of docetaxel. The fact that docetaxel is generally the main circulating compound and that the major metabolites are much less active than docetaxel indicates that parent drug analysis is an appropriate parameter for pharmacokinetic/pharmacodynamic studies of this drug.

## Preclinical toxicology

The initial investigations followed the recommendations of the US National Cancer Institute for preclinical toxicology studies on antineoplastic drugs,[69–71] and were performed with the objective to determine the clinical entry dose. These investigations included single-dose toxicity studies in CD® rats, $CD2F_1$ mice and beagle dogs, followed by 5-day toxicity studies in mice and dogs. Additional studies investigated the long-term effects of intermittent treatment in rats, dogs and monkeys as well as the reproductive toxicity in rats and rabbits. The genetic toxicity was evaluated in a series of *in vitro* and *in vivo* tests. The irritation and sensitization potential of the drug was investigated in guinea pigs, mice and rabbits. In all studies, a solution of docetaxel in polysorbate 80 or in polysorbate 80/ethanol was diluted with 5% aqueous glucose or 0.9% aqueous NaCl and was administered i.v. All studies included control groups which were treated with the corresponding vehicle containing appropriate concentrations of formulation components of docetaxel, i.e. polysorbate 80 or polysorbate 80/ethanol. In the following, doses are expressed as mg/m$^2$ body surface area. Conversion factors (dosage mg/kg to mg/m$^2$) were 40, 20, 12, 12, 6 and 3 for man, dog, monkey, rabbit, rat and mouse, respectively.[73]

### Single-dose toxicity studies

The results of single-dose toxicity studies are summarized in Table 6. In rats, mortality occurred fol-

**Table 6.** Results of single dose toxicity studies in mice, rats and dogs (doses in mg/m$^2$ body surface area)

| Parameter | Mouse | Rat | Dog |
|---|---|---|---|
| TDL[a] | NA[e] | NA | 15 |
| HNTD[b] | <285 | <60 | <15 |
| HNLD[c] | 285 | 60 | 30 |
| $LD_{10}$ | 345 | NA | NA |
| $LD_{50}$ | 414 | NA | NA |
| LD[d] | ≥414 | 240 | ≥50 |

[a] Toxic dose low.
[b] Highest non-toxic dose.
[c] Highest non-lethal dose.
[d] Lethal dose.

lowing single doses of 120 mg/m$^2$ of docetaxel and above. A single dose of 60 mg/m$^2$ docetaxel was the 'highest non-lethal dose' (HNLD). Principal effects in rats included alopecia, testicular atrophy (at 120 mg/m$^2$ and above) and hematopoietic toxicity (bone marrow aplasia, depletion of lymphoid organs, leukopenia, reduced red blood cell (RBC) parameters) at 60 mg/m$^2$ and above. Abnormal mitosis and single cell necrosis was observed in several tissues 4 days after treatment but were no longer present 29 days following treatment. Leukopenia recovered within 24 days following treatment.

In mice, the single dose $LD_{50}$ of docetaxel was 414 mg/m$^2$ and the highest non-lethal dose was 285 mg/m$^2$. Principal toxic effects in mice were hematopoietic toxicity (bone marrow aplasia, leukopenia, depletion of lymphoid organs), neurotoxicity noted at doses of 144 mg/m$^2$ or above (paresis of hind limbs, axonal and myelin degeneration of the sciatic nerve) and testicular atrophy (at 285 mg/m$^2$ or above). Hematopoietic toxicity was reversible, neurotoxicity was partially reversible and testicular effects were not reversible within the 28 day recovery period following treatment.

In dogs, single doses of 70 or 50 mg/m$^2$ were severely toxic and/or lethal. A dose of 30 mg/m$^2$ producing moderate diarrhea and white blood cell (WBC) reduction was considered to be the 'toxic dose high' (TDH). A dose of 15 mg/m$^2$ which produced only a mild reduction in WBC count was considered to be the 'toxic dose low' (TDL).[72] The principal toxicities noted in dogs were gastrointestinal and hematopoietic. Gastrointestinal effects were moderate (at 30 mg/m$^2$) or severe (at 50 mg/m$^2$ and above) and included emesis, diarrhea and feces containing blood. At high doses (50 mg/m$^2$ and above), treated dogs had severe diarrhea associated with necrosis of the intestinal epithelium. These gastrointestinal effects were considered to be the dose-limiting toxicity in dogs. The hematopoietic toxicity was mild at 15 mg/m$^2$, moderate at 30 mg/m$^2$ and marked at 50 mg/m$^2$ and above, and included bone marrow aplasia, leukopenia and lymphoid depletion. WBC count depression recovered within 14 days following treatment. In addition, dogs at all dose levels as well as vehicle-treated control dogs displayed during or immediately after infusion, marked and immediate clinical and cardiovascular signs such as facial swelling, general erythema and decreased blood pressure associated with increased heart rates.

Overall, docetaxel principally affected tissues with a high cell turnover such as the gastrointestinal epithelium (dog), hematopoietic and lymphatic organs (all species), and testis (rodents). These effects are consistent with the toxicities commonly observed following administration of antimitotic antineoplastic drugs.[73] In mice, peripheral nerves were an additional target tissue for toxicity of docetaxel, possibly due to an adverse effect upon microtubules in axons. Microtubule-disrupting agents such as paclitaxel or vinca alkaloids have been reported to produce peripheral neurotoxicity.[74–76] Gastrointestinal and hematopoietic effects of docetaxel were reversible, whereas testicular and neurotoxic effects in rodents were partially reversible or irreversible within the duration of the recovery periods of the toxicology studies. The cardiovascular effects observed in dogs were attributed to histamine release in response to i.v. administration of polysorbate 80, a vehicle component of docetaxel which has been reported to produce histamine release in dogs.[77,78] Similar effects were reported following treatment of dogs with paclitaxel;[79] paclitaxel contains Cremophor® EL which produces histamine release in dogs following i.v. administration.[80]

## Five-day repeated-dose toxicity studies

The results of 5-day repeated toxicity studies in mice and dogs are summarized in Table 7. The studies revealed effects comparable to those observed after single-dose administration. In mice, mortality, severity and incidence of neurotoxicity and leukopenia occurred at cumulative dose levels which were comparable to the corresponding single-dose levels, e.g. the cumulative 5-day $LD_{50}$ was 450 mg/m$^2$ (5 × 90 mg/m$^2$) which correlates well with the single-dose $LD_{50}$ of 414 mg/m$^2$. In dogs, gastrointestinal toxicity and leukopenia of cumulative daily

**Table 7.** Results of 5-day subacute studies in mice and dogs (doses in mg/m$^2$ body surface area)

| | Dog | | Mouse | |
|---|---|---|---|---|
| | daily dose | cumulative dose | daily dose | cumulative dose |
| HNTD[a] | 3 | 15 | 6 | 30 |
| HNLD[b] | 6 | 30 | 30 | 150 |
| $LD_{10}$ | NA[d] | NA | 60 | 300 |
| $LD_{50}$ | NA | NA | 90 | 450 |
| LD[c] | 15 | 75 | 136 | 680 |

[a] Highest non-toxic dose.
[b] Highest non-lethal dose.
[c] Lethal dose.
[d] Not applicable.

doses were of a severity corresponding to comparable single-dose levels: five daily doses of 6 mg/m$^2$ produced adverse effects comparable to those following a single dose of 30 mg/m$^2$.

The results of repeated-dose toxicity studies in dogs and mice demonstrated the cumulative toxicity of docetaxel when administered as a daily dosing regimen. Cumulative toxicity has also been reported for paclitaxel.[76] The results of the repeated-dose studies indicated that docetaxel should preferably be administered using an intermittent-dose regimen to allow for recovery of hematopoietic effects.

## Intermittent-dose toxicity studies

To investigate potential long-term effects of docetaxel treatment, intermittent-dose studies were performed in CD® rats, beagle dogs and cynomolgus monkeys. In these studies, docetaxel was administered at 21 day intervals for a duration of up to 10 (rat, dog) or 12 (monkey) subsequent treatment courses.

Intermittent-dose treatment in rats at dose levels of up to 60 mg/m$^2$ over three or 10 treatment courses, resulted in hematopoietic toxicity (bone marrow hypoplasia, leukopenia and thrombocytopenia), thymic and testicular atrophy. The affected hematological parameters recovered during the 21 day interval between treatments and no increase in severity of adverse effects was noted with the progression of the studies.

In dogs, administration of docetaxel over five or 10 intermittent treatment courses at dose levels of up to 30 mg m$^2$ produced clinical signs resembling those observed in the single-dose and repeated-dose studies. Gastrointestinal toxicity (emesis, diarrhea, feces containing blood and intestinal epithelial single cell necrosis), hematopoietic toxicity (neutropenia/lymphopenia) and dermal effects (alopecia, increased mitosis and epidermal single cell necrosis) were the most prominent toxicities. The affected hematological parameters recovered well during the 21 day recovery interval between treatments and, with the possible exception of dermal effects, there was no increase in the incidence or severity of adverse effects with the progression of the studies.

The results of a preliminary toxicity study in cynomolgus monkeys indicated that a single dose of 50 mg/m$^2$ was in the range of the MTD; leukopenia (neutropenia/lymphopenia) and gastrointestinal signs (diarrhea) were the principal toxicities. Therefore, a subsequent intermittent-dose study in monkeys used initial dose levels of 25 or 50 mg/m$^2$ per group.[81,82] However, the dose level of 50 mg/m$^2$ produced severe toxicity (severe leukopenia, marked clinical signs) and treatment at this dose level was discontinued after the first treatment course. The principal effects noted in monkeys receiving 25 mg/m$^2$ over 12 treatment courses consisted of moderate neutropenia/lymphopenia, a mild reduction in RBC parameters and moderate gastrointestinal toxicity (diarrhea). The severity of diarrhea was moderate to marked, but was not considered dose-limiting. All affected hematological parameters recovered within the 21 day intervals between treatments and there was no increase in severity or incidence of adverse effects with the progression of the study.

The results of the intermittent-dose studies demonstrated that docetaxel may be administered for up to 12 treatment courses without increasing the severity of principal (particularly hematopoietic) toxicities.

## Reproductive toxicity studies

The reproductive toxicity of docetaxel was investigated in CD® rats and NZW rabbits. In a fertility study, male rats were treated for 70 days prior to and through mating with daily doses of up to 1.8 mg/m$^2$ docetaxel, while females received daily doses of up to 1.35 mg m$^2$ from 15 days prior to mating through day 7 of gestation.

At the intermediate (0.9 mg m$^2$) and (1.35 or 1.8 mg m$^2$) high dose levels, docetaxel produced maternal and paternal toxicity associated with a reduction in mean body weight (males and females),

testicular weight, and a reduction in fertility parameters, such as the number of corpora lutea and implantations, litter size and number of live fetuses associated with increased resorptions. Therefore, the drug was considered to be embryotoxic at daily doses of 0.9 mg/m$^2$ and above. However, no external fetal malformations were observed at any dose level. The no-effect level in this study was 0.3 mg/m$^2$/day.

Pregnant rats and rabbits were treated with daily doses of up to 1.8 mg/m$^2$ (rat) or 2.4 mg/m$^2$ (rabbit) during the organogenesis phase of gestation. In rats, 1.8 mg/m$^2$/day produced an increase in post-implantation loss, reduced fetal weight and a delay in fetal ossification. No treatment-related external, skeletal or visceral malformations were observed and post-natal development was not affected in any group. In rabbits, 2.4 mg/m$^2$/day was lethal to most of the dams. A dose of 1.2 mg/m$^2$ produced marked maternal toxicity, an increased incidence in abortions and reduced fetal weight associated with a delay in fetal ossification. No compound-related external, skeletal or visceral malformations were observed in this study. No adverse embryo–fetal effects were noted following maternal exposure to 0.18 mg/m$^2$ (rat) or 0.36 mg/m$^2$ (rabbit).

In conclusion, docetaxel was non-teratogenic, but was embryo- and fetotoxic. The increased abortion rate in rabbits treated with daily doses of 1.2 mg/m$^2$ and above is a common response of rabbits to maternal toxicity.[83] In addition, the results of a fertility study and the adverse effects observed in the testes of rats and mice indicate that docetaxel, like paclitaxel or other antimitotic drugs, has the potential to affect fertility.[76,84]

## Genetic toxicity studies

Docetaxel was evaluated for potential genetic toxicity in a battery of standard tests. All *in vitro* tests were performed in the presence and the absence of an appropriate metabolic activation system. Docetaxel has no mutagenic activity in five strains of *Salmonella typhimurium* or in the WP2 *uvrA* strain of *Escherichia coli*. No evidence of mutagenic activity was found in the hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) test in Chinese hamster ovary cells (CHO-K1). In the *in vitro* micronucleus test in CHO-K1 cells, docetaxel induced, in the presence and absence of metabolic activation, a dose-dependent increase in the number of micronucleated cells at concentrations of 0.3 μg/ml and above. In an *in vitro* chromosomal aberration test using CHO-K1 cells, docetaxel had no clastogenic effect, but induced pronounced polyploidy at concentrations of 0.5 μg/ml and above without metabolic activation, and at 0.1 μg/ml and above in presence of metabolic activation. In the *in vivo* micronucleus test, mice were treated with two doses ranging from 0.195 to 7.2 mg/kg within a 24 h interval; in this test docetaxel induced an increase in the number of polychromatic micronucleated erythrocytes in the bone marrow at a total dose of 1.54 mg/kg and above. The results of genotoxicity tests indicated that docetaxel was non-mutagenic and non-clastogenic, but significantly increased the incidence of micronucleated, aneuploid and polyploid cells *in vitro* and *in vivo*. This effect is consistent with the pharmacological activity of the drug on microtubules and has been reported for paclitaxel, the prototype taxoid, and for spindle poisons such as vincristine.[85,86]

## Local tolerance and sensitization studies

External or histopathological evaluation of injection sites revealed no evidence of irritation in the single-dose, repeated-dose or intermittent-dose studies. A tolerance study in rabbits used i.v., paravenous (p.v.; intradermal) and intra-arterial (i.a.) administration of solutions of docetaxel in 0.9% aqueous NaCl containing polysorbate 80 at concentrations which were up to three times higher than those used in man; the results of this study indicated good tolerance by the i.a. and i.v. route and adequate tolerance by the p.v. route. Sensitization studies in mice, guinea pigs and rabbits revealed no evidence for sensitization, whereas a mild reaction was observed following intradermal re-challenge of docetaxel-pre-treated rabbits. In conclusion, the results of irritation and sensitization studies suggest a good tolerance and the absence of a significant sensitization potential of the i.v. formulations of docetaxel.

Overall, the results of the preclinical toxicity studies indicate that the principal toxicological effects of docetaxel are compatible with the pharmacological (antimitotic) activity of the taxoid class of antineoplastic compounds.[87] However, there were species-specific differences in the tolerance to the drug and in the susceptibility of principal target tissues. The results of the preclinical safety evaluations suggest that the monkey, in which hematopoietic effects (neutropenia) are the dose-limiting toxicity and in which i.v. administration of solutions

containing polysorbate 80 are well tolerated, may be the most suitable large animal model for predicting the principal toxicities of docetaxel in man.[82]

The results of the acute toxicity study in dogs served as a basis for the entry dose in man. Following recommendations of the National Cancer Institute[69,70] one third of the 'toxic dose low' in dogs (15 mg/m$^2$), i.e. 5 mg/m$^2$, was selected as the initial dose level for phase I clinical trials.

## Conclusion

In conclusion, docetaxel obtained through the development of an efficient semisynthesis process represents a new chemical entity with a broad spectrum of antitumor activity and a unique mechanism of action. The compound entered phase I clinical trials in 1990. It is now completing an extensive phase II program and broad spectrum of activity is being reported in breast and non-small cell lung cancer, but also in head and neck, ovary, and pancreas cancers.[7,87]

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## References

See reference list at end of Part II.

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