Liposomal encapsulated anti-cancer drugs

Ralf-Dieter Hofheinz[a], Senta Ulrike Gnad-Vogt[a], Ulrich Beyer[b] and Andreas Hochhaus[a]

Among several drug delivery systems, liposomal encapsulated anti-cancer agents represent an advanced and versatile technology. Several formulations of liposomal anthracyclines are approved, e.g. for the treatment of metastatic breast cancer (pegylated and non-pegylated liposomal doxorubicin) or AIDS-related Kaposi's sarcoma (pegylated liposomal doxorubicin and liposomal daunorubicin). Meanwhile, virtually all anti-cancer drugs have been encapsulated in liposomes using different technologies. This review will summarize preclinical and clinical data of approved and exemplary emerging liposomal anti-cancer agents. *Anti-Cancer Drugs* 16:691–707 © 2005 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2005, **16**:691–707

[a]Onkologisches Zentrum, III. Medizinische Klinik, Fakultät für Klinische Medizin Mannheim, Universität Heidelberg, Mannheim, Germany and [b]medac GmbH, Wedel, Germany.

Correspondence to R.-D. Hofheinz, Onkologisches Zentrum, III. Medizinische Klinik, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg, Theodor-Kutzer-Ufer 1–3, 68167 Mannheim, Germany.
Tel: +49 621 383 2855; fax: +49 621 383 3833;
e-mail: ralf.hofheinz@med3.ma.uni-heidelberg.de

Received 20 April 2005 **Accepted** 2 May 2005

Introduction

A variety of novel drug delivery systems have been developed in an attempt to address some of the problems associated with the lack of tumor selectivity and stability of conventional cytostatic drugs. Of these, liposomal drug carrier systems represent an advanced, mature and versatile technology, and several liposomal formulations of anti-cancer drugs have been approved for cancer chemotherapy or are in advanced stages of clinical development (Table 1).

Liposomes are self-assembling closed colloid structures composed of lipid bilayers. They were first described by Bangham *et al.* in the mid-1960s and were originally called *phospholipid spherules* [1]. Initially, they were used as a model system to study biological membranes. The term *liposomes* was introduced in 1968 by Sessa and Weissman [2]. The potential value as vehicles for targeted drug delivery for a broad spectrum of disease was recognized early.

Liposomes can be classified in two ways. (i) *Classification according to lamellarity and size* [3]. Unilamellar vesicles comprising one lipid bilayer have diameters of 50–250 nm. They contain a large aqueous core and are used for the encapsulation of water-soluble drugs. Multilamellar vesicles composed of several concentric lipid bilayers in an onion-skin arrangement have diameters of 1–5 μm. The high lipid content allows these multilamellar vesicles to passively entrap lipid-soluble drugs. (ii) *Classification according to a phylogenetic scheme* [4]. Classical or conventional liposomes (i.e. simple mixtures of phospholipids and cholesterol) target the reticuloendothelial system (RES) and are called *RES-targeted liposomes*. Vesicle size in liposomes of similar lipid composition is usually inversely correlated with the amount of RES uptake [5]. While this strategy has several advantages, and various strategies to exploit this phenomenon are in use, in some aspects the RES is considered an obstacle to more specific drug delivery. This led to several approaches to develop *RES-avoiding* and sterically stabilized, surface-modified liposomes (e.g. by coating liposomes with polyethylene glycol; STEALTH technology). For a detailed overview of the preclinical development and pharmaceutical properties of liposomes, see [6,7].

Liposomal formulations aim at reducing the toxic side-effects of conventional cytostatic drugs without hampering the efficacy. From a theoretical point of view these goals may be reached by two strategies: (i) the encapsulated drug is impeded from reaching healthy tissue ('site avoidance') and/or (ii) drug concentrations are mainly delivered to neoplastic tissue ('drug targeting'). *Active targeting* occurs by coating liposomes with antibodies ('immunoliposomes') or ligands in order to specifically recognize epitopes or receptors of malignant cells. *Passive targeting* is a result of the physicochemical properties of liposomes which may lead to preferential accumulation in tumor tissue.

After administration, RES-avoiding pegylated liposomes, for instance, circulate in the plasma for several days without being opsonized by plasma proteins and recognized by the immune system (overview in [8]). In most normal tissues uptake of particles from the blood stream

0959-4973

Table 1 Approved and examplary emerging liposomal encapsulated anti-cancer drugs

Ecapsulated drug	Company	Indication	Status
PLD (Doxil, Caelyx)	Essex (Europe), Ortho Biotech (US)	ovarian and breast cancer, Kaposi's sarcoma	approved
NPLD (Myocet)	Medeus	breast cancer	approved (Europe)
DNX (DaunoXome)	Gilead	Kaposi's sarcoma	approved
Mitoxantrone	NeoPharm	advanced cancer	phase I/II
Pegylated liposomal cisplatin (SPI-077)	Sequus	advanced cancer	phase I/II
Cisplatin (Lipoplatin)	Regulon	e.g. lung cancer	phase III
Oxaliplatin (Aroplatin)	Antigenics	e.g. colorectal cancer	phase II
Vincristine (Marqibo)	Inex/Enzon	non-Hodgkin's lymphoma, acute lymphoblastic leukemia, Hodgkin's lymphoma	phase II/III
Cytarabine (DepoCyte)	Enzon/Skye Pharma	lymphomatous meningitis	approved (intrathecal application)
All-*trans* retinoic acid (Atragen)	Aronex	leukemia, non-Hodgkin's lymphoma	phase I/II
Lurtotecan (OSI-211)	OSI	ovarian cancer, small cell lung cancer	phase III
Irinotecan metabolite SN 38	NeoPharm	colorectal and lung cancer	phase I/II
Topotecan TCS (INX-0076)	Inex	advanced cancer	phase I/II
Paclitaxel LEP ETU	NeoPharm	breast, lung, ovarian cancer	phase I/II
Paclitaxel (EndoTAG-1; formerly: MBT-0206)	MediGene	pancreatic and prostate cancer	phase II
Vinorelbine (INX-0125)	Inex	advanced cancer	phase I

is limited by a continuous, non-fenestrated endothelium of the vasculature. Tight endothelial junctions (around 5 nm) between endothelial cells prevent extravasations of even small unilamellar vesicles. Furthermore, the basal lamina inhibits the extravasation of macromolecules. In contrast, tissues with sinusoids (like the spleen) or fenestrated capillaries (bone marrow or liver), or tumor tissue with abnormally formatted blood vessels, are porous and allow the escape of macromolecules with diameters up to 100 nm. In particular, tumor vessels are inherently leaky and intact liposomes extravasate through the fenestrae into the interstitial space, lodging between tumor cells. Drug molecules are then released from the extravasated liposomes and enter tumor cells. This passive targeting, relying on enhanced permeability and retention (EPR 'effect') of molecules in the microenvironment of the tumor, has been demonstrated experimentally with long-circulating liposomes. This might be achieved by pegylation, for example.

Another option of passive targeting is based on the fact that the environment of tumor cells may show a decreased pH value due to the hypoxic metabolism. pH-sensitive liposomes were designed to be stable at pH values of 7.2, but degraded at lower pH [9]. Furthermore, thermolabile liposomes have been made which can be preferentially guided by local hyperthermia towards tumor tissue [10]. Another mechanism of passive targeting is the development of cationic liposomes, which show an affinity towards endothelial cells of newly sprouting tumor vessels, suggesting a possible targeting of malignant angiogenesis [11].

Approved and emerging formulations of liposomes differ substantially with regard to production technology, composition of the lipid layers and consequently pharmacokinetics as well as efficacy. The improvement of liposome technology is an ongoing process and liposomal formulations of virtually all anti-cancer drugs are under investigation. This review will – without aiming to be encyclopedic – primarily focus on the clinical data of established and exemplary emerging liposomal anti-cancer drugs.

Liposomal anthracyclines

Three liposomal formulations of anthracyclines are available in the clinic: pegylated liposomal doxorubicin (PLD; Doxil in the US, Caelyx outside the US), non-pegylated liposomal doxorubicin (NPLD; Myocet) and liposomal daunorubicin (DNX; DaunoXome). They differ in terms of primary target, liposome size and lipid composition, rate of drug release, liposome technology, and the encapsulated drug (Table 2). These differences result in different pharmacokinetic properties, toxicity profiles and dosages. Therefore, especially in the case of PLD and NPLD, it is impossible to extrapolate clinical results from one product to another.

The special interest in liposomal encapsulation of anthracyclines derives from the fact that this group of cytostatics bears the risk of acute and, more importantly, cumulative cardiotoxicity [12]. Congestive cardiomyopathy has been associated with high peak plasma concentrations of anthracyclines as well as with the cumulative ('lifetime') dose administered. Strategies to overcome this dilemma focused on: (i) prolonged, continuous anthracycline administration avoiding high plasma peaks [13], (ii) the co-administration of dexrazoxan to prevent free radical formation [14], (iii) the development of newer anthracycline- or anthracenedione analogs [15,16] and (iv) the liposomal encapsulation of anthracyclines in order to alter the pharmacokinetics with the aim of improving the therapeutic index.

Table 2 Characteristics of doxorubicin and liposomal encapsulated anthracyclines

	Doxorubicin [124]	PLD [125]	NPLD [124]	DNX [46]
Liposome diameter (nm)	NA	100	150	45
Layers	NA	unilamellar plus PEG	unilamellar	unilamellar
Lipid composition	NA	soy-bean-derived phosphatidyl-choline, cholesterol, PEG	egg-yolk-derived phosphatidyl-choline, cholesterol	Distearoyl-phosphatidylcholine, cholesterol
RES	NA	avoiding	avoiding	targeting
Pegylation	NA	yes	no	no
Half-life (h)	0.2	41–70	2.0–3.0	5.1
Clearance (ml/h)	46409	16–29	4880	1260
AUC (μg/ml·h)	3.81	902	46	62
Safety (versus doxorubicin/daunorubicin)				
alopecia	NA	reduced	slightly reduced	reduced
cardiotoxicity	NA	reduced	reduced	reduced
myelosuppression	NA	slightly reduced	dose limiting	dose limiting
PPE	NA	+ +, dose-limiting	rarely observed	rarely observed
approved indications	broad variety of tumors	first-line monotherapy of breast cancer (US, EU); platinum-pre-treated ovarian cancer, HIV-associated Kaposi's sarcoma	first-line therapy breast cancer (+ cyclophosphamide)	HIV-associated Kaposi's sarcoma (first-line therapy)

PLD

PLD consists of doxorubicin encapsulated in small unilamellar vesicles with a diameter of about 100 nm. The bilayer comprises hydrogenated soy phosphatidyl-choline and cholesterol as well as *N*-(carbonyl-methoxy-polyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (PEG-DSPE). The PEG-DSPE coating ('pegylation' using STEALTH technology) is the unique feature that prevents opsonization by plasma proteins and diminishes the uptake by the RES (RES-avoiding liposome). The sterical stabilization adds to longer plasma circulation, resulting in decreased plasma clearance and higher area-under-the-curve (AUC) values compared with NPLD. Extravasation through capillary leaks occurs preferentially in areas with fenestrated microvessels (e.g. tumor tissue). Given as monotherapy, a dose of 40–50 mg/m^2 PLD applied over 1 h at intervals of 4 weeks is usually recommended. The main toxicities of PLD are the palmar–plantar skin reaction (PPE) and stomatitis/mucositis. In comparison with conventional doxorubicin, equieffective doses of PLD show a decreased rate of cardiotoxicity, myelosuppression, alopecia and nausea/vomiting.

Three major indications of PLD have emerged during the past decade: ovarian and breast cancer, as well as AIDS-related Kaposi's sarcoma.

In phase II trials including 146 patients with platinum/paclitaxel (± topotecan)-pretreated *ovarian cancer*, a cumulative response rate of 14.4% has been observed and a further 36.3% of patients had stable disease upon treatment with PLD [17]. Consequently, PLD was compared with topotecan in a randomized phase III trial in patients with pretreated epithelial ovarian cancer that failed or recurred upon platinum-based combination chemotherapy [18]. Patients were randomly assigned to receive either PLD 50 mg/m^2 (day 1, 1-h infusion every 4 weeks) or topotecan 1.5 mg/m^2 (days 1–5, 30-min infusion every 3 weeks). The primary study endpoint was progression-free survival (PFS). Baseline demographic data, disease characteristics and risk stratification were well balanced between both arms. A total of 474 patients was assessable. The first analysis in 2001 [18] showed that the response rates did not differ significantly (PLD 19.7%; topotecan 17.0%) and the overall PFS was comparable between both patient groups (PLD 16.1 weeks; topotecan 17.0 weeks). The PLD subgroup of patients with platinum-sensitive patients had a statistically longer PFS (28.9 versus 23.2 weeks) and overall survival (108 versus 72 weeks). An update of this study with mature follow-up ($n = 413$ deaths) confirmed these data [19]. An 18% reduction of the risk of deaths for the PLD group was described [hazard ratio (HR) 1.216; 95% confidence interval (CI) 1.000–1.478, $p = 0.05$]. The risk reduction in the subgroups of platinum-sensitive patients was calculated as 30% (HR 1.432; 95% CI 1.066–1.923, $p = 0.017$). The toxicity profile differed, with PLD preferentially causing stomatitis in 40% (8% CTC grades 3–4) and skin toxicity in 49% (23% CTC grades 3–4) of patients. In contrast, the use of topotecan was associated with pronounced hematotoxicity [leukopenia 63% (50% grades 3–4), thrombocytopenia 65% (34% grades 3–4)], and led to a significantly higher rate of dose reductions (52 versus 27%) and a higher proportion of patients requiring treatment with hematopoietic growth factors (29 versus 5%). Overall, 71% of patients treated with topotecan, but only 17% treated with PLD, were reported to have a grade 4 adverse event. Overall, this study established the usefulness of PLD as first-choice salvage regimen in platinum pretreated ovarian cancer.

Another phase III trial compared PLD 50 mg/m^2 (every 4 weeks) with paclitaxel 175 mg/m^2 (every 3 weeks) in patients with relapse after first-line platinum-based chemotherapy without prior taxane treatment [20]. A total of 214 patients were recruited into the study.

Neither the response rates (PLD versus paclitaxel: 17.8 versus 22.4%) nor PFS (21.7 versus 22.4 weeks) and overall survival (45.7 versus 56.1 weeks) data were statistically significantly different. Considering all grades of toxicity, PLD significantly improved the rate of arthralgia/myalgia, paresthesia and alopecia, while skin toxicity and stomatitis occurred significantly more often in the PLD group. The rates of hematotoxicity and gastrointestinal adverse events were comparable. In conclusion, the results of this study suggest that PLD may be considered an alternative to paclitaxel in non-taxane, but platinum pretreated patients with ovarian cancer.

Current efforts focus on PLD combination treatment either as part of intensified first-line [21], second-line [22,23] or as monotherapy in consolidation regimen [24].

Anthracyclines are among the most effective drugs in the adjuvant or palliative treatment of *breast cancer* [25]. Thus, the intensive clinical research done in this tumor with either liposomal formulation, PLD and NPLD, may not be surprising. PLD at varying doses (3-weekly 45–60 mg/m^2 or 4-weekly 45 mg/m^2) has demonstrated substantial activity in a multicenter phase II trial in patients without or with one previous regimen for advanced breast cancer: 31% objective remissions were noted and an additional 31% of patients had a tumor stabilization upon PLD [26]. Further phase II studies with PLD administered as first- or second-line therapy confirmed these results [27,28].

Two pivotal phase III studies published in 2004 have established the role of PLD in the treatment of advanced breast cancer. The first trial, conducted by Keller *et al.* [29], randomized 301 patients with taxane-resistant breast cancer between PLD 50 mg/m^2 and either weekly vinorelbine 30 mg/m^2 or vinblastine 5 mg/m^2 (day 1, repeated every other week for 2 cycles and beginning with cycle 3 on day 22) in combination with mitomycin C 10 mg/m^2 (day 1 repeated every 6–8 weeks depending on hematological recovery). The choice of the comparator regimen was left to each center, but this regimen had to be used in all patients recruited onto the study. The primary efficacy variable was PFS. A considerable proportion of poor-prognosis patients were allocated: 18% had a Karnofsky index below 70%, 30% had three or more metastatic sites, 41% had two or more chemotherapy regimens for advanced disease and 37% had anthracycline resistance (progression while receiving or within 6 months after completing an anthracycline-containing regimen).

The PFS values for PLD (2.9 months) and the comparator regimens (2.5 months) did not show any statistical difference ($p = 0.11$) and the overall survival was comparable (PLD 10.4 versus comparator regimens 9.0 months; $p = 0.57$). Secondary efficacy variables such as response rate (PLD 10 versus comparator 12%), response duration (PLD 5.7 versus comparator 6.0 months) and clinical benefit rate (PLD 10 versus comparator 7.9%) were almost identical. The safety profile observed in this study with PLD compared well with previous trials. In addition to skin toxicity (37% all grades; grade 3–4 18%), the rate of stomatitis (22 versus 4% all grades) and mucositis (14 versus 1%) was higher in the PLD arm, while the lower rate of myelosuppression (neutropenia 3 versus 14%) favored the PLD regimen. Of note, 83% of the patients had been pretreated with anthracyclines and 37% entered the study with primary anthracycline-resistant disease. However, PLD exhibited some efficacy even in anthracycline-resistant patients, indicating that PLD is not completely cross-resistant to anthracyclines. The analysis of subgroups revealed that in anthracycline-naive patients the PFS was longer with PLD relative to comparator regimens (5.8 versus 2.1 months; $n = 44$). Overall, the efficacy of the convenient monthly PLD regimen was comparable with commonly used salvage regimens for taxane-refractory metastatic breast cancer. PLD may serve as a salvage regimen especially in anthracycline-naive patients with taxane pretreatment.

The second phase III trial conducted with PLD was performed in the first-line setting in 509 women with metastatic breast cancer [30]. Patients received either conventional doxorubicin 60 mg/m^2 every 3 weeks or PLD 50 mg/m^2 every 4 weeks. The primary endpoints of this study were to test: (i) non-inferiority of PLD regarding the PFS and (ii) whether significantly less cardiotoxicity was observed with PLD as defined by clinical parameters as well as multiple-gated acquisition (MUGA) scan. Both groups were well balanced with regard to demographic and disease characteristics; 15% (PLD group) and 16% (doxorubicin group) of patients had received prior adjuvant anthracyclines. The median PFS values of 6.9 (PLD) and 7.8 months (doxorubicin group) were not statistically different (HR 1.00; 95% CI 0.81–1.20). Overall survival was 21 months with PLD and 22 months with conventional doxorubicin (HR 0.92; 95% CI 0.74–1.19). Of 410 patients with measurable disease, overall response (PLD 33%, doxorubicin 38%) as well as disease stabilization rates (25% in each arm) were comparable. Skin toxicity (mainly PPE) occurred in 48% of patients treated with PLD. Nausea and vomiting were more often associated with doxorubicin treatment, while stomatitis and mucositis (23 and 22%, all grades, respectively) occurred more often in the PLD arm (doxorubicin 13 and 15%, respectively). Pronounced or total hair loss was reported in 7% of PLD patients compared with 54% of doxorubicin patients. Grade 3 and 4 leukopenia was observed in 9% of patients treated with doxorubicin and in 1% treated with PLD.

In all, PLD reduced some of the adverse events observed under conventional doxorubicin, especially hair loss and leukopenia, at the cost of skin toxicity and a higher rate of stomatitis/mucositis. Nevertheless, the favorable cardiac safety profile as discussed below of PLD makes it a good alternative drug choice for patients at cardiac risk or after adjuvant anthracycline treatment. Current studies focus on the use of PLD in the adjuvant setting as well as in combination with trastuzumab for HER-2 overexpressing breast cancer [31] in order to minimize the known cardiotoxicity associated with the combination of free doxorubicin and trastuzumab [32].

Furthermore, several combination studies with PLD and cyclophosphamide, paclitaxel or docetaxel, vinorelbine and gemcitabine have been conducted (reviewed in [25]), and randomized studies are awaited to define the potential benefit of these combinations in the sequence therapy of advanced breast cancer.

Kaposi's sarcoma is still considered a major problem among patients with acquired immunodeficiency syndrome (AIDS), although its incidence has dramatically declined since highly active antiretroviral therapy (HAART) has become standard of care for HIV-infection (reviewed in [33]). A standard therapy of this sarcoma cannot be defined; chemotherapy with doxorubicin, bleomycin, etoposide and vinca alkaloids induces tumor shrinkage [34]. Initially, PLD was used in AIDS-related Kaposi's sarcoma in escalating doses. Later on, PLD 20 mg/m^2 (repeated day 15 or 22) became the recommended dose to avoid myelosuppression, especially neutropenia. Northfelt *et al.* investigated PLD after failure or intolerance of standard chemotherapy (bleomycin, vincristine ± doxorubicin; i.e. ABV or BV regimen) in AIDS-related Kaposi's sarcoma in 53 patients and found a remission rate of 36%. Another 36% of patients had stable disease as their best response. PLD was shown to be effective in relieving tumor symptoms [35]. The role of PLD in first-line therapy was established by two randomized trials. Steward *et al.* randomized 241 patients between PLD 20 mg/m^2 and BV (bleomycin 15 mg/m^2 and vincristine 1.4 mg/m^2) administered every 3 weeks, respectively [36]. The overall response rate was significantly higher with PLD (58.7 versus 23.3%). In contrast to the BV regimen, PLD induced a significant relief of symptoms caused by pulmonary metastases. Furthermore, PLD produced a greater improvement with respect to lesion thickness, color, pain, edema, size and nodularity. In a second first-line trial, patients were allocated either to 2-weekly PLD 20 mg/m^2 or 2-weekly ABV (doxorubicin 20 mg/m^2, bleomycin 10 mg/m^2 and vincristine 1.0 mg/m^2) [37]. PLD had a significantly higher overall response rate in comparison with the three-drug regimen (45.9 versus 24.8%). Furthermore, secondary parameters of improved tolerability and symptom relief (treatment discontinuation 1 versus 37% with ABV, better improvement of pulmonary symptoms, and head and limb motility) were in favor of PLD. Both trials were conducted before the implementation of HAART. Therefore, most patients died of opportunistic infections rather than of tumor progression and survival as study endpoint is not reliable in these trials. Another trial has been conducted to determine whether the addition of other agents might improve the results obtained with PLD monotherapy [38]. Patients were randomized to receive PLD ± BV every 2 weeks (in doses as used in the ABV regimen). The overall response rates (79 and 80%) and the median time to tumor progression or death (29 and 32 weeks) were identical. Thus far, no additional benefit has been obtained by combining PLD monotherapy with other anti-cancer drugs in AIDS-related Kaposi's sarcoma.

Meanwhile, PLD has been studied in virtually all anthracycline-sensitive tumors. While the main target indications remain breast and ovarian cancer, as well as AIDS-related Kaposi's sarcoma, interesting data for PLD monotherapy have been reported for multiple myeloma [39], for example. In addition, a wealth of combination therapies with other cytostatic agents have been published [40].

NPLD

The formulation of NPLD is composed of a doxorubicin–citrate complex encapsulated in unilamellar vesicles with a diameter of about 150 nm. The bilayer contains egg phosphatidylcholine/cholesterol in a molar ratio of 55:45. The pharmacokinetic features are shown in Table 2. NPLD liposomes are targeted at the RES. They leave the blood vessels preferably through capillary leaks. The main toxicities of NPLD are myelosuppression and alopecia. In comparison with conventional doxorubicin, equieffective doses of PLD show a decreased rate of cardiotoxicity and gastrointestinal toxicity with a slight reduction of alopecia. The main indication of NPLD is breast cancer; NPLD is approved in Europe for the first-line treatment of this cancer in combination with cyclophosphamide.

In a first-line trial comparing monotherapy with NPLD and conventional doxorubicin in advanced breast cancer, 224 patients were randomly allocated to receive either NPLD at a dose of 75 mg/m^2 every 3 weeks or doxorubicin at the same dose and schedule [41]. The study was powered to test the hypothesis that the liposomal formulation would result in significantly less cardiotoxicity while preserving the anti-tumor efficacy. The overall response rates (26%) and the PFS (NPLD 3.8 versus doxorubicin 4.3 months) were not statistically different. Nevertheless, a trend towards improved overall survival in the conventional doxorubicin group was observed (NPLD 16 versus 20 months). This might have

been caused by different subsequent treatment regimens, but the study protocol did not collect post-progression treatment data and this information could not be collected retrospectively. No significant differences were noted between the two treatment groups with regard to grade 3 or 4 toxicities with the exception of cardiotoxicity (as discussed below). PPE was seen in a single patient.

Two randomized trials in breast cancer compared the combination of NPLD and cyclophosphamide and either doxorubicin or epirubicin and cyclophosphamide. In the pivotal trial by Batist *et al.*, 297 patients without prior chemotherapy for metastatic disease were randomized to either receive NPLD (60 mg/m^2) or doxorubicin (60 mg/m^2) every 3 weeks, respectively, in combination with cyclophosphamide (600 mg/m^2) [42]. Ten percent in each group had received prior adjuvant anthracycline treatment. The overall response rate was 43% for both treatment groups, and the duration of response was estimated as 9.6 months in the NPLD group (NPLD-C) and 9.1 months in the conventional doxorubicin group (D-C). The time to tumor progression was 5.1 months with NPLD-C versus 5.5 months with D-C. The median overall survival slightly favored the NPLD-C group (19 versus 16 months with D-C), but this was not statistically significant. With the exception of cardiotoxicity and grade 4 neutropenia (NPLD-C 61 versus D-C 75%; $p = 0.017$), the grade 3 or 4 adverse events recorded did not differ between both groups. In all, this study demonstrated that the NPLD-C regimen improved the therapeutic index by reducing cardiotoxicity and neutropenia grade 4, and provided comparable anti-tumor efficacy. Following the results of this trial, the NPLD-C regimen was approved in Europe for the first-line treatment of metastatic breast cancer.

In a second randomized study, the NPLD-C regimen was compared with the EC regimen (epirubicin 75 mg/m^2 and cyclophosphamide 600 mg/m^2). This trial (only published as an abstract thus far [43]) has randomized 160 patients for first-line treatment. Again, the overall response rates were nearly identical (NPLD-C 46 versus EC 39%), while the median time to progression favored the NPLD-C regimen (7.6 versus 6.0 months; $p < 0.05$). The rates of neutropenia grades 3 and 4 were higher in the NPLD-C group (47 versus 36% with EC).

In summary, both trials showed that replacement of conventional anthracyclines by NPLD in combination with cyclophosphamide does not result in decreased efficacy parameters, but in a significantly reduced risk of cardiotoxicity. The combination of NPLD (± paclitaxel) and trastuzumab has been investigated in phase I and II studies, and was found to be highly active and well tolerated with an acceptable cardiac safety profile [44,45].

DNX

Although conventional daunorubicin is not as widely used as doxorubicin, it has demonstrated efficacy in the treatment of different tumors like soft tissue sarcoma (around 20%), rhabdomyosarcoma (around 20%) and Hodgkin's lymphoma (around 20%) [46]. Daunorubicin differs from doxorubicin in that it lacks a hydroxyl-group at the C-14 position. Daunorubicin had been selected early for liposomal encapsulation due to its increased stability in the aqueous environment. DNX is a small unilamellar vesicle containing daunorubicin–citrate complex with a diameter of only 45 nm. The bilayer consists of distearoyl-phosphatidylcholine and cholesterol in a 2:1 molar ratio. Until now, DNX has been the only pure-lipid RES-avoiding liposomal formulation. This is achieved by the entrapment of daunorubicin in the inner aqueous space of the liposome as a citrate salt, leading to a decreased negative charge within the membrane resulting in delayed uptake by the RES and thus improved tumor uptake. The pharmacokinetic properties are shown in Table 2. In clinical trials, DNX produced a 35-fold increase in the plasma AUC compared with conventional daunorubicin. The main toxicity of DNX is myelosuppression, especially neutropenia. Alopecia occurs in about 8% of the patients. The approved indication of DNX is AIDS-associated Kaposi's sarcoma.

This approval was based on a randomized phase III trial including 232 patients with chemotherapy-naive advanced Kaposi's sarcoma [47]. The patients received either 2-weekly DNX 40 mg/m^2 or a modified 2-weekly ABV regimen (doxorubicin 10 mg/m^2, bleomycin 15 U and vincristine 1.0 mg/m^2). The overall response rates were not significantly different between DNX (25%) and the ABV regimen (28%). The median survival time was 369 days for DNX patients and 342 days for ABV patients ($p = 0.19$). The median time to treatment failure was 115 days for DNX and 99 days for ABV ($p = 0.13$). ABV patients experienced significantly more alopecia and neuropathy ($p < 0.0001$). DNX patients experienced more grade 4 neutropenia ($p = 0.021$). Cardiac function remained stable, with no instances of congestive heart failure on either treatment arm. The efficacy and safety of DNX as first-line treatment for advanced AIDS-related Kaposi's sarcoma was clearly established in this large phase III trial.

An exploratory post-approval trial was undertaken in order to compare PLD with DNX using a 3:1 randomization in Kaposi's sarcoma [48]. This trial was double blind, but was rather small (PLD $n = 60$; DNX $n = 19$). Patients received either PLD (20 mg/m^2) or DNX 40 mg/m^2 every 2 weeks for up to 6 cycles. They were evaluated for clinical benefit, defined as improvement in at least one of five symptom categories. Each patient made a biweekly assessment of the symptom categories using an 11-item questionnaire. An independent blinded reviewer

evaluated photographs for Kaposi's sarcoma disfiguring lesions and edema. Baseline characteristics were well balanced. The majority of patients had newly diagnosed Kaposi's sarcoma. Forty-eight of 60 PLD patients and 12 of 19 DNX patients achieved clinical benefit in at least one of the symptom categories. Partial tumor remission was observed in 55% in the PLD arm and 32% in the DNX arm. The most common adverse events were neutropenia, nausea, asthenia, anemia and paresthesia. In all, this study confirmed the data of previous studies for both drugs, but the study did not have the power to detect any differences and the design precluded the calculation of p values.

With respect to other tumor entities, studies of DNX either as a single agent or in combination schedules have also been conducted in breast cancer [49], multiple myeloma [50] and non-Hodgkin's lymphoma [51]. Nevertheless, to our knowledge, randomized trials have thus far not been reported.

Cardiac safety of liposomal anthracycline formulations

The problem of cumulative rather than acute cardiotoxicity is the major reason for the ongoing interest in liposomal encapsulated anthracyclines. Naturally, the best evidence for a reduced rate of cardiomyopathy comes from direct comparisons of the left ventricular ejection fraction (LVEF) of patients receiving liposomal formulations instead of conventional anthracyclines. Measurement of the LVEF in clinical trials is usually done by MUGA scan. Moreover, morphologic changes such as vacuolization of myocytes and myofibrillar loss using the Billingham score have been used as a surrogate grading system for anthracycline cardiotoxicity [52].

No preclinical or clinical direct comparisons of cardiac safety measures between free and liposomal encapsulated daunorubicin have been published. In contrast, both commercially available liposomal doxorubicin formulations, PLD and NPLD, showed preclinical evidence of reduced cardiomyopathy rates [53,54]. In a phase III trial in metastatic breast cancer [41] examining a head to head comparison between doxorubicin and NPLD, significantly fewer cardiac events were noted in the NPLD arm (13 versus 29%; $p = 0.0001$). Moreover, the median cumulative dose at the onset of cardiotoxicity was higher with NPLD (785 versus 570 mg/m^2; $p = 0.0001$). Furthermore, clinical symptoms of congestive heart failure were observed in a significantly lower proportion of patients in the NPLD arm (2 versus 8%; $p = 0.0001$) and endomyocardial biopsies revealed that fewer NPLD-treated patients (26 versus 71%) had a Billingham score of 2.5 or above (i.e. 26–35% of all myocytes affected by the above mentioned morphologic changes).

The phase III pivotal trial by Batist *et al.* [42] in breast cancer (NPLD or doxorubicin plus cyclophosphamide) also confirmed these findings for combination treatment: changes of LVEF (defined as a decrease of at least 20% from baseline values in the case of resting LVEF values of 50% or above, or a decrease of 10% or more in the case of resting values below 50%) were observed in 6% of patients treated with NPLD and 21% with conventional doxorubicin ($p = 0.0001$). Again, the onset of LVEF decrease was seen at higher median cumulative doses for NPLD (estimated at 2200 or above versus 480 mg/m^2). No NPLD-treated patient developed clinical symptoms of congestive heart failure ($n = 5$ patients treated with doxorubicin).

For the case of PLD, the final results of a randomized phase III trial in the first-line treatment of metastatic breast cancer [30] have recently confirmed retrospective data obtained from eight phase I and II trials in advanced cancer [55]. In this phase III trial a head to head comparison between PLD and doxorubicin was carried out with cardiac safety being a primary endpoint. MUGA scans were scheduled at baseline, after 300 mg/m^2 cumulative doxorubicin and after an additional 100 mg/m^2 (PLD) or 120 mg/m^2 (conventional doxorubicin). The per-protocol definition for cardiac events was similar to the above-mentioned criteria of Harris *et al.* [41]. In all, 339 patients were included in the cardiac safety analysis. Ten patients in the PLD group and 48 patients receiving conventional doxorubicin met the protocol-defined criteria for cardiotoxicity according to a LVEF decrease. This increased risk in the conventional doxorubicin group was observed in all subgroups (patients with prior adjuvant anthracycline treatment had a 7-fold higher risk). None of the 10 PLD patients with a LVEF decrease had clinical symptoms of congestive heart failure, whereas 10 out of 48 doxorubicin-treated patients developed symptoms.

In summary, clear evidence comes from several randomized trials that liposomal encapsulated doxorubicin bears a significantly lower risk of cardiotoxicity in comparison with equieffective doses of conventional doxorubicin. Moreover, encouraging preliminary data suggest minimal cardiotoxicity even in the combination with trastuzumab in the treatment of patients with HER-2 overexpression [31,44].

Liposomal encapsulated platinum compounds

The cytotoxicity of cisplatin [*cis*-dichlorodiammine platinum(II)] is mediated by induction of DNA cross-links leading to cell apoptosis (reviewed in [56]). It is an important compound for the treatment of a variety of human tumors, like cancer of the lung, head, and neck, ovary, esophagus, stomach, bladder, testis, and cervix. Its application is impeded, especially in the elderly and in

patients with substantial comorbidities, by a number of toxicities, i.e. nausea/vomiting, renal toxicity, ototoxicity, and peripheral neurotoxicity. In order to improve the therapeutic index, newer platinum analogs like carboplatin and oxaliplatin with a more favorable toxicity profile have been developed. Yet the spectrum of anti-tumor activity is partly different (e.g. oxaliplatin in colorectal cancer).

The preclinical assessment of liposomal encapsulation of platinum analogs has mainly focused on two issues: (i) a reduction of toxic effects and (ii) encapsulation of lipophilic platinum analogs that cannot be administered in the aqueous phase [6]. Three liposomal formulations have been investigated in a larger-scale clinical program: (i) pegylated liposomal cisplatin (STEALTH technology) SPI-077 (Sequus Pharmaceuticals, Menlo Park, California, USA), (ii) pegylated liposomal cisplatin Lipoplatin (Regulon. Mountain View, California, USA) and (iii) liposomal *cis*-bis-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum(II) Aroplatin (Antigenics, New York, New York, USA).

SPI-077

SPI-077 is a pegylated, sterically stabilized liposomal formulation of cisplatin using the same STEALTH technology as PLD. The bilayer comprises hydrogenated soy phosphatidylcholine and cholesterol as well as PEG-DSPE. SPI-077 has been found to be significantly more active than cisplatin in two murine (C26 colon carcinoma and Lewis lung) tumor models [57]. SPI-077 exhibited a 55-fold lower volume of distribution and a 60-fold larger plasma AUC. Furthermore, evidence for tumor targeting was seen with SPI-077 having a 28-fold higher tumor AUC than conventional cisplatin. Similarly, the anti-tumor efficacy of SPI-077 in a HT29 colon cancer xenograft model was significantly higher using equivalent doses of conventional cisplatin [58]. In cynomolgus monkeys repeated applications of SPI-077 (until 25 mg/kg) produced minimal side-effects, with cyclical decreases in hematology parameters being most notable [59]. Animals treated with 10-fold lower doses of conventional cisplatin (2.5 mg/kg) in contrast exhibited typical side-effects (nephro-, neurotoxicity, etc.). In a phase I study in pediatric patients, doses ranging between 40 and 320 mg/m^2 were applied every 4 weeks [60]. SPI-077 displayed a half-life of 134 h and less than 4% of the applied dose was found during the first 72 h in the urine. No response was observed among 17 treated patients. A parallel phase I study in 27 adult patients used dosages between 40 and 420 mg/m^2 every 4 weeks [61]. Again, minimal toxicity was observed: elevation of cholesterol concentrations and mild myelosuppression. Also in this study, no anti-tumor efficacy was seen and relatively low levels of platinum–DNA adducts were observed in tumor samples obtained from two patients treated at the highest dose levels. Furthermore, only limited activity of SPI-077 was seen in a phase I/II study in head and neck cancer (two partial remissions in 18 patients [62]) and a phase II study in lung cancer [63]. Summarizing the experience from several trials with SPI-077, the toxicity profile is promising, but the therapeutic efficacy might be hampered by unsatisfactory release of cisplatin from the liposomes. Reformulations of SPI-077 might be necessary in order to obtain the desired balance between encapsulation and release.

Lipoplatin

Lipoplatin is another pegylated liposomal formulation of cisplatin in small unilamellar vesicles (110 nm diameter). The anionic lipid bilayer contains dipalmitoyl phosphatidylglycerol (DPPG), soy phosphatidylcholine and cholesterol in a molar ratio of 2:2:1. In accordance with other studies [64], the anionic lipid DPPG is thought to improve Lipoplatin's fusogenic properties with respect to entrance through the cell membrane. This is considered an essential difference to SPI-077 or PLD shells, which are unable to cross the cell membrane barrier and accumulate in the extracellular spaces until lipases degrade the bilayers and the cytostatic drug is released [65]. Apart from that, the total lipid to cisplatin ratio for Lipoplatin is 10.24 mg/mg cisplatin, whereas SPI-077 contains 7-times more lipid (71.43 mg/mg cisplatin) than Lipoplatin.

Lipoplatin showed a substantially reduced renal toxicity compared to cisplatin in mice and rats after i.p. administration [66]. Anti-tumor effects were observed in xenografts of human breast, prostate and pancreatic cancer [65]. A phase I clinical trial including 19 patients with advanced cancer showed a plasma half-life of 36 h following an 8-h infusion. The side-effects of Lipoplatin using 100 mg/m^2 every 2 weeks included mild myelosuppression (grade 1–3 in 30% of patients), gastrointestinal toxicity and nausea/vomiting (20% grades 1–3). No oto-, neuro- or nephrotoxicity occurred and two out of 19 patients had partial remissions (gastric and pancreatic cancer; $n = 1$, respectively). Another 53% of the patients had stable disease [56]. A phase I trial in 27 patients using biweekly Lipoplatin (25–125 mg/m^2) found no renal toxicity [67]. Measurement of platinum plasma levels showed that a maximum is attained at 6–8 h. The half-life of Lipoplatin in these patients was 60–117 h depending on the dose administered. Urine platinum excretion reached about 40% of the administered dose during the first 72 h.

Several phase II and III trials with Lipoplatin are under way in various cancer types and results are eagerly awaited.

Aroplatin

Aroplatin is a liposomal formulation of *cis*-bis-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum(II), a

hydrophobic structural analog of oxaliplatin, encapsulated in conventional multilamellar vesicles. Several different lipophilic platinum analogs were tested in a series of studies and Aroplatin proved to be the most promising of them [68–70]. The liposomes are composed of dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol in a molar ratio of 7:3. In preclinical studies, activity against several tumor models has been shown including L1210 leukemia, M5076 reticulosarcoma and B16 melanoma. Aroplatin exhibited either equivalent or superior anti-tumor activity to cisplatin with significantly reduced nephrotoxicity.

A phase I study with escalating doses of Aroplatin every 4 weeks in 39 patients with advanced cancer reported the maximum tolerated dose to be 312.5 mg/m^2 [71]. The dose-limiting toxicities were myelosuppression; other significant toxicities included nausea/vomiting and diarrhea. More recently, interim results of a phase II trial with Aroplatin every 3 weeks in 5-fluorouracil/irinotecan pretreated colorectal cancer patients have been reported [72]. The starting dose level was 300 mg/m^2 and intrapatient escalation up to 375 and 470 mg/m^2 was allowed. One of 15 evaluable patients had confirmed partial remission, two patients had stable disease for at least 3 months and 11 patients progressed. Hematologic toxicity was mild, and non-hematological adverse events included infusion-related pain ($n = 7$), elevation of the activity of liver aminotransferases ($n = 14$) and sensory neuropathy ($n = 2$).

Given favorable pharmacokinetic properties after intracavitary administration in rats [73], intrapleural treatment with Aroplatin in patients with malignant effusion has been carried out. In a group of 20 patients with malignant mesothelioma, 11 out of 15 patients treated with Aroplatin 450 mg/m^2 every 3 or 4 weeks had a major tumor remission [74]. Two patients in this trial died soon after the first drug administration owing to local complications.

To date, 175 patients have been treated with Aroplatin and initial results suggest that Aroplatin might be a promising liposomal formulation. Current efforts focus on the improvement of formulations in order to enhance the convenience and efficacy of use (e.g. by intraliposomal conversion of the platinum complex; see US patent 6,696,079 B2). Larger studies with Aroplatin in oxaliplatin-sensitive tumors are warranted.

Liposomal encapsulated cytarabine (Ara-C) for intrathecal use

The treatment of disseminated lymphomatous meningitis, which may compromise up to 25% of high-grade lymphoma patients, requires a long exposure of the malignant cells to a high concentration of anti-neoplastic agent to achieve a sufficiently cytostatic effect [75]. The main strategy to achieve effective levels of chemotherapeutic agents in the cerebrospinal fluid (CSF) has become intrathecal injection. Many anti-neoplastic agents cause substantial neurotoxicity after long exposure times and are not suitable for intrathecal administration. In contrast, the anti-metabolites methotrexate (MTX) and Ara-C are agents of choice for intrathecal chemotherapy. Given the low proliferation index of malignant cells in the central nervous system, their susceptibility to anti-metabolite treatment is theoretically increased by longer exposure [75]. The removal of MTX or Ara-C from the CSF is slow, which provides the rationale for the intrathecal use of these drugs.

Until recently, the application of MTX has been preferred over Ara-C because of its even lower CSF clearance, and deeper penetration into the meninges and CNS parenchyma [75]. A sufficient, effective drug concentration in the CSF requires two to three intrathecal applications per week.

The development of a liposome-based, slow-release formulation of Ara-C appears to be a step forward in the treatment of lymphomatous meningitis [76]. This agent, DTC 101 (DepoCyte), is an intrathecally injectable suspension of Ara-C encapsulated in multivesicular lipid-based particles using DepoFoam technology [77]. Each multivesicular DepoFoam particle has a diameter of approximately 3–30 μm and consists of numerous non-concentric vesicules (aqueous chambers containing Ara-C) in a honeycomb arrangement. The chambers are separated from each other by lipid bilayers consisting of dioleyl-phosphatidycholine, dipalmitoyl-phosphatidylglycerol, cholesterol and triolein. DepoFoam particles are therefore much larger than conventional uni- or multilamellar liposomes bearing a high drug-loading capacity. At storage temperatures of 2–8°C the particles are stable for 12 months; however, after intrathecal injection they slowly release Ara-C. The lipid compounds enter the normal lipid metabolism pathways. DepoCyte has a mean half-life of 130–277 h compared to 3–4 h for conventional Ara-C [78]. No Ara-C was found in blood plasma after intrathecal administration of 50 mg of DepoCyte. Therefore, no interference with concomitant systemic chemotherapy occurs. A biweekly dosing schedule of 50 mg DepoCyte for induction therapy has been established (consolidation and maintenance therapy in 4-weekly intervals). Using this schedule, cytotoxic CSF levels of Ara-C were found up to 14 days regardless of the site of drug injection (ventricular or lumbar) [79].

Liposomal and free Ara-C were compared in a randomized trial in 28 patients with lymphomatous meningitis [80]. One treatment arm consisted of biweekly 50 mg

DepoCyte and the reference treatment was Ara-C 50 mg twice weekly for a 1-month induction period. In case of a response (CSF clearing of lymphoblasts), consolidation and maintenance cycles with longer application intervals were added. Response rates (i.e. clearing of CSF and absence of neurological progression) were statistically significantly higher in the DepoCyte arm (71 versus 15%; $p < 0.006$). A strong trend in favor of DepoCyte in terms of time to neurologic progression (78 versus 42 days; NS) and overall survival (99 versus 63 days; NS) was observed. DepoCyte treatment was associated with an improvement of Karnofsky status at the end of the induction treatment. The main side-effects of both treatment arms were headache and arachnoiditis. Consistent with the higher CSF levels of Ara-C in the DepoCyte group, headache (grades 1–3) occurred in more treatment cycles (27 versus 2%). Arachnoiditis (grades 1–3) was reported to occur in 22% of DepoCyte cycles (13% with Ara-C). Concomitant oral dexamethasone treatment (4 mg bid days 1-5) is recommended to prevent or mitigate this adverse event.

Another randomized trial compared biweekly DepoCyte 50 mg (up to six applications) with intrathecal MTX 10 mg twice weekly (up to 16 applications) in patients with cytologically proven neoplastic meningitis derived from solid tumors [81]. A total of 61 patients were accrued to receive the drugs either via lumbar puncture or an intraventricular Ommaya reservoir. Responses occurred in 26% of patients in the DepoCyte group and 20% in the MTX group. Median survival was not significantly different (105 days with DepoCyte versus 78 days with MTX), but a greater median time to neurological progression was obtained with DepoCyte (58 versus 30 days; $p = 0.007$). The grades and extent of adverse events observed were comparable between both groups.

Meanwhile, several phase II studies with DepoCyte in patients with solid tumor neoplastic meningitis have been reported, and cytologic response rates in the range of 20–27% and a time to neurologic progression of 49–55 days were consistently noted [82,83].

DepoCyte appears to be at least equieffective in comparison with MTX or conventional Ara-C for meningeosis either from solid tumors or lymphoma. At present, it is approved in the US and Europe only for the intrathecal treatment of lymphomatous meningitis. Clinical trials for meningeosis of acute lymphoblastic leukemia are ongoing. Future directions could be the prophylactic administration of DepoCyte in this disease. Open questions are the optimal duration of treatment after the initial clearing of CSF from malignant cells, the best dosing regimen for consolidation therapy, as well as (with respect to planned trials administering DepoCyte prophylactically) the long-term side-effects.

Liposomal encapsulated paclitaxel

Paclitaxel is one of the most widely used cytotoxic agents and has activity in a range of tumors, including ovarian, breast, non-small-cell lung, gastric and esophageal cancer. It is relatively hydrophobic and requires the polyoxyethylated castor oil vehicle, Cremophor EL, which is responsible for side-effects such as hypersensitivity reactions and neurotoxicity. Thus, premedication with corticosteroids and H_1 or H_2 receptor antagonists is required. Some paclitaxel toxicities are related to its peak plasma concentration, but the efficacy appears to be mainly associated with the AUC values. The current formulation of paclitaxel therefore is prone to toxicity. In order to mitigate this and to further improve pharmacokinetic properties like AUC, paclitaxel would be a good theoretical candidate for liposomal encapsulation.

While other macromolecular conjugations of paclitaxel [e.g. polymer conjugates like CT-2103 (Xyotax)] are in advanced stages of clinical development [84,85], liposomal formulations of paclitaxel are still encountering obstacles, e.g. in forming stable liposomes given paclitaxel's hydrophobic nature. A phase I trial with liposomal paclitaxel found dose-limiting toxicities at the dose level of 150 mg/m^2/week at less than 70% of the intended cumulative dose [86]. Moreover, the whole blood clearance of paclitaxel was similar for liposomal (15.3 ± 8.98 l/h/m^2) and conventional (17.5 ± 3.43 l/h/m^2) paclitaxel.

'Liposomal encapsulated paclitaxel easy-to-use' (LEP-ETU; NeoPharm, Waukegan, Illinois, USA) is a new formulation using NeoLipid technology [87]. The liposome shell consists of a 1,2-dioleyl-*sn*-glycero-3-phosphocholine, cholesterol and cardiolipin in a molar ratio of 90:5:5. Paclitaxel is encapsulated at a lipid:drug molar ratio of 33:1 with a paclitaxel concentration of 2 mg/ml. The mean vesicle size is about 150 nm. This formulation was physically and chemically stable for at least 1 year at both 2–8 and 25°C. Toxicology studies have shown that LEP-ETU is less toxic compared to paclitaxel. Their therapeutic efficacy was compared in a SCID mouse xenograft model of human ovarian (OVCAR-3), human lung (A-549), breast (MX-1) and prostate (PC-3) cancers [88]. At the 25 mg/kg dose level at least 2–3 times greater tumor growth inhibition was observed in comparison with paclitaxel. The time to reach a predefined growth endpoint (1000 mm^2) in A-549 was significantly delayed with LEP-ETU (70 days) in comparison with paclitaxel (49 days).

Twenty-five patients were treated in a phase I study with escalating doses of LEP-ETU (90 min) 135–375 mg/m^2. Five out of 23 evaluable patients had neutropenia grade 3 or 4, grade 1 or 2 neuropathy was observed in nine patients. The majority of patients tolerated multiple infusions without premedication with H_1 or H_2 receptor

antagonists. At the time of the report, the maximum tolerated dose had not yet been reached. Evidence of anti-tumor activity in several cancer types was observed with three partial remissions (ovarian and endometrial cancer, and adenocarcinoma of unknown primary, $n = 1$, respectively) and 11 patients with stable disease [89].

Another paclitaxel-based formulation comprises paclitaxel encapsulated in cationic liposomes (EndoTag-1, formerly MBT-0206; MediGene, formerly Munich Biotech, Munich, Germany). The lipid bilayers of these multilamellar liposomes consist of 1,2-dioleyl-*sn*-glycero-3-phosphocholine and 1,2-dioleyl-3-trimethylammonium-propane in a molar ratio of about 1:1 [90]. The liposome's size is about 180–200 nm. The unique feature of cationic liposomes is the fact that they selectively accumulate at activated tumor microvessels [11]. In preclinical models of Syrian golden hamsters bearing syngeneic A-Mel-3 melanomas [90,91], tumors were either treated with conventional paclitaxel or with MBT-0206. Compared with conventional paclitaxel, tumor growth was significantly retarded and analysis of intratumoral microvessel density revealed a superior reduction in the MBT-0206 treated animals. A superior reduction of intratumoral blood volume by MBT-0206 in comparison with conventional paclitaxel using Syrian golden hamsters with A-Mel-3 melanoma was also demonstrated using dynamic MRT scans [92]. In all, these data demonstrate that MBT-0206 may increase the anti-tumor efficacy in comparison with paclitaxel. Profound effects on the tumor microvessels indicating a possible anti-angiogenesis mechanism were observed. Thus, this neovascular targeting may add to the drug's efficacy by widening the therapeutic targets. In xenograft models of human pancreatic cancer (L3.6pl), MBT-0206 was as effective as gemcitabine. Moreover, the combination therapy of both drugs led to a significant enhancement of anti-tumor efficacy [93].

To date, over 150 patients have been treated within the frame of clinical trials including breast, prostate and colorectal cancer, and melanoma. In a phase I trial, MBT-0206 was administered at four dose levels (0.28, 0.55, 1.1 and 1.65 mg/kg body weight, respectively) on days 1, 3 and 5 of a 4-week cycle to patients with advanced gastrointestinal cancer ($n = 31$) [94]. The dose-limiting toxicities were fatigue and hypersensitivity reactions. Hematologic adverse events were restricted to the highest dose level. The maximum tolerated dose was defined as 1.1 mg/kg body weight. The assessment of tumor response resulted in 13% stable diseases.

In a phase Ib study in patients with anthracycline pretreated breast cancer, MBT-0206 was applied as a short infusion on days 1, 3 and 5 of a 22-day cycle at two different dosages to 18 patients per group [95]. In group A (0.55 mg/kg body weight) tumor remission was observed in 6% and stable disease in 28% of the patients; 56% of the patients treated in group B (1.1 mg/kg body weight) had stable disease. The main toxicities in group A were vomiting 28%, nausea 22%, anemia 17% and infusion-related reactions 56%; for group B, thrombocytopenia 44%, neutropenia 33%, asthenia 22% and infusion-related events 28% were reported. Of note, no neuropathy was observed and alopecia affected only 11%.

MBT-0206 is a promising new cationic liposomal formulation of paclitaxel with anti-angiogenic properties. Due to its favorable safety profile MBT-0206 appears to be a suitable combination partner for established cytostatics. MBT-0206 was originally developed by Munich Biotech, which has been taken over by MediGene in 2004. Nonetheless, the clinical development of MBT-0206 (under the new development name: EndoTag1) is ongoing and larger-scale studies are planned.

Liposomal camptothecin derivatives

Commercially available camptothecin-based cytostatics like irinotecan or topotecan have substantial anti-tumor activity in a variety of cancer types. Other camptothecin derivates such as lurtotecan [96] or gimatecan [97] are also under clinical investigation. Several liposomal formulations of approved or emerging camptothecin derivatives have been investigated in preclinical tumor models and some of them are about to enter phase III trials.

Irinotecan is a water-soluble analog of the natural alkaloid camptothecin, which acts as a topoisomerase I inhibitor. *In vivo* irinotecan is enzymatically converted by the carboxylesterase to its most active metabolite SN-38 (7-ethyl-10-hydroxy-camptothecin), which exerts its cytotoxicity only as the closed lactone ring. SN-38 establishes an equilibrium between this closed lactone ring and the inactive open ring hydroxy acid form. Various formulations of liposomal irinotecan have been investigated preclinically [98,99]. Messerer *et al.* recently reported successful encapsulation of irinotecan in small unilamellar vesicles (diameter around 100 nm) within a bilayer comprising 1,2,-distearoyl-*sn*-glycero-3-phosphocholine and cholesterol at a molar ratio of 55:45 resulting in a drug:lipid weight ratio of 0.3 [99]. Plasma elimination studies revealed that irinotecan was steadily released from the liposomes over a 24-h period, resulting for instance in 100-fold greater circulating levels in comparison with the free drug at 1 h after i.v. administration. Moreover, analyses of plasma samples indicated that the liposomal formulation of irinotecan was protected from the conversion to the inactive carboxylate form, which usually occurs rapidly at physiologic pH values. Therapeutic activity was demonstrated in SCID/Rag-2M mice bearing LS180 tumors or orthotopic LS174T colorectal metastases. It could be demonstrated that the liposomal

formulations administered at the same doses significantly prolonged survival compared with animals treated with conventional irinotecan [99]. To our knowledge, clinical studies with this formulation have not been reported.

In contrast, a liposomal formulation of SN-38, which is 200- to 2000-fold more cytotoxic than irinotecan has recently entered clinical trials. Despite its promising anticancer potential, SN-38 had not been exploited as an anti-cancer drug due to its poor solubility in any pharmaceutically acceptable solvent. Using NeoLipid technology, SN-38 has successfully been encapsulated in liposomes (LE-SN38; NeoPharm, Waukegan, Illinois, USA) [100]. The potency of LE-SN38 and irinotecan was evaluated against several cancer cell lines (e.g. HT-29 colon cancer and A549 lung cancer). The 50% growth inhibition values of all cell lines with LE-SN38 were 300–1700 times less than irinotecan [101]. In P388 murine leukemia-bearing mice, median survival was significantly improved with LE-SN38 in comparison with irinotecan [102]. Phase I clinical studies have recently been launched [103].

The most advanced clinically investigated liposomal formulation is lurtotecan. The conventional form of this water-soluble camptothecin analog was investigated in the mid 1990s in a series of phase II trials, and has been recognized as effective in the second-line treatment of ovarian and small cell lung cancer [104,105]. A stable liposomal formulation of lurtotecan (OSI-211, formerly NX211) was created using proprietary methodology similar to DNX. These small unilamellar vesicles with diameters of 50–100 nm yield a lipid:drug ratio of 20:1 and a lipid composition of 2:1 fully hydrogenated soy phosphatidylcholine and cholesterol [106]. A clear advantage of liposomal over free lurtotecan could be shown in nude mice and several xenograft models with respect to the 1500-fold increased plasma AUC, restricted distribution and consequently improved antitumor activity [106]. OSI-211 is sensitive to rapid degradation if exposed to light [107].

Twenty patients with advanced leukemia were treated at escalating doses (30-min infusion of 1.5–4.5 mg/m^2, days 1–3 of a 3-week cycle). The maximum tolerated dose was determined as 3.7 mg/m^2. One patient had a complete response and 14 out of 18 evaluable patients had transient bone marrow aplasia. Nevertheless, no evident correlation between exposure (as determined by plasma pharmacokinetics) and clinical response or toxicities was observed [108].

The maximum tolerated dose of the same regimen applied to patients with solid tumors was found to be 2.1 mg/m^2 in minimally pre-treated patients and 1.8 mg/m^2 in heavily pre-treated patients [109]. Again, the primary targets of toxicity were thrombocytes and neutrophils. Two partial remissions were observed (breast and ovarian cancer, $n = 1$, respectively).

Another phase I trial investigated OSI-211 given as a single 30-min infusion every 3 weeks in patients with advanced refractory solid tumors [110]. Twenty-nine patients were accrued to receive doses between 0.4 and 4.3 mg/m^2. Neutropenia and thrombocytopenia were dose limiting. The recommended dose was 3.8 mg/m^2 once every 3 weeks. In contrast to systemic exposure measures (plasma and whole-blood clearance), the dose excreted in urine (median urine recovery 10.1% ranging between 4.9 and 18.9%) was significantly related to the percent decrease in neutrophil and platelet count. Nine patients had stable disease while undergoing OSI-211 treatment.

Liposomal lurtotecan has been investigated in 50 patients with recurrent squamous cell cancer of the head and neck (2.4 mg/m^2 as a 30-min infusion days 1 and 8 of a 3-week cycle), but only minimal activity was observed with one patient achieving a partial remission [111].

The main focus of clinical investigation of liposomal lurtotecan is ovarian cancer since OSI-211 exhibited levels of activity in preclinical models similar to topotecan, which is an established drug in the treatment of recurrent ovarian cancer [112].

Twenty-two women with ovarian cancer resistant to topotecan were treated in a phase II trial with OSI-211 (2.4 mg/m^2 as a 30-min infusion days 1 and 8 of a 3-week cycle). Treatment was generally well tolerated with mild to moderate thrombocytopenia, neutropenia and gastrointestinal toxicity, but no response was observed [113].

Recently, a randomized phase II trial including women with relapsed ovarian cancer was reported [114]. Patients were randomly assigned to receive OSI-211 as a 30-min infusion either at a dose of: (i) 1.8 mg/m^2 days 1–3 (arm A) or (ii) 2.4 mg/m^2 days 1 and 8 (arm B). The cycles were repeated on day 22, respectively. A 'pick the winner' statistical design was chosen with the response rate being the primary study endpoint. Eighty-one eligible patients with a median age of 58 years (range 31–79) were accrued. The majority of patients had received two or more prior regimen including platinum. Hematologic side-effects were more pronounced in arm A (grade 4 neutropenia 51 versus 22%; grade 3/4 thrombocytopenia 51 versus 26%). The rate of febrile neutropenia was 10-fold higher in arm A (26 versus 2.4%). Nevertheless, the response rates significantly favored arm A with 15.4% patients (95% CI 6–30%) responding to OSI-211, while only 4.9% responded in arm B (95% CI 1–17%). A total of 41 patients had stable disease (median duration 5.7 months). The median progression-free survival was 4.9

months in arm A and 2.7 months in arm B. In accordance with observations made during phase I trials, the pharmacokinetics of OSI-211 in this trial exhibited significant interpatient variability. In all, this study suggests that (despite the prolonged plasma exposure of the liposomal formulation) repeated daily doses of OSI-211 as used in arm A cannot be avoided and appear to be crucial for anti-tumor activity. A randomized trial to address the question of whether OSI-211 is superior to topotecan in relapsed ovarian cancer has recently completed accrual.

Liposomal vincristine

Vincristine is a cell-cycle-specific active agent in lymphoma treatment, but its use is hampered by the development of neurotoxicity. Its cell-cycle specificity is based on the tubulin depolymerization, which arrests cells in the metaphase. Cell culture studies have shown that prolonged exposure of malignant cells to vincristine results in a significantly higher rate of cell killing [115]. Therefore, in order to prolong the plasma half-life, research on liposomal formulations of vincristine commenced more than a decade ago. Liposomal vincristine sulfate (OncoTCS, Marqibo; Inex Pharmaceuticals, Vancouver, British Columbia, Canada) is the most advanced liposomal formulation of vincristine. It has clearly shown toxicology advantages over free vincristine [116]. The unilamellar vesicles have a median diameter of 120 nm and comprise vincristine encapsulated in a lipid bilayer consisting of distearoyl-phosphatidylcholine and cholesterol.

In a phase I trial, 25 patients with refractory malignancies were treated with escalating doses of OncoTCS (1.0–2.8 mg/m^2 once every 3-weeks) [117]. A dose of 2.4 mg/m^2 was defined as maximum tolerable and 2.0 mg/m^2 was the recommended dose for further studies. Pain and constipation were dose-limiting events, while hematological toxicity was mild. Tumor shrinkage was noted in three patients (one fulfilling the criteria for partial remission). The results of this clinical trial indicate that by using OncoTCS higher doses of vincristine can be administered. The total systemic exposure (as reflected by AUC values) was 150-fold higher with OncoTCS than that previously described for free vincristine.

A phase II trial of OncoTCS (2.0 mg/m^2 biweekly over 60 min) in relapsed non-Hodgkin's lymphoma indicated substantial activity [118]. At the time of the report, 35 of 51 registered patients were evaluable for response and the response rate was reported to be 41%. Of note, these patients had been pretreated with a median of three prior chemotherapy regimen including vincristine in 100% of patients. Grade 3 or 4 motor or sensory neuropathy was seen in 11 patients and five of them had to stop treatment. Nonetheless, all five patients had pre-existing treatment-related neuropathy.

Early results of a combination study in chemotherapy-naive patients with aggressive B cell non-Hodgkin's lymphoma confirmed the promising data for single-agent treatment [119]. In this trial, elderly patients (over 60 years old) received the standard CHOP regimen plus rituximab, but vincristine was replaced by OncoTCS (2.0 mg/m^2). Eighteen of 23 included patients had undergone restaging procedures at the time of the report; 17 attained a complete and one patient a partial remission. Neuropathy did not exceed grade 2. No tumor recurrences had been observed within the median follow-up period of 6 months.

Unpublished data from a multicenter phase II/III trial in 119 pre-treated patients with aggressive non-Hodgkin's lymphoma showed a 25% overall response. Preferentially relying on these data, Inex and Enzon (which set up a strategic partnership in development and commercialization of Marqibo in January 2004) submitted the third and last section of a 'rolling submission' of a New Drug Application to the FDA seeking approval for single-agent salvage treatment in March 2004. Following the vote of the Oncologic Drug Advisory Committee (ODAC) in December 2004, the FDA announced in January 2005 that Marqibo was refused an accelerated approval based on phase II data. Therefore, the results of further randomized trials are required to enable a re-application for approval. As a consequence of the FDA 'not approval letter' the strategic partnership between Enzon and Inex was terminated on 17 March 2005.

Summary and outlook

In addition to the liposomal anthracycline derivatives, several other formulations of liposomal anti-cancer drugs have been approved in the past decade or are about to enter the clinic. The optimized pharmacokinetic properties of liposomal encapsulated anti-cancer drugs resulting in different, but in all cases improved, toxicity profile is still the main argument for the use of liposomal agents. Nonetheless, for the case of liposomal anthracyclines, for example, superior anti-tumor efficacy has never been proven in a randomized trial. Thus, with the advent of an army of liposomal agents on the verge of clinical use, cost-effectiveness analyses are demanded in view of the expanding costs of healthcare providers and decreasing resources. For liposomal anthracyclines, for example, analyses in the UK and Sweden have shown that the cost to achieve an objective response in Kaposi's sarcoma was more than twice as high for DNX than for PLD [120] and for platinum-refractory ovarian cancer the use of PLD was associated with net cost-savings compared with topotecan [120]. Despite higher acquisition costs of DepoCyte in comparison with conventional Ara-C, the quality-adjusted life years were significantly improved by the liposomal DepoCyte formulation, suggesting cost-effectiveness [121]. Future studies should especially

implement and emphasize the need for thoroughly planned cost-effectiveness analyses in order to facilitate decisions for the allocation of healthcare costs.

New insights into the biology and pharmacokinetic behavior of liposomes, e.g. the anti-angiogenic properties of cationic liposomes [11], offer an expanded therapeutic repertoire of these drug carrier systems and indicate changes for broader targeted therapies. In addition, in analogy to the expanding use of antibodies in solid tumor treatment (such as trastuzumab, bevacizumab or cetuximab), efforts are being made to develop antibody-coated liposomes (immunoliposomes) or antisense oligonucleotides in order to deliver the encapsulated cytostatic drugs more specifically [122,123].

In all, modern liposome technologies have undergone substantial optimization and an improvement in their therapeutic gains is expected. It is anticipated that liposomal anti-cancer drugs may play an increasing role in the field of targeted tumor treatment.

References

1 Bangham AD, Standish HM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 1965; **13**: 238–252.
2 Sessa G, Weissmann G. Phospholipid spherules (liposomes) as a model for biological membranes. *J Lipid Res* 1968; **9**:310–318.
3 Perez-Soler R. Liposomes as carriers of antitumor agents: towards a clinical reality. *Cancer Treat Rev* 1989; **16**:67–82.
4 Martin FJ. Pegylated liposomal doxorubicin: scientific rationale and preclinical pharmacology. *Oncology* 1997; **11**(**suppl 11**):11–20.
5 Senior J, Gregoriadis G. Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol components. *Life Sci* 1982; **30**:2123–2136.
6 Harrington KJ, Lewanski CR, Stewart JSW. Liposomes as vehicles for targeted therapy of cancer. Part 1: preclinical development. *Clin Oncol* 2000; **12**:2–15.
7 Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumours. *Pharmacol Rev* 1999; **51**:691–743.
8 Vail DM, Amantea MA, Colbern GT, Martin FJ, Hilger RA, Working PK. Pegylated liposomal doxorubicin: proof of principle using preclinical animal models and pharmacokinetic studies. *Semin Oncol* 2004; **31**(**suppl 13**):16–35.
9 Yatvin MB, Kreutz W, Horwitz BA, Shinitzky M. pH-sensitive liposomes: possible clinical implications. *Science* 1980; **210**:1253–1255.
10 Huang SK, Stauffer PR, Hong K, Guo JW, Philips TL, Huang A, *et al.* Liposomes and hyperthermia in mice: increased tumor uptake and therapeutic efficacy of doxorubicin in sterically stabilized liposomes. *Cancer Res* 1994; **54**:2186–2191.
11 Thurston G, McLean JW, Rizen M, Baluk P, Haskell A, Murphy TJ, *et al.* Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice. *J Clin Invest* 1998; **101**:1401–1413.
12 Swain S, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin. A retrospective analysis of three trials. *Cancer* 2003; **97**:2869–2879.
13 Workman P. Infusional anthracyclines: is slower better? If so, why? *Ann Oncol* 1992; **3**:591.
14 Swain SM, Vici P. The current and future role of dexrazoxane as a cardioprotectant in anthracycline treatment: expert panel review. *J Cancer Res Clin Oncol* 2004; **130**:1–7.
15 Behringer DM, Engelhart R, Hofheinz RD, Hochhaus A, Herrmann R, Stern A, *et al.* A phase I dose escalation study on the tolerability and activity of BBR 3438 in patients with advanced solid tumours. *Eur J Cancer* 2002; **38**(**suppl**):82P (abstr).
16 Hofheinz RD, Porta C, Hartung G, Santoro A, Hanauske AR, Kutz K, *et al.* BBR 3438, a novel 9-aza-anthrapyrazole, in patients with advanced gastric cancer: a phase II study group trial of the Central European Society of Anticancer-drug Research (CESAR). *Invest New Drugs* 2005; in press.
17 Markman M, Gordon AN, McGuire WP, Muggia FM. Liposomal anthracycline treatment for ovarian cancer. *Semin Oncol* 2004; **31**(**suppl 13**):91–105.
18 Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME, Lacave AJ. Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. *J Clin Oncol* 2001; **19**:3312–3322.
19 Gordon AN, Tonda M, Sun S, Rackoff W on behalf of the Doxil Study 30–49 investigators. Long-term survival advantage for women treated with pegylated liposomal doxorubicin compared with topotecan in a phase 3 randomized study of recurrent and refractory epithelial ovarian cancer. *Gynecol Oncol* 2004; **95**:1–8.
20 O'Byrne KJ, Bliss P, Graham JD, Gerber J, Vasey PA, Khanna S, *et al.* A phase III study of Doxil/Caelyx versus paclitaxel in platinum treated, taxane-naive relapsed ovarian cancer. *Proc Am Soc Clin Oncol* 2002; **21**:808 (abstr).
21 Potamianou A, Polyzos A, Boukovinas J, Latoufis C, Andoulakis N, Agelidou S, *et al.* Sequential combination of paclitaxel, carboplatin and paclitaxel liposomal doxorubicin as 1st line treatment in patients with ovarian cancer: a multicenter phase II trial. *Proc Am Soc Clin Oncol* 2004; **23**:5018 (abstr).
22 Ferrero JM, Weber B, Lepille D, Orfeuvre H, Combe M, Mayer F, *et al.* Carboplatin (PA) and pegylated liposomal doxorubicin (CA; PACA regimen) in patients with advanced ovarian cancer in late (>6 months) relapse (AOCLR): survival results of a GINECO phase II trial. *Proc Am Soc Clin Oncol* 2004; **23**:5022 (abstr).
23 Holloway RW, Finkler NJ, Nye LP, Bigsby GE, Ortiz BH. Doxil and gemcitabine combination therapy for recurrent ovarian cancer: results of a phase II trial. *Proc Am Soc Clin Oncol* 2004; **23**:5090 (abstr).
24 Straughn JM, Rocconi RP, Leath CA, Kilgore LC, Huh WK, Alvarez RD. Feasibility study of liposomal doxorubicin consolidation therapy after platinum/paclitaxel based chemotherapy for suboptimally debulked stage IIIC/IV ovarian cancer patients. *Proc Am Soc Clin Oncol* 2004; **23**:5044 (abstr).
25 Robert NJ, Vogel CL, Henderson IC, Sparano JA, Moore MR, Silveman P, *et al.* The role of liposomal anthracyclines and other systemic therapies in the management of advanced breast cancer. *Semin Oncol* 2004; **31** (**suppl 13**):106–146.
26 Ranson MR, Carmichael J, O'Byrne K, Stewart S, Smith D, Howell A. Treatment of advanced breast cancer with sterically stabilized liposomal doxorubicin: results of a multicenter phase II trial. *J Clin Oncol* 1997; **15**:3185–3191.
27 Lyass O, Uziely B, Ben-Yosef R, Tzemch D, Heshing NI, Lotem M. Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in metastatic breast cancer. *Cancer* 2000; **89**: 1037–1047.
28 Mlineritsch B, Mayer P, Rass C, Reiter E, Russ G, Vesenmayer G, *et al.* Phase II study of single-agent pegylated liposomal doxorubicin HCL (PLD) in metastatic breast cancer after first-line treatment failure. *Onkologie* 2004; **27**:441–446.
29 Keller AM, Mennel RG, Georgoulias V, Nabholtz JM, Erazo A, Lluch A, *et al.* Randomized phase III trial of pegylated liposomal doxorubicin versus vinorelbine or mitomycin C plus vinblastine in women with taxane-refractory advanced breast cancer. *J Clin Oncol* 2004; **22**:3893–3901.
30 O'Brien MER, Wigler N, Inbar M, Rosso R, Grischke E, Santoro A, *et al.* Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCL (CAELYX™/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann Oncol* 2004; **15**:440–449.
31 Chia SK, Clemons M, Martin LA, Rodgers A, Gelmon K, Panasci L. A multicentre phase II trial of pegylated liposomal doxorubicin and trastuzumab in HER-2 overexpressing metastatic breast cancer (MBC). *Proc Am Soc Clin Oncol* 2004; **23**:630 (abstr).
32 Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, *et al.* Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpress HER2. *N Engl J Med* 2001; **344**:783–792.
33 Gallafent JH, Buskin SE, De Turk PB, Aboulafia DM. Profile of patients with Kaposi's sarcoma in the era of highly active antiretroviral therapy. *J Clin Oncol* 2005; **23**:1253–1260.
34 Krown SE, Northfelt DW, Osoba D, Stewart JS. Use of liposomal anthracyclines in Kaposi's sarcoma. *Semin Oncol* 2004; **31**(**suppl 13**): 36–52.

35 Northfelt DW, Dezube BJ, Thommes JE, Levine R, von Roenn JH, Dosik GM, *et al.* Efficacy of pegylated-liposomal doxorubicin in the treatment of AIDS-related Kaposi's sarcoma after failure of standard chemotherapy. *J Clin Oncol* 1997; **15**:653–659.

36 Stewart S, Jablonowski H, Goebel FD, Arasteh K, Spittle M, Rios A, *et al.* Randomized comparative trial of pegylated liposomal doxorubicin versus bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma. International pegylated liposomal doxorubicin study group. *J Clin Oncol* 1998; **16**:683–691.

37 Northfelt DW, Dezube BJ, Thommes JE, Miller BJ, Fischel MA, Friedmann-Kien A, *et al.* Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomized phase III clinical trial. *J Clin Oncol* 1998; **16**:2445–2451.

38 Mitsuyasu RT, van Roenn J, Krown S, Kaplan L, Testa M. Comparison study of liposomal doxorubicin (DOX) alone or with bleomycin and vincristine (DBV) for treatment of AIDS-related Kaposi's sarcoma (AIDS-KS): AIDS clinical trial group (ACTG) protocol 286. *Proc Am Soc Clin Oncol* 1997; **16**:191 (abstr).

39 Hussein MA, Anderson KC. Role of liposomal anthracyclines in the treatment of multiple myeloma. *Semin Oncol* 2004; **31**(**suppl 13**): 147–160.

40 Alberts DS, Muggia FM, Carmichael J, Winer EP, Jahanzeb M, Venook AP, *et al.* Efficacy and safety of liposomal anthracyclines in phase I/II clinical trials. *Semin Oncol* 2004; **31**(**suppl 13**):36–52.

41 Harris L, Batist G, Belt R, Rovira D, Navari R, Azarnia N, *et al.* Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer* 2002; **94**:25–36.

42 Batist G, Ramakrishnan G, Rao CS, Chandrasekharan, Gutheil J, Guthrie T, *et al.* Reduced cardiotoxicity and preserved anti-tumour efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. *J Clin Oncol* 2001; **19**: 1444–1454.

43 Chan S, Davidson N, Juozaityte E, Erdkamp F, Azarnia N, Lee LW on behalf of the myocet study group. Liposome-encapsulated doxorubicin (myocet) and cyclophosphamide is superior to epirubicin and cyclophosphamide in first-line therapy of metastatic breast cancer. *Eur J Cancer* 2001; **37**(**suppl 5**):0–80 (abstr).

44 Trigo J, Climent MA, Lluch A, Gascon P, Hornedo J, Gil M, *et al.* Liposomal doxorubicin Myocet in combination with Herceptin and paclitaxel is active and well tolerated in patients with HER-2 positive locally advanced or metastatic breast cancer: a phase II study. *Breast Cancer Res Treat* 2003; **82**(**suppl 1**):351 (abstr).

45 Theodoulou M, Campos SM, Batist G, Winer E, Norton L, Hudis C, *et al.* TLC D99 (D, Myocet) and Herceptin (H) is safe in advanced breast cancer (ABC): final cardiac safety and efficacy analysis. *Proc Am Soc Clin Oncol* 2002; **21**:216 (abstr).

46 Forssen EA. The design and development of DaunoXome for solid tumor targeting *in vivo*. *Adv Drug Deliv Rev* 1997; **24**:133–150.

47 Gill PS, Wernz J, Scadden DT, Cohen P, Mukwaya GM. Randomized phase III trial of liposomal daunorubicin versus doxorubicin, bleomycin, and vincristine in AIDS-related Kaposi's sarcoma. *J Clin Oncol* 1996; **14**:2353–2364.

48 Martin F, Cooley T, Volberding P, Martin F, Tonda M, Kroll M. Final results of a phase III randomized trial of Doxil versus DaunoXome in patients with AIDS-related Kaposi's sarcoma (KS). *Proc Am Soc Clin Oncol* 2002; **21**:411 (abstr).

49 O'Byrne KJ, Thomas AL, Sharma RA, DeCatris M, Shields F, Beare S, Steward WP. A phase I dose-escalating study of DaunoXome, liposomal daunorubicin, in metastatic breast cancer. *Br J Cancer* 2002; **87**:15–20.

50 Eucker J, Eikel D, Heider U, Jakob C, Zavrski I, Gatz F, *et al.* Liposomal daunorubicin (DaunoXome) in multiple myeloma: a modified VAD regimen using short-term infusion. *Anticancer Drugs* 2003; **14**:793–799.

51 Tulpule A, Rarick MU, Kolitz J, Bernstein J, Myers A, Buchanan LA, *et al.* Liposomal daunorubicin in the treatment of relapsed or refractory non-Hodgkin's lymphoma. *Ann Oncol* 2001; **12**:457–462.

52 Billingham M, Bristow M. Evaluation of anthracycline cardiotoxicity: predictive ability and functional correlation of endomyocardial biopsy. *Cancer Treat Symp* 1984; **3**:71–76.

53 Kanter PM, Bullard GA, Ginsberg RA, Pilkiewicz FG, Mayer LD, Cullis PR, Pavelic ZP. Comparison of the cardiotoxic effects of liposomal doxorubicin (TLC D-99) versus free doxorubicin in beagle dogs. *In vivo* 1993; **7**:17–26.

54 Working PK, Newman MS, Sullivan T, Yarrington J. Reduction of the cardiotoxicity of doxorubicin in rabbits and dogs by encapsulation in long-circulating, pegylated liposomes. *J Pharmacol Exp Ther* 1999; **289**: 1128–1133.

55 Safra T, Muggia F, Jeffers S, Tsao-Wei DD, Groshen S, Lyass O, *et al.* Pegylated liposomal doxorubicin (Doxil): reduced clinical cardiotoxicity in patients reaching or exceeding cumulative doses of 500 mg/m^2. *Ann Oncol* 2000; **11**:1029–1033.

56 Boulikas T, Vougiouka M. Cisplatin and platinum drugs at the molecular level. *Oncol Rep* 2003; **10**:1663–1682.

57 Newman MS, Colbern GT, Working PK, Engbers C, Amantea MA. Comparative pharmacokinetics, tissue distribution, and therapeutic effectiveness of cisplatin encapsulated in long-circulating, pegylated liposomes (SPI-077) in tumor bearing mice. *Cancer Chemother Pharmacol* 1999; **43**:1–7.

58 Vaage J, Donovan D, Wipff E, Abra R, Colbern G, Uster P, Working P. Therapy of a xenografted human colonic carcinoma using cisplatin or doxorubicin encapsulated in long-circulating pegylated stealth liposomes. *Int J Cancer* 1999; **80**:134–137.

59 Working PK, Newman MS, Sullivan T, Brunner M, Podell M, Zahenk Z, *et al.* Comparative intravenous toxicity of cisplatin solution and cisplatin encapsulated in long-circulating, pegylated liposomes in cynomolgus monkeys. *Toxicol Sci* 1998; **46**:155–165.

60 Veal GJ, Griffin MJ, Price E, Parry A, Dick GS, Little MA, *et al.* A phase I study in paediatric patients to evaluate the safety and pharmacokinetics of SPI-077, a liposome encapsulated formulation of cisplatin. *Br J Cancer* 2001; **84**:1029–1035.

61 Meerum Terwogt JM, Groenewegen G, Pluim D, Maliepaard M, Tibben MM, Huisman A, *et al.* Phase I and pharmacokinetic study of SPI-77, a liposomal encapsulated dosage form of cisplatin. *Cancer Chemother Pharmacol* 2002; **49**:201–210.

62 Harrington KJ, Lewanski CR, Northcote AD, Whittaker J, Wellbank H, Vile RG, *et al.* Phase I–II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. *Ann Oncol* 2001; **12**: 493–496.

63 Kim ES, Lu C, Khuri FR, Tonda M, Glisson BS, Liu D, *et al.* A phase II study of STEALTH cisplatin (SPI-77) in patients with advanced non-small cell lung cancer. *Lung Cancer* 2001; **23**:427–432.

64 Sen A, Zhao Y, Zhang L, Hui SW. Enhanced transdermal transport by electroporation using anionic lipids. *J Control Release* 2002; **82**:399–405.

65 Boulikas T. Low toxicity and anticancer activity of a novel liposomal cisplatin (Lipoplatin) in mouse xenografts. *Oncol Rep* 2004; **11**:1–10.

66 Devarajan P, Tarabishi R, Mishra J, Ma Q, Kourvetaris A, Vougiouka M, *et al.* Low renal toxicity of lipoplatin compared to cisplatin in animals. *Anticancer Res* 2004; **24**:2193–2200.

67 Stathopoulos GP, Boulikas T, Vougiouka M, Deliconstantinos G, Rigatos S, Darli E, *et al.* Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): phase I study. *Oncol Rep* 2005; **13**:589–595.

68 Khoakhar AR, al-Baker S, Krakoff IH, Perez-Soler R. Toxicity and antitumour activity of *cis*-bis carboxylato(*trans-R,R*-1,2-diaminocyclohexane) platium (II) complexes entrapped in liposomes. *Cancer Chemother Pharmacol* 1989; **23**:219–224.

69 Perez-Soler R, Khoakhar AR, Bernstein G. Treatment and prophylaxis of experimental liver metastases of M5076 reticulosarcoma with *cis*-bis-neodecanoato *trans-R,R*-1,2-diaminocyclohexane platinum-(II) entrapped in multilamellar vesicles. *Cancer Res* 1987; **47**:6462–6466.

70 Perez-Soler R, Young LY, Drewinko B, Lautersztain J, Khoakar AR. Increased cytotoxicity and reversal of resistance to cisplatin with entrapment of *cis*-bis-neodecanoato *trans-R,R*-1,2-diaminocyclohexane platinum(II) in multilamellar vesicles. *Cancer Res* 1988; **48**:4509–4512.

71 Perez-Soler R, Lopez-Berestein G, Lautersztain J, al-Baker S, Francis K, Macias-Kiger D, *et al.* Phase I clinical and pharmacological study of liposome-entrapped *cis*-bis-neodecanoato-*trans-R,R*-1,2-diaminocyclohexane platinum(II). *Cancer Res* 1990; **50**:4254–4259.

72 Dragovich T, Mendelson D, Hoos A, Lewis J, Richardson, von Hoff D. A phase II trial of aroplatin (L-NDDP), a liposomal DACH platinum, in patients with metastatic colorectal cancer (CRC): a preliminary report. *Proc Am Soc Clin Oncol Gastrointestinal Cancer Symp* 2004; **260** (abstr).

73 Vadiei K, Siddik ZH, Khokhar AR, al-Baker S, Sampedro F, Perez-Soler R. Pharmacokinetics of liposome entrapped *cis*-bis-neodecanoato *trans-R,R*-1,2-diaminocyclohexane platinum (II) and cisplatin given i.v. and i.p. in the rat. *Cancer Chemother Pharmacol* 1992; **30**:365–369.

74 Perez-Soler R, Walsh GL, Swisher SG, Shin HJ, Kemp BL, Terry K, *et al.* Phase II study of liposome entrapped cisplatin-analog (L-NDDP) administered intrapleurally in patients with malignant pleural mesothelioma. *Proc Am Soc Clin Oncol* 1999; **18**:421 (abstr).

75 Bleyer WA. Intrathecal depot cytarabine therapy: a welcome addition to a limited armamentarium. *Clin Cancer Res* 1999; **5**:3349–3351.

76 Murry DJ, Blaney SM. Clinical pharmacology of encapsulated sustained-release cytarabine. *Ann Pharmacother* 2000; **34**:1173–1178.

77 Ye Q, Asherman J, Stevenson M, Brownson E, Katre NV. DepoFoam technology: a vehicle for controlled delivery of protein and peptide drugs. *J Control Rel* 2000; **64**:155–166.

78 Zimm S, Collins JM, Miser J, Chatterji D, Poplack DG. Cytosine arabinoside cerebrospinal fluid kinetics. *Clin Pharmacol Ther* 1984; **35**:826–830.

79 Kim S, Chatelut E, Kim JC, Howell SB, Cates C, Kormanik PA, *et al.* Extended CSF cytarabine exposure following intrathecal administration of DTD 101. *J Clin Oncol* 1993; **11**:2186–2193.

80 Glantz MJ, LaFollette S, Jaeckle K, Shapiro W, Swinnen L, Rozental J, *et al.* Randomized trial of a slow-release versus a standard formulation of cytarabine for the intrathecal treatment of lymphomatous meningitis. *J Clin Oncol* 1999; **17**:3110–3116.

81 Glantz MJ, Jaeckle KA, Chamberlain MA, Phuphanich S, Recht L, Swinnen LJ, *et al.* A randomized controlled trial comparing intrathecal sustained release cytarabine (DepoCyte) to intrathecal methotrexate in patients with neoplastic meningitis from solid tumours. *Clin Cancer Res* 1999; **5**: 3394–3402.

82 Jaeckle KA, Phuphanich S, Bent MJ, Aiken R, Batchelor T, Campbell T, *et al.* Intrathecal treatment of neoplastic meningitis due to breast cancer with a slow-release formulation of cytarabin. *Br J Cancer* 2001; **84**:157–163.

83 Jaeckle KA, Batchelor T, O'Day SJ, Phuphanich S, New P, Lesser G, *et al.* An open label trial of sustained-release cytarabine (DepoCyt) for the intrathecal treatment of solid tumour neoplastic meningitis. *J Neuro-Oncol* 2002; **57**: 231–239.

84 Langer CJ. Dilemmas in management: the controversial role of chemotherapy in PS 2 advanced NSCLC and the potential role of CT-2103 (Xyotax™). *The Oncologist* 2004; **9**:398–405.

85 Singer JW, Shaffer S, Baker B, Bernareggi A, Stromatt S, Nierstedt D, Besman M. Paclitaxel poliglumex (XYOTAX; CT-2103): an intracellularly targeted taxane. *Anticancer Drugs* 2005; **16**:243–254.

86 Soepenberg O, Sparreboom A, de Jonge MJA, Planting AST, de Heus G, Loos WJC, *et al.* Real-time pharmacokinetics guiding clinical decisions: phase 1 study of a weekly schedule of liposome encapsulated paclitaxel in patients with solid tumors. *Eur J Cancer* 2004; **40**:681–688.

87 Zhang JA, Anyarambhatla G, Ma L, Ugwu S, Xuan T, Sardone T, Ahmad I. Development and characterisation of a novel Cremophor EL free liposome-based paclitaxel (LEP-ETU) formulation. *Eur J Pharm Pharm Biopharm* 2005; **59**:177–187.

88 Ali S, Lei S, Pal A, Kamath N, Sarkar A, Sheikh S, *et al.* Enhanced anticancer effects of a novel liposome-based formulation of paclitaxel in human and murine tumour models in mice. *Proc Am Assoc Cancer Res* 2003; **44**:1345 (abstr.)

89 Fishman MN, Elsayed YA, Damjanov N, Steinberg JL, Mahany JJ, Nieves JA, *et al.* Safety and tolerability of liposome entrapped paclitaxel (LEP-ETU) administered with and without premedication in patients with advanced cancer. *Ann Oncol* 2004; **15**(**suppl 3**):402 (abstr).

90 Schmidt-Sody M, Strieth S, Krasnici S, Sauer B, Schulze B, Teifel M, *et al.* Neovascular targeting therapy: paclitaxel encapsulated in cationic liposomes improves antitumoral efficacy. *Clin Cancer Res* 2003; **9**: 2335–2341.

91 Strieth S, Eichhorn ME, Sauer B, Schulze B, Teifel M, Michaelis U, *et al.* Neovascular targeting chemotherapy: encapsulation of paclitaxel in cationic liposomes impairs functional tumor microvasculature. *Int J Cancer* 2004; **110**:117–124.

92 Griebel J, Eichhorn ME, Becker S, Werner A, Michaelis U, Ruhstorfer H, *et al.* Dynamische Magnetresonanztomographie zum Monitoring der Effektivität einer antivasculären Tumortherapie durch Paclitaxel enkapsuliert in kationische Lipidkomplexe. *Röfo Fortsch Geb Röntgenstr* 2004; **176**(**suppl 1**):P164 (abstr).

93 Papyan A, Werner A, Ischenko I, Teifel M, Michaelis U, Jauch KW, *et al.* Combination of standard chemotherapy with MBT-0206 enhances the anti-tumor efficacy in a highly metastatic human pancreatic cancer mouse model. Presented at 121 Kongress der Deutschen Gesellschaft für Chirurgie, Berlin; 2004, available http://www.egms.de/en/meetings/dgch2004/04dgch235.shtml (accessed 18.03.2005).

94 Ridwelski K, Kettner E, Fahlke J, Bartelheim K, Hitzl M, Schulze B, *et al.* Phase I study of the anti-neovascular agent MBT-0206 to evaluate safety and efficacy in patients with metastatic or advanced unresectable gastrointestinal cancer (GI cancer). *Proc Am Soc Clin Oncol* 2004; **22**:3163 (abstr).

95 Bartelheim K, Ognerubov A, Semiglazov VF, Vtoraya OM, Kaletta C, Reichenberger I, *et al.* Phase Ib study of the anti-neovascular agent MBT-0206 to evaluate safety and efficacy in patients with metastatic breast cancer. *Proc Am Soc Clin Oncol* 2004; **22**:3079 (abstr).

96 Gerrits CJ, Creemers GJ, Scheelens JHM, Wissel P, Planting AS, Kunka R, *et al.* Phase I and pharmacological study of the new topoisomerase inhibitor GI147211 using a daily $\times 5$ intravenous administration. *Br J Cancer* 1996; **73**:744–750.

97 Pratesi G, Beretta GL, Zunino F. Gimatecan, a novel camptothecin with a promising preclinical profile. *Anticancer Drugs* 2004; **15**:545–552.

98 Sadzuka Y, Hirotsu S, Hirota S. Effective irinotecan (CPT-11)-containing liposomes: intraliposomal conversion to the active metabolite SN-38. *Jpn J Cancer Res* 1999; **90**:226–232.

99 Messerer CL, Ramsay EC, Waterhouse D, Ng R, Simms EM, Harasym N, *et al.* Liposomal irinotecan: formulation development and therapeutic assessment in murine xenograft models of colorectal cancer. *Clin Cancer Res* 2004; **10**:6638–6649.

100 Zhang JA, Xuan T, Parmar M, Ma L, Ugwu S, Ali S, *et al.* Development and characterization of a novel liposome-based formulation of SN-38. *Int J Pharm* 2004; **270**:93–107.

101 Chien PY, Sheikh S, Zhang A, Ali S, Ahmad I. Cytotoxicity evaluation of a liposome-based formulation of SN38 in human and murine cancer cell lines. *Proc Am Ass Cancer Res* 2003; **44**:1607 (abstr).

102 Kamath N, Sarkar AK, Sheikh S, Zhang A, Ali S, Ahmad I. Therapeutic efficacy of liposome-based formulation of SN38 against leukemia model in CD2F1 mice. *Proc Am Ass Cancer Res* 2003; **44**:1784 (abstr).

103 Fishman M, LoRusso P, Kraut E, Fleming C, Paulson S, Steinberg J, *et al.* Phase I study of liposome encapsulated SN38 (LE-SN38) in patients with advanced cancer. *Proc Am Soc Clin Oncol* 2003; **22**: (abstr).

104 Wanders J, van Oosterom AT, Gore M, Calvert HA, Ten-Bokkel Huinink WA, Hansen HH, *et al.* A phase II study with GI147211 in ovarian cancer. *Eur J Cancer* 1997; **33**(**suppl**):118 (abstr).

105 Wanders J, Dombernowsky P, Nielsen D, Morant R, Drings P, Sessa C, *et al.* A phase II study with GI147211 (GW211) in small cell lung cancer. *Proc Am Soc Clin Oncol* 1998; **17**:1823 (abstr).

106 Emerson DL, Bendele R, Brown E, SuMing C, Desjardins JP, Dihel LC, *et al.* Antitumor efficacy, pharmacokinetics, and biodistribution of NX 211: a low-clearance liposomal formulation of lurtotecan. *Clin Cancer Res* 2000; **6**:2903–2912.

107 Loos WJ, Verweij J, Kehrer DFS, de Bruijn P, de Groot FMH, Hamilton M, *et al.* Structural identification and biological activity of 7-methyl-10,11-ethylenedioxy-20(*S*)-camptothecin, a photodegradant of lurtotecan. *Clin Cancer Res* 2002; **8**:856–862.

108 Giles FJ, Taliman MS, Garcia-Manero G, Cortes JE, Thomas DA, Wierda WG, *et al.* Phase I and pharmacokinetic study of a low-clearance, unilamellar liposomal formulation of lurtotecan, a topoisomerase 1 inhibitor, in patients with advanced leukemia. *Cancer* 2004; **100**:1449–1458.

109 Gelmon K, Hirte H, Fisher B, Walsh W, Ptaszynski M, Hamilton M, *et al.* A phase 1 study of OSI-211 given as an intravenous infusion days 1, 2, and 3 every three weeks in patients with solid cancer. *Inv New Drugs* 2004; **22**:263–275.

110 Kehrer DFS, Bos AM, Verweij J, Groen HJ, Loos WJ, Sparreboom A, *et al.* Phase I and pharmacologic study of liposomal lurtotecan, NX 211: urinary excretion predicts hematologic toxicity. *J Clin Oncol* 2002; **20**: 1222–1231.

111 Duffaud F, Borner M, Chollet P, Vermorken JB, Bloch J, Degardin M, *et al.* Phase II study of OSI-211 (liposomal lurtotecan) in patients with metastatic or loco-regional recurrent squamous cell carcinoma of the head and neck. An EORTC early drug development group study. *Eur J Cancer* 2004; **40**:2748–2752.

112 Desjardins JP, Abbott EA, Emerson DL, Tomkinson BE, Leray JD, Brown EN, *et al.* Biodistribution of OSI-211, liposomal lurtotecan, in tumor-bearing mice. *Anticancer Drugs* 2001; **12**:235–245.

113 Seiden MV, Muggia F, Astrow A, Matulonis U, Campos S, Roche M, *et al.* A phase II study of liposomal lurtotecan (OSI-211) in patients with topotecan resistant ovarian cancer. *Gynecol Oncol* 2004; **93**:229–232.

114 Dark GG, Calvert AH, Grimshaw R, Poole C, Swenerton K, Kaye S, *et al.* Randomized trial of two intravenous schedules of the topoisomerase I inhibitor liposomal lurtotecan in women with relapsed epithelial ovarian cancer: a trial of the National Cancer Institute of Canada clinical trials group. *J Clin Oncol* 2005; **23**:1859–1866.

115 Boman NL, Bally BB, Cullis PR. Encapsulation of vincristine in liposomes reduces its toxicity and improves its antitumor efficacy. *J Liposome Res* 1995; **5**:523–541.

116 Kanter PM, Klaich GM, Bullard GA, Pilkiewicz FG, Mayer LD, Culiis PR, *et al.* Liposome encapsulated vincristine: preclinical toxicologic and pharmacologic comparison with free vincristine and empty liposomes in mice, rats and dogs. *Anticancer Drugs* 1994; **5**:579–590.

117 Gelmon KA, Tolcher A, Diab AR, Bally MB, Embree L, Hudon N, *et al.* Phase I study of liposomal vincristine. *J Clin Oncol* 1999; **17**:697–705.

118 Sarris AH, Hagemeister F, Romaguera J, Rodriguez MA, McLaughlin P, Tsimberidou AM, *et al.* Liposomal vincristine in relapsed non-Hodgkin's lymphomas: early results of an ongoing phase II trial. *Ann Oncol* 2000; **11**:69–72.
119 Rodrigues MA, Sarris A, East K, Dang NH, Fayad L, Goy A, *et al.* A phase II study of liposomal vincristine in CHOP with rituximab for elderly patients with untreated aggressive B-cell non-Hodgkin's lymphoma. *Proc Am Soc Clin Oncol* 2004; **20**:1132 (abstr).
120 Bennett CL, Caljoun EA. Pharmacoeconomics of liposomal anthracycline therapy. *Semin Oncol* 2004; **31**(**suppl 13**):191–195.
121 Moeremans K, Annemans L, Morris JD. Liposomal cytarabine (DepoCyte™ injection) is cost-effective compared with standard cytarabine for the intrathecal treatment of patients with lymphomatous meningitis. *Blood* 2004; **104**(**suppl**):267 (abstr).
122 Mamot C, Drummond DC, Greisser U, Hong K, Kirpotin DB, Marks JD, *et al.* Epidermal growth factor receptor (EGFR)-targeted immunoliposomes mediate specific and efficient drug delivery to EGFR- and EGFRvIII-overexpressing tumor cells. *Cancer Res* 2003; **63**: 3154–3161.
123 Park JW, Benz CC, Martin FJ. Future directions of liposome- and immunoliposome-based cancer therapeutics. *Semin Oncol* 2004; **31**(**suppl 13**):196–205.
124 Swenson CE, Perkins WR, Roberts O, Janoff AS. Liposome technology and the development of Myocet (liposomal doxorubicin citrate). *The Breast* 2001; **10**(**suppl 2**): 1–7.
125 Richly H, Oberhoff C, Strumberg D, Grubert M, Kredtke S, Hartlapp J, *et al.* Pharmacokinetics (PK) of the liposomal encapsulated fraction (Caelyx, Doxil) as well as released doxorubicin after intravenous infusion of pegylated liposomes: PK based evidence for an indirect tumor targeting and a high systemic disposition of the released drug. *Proc Am Soc Clin Oncol* 2002; **32**:478 (abstr).