

# Biological and clinical characterization of paclitaxel poliglumex (PPX, CT-2103), a macromolecular polymer–drug conjugate

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**Abstract:** Paclitaxel is a widely used chemotherapeutic agent; however, its therapeutic index is limited by low tumor exposure and high systemic exposure. Paclitaxel poliglumex (PPX) is macromolecular drug conjugate that links paclitaxel with a biodegradable polymer, poly-L-glutamic acid. PPX enhances tumor exposure by taking advantage of the hyperpermeable vasculature and suppressed lymphatic clearance characteristic of tumor tissue. The release of paclitaxel from the polymeric backbone is, at least in part, dependent on the metabolism of PPX by the lysosomal protease cathepsin B, which is upregulated in many tumor types. Retrospective analysis of clinical data from two phase III trials in advanced lung cancer suggests that PPX activity may be modulated by estradiol: a trend toward improved survival in the PPX arm compared with the control arm was observed in female, but not in male patients. Estrogens are known to induce cathepsin B activity; cathepsin B-mediated proteolysis is a key enzymatic processing step in PPX metabolism. The association between estrogens and PPX activity is being further explored in ongoing preclinical studies. An additional phase III trial will enroll women with advanced NSCLC to prospectively evaluate the efficacy of PPX in relation to pre- and post-menopausal estrogen levels.

**Keywords:** paclitaxel, poly-L-glutamic acid, lung neoplasms, estradiol

## Introduction

Paclitaxel, one of the most widely used cytotoxic agents, induces mitotic arrest and apoptosis in proliferating cells by targeting tubulin, a component of the mitotic spindle (Manfredi et al 1982; Bhalla 2003). Like other small, hydrophobic agents, paclitaxel binds extensively to plasma proteins, and its pharmacokinetic profile is characterized by a short plasma elimination half-life with a broad tissue distribution (Kumar et al 1993; Sonnichsen and Relling 1994). These unfavorable pharmacokinetic characteristics are associated with limited tumor and high systemic tissue exposure, thus reducing the therapeutic index of paclitaxel. In addition, the intravenous administration of hydrophobic agents requires the use of solubilizing agents, such as Cremophor<sup>®</sup> EL/ethanol. Cremophor EL is a biologically and pharmacologically active compound, and its use is associated with acute hypersensitivity reactions (Gelderblom et al 2001).

As a chemotherapeutic agent, paclitaxel is indicated for first-line platinum-based combination treatment in advanced ovarian carcinoma and non-small cell lung carcinoma (NSCLC). Paclitaxel is also indicated for second-line treatment of ovarian and breast carcinoma. The toxicity profile of paclitaxel is characterized by bone marrow suppression, neuropathy, and alopecia (Rowinsky and Donehower 1995). Hematological toxicities can be managed with the prophylactic use of hematopoietic growth factors, particularly in patients at risk for myelosuppression (Markman 2003). Also patients require pretreatment corticosteroids and anti-histamines to prevent acute hypersensitivity reactions. The administration of paclitaxel typically requires a 3-hour

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infusion, on a 3-week schedule. Weekly taxane schedules are currently being investigated, as more frequent administration at a lower dose may reduce myelosuppression and febrile neutropenia (Seidman 2005).

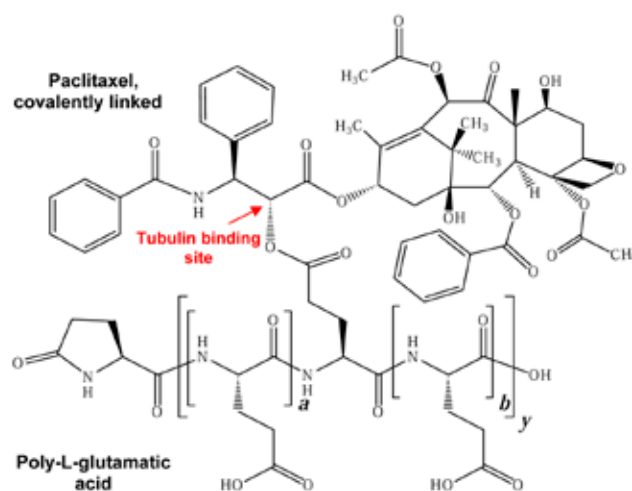
Biodegradable, macromolecular polymer–drug conjugates allow for a more sustained and targeted delivery of chemotherapeutic agents. The performance of these nano-sized (5–100 nm) polymer-based pharmaceuticals is influenced by morphological characteristics, surface chemistry, and molecular weight (Langer 1998; Bala et al 2004). When well-designed, these polymer–drug conjugates preferentially deliver active drug to tumor tissue, limiting exposure of normal tissues. In addition, the slow release of drug from the polymer yields lower peak plasma concentrations of active drug. Paclitaxel polyglumex, a polymer–drug conjugate of paclitaxel and poly-L-glutamic acid, was designed to enhance the therapeutic index of paclitaxel by improving its pharmacokinetic profile, and to provide a water-soluble alternative to the standard paclitaxel formulation.

## Paclitaxel polyglumex A macromolecular polymer–drug conjugate

Paclitaxel polyglumex (PPX) is a polymer–drug conjugate that links paclitaxel to a biodegradable polymeric backbone consisting of L-glutamic acid residues (Singer et al 2005). Paclitaxel is conjugated by ester linkage to the  $\gamma$ -carboxylic acid side chains of poly-L-glutamic acid. Because the conjugation site is through the 2' hydroxyl of paclitaxel, a site crucial for tubulin binding, conjugated paclitaxel does not interact with  $\beta$ -tubulin and is biologically inactive (Gueritte-Voegelein et al 1991). The median molecular weight of PPX is 38.5 kDa. Conjugated paclitaxel represents approximately 36% by weight of PPX, equivalent to about one paclitaxel ester linkage per 11 glutamic acid units (Figure 1).

The rate of release of paclitaxel from PPX by hydrolysis in buffered saline solution and in mouse or human plasma was evaluated. Incubation of PPX in buffered saline or plasma for 24 hours at 37°C showed that less than 14% of the bound paclitaxel had been hydrolyzed. This indicates that PPX is relatively resistant to plasma esterases and is unlikely to release substantial amounts of paclitaxel in the circulation, even with prolonged clearance times.

The characteristics of PPX were evaluated using gel-permeation chromatography in an organic mobile phase. When combined with multiple angle laser-light scattering and reflective index detection, the elution profile provides

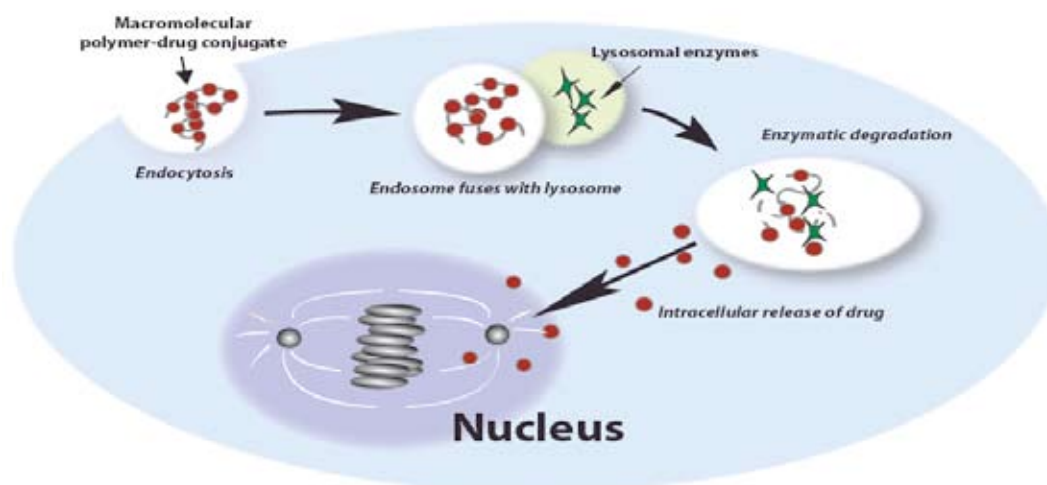


**Figure 1** Schematic representation of PPX. The structure shown is illustrative of a fragment of the molecule, but specific conjugation sites are not implied. On average there are approximately 10.4 non-conjugated monomer glutamic acid units ( $a + b$ ) for every molecule conjugated to a paclitaxel molecule ( $y$ ). The  $a$ -poly-L-glutamic degree of polymerization and the number of conjugation sites with paclitaxel are variable within the drug substance's specifications.

information on the apparent average molecular weight and polydispersity of the polymer. Nuclear magnetic spectroscopy confirmed the conjugation of paclitaxel to the  $\gamma$  and  $\alpha$  positions of polyglutamate. Spectral changes were consistent with conjugation through the paclitaxel 2'- position with additional minor amounts of conjugation in the 7'-position. The distribution of paclitaxel on the polyglutamate backbone was studied through limited proteolysis of PPX with pronase. The resulting peptide mixtures were analyzed by reverse-phase HPLC and mass spectrometry. A wide range of peptide species were observed in the enzyme digest; they can be described as  $(\text{Glu})_n\text{-(paclitaxel)}_x$ , where  $n = 1\text{--}20$  and  $x = 1\text{--}6$ . This is in good agreement with a distribution of paclitaxel that would be expected if the conjugation occurred in a non-directed fashion (Singer et al 2005).

## Tumor accumulation

Polymer–drug conjugates passively accumulate in tumor tissue by taking advantage of the hyperpermeable tumor vasculature and reduced lymphatic clearance, a phenomenon known as the enhanced permeation and retention (EPR) effect. Tumor vasculature is more permeable to macromolecules than normal vasculature because of structural differences between the neovasculature in tumors and the mature vasculature in normal organs (Gerlowski and Jain 1986; Roberts and Palade 1997). The paucity of lymphatic vessels in tumor tissue allows the retention of these macromolecules in the interstitial space,

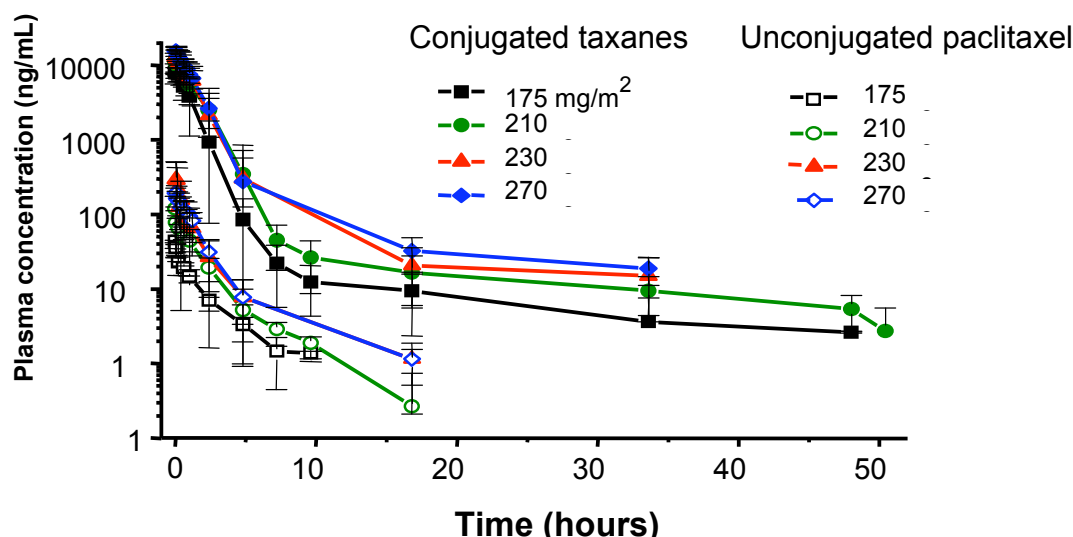


**Figure 2** Internalization of macromolecules by endocytosis and release of active drug. Macromolecules are internalized through endocytosis. Enzymatic degradation of the polymeric backbone by lysosomal enzymes mediates the release of active drug.

resulting in a 10- to 100-fold increase in intra-tumoral drug concentrations when compared with an equivalent dose of the drug given conventionally (Matsumura and Maeda 1986; Greish et al 2003). Macromolecules, characterized by high molecular weight, cannot be internalized into cells by simple diffusion; instead, macromolecules enter cells through endocytosis. Following internalization through endocytosis, polymer–drug conjugates are transported via the endosomal compartment to the lysosomes. Active drug is released through degradation by lysosomal enzymes, followed by diffusion of the active agent into the cytoplasm or nucleus (Figure 2) (Duncan 1992, 2003).

To take advantage of the EPR effect, macromolecules have to remain in circulation for at least 6 hours (Matsumura

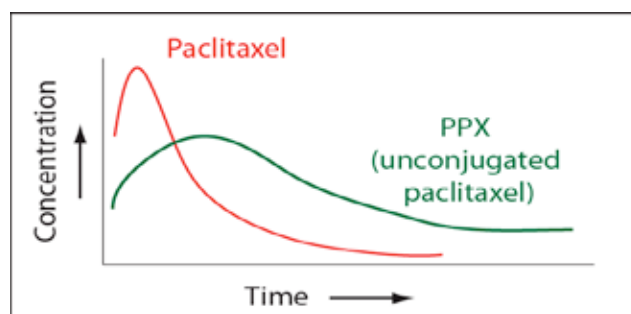
and Maeda 1986). Clinical plasma pharmacokinetics of PPX show a biphasic decline with a prolonged distribution phase, and an elimination phase with a long terminal half-life. Following a 10- to 30-minute infusion of single-agent PPX, plasma concentrations of conjugated taxanes decline biphasically (Figure 3A). The distribution phase is prolonged and the elimination phase, which appears approximately 48 hours after drug administration, is characterized by a long terminal half-life,  $t_{1/2,z}$ , of 108–261.5 hours (Bernareggi et al 2005). PPX is relatively stable in circulation; the area under the curve (AUC) of unconjugated paclitaxel is 1%–2% of the AUC of conjugated paclitaxel. The total systemic exposure to unconjugated paclitaxel is similar after administration of equivalent doses of PPX and standard paclitaxel; however, the



**Figure 3A** Clinical plasma pharmacokinetics. Plasma samples were collected from patients receiving PPX as a 10- to 30-minute infusion once every 3 weeks. Conjugated and unconjugated paclitaxel were measured by validated HPLC/MS methods. The plasma concentration of conjugated paclitaxel declines biphasically and is characterized by a prolonged distribution phase and an elimination phase with a long terminal half-life.

$C_{max}$  values for paclitaxel are significantly lower in patients treated with PPX (Figure 3B).

The prolonged circulation time of PPX facilitates tumor accumulation through the EPR effect, as was demonstrated in model animals (Singer et al 2005). To determine the pharmacokinetic profile of PPX and its tissue distribution, female mice with subcutaneous B16 murine melanomas were given equivalent doses of tritium-labeled paclitaxel 40 mg/kg iv, either as [ $^3$ H]paclitaxel in Cremophor EL/ethanol or as [ $^3$ H]paclitaxel poliglumex in phosphate buffer. Tumor samples were collected at regular intervals up to 144 hours after the injection, and the concentrations of PPX and paclitaxel were determined by LC/MS analysis. Tumor exposure of B16 melanomas to total taxanes was increased by a factor of 3 ( $C_{max}$ ) or factor of 12 (AUC) in mice treated with [ $^3$ H]PPX compared with animals treated with [ $^3$ H]paclitaxel (Table 1; Figure 4). Distribution of paclitaxel to the tumor was faster with [ $^3$ H]paclitaxel, but overall tumor exposure to paclitaxel, with steady concentrations between 24 and 120 hours, was higher after administration of [ $^3$ H]PPX. In addition to tumor tissue, PPX also accumulates in tissues with abundant reticular endothelial systems through active phagocytosis (eg, liver, spleen).

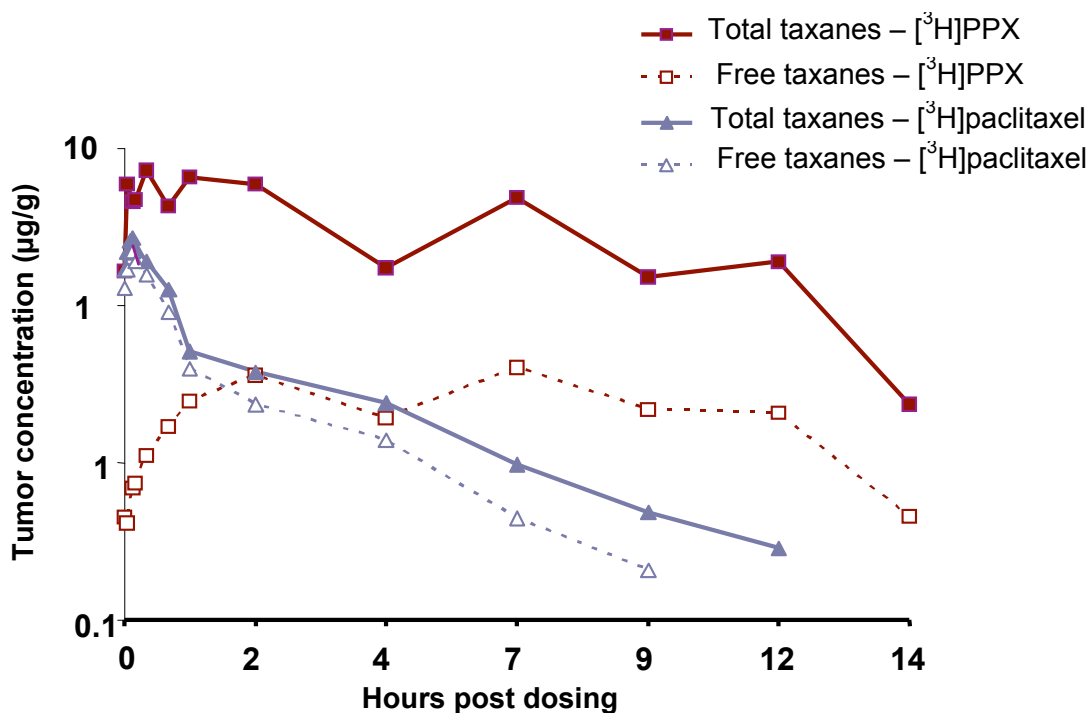


**Figure 3B** Schematic representation of the plasma pharmacokinetics of paclitaxel vs PPX (unconjugated paclitaxel). Total systemic exposure to PPX (unconjugated paclitaxel) and standard paclitaxel are similar; however,  $C_{max}$  of PPX (unconjugated paclitaxel) is lower than equivalent doses of standard paclitaxel.

**Table 1** Preclinical tumor pharmacokinetics

	$C_{max}$ ( $\mu$ g/g)	$T_{max}$ (h)	$AUC_{last}$ ( $\mu$ g h/g)	MRT (h)
<b>[<math>^3</math>H]PPX</b>				
Total taxanes	72.0	4	4547	51
Paclitaxel	4.0	72	345	66
<b>[<math>^3</math>H]paclitaxel</b>				
Total taxanes	26.7	1.5	384	23
Paclitaxel	22.4	1.5	261	17

**Notes:**  $C_{max}$ , highest drug concentration;  $T_{max}$ , time at which highest drug concentration occurs following administration;  $AUC_{last}$ , area under the drug concentration-time curve; MRT, mean retention time.



**Figure 4** Preclinical tumor pharmacokinetics. To determine the tissue pharmacokinetic profile of PPX, female mice with subcutaneous B16 murine melanomas were given tritium-labeled paclitaxel 40 mg/kg intravenously, either as [ $^3$ H]paclitaxel in polyoxyethylated castor oil/ethanol or [ $^3$ H]PPX in phosphate buffer. Samples were collected from 0 to 144 hours; the concentration of extractable taxanes was determined by HPLC/MS.

## Metabolism

Anti-tumor activity requires the release of paclitaxel from the conjugate through the proteolysis of the polymeric backbone. Initially, two PPX metabolites, monoglutamyl-paclitaxel isomers Glu-2'-TXL (2'-[L- $\gamma$ -glutamyl]-paclitaxel) and Glu-7'-TXL (7'-[L- $\gamma$ -glutamyl]-paclitaxel), were identified in tumor tissue from B16 tumor-bearing mice exposed to [ $^3$ H]-labeled PPX. Subsequent pharmacokinetic analysis indicated a gradual increase of [ $^3$ H]-labeled PPX metabolites in tumor tissue, reaching  $T_{max}$  at 72 hours post-administration. In comparison, administration of [ $^3$ H]-paclitaxel resulted in a  $T_{max}$  at 1.5 hours. The gradual accumulation of PPX in tumor tissue was associated with prolonged tumor exposure to PPX metabolites Glu-2'-TXL and paclitaxel. These observations demonstrate the intra-tumoral degradation of the poliglutamate backbone of PPX in association with the release of paclitaxel (Singer et al 2005).

PPX biodegradation was further characterized in vitro and in vivo using qualitative and quantitative LC/MS analysis (Shaffer et al 2007). The intracellular, time-dependent generation of 5 PPX metabolites was observed in RAW 264.7 monocytes as well as the human HT-29 colon adenocarcinoma and NCI-H460 non-small lung carcinoma cell lines. In cell-free assays, mainly the carboxy exopeptidases, cathepsin B and X, could mediate PPX metabolism. In vitro, the release of conjugated paclitaxel was dependent on the activity of cathepsin B. Data from a cathepsin B deficient animal model confirmed that cathepsin B is an important in vivo mediator of PPX metabolism and the subsequent release of paclitaxel; however, other proteolytic pathways may contribute as well.

In normal physiological conditions, cathepsin B expression and activity is tightly regulated (Yan and Sloane 2003). In malignant tumors and premalignant lesions, cathepsin B expression is increased, which may also be associated with secretion and localization at the cell membrane (Poole et al 1978; Spiess et al 1994; Linebaugh et al 1999). Both intracellular and membrane-bound cathepsin B contributes to the degradation of the extracellular matrix, a crucial step in the process of tumor cell invasion (Szpaderska and Frankfater 2001; Premzl et al 2003).

## Clinical development

Performance status (PS) measures the impact of tumor-related symptoms and co-morbidities, such as co-existing illnesses and older age, on a patient's functional status; poor PS is defined by a score of 2 or more on the Eastern Cooperative Oncology Group (ECOG) performance scale. In NSCLC, poor PS is associated with a poor prognosis and an increased

vulnerability to chemotherapy-related toxicities (Sweeney et al 2001; Crinò et al 2002). In the absence of clinical trials enrolling significant numbers of poor PS patients, no standard of care has been established for the treatment of patients with advanced NSCLC and poor PS. When considering treatment options for patients with advanced NSCLC and poor PS, meaningful clinical benefit is not only determined by improved survival, but also symptom relief and tolerability (Blackhall et al 2005). Two phase III studies evaluated the efficacy and safety of PPX in chemotherapy-naïve patients with advanced NSCLC and a poor performance status. In STELLAR 3, PPX in combination with carboplatin was compared with paclitaxel in combination with carboplatin, and in STELLAR 4, PPX as a single agent was compared with physician's choice of either gemcitabine or vinorelbine. A third phase III study, STELLAR 2, compared PPX with docetaxel in patients with relapsed/refractory NSCLC. The primary efficacy endpoint for all three trials was survival, and PPX was shown to be as effective as current treatment options in NSCLC (Table 2).

Compared with docetaxel, PPX was associated with a favorable hematological toxicity profile, reducing the incidence of severe neutropenia and infection (Table 3). The reduced hematologic toxicity was clinically relevant as patients in the PPX arm showed a significant decrease in the requirement for supportive care (Figure 5). The non-hematologic toxicity profile of PPX is characterized by minimal hair loss, a distressing side-effect of chemotherapy associated with a lowered self-esteem and a negative body

**Table 2** PPX phase III efficacy summary (no statistically significant differences between the treatment arms were observed in STELLAR 2, 3, and 4)

STELLAR 3	PPX/Carbo	Pac/Carbo
N	199	201
Median OS (mo)	7.8	7.9
1-yr survival	31%	31%
2-yr survival	13%	11%
STELLAR 4	PPX	Gem or Vin
N	191	190
Median OS (mo)	7.3	6.6
1-yr survival	26%	26%
2-yr survival	15%	13%
STELLAR 2	PPX	Docetaxel
N	422	416
Median OS (mo)	6.9	6.9
1-yr survival	25%	29%
2-yr survival	9%	12%

**Abbreviations:** Carbo, carboplatin; gem, gemcitabine; pac, paclitaxel; vin, vinorelbine.

**Table 3** Hematologic toxicity profile: PPX vs docetaxel (STELLAR 2)

	PPX	Docetaxel	p value
Anemia, All	17%	26%	0.002
Grade 3/4 <sup>a</sup>	5%	4%	0.865
Neutropenia	21%	44%	<0.001
Grade 3/4 <sup>a</sup>	14%	37%	<0.001
Thrombocytopenia	7%	4%	0.077
Grade 3/4 <sup>a</sup>	2%	<1%	0.090
Febrile Neutropenia <sup>a</sup>	2%	6%	0.002
Infection	25%	32%	0.032
Grade 3/4 <sup>a</sup>	7%	11%	0.088

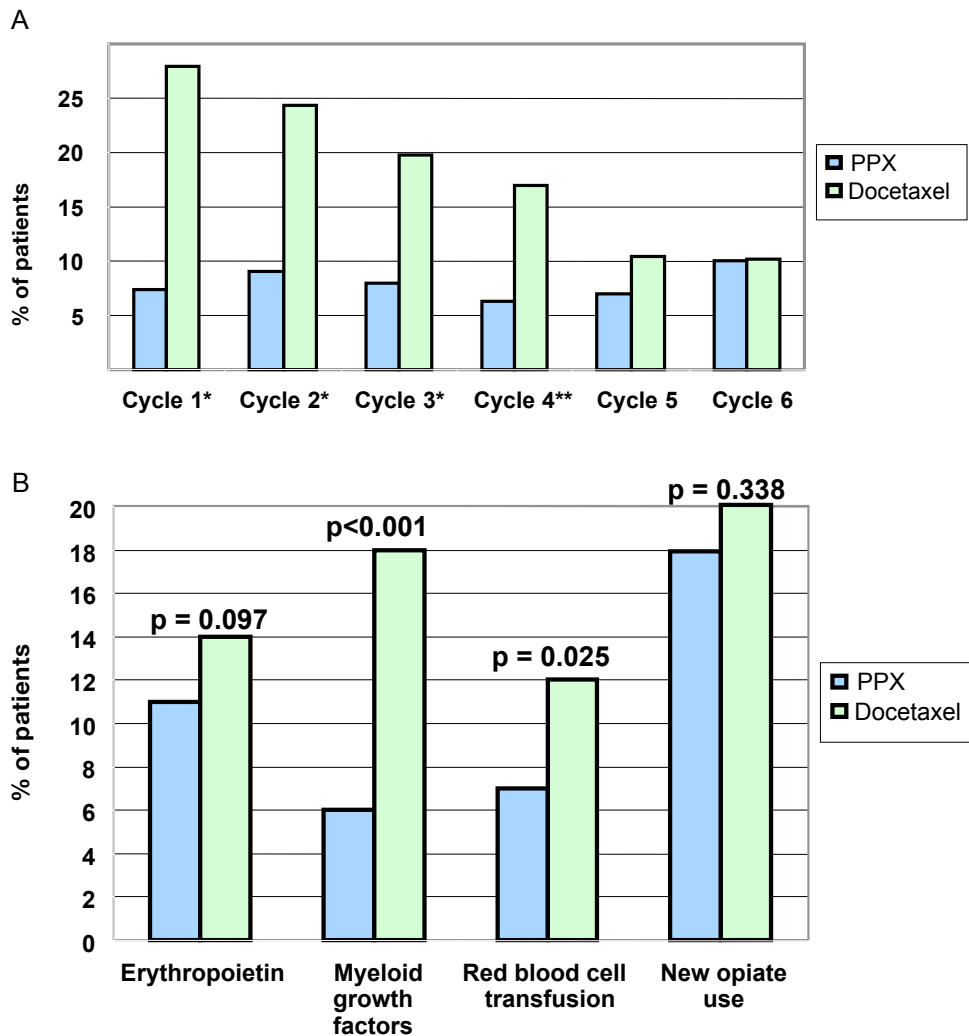
<sup>a</sup>Reported adverse events.

image. In general, PPX-related non-hematologic severe (grade 3 or 4) adverse events are similar to those of other taxanes, with dose-related neuropathy as the most clinically important issue (Langer et al 2005; O’Byrne 2005a, b).

### Future directions

In the phase III studies, chemo-naïve patients receiving PPX had similar overall survival compared with patients in the control arms. However, in STELLAR 4, a trend towards improved survival was noted for female patients receiving PPX compared with female patients in the control arm: 49 women received PPX and 56 received gemcitabine or vinorelbine; the median survival for females in the PPX arm was 312 days compared with 209 days for females in the control arm (log rank  $p=0.069$ ; hazard ratio=0.65). In contrast, survival was similar for male patients, regardless of treatment, indicating a potential gender-specific benefit associated with PPX for the treatment of lung cancer (Ross 2006).

The comparison of lung tumors from male and female patients has identified various tumor characteristics that may affect tumor etiology in a gender-specific manner (Patel 2005; Thomas et al 2005). Particularly, lung tumorigenesis



**Figure 5** Clinical benefits of reduced myelosuppression. A: Percentage of patients with grade 3/4 neutropenia (by treatment cycle) in STELLAR 2. B: Reduced need for supportive measures to manage effects of myelosuppression. \* $p < 0.0001$ ; \*\* $p < 0.01$ .

may be modulated depending on exposure to estrogen. An epidemiologic link between estrogen exposure and incidence of NSCLC has been suggested in Japanese non-smoking women (Liu et al 2005). Younger, presumably pre-menopausal, women appear to have shorter survival than older women: in an analysis of patients with advanced NSCLC enrolled in Southwest Oncology Group (SWOG) trials, women over 70 had a 34% 1-year survival compared with 11% for those under 45 (Albain et al 1991).

Estrogen-induced cell proliferation is a critical step in the etiology and progression of a variety of tumor types (Deroo and Korach 2006). The cellular response to estrogen is mediated by estrogen receptor alpha (ER-alpha) and ER-beta. These receptor proteins function as ligand-dependent transcription factors and regulate the expression of genes implicated in cell cycle control, signal transduction, and cell survival (Frasor et al 2003; Edwards 2005). In animal models, estrogen plays a role in normal pulmonary physiology (Massaro and Massaro 2004, 2006); adult females have a larger number of alveoli that are smaller in size, than males. This difference develops as animals reach sexual maturity and seems to be mediated mainly by estrogens (Massaro et al 1996). In vivo studies show that ER-beta is abundantly expressed in lung tissue and controls the transcription of platelet-derived growth factor A (PDGF-A), which plays a pivotal role in alveolar formation, and granulocyte macrophage colony-stimulating factor (GM-CSF), a key regulator of surfactant homeostasis (Patrone et al 2003). ER-beta, and to a lesser extent ER-alpha, are expressed in lung tumors from both men and women; in a large series of surgically resected NSCLC tumors, ER-beta was detected in 45.8% of cases. Overexpression of ER-beta was significantly more common in tumors from non-smokers (53.5%) than smokers (36.6%,  $p < 0.004$ ). Among non-smokers, higher ER-beta expression was observed significantly more frequently in female patients (58.3%) than in male patients (40.9%) (Wu et al 2005). The complex biological effects

mediated by ERs involve communication between many proteins and signaling pathways, including lysosomal vesicle trafficking.

Under the control of estrogen, intracellular trafficking of cathepsin B may be altered in malignant tumors, resulting in the increased secretion of precursor and active forms of the enzyme (Poole et al 1978; Achkar et al 1990; Linebaugh et al 1999), its redistribution from perinuclear lysosomes to peripheral vesicles (Sloane et al 1994), and its association with the cell membrane (Spiess et al 1994; Sameni et al 1995; Cavallo-Medved et al 2005). Cathepsin B localizes with other proteases at the tumor cell surface in caveolae, mediating cell-surface proteolytic events associated with invasion (Roshy et al 2003; Cavallo-Medved et al 2005). The abundance of cathepsin B in tumor tissue is relevant for the efficient degradation of PPX. The interaction of lung cancer cells and infiltrating immune cells, particularly phagocytic monocytes, can further promote tumor development and metastasis. Homeostasis of human monocytes is regulated by estrogen, and monocytes are known to express both ER-alpha and ER-beta (Phiel et al 2005). Interestingly, cathepsin X is upregulated in tumor-infiltrating immune cells, particularly in phagocytic monocytes (Kos et al 2005).

## Summary

Factors limiting the therapeutic index of paclitaxel include low solubility, high systemic exposure, poor pharmacokinetic characteristics, and a lack of selective tumor uptake. The rationale for developing paclitaxel poliglumex (PPX), a macromolecular polymer–drug conjugate, was to improve standard chemotherapy with paclitaxel by overcoming some of these limitations (Table 4). Tumor pharmacokinetics show that tumor distribution is faster with paclitaxel, but overall tumor exposure is higher with PPX. The available data support a model in which PPX accumulates in tumor tissue through the EPR effect, followed by the cathepsin B-mediated release of paclitaxel.

**Table 4** Paclitaxel vs PPX

Characteristic	Paclitaxel	PPX
Solubility	Requires toxic solubilizing agents	Water-soluble
Administration	3- to 24-hr infusion with routine premedications	10- to 20-minute infusion, no routine premedication
Systemic exposure	Yes	Reduced $C_{max}$ ; gradual paclitaxel release from inactive drug conjugate
Pharmacokinetics	Short elimination half-life	Prolonged distribution phase, elimination phase with long terminal half-life
Tumor selectivity	No	Passive tumor accumulation



The cathepsin-mediated release of paclitaxel may have therapeutic implications as cathepsin B is upregulated in malignant cells, particularly during tumor progression. Estrogen may play an important role in lung tumor biology and is a modulator of cathepsin B activity. A possible association between estrogen levels and PPX activity will be further explored in both preclinical and clinical studies.

## References

- Achkar C, Gong QM, Frankfater A, et al. 1990. Differences in targeting and secretion of cathepsins B and L by BALB/3T3 fibroblasts and Moloney murine sarcoma virus-transformed BALB/3T3 fibroblasts. *J Biol Chem*, 265:13650-4.
- Albain KS, Crowley JJ, Leblanc M, et al. 1991. Survival determinants in extensive-stage non-small-cell lung cancer: the Southwest Oncology Group experience. *J Clin Oncol*, 9:1618-26.
- Bala I, Hariharan S, Kumar MN 2004. PLGA nanoparticles in drug delivery: the state of the art. *Crit Rev Ther Drug Carrier Syst*, 21:387-422.
- Bernareggi A, Oldham F, Baker B, et al. 2005. XYOTAX™ (paclitaxel poliglumex, PPX): tumor accumulation and prolonged exposure to paclitaxel. 11th World Congress on Lung Cancer. Barcelona, Spain.
- Bhalla KN. 2003. Microtubule-targeted anticancer agents and apoptosis. *Oncogene*, 22:9075-86.
- Blackhall FH, Bhosle J, Thatcher N. 2005. Chemotherapy for advanced non-small cell lung cancer patients with performance status 2. *Curr Opin Oncol*, 17:135-9.
- Cavallo-Medved D, Mai J, Dosesco J, et al. 2005. Caveolin-1 mediates the expression and localization of cathepsin B, pro-urokinase plasminogen activator and their cell-surface receptors in human colorectal carcinoma cells. *J Cell Sci*, 118:1493-503.
- Crinò L, Migliorino M. 2002. A phase III randomized trial comparing three platinum-based doublets in advanced non-small cell lung cancer (NSCLC): impact of PS = 2 vs 0 or 1 and age >70 vs <70 on chemotherapy outcome. *Proc Am Soc Clin Oncol*, 21:315a.
- Deroo BJ, Korach KS. 2006. Estrogen receptors and human disease. *J Clin Invest*, 116, 561-70.
- Duncan R. 1992. Drug-polymer conjugates: potential for improved chemotherapy. *Anticancer Drugs*, 3:175-210.
- Duncan R. 2003. The dawning era of polymer therapeutics. *Nat Rev Drug Discov*, 2:347-60.
- Edwards DP. 2005. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol*, 67:335-76.
- Frasor J, Danes JM, Komm B, et al. 2003. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. *Endocrinology*, 144:4562-74.
- Gelderblom H, Verweij J, Nooter K, et al. 2001. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer*, 37:1590-8.
- Gerlowski LE, Jain RK. 1986. Microvascular permeability of normal and neoplastic tissues. *Microvasc Res*, 31:288-305.
- Greish K, Fang J, Inutsuka T, et al. 2003. Macromolecular therapeutics: advantages and prospects with special emphasis on solid tumour targeting. *Clin Pharmacokinet*, 42:1089-105.
- Gueritte-Voegelein F, Guenard D, Lavelle F, et al. 1991. Relationships between the structure of taxol analogues and their antimetabolic activity. *J Med Chem*, 34:992-8.
- Kos J, Sekirnik A, Premzl A, et al. 2005. Carboxypeptidases cathepsins X and B display distinct protein profile in human cells and tissues. *Exp Cell Res*, 306:103-13.
- Kumar GN, Walle UK, Bhalla KN. et al. 1993. Binding of taxol to human plasma, albumin and alpha 1-acid glycoprotein. *Res Commun Chem Pathol Pharmacol*, 80:337-44.
- Langer CJ, Socinski MA, Ross H, et al. 2005. Paclitaxel poliglumex (PPX)/carboplatin vs paclitaxel/carboplatin for the treatment of PS2 patients with chemotherapy-naïve advanced non-small cell lung cancer (NSCLC): A phase III study. *J Clin Oncol, ASCO Annual Meeting Proceedings*, 23:7011.
- Langer R. 1998. Drug delivery and targeting. *Nature*, 392:5-10.
- Linebaugh BE, Sameni M, Day NA, et al. 1999. Exocytosis of active cathepsin B enzyme activity at pH 7.0, inhibition and molecular mass. *Eur J Biochem*, 264:100-9.
- Liu Y, Inoue M, Sobue T. et al. 2005. Reproductive factors, hormone use and the risk of lung cancer among middle-aged never-smoking Japanese women: a large-scale population-based cohort study. *Int J Cancer*, 117:662-6.
- Manfredi JJ, Parness J, Horwitz SB. 1982. Taxol binds to cellular microtubules. *J Cell Biol*, 94:688-96.
- Markman M. 2003. Managing taxane toxicities. *Support Care Cancer*, 11:144-7.
- Massaro D, Massaro GD. 2004. Estrogen regulates pulmonary alveolar formation, loss, and regeneration in mice. *Am J Physiol Lung Cell Mol Physiol*, 287:L1154-9.
- Massaro D, Massaro GD. 2006. Estrogen receptor regulation of pulmonary alveolar dimensions: alveolar sexual dimorphism in mice. *Am J Physiol Lung Cell Mol Physiol*, 290:L866-70.
- Massaro GD, Mortola JP, Massaro D. 1996. Estrogen modulates the dimensions of the lung's gas-exchange surface area and alveoli in female rats. *Am J Physiol*, 270:L110-4.
- Matsumura Y, Maeda H. 1986. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res*, 46:6387-92.
- O'Byrne KJ. 2005a. Paclitaxel poliglumex vs. gemcitabine or vinorelbine for the treatment of performance status (PS) 2 patients with chemotherapy-naïve advanced non-small cell lung cancer (NSCLC): the STELLAR 4 phase III study. *Eur J Cancer Suppl*, 3:324.
- O'Byrne KJ. 2005b. Paclitaxel poliglumex vs. docetaxel for the second-line treatment of non-small cell lung cancer (NSCLC): the STELLAR 2 phase III study. *Eur J Cancer Suppl*, 3:5.
- Patel JD. 2005. Lung cancer in women. *J Clin Oncol*, 23:3212-8.
- Patrone C, Cassel TN, Pettersson K, et al. 2003. Regulation of postnatal lung development and homeostasis by estrogen receptor beta. *Mol Cell Biol*, 23:8542-52.
- Phiel KL, Henderson RA, Adelman SJ, et al. 2005. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol Lett*, 97:107-13.
- Poole AR, Tiltman KJ, Recklies AD, et al. 1978. Differences in secretion of the proteinase cathepsin B at the edges of human breast carcinomas and fibroadenomas. *Nature*, 273:545-7.
- Premzl A, Zavasnik-Bergant V, Turk V, et al. 2003. Intracellular and extracellular cathepsin B facilitate invasion of MCF-10A neoT cells through reconstituted extracellular matrix in vitro. *Exp Cell Res*, 283:206-14.
- Roberts WG, Palade GE. 1997. Neovasculature induced by vascular endothelial growth factor is fenestrated. *Cancer Res*, 57:765-72.
- Roshy S, Sloane BF, Moin K. 2003. Pericellular cathepsin B and malignant progression. *Cancer Metastasis Rev*, 22:271-86.
- Ross H, Bonomi P, Langer C, et al. 2006. Effect of gender on outcome in two randomized phase III trials of paclitaxel poliglumex (PPX) in chemo-naïve pts with advanced NSCLC and poor performance status (PS2). *J Clin Oncol, ASCO Annual Meeting Proceedings*, 24:7039.
- Rowinsky EK, Donehower RC. 1995. Paclitaxel (taxol). *N Engl J Med*, 332:1004-14.
- Sameni M, Elliott E, Ziegler G, et al. 1995. Cathepsin B and D are localized at the surface of human breast cancer cells. *Pathol Oncol Res*, 1:43-53.



- Seidman AD. 2005. "Will weekly work"? Seems to be so. *J Clin Oncol*, 23:5873-4.
- Shaffer SA, Lee-Baker C, Kennedy, K., et al. 2007. In vitro and in vivo metabolism of paclitaxel poliglumex: Identification of metabolites and active proteases. *Cancer Chemother Pharmacol*. In press.
- Singer JW, Shaffer S, Baker B, et al. 2005. Paclitaxel poliglumex (XYOTAX; CT-2103): an intracellularly targeted taxane. *Anticancer Drugs*, 16:243-54.
- Sloane BF, Moin K, Sameni M, et al. 1994. Membrane association of cathepsin B can be induced by transfection of human breast epithelial cells with c-Ha-ras oncogene. *J Cell Sci*, 107:373-84.
- Sonnichsen DS, Relling MV. 1994. Clinical pharmacokinetics of paclitaxel. *Clin Pharmacokinet*, 27:256-69.
- Spiess E, Bruning A, Gack S, et al. 1994. Cathepsin B activity in human lung tumor cell lines: ultrastructural localization, pH sensitivity, and inhibitor status at the cellular level. *J Histochem Cytochem*, 42:917-29.
- Sweeney CJ, Zhu J, Sandler, et al. 2001. Outcome of patients with a performance status of 2 in Eastern Cooperative Oncology Group Study E1594: a Phase II trial in patients with metastatic nonsmall cell lung carcinoma. *Cancer*, 92:2639-47.
- Szpadarska AM, Frankfater A. 2001. An intracellular form of cathepsin B contributes to invasiveness in cancer. *Cancer Res*, 61:3493-500.
- Thomas L, Doyle LA, Edelman MJ. 2005. Lung cancer in women: emerging differences in epidemiology, biology, and therapy. *Chest*, 128:370-81.
- Wu CT, Chang YL, Shih JY, et al. 2005. The significance of estrogen receptor beta in 301 surgically treated non-small cell lung cancers. *J Thorac Cardiovasc Surg*, 130:979-86.
- Yan S, Sloane BF. 2003. Molecular regulation of human cathepsin B: implication in pathologies. *Biol Chem*, 384:845-54.