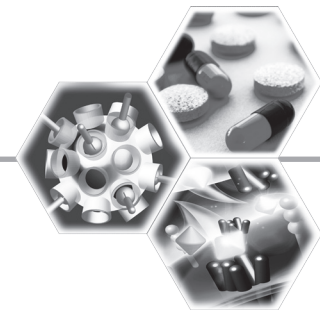


For reprint orders, please contact reprints@future-science.com

Thermosensitive liposomes for the delivery of cancer therapeutics

"*In principle, this approach combines the best of both worlds: the decreased systemic toxicity of chemotherapeutic drugs upon liposomal encapsulation, with the improved and triggered drug delivery and tumor sensitization by hyperthermia.*"

Liposomal nanocarriers: toxicity entrapped

Nanocarriers are being studied and developed as a means to improve delivery of anticancer drugs to solid tumors. Entrapping an anticancer drug in a nanocarrier can improve its therapeutic index by increasing the drug concentration at the target site in combination with decreasing the toxic side effects. Liposomes, phospholipid-based nano-sized vesicles, represent one of the best-studied types of nanocarriers for drug delivery purposes. They are being used clinically, for instance for the treatment of fungal infections (liposomal amphotericin B, Ambisome®) or cancer (liposomal daunorubicin, Daunoxome®, liposomal doxorubicin, Doxil®/Caelyx® or Myocet®). Besides these approved liposomal drug formulations, several others are in clinical trials, such as vincristin [1,2], lurtotecan [3,4], cisplatin [5], prednisolone [6] and combinations of irinotecan and floxuridine [7], and cytarabine and daunorubicin [8,9].

"*Entrapping an anticancer drug in a nanocarrier can increase the drug concentration at the target site and decrease the toxic side effects.*"

In general, liposomes offer the advantages that they are biocompatible, have a high drug payload and greatly improve pharmacokinetics and biodistribution of encapsulated drugs. Usually these result in increased blood-residence time, decreased drug toxicity and enhanced drug accumulation at tumor sites. Increased accumulation is due to the relative leakiness of tumor vasculature in comparison with normal vasculature. This allows extravasation of long-circulating small (100 nm) liposomes in animal tumor models [10,11], as well as in cancer patients [12,13]. Clinical studies, mainly performed with the PEGylated liposomal doxorubicin (PLD) formulation Doxil, have demonstrated strongly improved toxicity profiles compared with free doxorubicin administration [12,14], and therapeutic efficacy of PLD in several large randomized trials [15–17].

Two main challenges for future liposomal drug formulations for solid-tumor treatment are increased targeting to tumor sites and improved bioavailability of the encapsulated drugs to tumor cells.

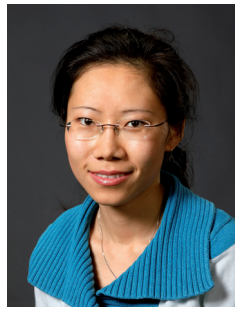
Increasing tumor targeting

Although tumor vessels can be permeable for liposome extravasation, preclinical and clinical studies demonstrated that levels of liposome accumulation in tumors vary strongly amongst different tumor types and even within a tumor [13,18,19]. Besides, tumor size appears to be inversely related with liposome accumulation [13] and to be a strong prognostic factor for response to treatment with PLD in ovarian cancer patients [20].

Successful strategies to improve accumulation of liposomal doxorubicin in solid tumors include those that aim to manipulate tumor vasculature. 'Abnormalizing' tumor vasculature [21] using low-dose TNF-α results in improved and more homogeneous accumulation of liposomes and liposomal drugs [19,22]. A similar strategy using hyperthermia, which is known to increase tumor perfusion, vascular permeability and microconvection in the tumor interstitial space, results in increased intra-tumoral liposome accumulation [23,24]. In contrast, vascular normalization through VEGF signaling blockade, which improves delivery of low-molecular-weight cytotoxic drugs [25], caused a decrease in tumor penetration of PLD [26]. Vascular modulation has a strong potency to increase drug delivery, but should be carefully fine tuned with regard to the drug delivery vehicle. Hyperthermia in this respect represents a clinically relevant strategy

Gerben A Koning

Author for correspondence:
Laboratory of Experimental Surgical Oncology, Section of Surgical Oncology, Department of Surgery, Erasmus Medical Center, Rotterdam, PO Box 2040, 3000 CA Rotterdam, The Netherlands
Tel.: + 31 107 043 963
Fax: + 31 107 044 746
E-mail: g.koning@erasmusmc.nl

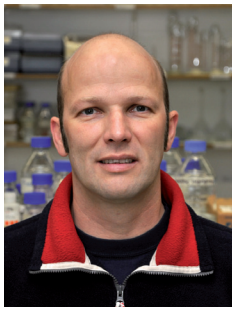


Li Li

Laboratory of Experimental Surgical Oncology, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands

Authors continued overleaf...

FUTURE SCIENCE part of fsg



Timo LM ten Hagen

Laboratory of Experimental Surgical Oncology, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands

that can improve liposome accumulation. The number of clinical studies combining hyperthermia with PLD are small, but results suggest a therapeutic benefit [27–29].

Improve drug bioavailability

Most liposomal drugs are formulated to remain stably entrapped in the carrier upon systemic administration and subsequent circulation. For instance, over 98% of doxorubicin administered as PLD remains entrapped during circulation for 7 days [13,30]. This characteristic strongly contributes to the observed favorable pharmacokinetics as the vast majority of the drug will remain confined to the blood circulation, causing a low volume of distribution in comparison to the free drug. Stable-drug entrapment also prevents major toxicity of the entrapped compound, for instance doxorubicin cardiotoxicity when formulated as PLD [13]. However, this important feature for circulating liposomes turns out to be a major drawback upon arrival at the tumor, where liposomal drugs need to become bioavailable to the tumor cells; this process is severely hampered by the stable formulation. In fact not much is known about the intra-tumoral fate of liposomal doxorubicin or about mechanisms involved in its release. Some studies claim involvement of tumor-associated macrophages, which either phagocytose the liposomes and release the drug in active form in the tumor area [31] or are involved in increasing the microvascular permeability leading to increased liposome accumulation [32]. Other publications demonstrated little to no effect of tumor-associated macrophages in therapeutic efficacy of PLD [33]. Recently, Seynhaeve *et al.* demonstrated significant levels of uptake of liposomal doxorubicin by tumor cells in living mice using intravital microscopy. Upon internalization liposomes remained in the cytoplasm and slowly released their doxorubicin content as evidenced by the nuclear doxorubicin appearance 2–3 days after administration [19]. Supportively, Laginha *et al.* quantified the bioavailability of PLD to be around 30–50% over several days after intravenous administration by measuring drug levels in isolated tumor cell nuclei [34].

Triggered drug release

The findings that the highly stable liposomal drug formulations consequently have a low drug bioavailability and slow drug release in the tumor call for further improvement. Development of controlled or triggered release systems represents a promising approach to deal with this challenge. Incorporating a functionality in liposomes that triggers drug release not only enhances drug bioavailability, but also introduces a control option within the liposome, by which drugs may only be released upon command in a localized area. Several different ways to introduce triggered release functionality into liposomes exist, varying from programmed enzymatic or pH-initiated destabilization of the liposomal bilayer or the PEGcoating surrounding the liposome to externally applied triggers such as light, heat and ultrasonic wave (reviewed elsewhere [35]). Currently the most well-developed approach for triggered release is the use of hyperthermia in combination with thermosensitive liposomes (TSL).

This approach relies on original findings by Yatvin *et al.*, who described the use of phospholipids that undergo a phase transition at temperatures of around 44°C to release compounds entrapped in liposomes composed of these phospholipids [36]. In later years this approach further developed into more advanced temperature-triggered release systems by applying efficient drug loading technology [37], PEGcoating of the liposomes [38–40], the inclusion of lysolipids to obtain burst-release properties [41], the development of novel lipids enhancing both circulation time and drug release [42] and by co-entrapment of imaging agents allowing for image-guided drug delivery [43–46]. To date, a TSL formulation called ThermoDox®, originally discovered by Needham and Dewhirst [41], has been further developed by the biopharmaceutical company Celsion and is currently undergoing clinical evaluation.

Hyperthermia & thermosensitive liposomes: a hot combination

The advantages of combining TSL with hyperthermia are multiple. Hyperthermia represents an established clinical treatment option in oncology either aiming at direct ablation of tumors at high temperatures or applied as mild hyperthermia (temperatures up to 43°C) in combination with radiotherapy and/or chemotherapy. In the latter approach, hyperthermia is known to strongly enhance the efficacy of radiotherapy and chemotherapy. This is related to increased perfusion and oxygenation of the tumor, which will increase the cytotoxic effects of radiation and chemotherapeutics. In addition, heat increases tumor vasculature permeability as well as interstitial convection and is able to sensitize tumor cells temporarily to the damaging effects of radiation or chemotherapeutics.

To date there is firm clinical evidence of increased efficacy of combined treatments of hyperthermia and chemotherapy [47–49]. A recent study by Issels *et al.* represented a large randomized EORTC trial in high-grade soft tissue sarcomas and demonstrated a strongly increased overall survival in the group of patients that completed the hyperthermia and chemotherapy protocol compared with chemotherapy alone [49].

Based on these important clinical data and with the knowledge that hyperthermia can now be applied in multiple centers in Europe and throughout the world, it represents a promising and feasible approach to further improve liposomal chemotherapy using thermosensitive liposomes. Liposomes in this approach function as carriers for the drug to be delivered and will prevent toxic side effects due to stable entrapment. Hyperthermia will add to liposomal chemotherapy by increasing tumor microvascular permeability and thus liposome extravasation. Upon arrival in the heated tumor area the bilayer of these liposomes will 'melt' to a fluid state and in that process liposomes rapidly release their water-soluble anticancer drugs. Upon release the synergistic effects of hyperthermia on chemotherapeutic drugs (as described previously) will also apply, including increased interstitial convection and increased tumor cell sensitivity. In principle, this approach combines the best of both worlds: the decreased systemic toxicity of chemotherapeutic drugs upon liposomal encapsulation, with the improved and triggered drug delivery and tumor sensitization by hyperthermia.

In this respect, the ongoing clinical trials using thermosensitive liposomal doxorubicin in combination with hyperthermia in patients with locoregional breast carcinoma of the chest wall or with radiofrequency ablation (RFA) in patients with hepatocellular carcinoma are of great importance to prove the validity of the approach. Some results from the RFA/ThermoDox clinical studies have been described by Poon *et al.* [50]. They concluded that the approach was safe and resulted in delivery of a high dose of doxorubicin to the tumor site treated with RFA, thereby improving the RFA efficacy. This outcome was promising enough to start a large Phase III clinical trial in patients with primary or metastatic liver cancer. It also became clear that the TSL in this study caused considerable high levels of free doxorubicin in circulation giving rise to side effects similar to free drug administration, although the overall toxicity profile was improved compared with free drug administration [50]. The free doxorubicin in circulation may in part be caused by the temperature-mediated release from circulating liposomes during RFA, but is most likely also related to the relative instability of the formulation, which was optimized for burst release upon heat. Therefore, challenges for current and novel formulations of TSL are to achieve a further improved stability at physiological temperature combined with rapid release at mild hyperthermic temperatures of approximately 41°C. In addition, in order to further expand the applicability of the heat triggered drug delivery approach to a wider range of solid tumors or possible combination treatments, novel TSL drug formulations should be developed. Examples of other interesting drugs formulated thus far in TSL are cisplatin [51,52], melphalan [53], taxol [54] and miltefosine [55]. These formulations are still in preclinical stages of development and need extensive testing before being applied in the clinic.

Imaging to improve

A final challenge lies in further optimizing this rather complicated drug delivery approach through image-guidance. Current developments aim at co-entrapment of imaging agents allowing for image-guided drug delivery [43–46]. This will render online information during treatment on accumulation of liposomes in the tumor area and will help to decide the optimal moment for triggering drug release using heat. During hyperthermia, MRI can be used to monitor tumor temperatures as well as drug release efficacy through co-release of the encapsulated contrast agents. Such image-guided approaches will help to further optimize the therapy for the individual patient.

In summary, liposomal formulations of chemotherapeutic drugs have proven clinical potency in both decreasing toxicity and increasing drug delivery. Two main approaches to further optimize liposomal chemotherapy aim at increasing liposome accumulation in the tumor and improving drug bioavailability. Applying hyperthermia in combination with thermosensitive liposomes can help to achieve these aims. The applied heat will first increase levels of liposome accumulation in the tumor and second, induce triggered release from the thermosensitive liposomes. Moreover, the applied heat might render tumor cells more sensitive to

the chemotherapeutic. This approach has now reached the phase of clinical testing and preliminary results from these studies are promising. Novel formulations with improved stability and different drugs are being developed to broaden the application to a wider range of tumor types. Image-guided drug delivery will ultimately help to optimize and personalize this promising cancer treatment.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Bibliography

1 Gelmon KA, Tolcher A, Diab AR *et al.* Phase I study of liposomal vincristine. *J. Clin. Oncol.* 17, 697–705 (1999).

2 Thomas DA, Sarris AH, Cortes J *et al.* Phase II study of sphingosomal vincristine in patients with recurrent or refractory adult acute lymphocytic leukemia. *Cancer* 106, 120–127 (2006).

3 Dark GG, Calvert AH, Grimshaw R *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.* 23, 1859–1866 (2005).

4 Duffaud F, Borner M, Chollet P *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 New Drug Development Group study. *Eur. J. Cancer* 40, 2748–2752 (2004).

5 Harrington KJ, Lewanski CR, Northcote AD *et al.* Phase I–II study of PEGylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. *Ann. Oncol.* 12, 493–496 (2001).

6 Barrera P, Mulder S, Smetsers AI *et al.* Long-circulating liposomal prednisolone versus pulse intramuscular methylprednisolone in patients with active rheumatoid arthritis. *Arthritis Rheum.* 58, 3976–3977 (2008).

7 Batist G, Gelmon KA, Chi KN *et al.* Safety, pharmacokinetics, and efficacy of CPX-1 liposome injection in patients with advanced solid tumors. *Clin. Cancer Res.* 15, 692–700 (2009).

8 Feldman EJ, Lancet JE, Kolitz JE *et al.* Phase I study of a liposomal carrier (CPX-351) containing an optimized, synergistic, fixed molar ratio of cytarabine (Ara-C) and daunorubicin (DNR) in advanced leukemias and myelodysplastic syndromes (MDS). *Blood* 110, 274A–275A (2007).

9 Lancet J, Feldman E, Kolitz J, Tallman M, Hogge DE, Louie A. Phase IIB randomized study of CPX-351 versus conventional cytarabine plus daunorubicin in newly diagnosed AML patients aged 60–75: an interim analysis. *Clin. Lymphoma Myeloma Leuk.* 10, E28 (2010).

10 Wu NZ, Da D, Rudoll TL, Needham D, Whorton AR, Dewhirst MW. Increased microvascular permeability contributes to preferential accumulation of STEALTH liposomes in tumor tissue. *Cancer Res.* 53, 3765–3770 (1993).

11 Charrois GJ, Allen TM. Rate of biodistribution of STEALTH liposomes to tumor and skin: influence of liposome diameter and implications for toxicity and therapeutic activity. *Biochim. Biophys. Acta* 1609, 102–108 (2003).

12 Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. *Clin. Pharmacokinet.* 42, 419–436 (2003).

13 Harrington KJ, Mohammadtaghi S, Uster PS *et al.* Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled PEGylated liposomes. *Clin. Cancer Res.* 7, 243–254 (2001).

14 Ewer MS, Martin FJ, Henderson C, Shapiro CL, Benjamin RS, Gabizon AA. Cardiac safety of liposomal anthracyclines. *Semin. Oncol.* 31, 161–181 (2004).

15 Gordon AN, Tonda M, Sun S, Rackoff W. Long-term survival advantage for women treated with PEGylated liposomal doxorubicin compared with topotecan in a Phase III randomized study of recurrent and refractory epithelial ovarian cancer. *Gynecol. Oncol.* 95, 1–8 (2004).

16 Northfelt DW, Dezube BJ, Thommes JA *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.* 16, 2445–2451 (1998).

17 Alberts DS, Liu PY, Wilczynski SP *et al.* Randomized trial of PEGylated liposomal doxorubicin (PLD) plus carboplatin versus carboplatin in platinum-sensitive (PS) patients with recurrent epithelial ovarian or peritoneal carcinoma after failure of initial platinum-based chemotherapy (Southwest Oncology Group Protocol S0200). *Gynecol. Oncol.* 108, 90–94 (2008).

18 Dvorak HF, Nagy JA, Dvorak JT, Dvorak AM. Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. *Am. J. Pathol.* 133, 95–109 (1988).

19 Seynhaeve AL, Hoving S, Schipper D *et al.* TNF-α mediates homogeneous distribution of liposomes in murine melanoma that contributes to a better tumor response. *Cancer Res.* 67, 9455–9462 (2007).

20 Safra T, Groshen S, Jeffers S *et al.* Treatment of patients with ovarian carcinoma with PEGylated liposomal doxorubicin: analysis of toxicities and predictors of outcome. *Cancer* 91, 90–100 (2001).

21 Seynhaeve AL, Eggermont AM, ten Hagen TL. TNF and manipulation of the tumor cell-stromal interface: "ways to make chemotherapy effective". *Front. Biosci.* 13, 3034–3045 (2008).

22 ten Hagen TL, Van Der Veen AH, Nooijen PT, van Tiel ST, Seynhaeve AL, Eggermont AM. Low-dose TNF-α augments anti-tumor activity of stealth liposomal doxorubicin (DOXIL) in soft tissue sarcoma-bearing rats. *Int. J. Cancer* 87, 829–837 (2000).

23 Kong G, Braun RD, Dewhirst MW. Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. *Cancer Res.* 61, 3027–3032 (2001).

24 Kong G, Braun RD, Dewhirst MW. Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size. *Cancer Res.* 60, 4440–4445 (2000).

25 Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor

2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. *Cancer Res.* 64, 3731–3736 (2004).

26 Tailor TD, Hanna G, Yarmolenko PS *et al.* Effect of pazopanib on tumor microenvironment and liposome delivery. *Mol. Cancer Ther.* 9, 1798–1808 (2010).

27 Kouloulias VE, Dardoufas CE, Kouvaris JR *et al.* Liposomal doxorubicin in conjunction with re-irradiation and local hyperthermia treatment in recurrent breast cancer: a Phase I/II trial. *Clin. Cancer Res.* 8, 374–382 (2002).

28 Dvorak J, Zoul Z, Melichar B *et al.* PEGylated liposomal doxorubicin in combination with hyperthermia for treatment of skin metastases of breast carcinoma: a case report. *Onkologie.* 24, 166–168 (2001).

29 Vujaskovic Z, Kim DW, Jones E *et al.* A Phase I/II study of neoadjuvant liposomal doxorubicin, paclitaxel and hyperthermia in locally advanced breast cancer. *Int. J. Hyperthermia* 26, 514–521 (2010).

30 Gabizon A, Catane R, Uziely B *et al.* Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res.* 54, 987–992 (1994).

31 Storm G, Steerenberg PA, Emmen F, Van Borssum WM, Crommelin DJ. Release of doxorubicin from peritoneal macrophages exposed *in vivo* to doxorubicin-containing liposomes. *Biochim. Biophys. Acta* 965, 136–145 (1988).

32 Mayer LD, Dougherty G, Harasym TO, Bally MB. The role of tumor-associated macrophages in the delivery of liposomal doxorubicin to solid murine fibrosarcoma tumors. *J. Pharmacol. Exp. Ther.* 280, 1406–1414 (1997).

33 Banciu M, Schiffelers RM, Storm G. Investigation into the role of tumor-associated macrophages in the antitumor activity of Doxil. *Pharm. Res.* 25, 1948–1955 (2008).

34 Laginha KM, Verwoert S, Charrois GJ, Allen TM. Determination of doxorubicin levels in whole tumor and tumor nuclei in murine breast cancer tumors. *Clin. Cancer Res.* 11, 6944–6949 (2005).

35 Koning GA, Krijger GC. Targeted multifunctional lipid-based nanocarriers for image-guided drug delivery. *Anticancer Agents Med. Chem.* 7, 425–440 (2007).

36 Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. *Science* 202, 1290–1293 (1978).

37 Maruyama K, Unezaki S, Takahashi N, Iwatsuru M. Enhanced delivery of doxorubicin to tumor by long-circulating thermosensitive liposomes and local hyperthermia. *Biochim. Biophys. Acta* 1149, 209–216 (1993).

38 Unezaki S, Maruyama K, Takahashi N *et al.* Enhanced delivery and anti-tumor activity of doxorubicin using long-circulating thermosensitive liposomes containing amphipathic polyethylene glycol in combination with local hyperthermia. *Pharm. Res.* 11, 1180–1185 (1994).

39 Gaber MH, Hong K, Huang SK, Papahadjopoulos D. Thermosensitive sterically stabilized liposomes: formulation and *in vitro* studies on mechanism of doxorubicin release by bovine serum and human plasma. *Pharm. Res.* 12, 1407–1416 (1995).

40 Li L, ten Hagen TL, Schipper D *et al.* Triggered content release from optimized stealth thermosensitive liposomes using mild hyperthermia. *J. Control. Release* 143, 274–279 (2010).

41 Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model. *Cancer Res.* 60, 1197–1201 (2000).

42 Lindner LH, Eichhorn ME, Eibl H *et al.* Novel temperature-sensitive liposomes with prolonged circulation time. *Clin. Cancer Res.* 10, 2168–2178 (2004).

43 Lindner LH, Reinl HM, Schlemmer M, Stahl R, Peller M. Paramagnetic thermosensitive liposomes for MR-thermometry. *Int. J. Hyperthermia* 21, 575–588 (2005).

44 Ponce AM, Viglianti BL, Yu D *et al.* Magnetic resonance imaging of temperature-sensitive liposome release: drug-dose painting and anti-tumor effects. *J. Natl Cancer Inst.* 99, 53–63 (2007).

45 de Smet M, Langereis S, van den BS, Grull H. Temperature-sensitive liposomes for doxorubicin delivery under MRI guidance. *J. Control. Release* 143, 120–127 (2010).

46 Djanashvili K, ten Hagen TL, Blange R, Schipper D, Peters JA, Koning GA. Development of a liposomal delivery system for temperature-triggered release of a tumor targeting agent, Ln(III)-DOTA-phenylboronate. *Bioorg. Med. Chem.* DOI: 10.1016/j.bmc.2010.06.036 (2010) (Epub ahead of print).

47 Colombo R, Da Pozzo LF, Salonia A *et al.* Multicentric study comparing intravesical chemotherapy alone and with local microwave hyperthermia for prophylaxis of recurrence of superficial transitional cell carcinoma. *J. Clin. Oncol.* 21, 4270–4276 (2003).

48 Franckena M, De Wit R, Ansink AC *et al.* Weekly systemic cisplatin plus locoregional hyperthermia: an effective treatment for patients with recurrent cervical carcinoma in a previously irradiated area. *Int. J. Hyperthermia* 23, 443–450 (2007).

49 Issels RD, Lindner LH, Verweij J *et al.* Neo-adjuvant chemotherapy alone or with regional hyperthermia for localized high-risk soft-tissue sarcoma: a randomized Phase III multicentre study. *Lancet Oncol.* 11, 561–570 (2010).

50 Poon RT, Borys N. Lyso-thermosensitive liposomal doxorubicin: a novel approach to enhance efficacy of thermal ablation of liver cancer. *Expert. Opin. Pharmacother.* 10, 333–343 (2009).

51 Iga K, Hamaguchi N, Igari Y *et al.* Enhanced anti-tumor activity in mice after administration of thermosensitive liposome encapsulating cisplatin with hyperthermia. *J. Pharmacol. Exp. Ther.* 257, 1203–1207 (1991).

52 Woo J, Chiu GN, Karlsson G *et al.* Use of a passive equilibration methodology to encapsulate cisplatin into preformed thermosensitive liposomes. *Int. J. Pharm.* 349, 38–46 (2008).

53 Chelvi TP, Ralhan R. Hyperthermia potentiates anti-tumor effect of thermosensitive-liposome-encapsulated melphalan and radiation in murine melanoma. *Tumour. Biol.* 18, 250–260 (1997).

54 Sharma D, Chelvi TP, Kaur J, Ralhan R. Thermosensitive liposomal taxol formulation: heat-mediated targeted drug delivery in murine melanoma. *Melanoma Res.* 8, 240–244 (1998).

55 Lindner LH, Hossann M, Vogeser M *et al.* Dual role of hexadecylphosphocholine (miltefosine) in thermosensitive liposomes: active ingredient and mediator of drug release. *J. Control. Release* 125, 112–120 (2008).