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Thermosensitive Liposomes

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Abstract

Thermosensitive liposomes (TSLs) are a drug delivery system for targeted delivery that release the encapsulated drug when heated to fever temperatures (~40–42°C). Combined with localized hyperthermia, TSLs allow precise drug delivery to a targeted region. While mostly investigated as cancer therapy, other applications including treatment of local infections and wound healing have been explored. Over the last ~40 years, numerous TSL formulations and payloads have been investigated. As with other nanoparticles, the addition of targeting molecules to TSL has been examined to improve targeted delivery. TSL release kinetics and plasma stability are two important factors that affect efficacy, and new formulations often aim to further improve on these properties. The possibility of encapsulating a magnetic resonance (MR) contrast agent that is released together with the encapsulated drug allows for visualization of drug delivery with MR imaging. Various heating modalities have been examined in combination with TSL. Since the goal is to expose a defined tissue region to uniform temperatures within the range where TSLs release (typically ~40–43°C), the choice of an appropriate heating modality has considerable impact on treatment efficacy. Several ongoing clinical trials with TSL as cancer therapy suggest the potential for clinical impact in the near future.

Keywords: liposomes, thermosensitive liposomes, triggered release, hyperthermia, drug targeting, drug delivery

1. Introduction

Surgical resection, radiation therapy, and chemotherapy are the three primary cancer treatment modalities. While chemotherapy is used in the treatment of almost all cancers, it has challenges and limitations. Most of the chemotherapeutic agents are highly cytotoxic to both cancer and normal tissues. Often chemotherapy is administered systemically, meaning it is

not directed to the cancerous tissues. The drug uptake by normal tissues causes off target effects including severe toxicities to different organs such as heart, liver, or kidneys, immune system, and others. In quite a number of cases, the toxicity profile of the drug limits the maximum tolerated dose (MTD) that can be administered. It is well known that inadequate dose is a primary cause for tumor recurrence and development of drug resistance. Thus, typically the highest possible dose is given to a patient to maximize the amount taken up by the cancerous tissues. All these factors have led to the development of methods to direct the drug to the tumor tissue, including various nanoparticles such as liposomes.

Liposome-encapsulated drug has evolved as a very potent source of directing the drug to the site of tumor. There are several ways by which a drug can be targeted to the tumor using liposomes. Kunjachan et al. review the various methods by which liposomes can be used to target the tumors [1]. Standard chemotherapy involves the administration of free (i.e., unencapsulated) drug (**Figure 1A**). Encapsulating the drug within a liposomal formulation allows prolonged blood circulation with very limited tissue uptake. Liposomes and other nanoparticles are most often based on passive targeting (**Figure 1B**). That is, they rely on the enhanced permeability and retention (EPR) effect resulting from leaky tumor vessels combined with absent lymph drainage in most tumors [2]. As a result, liposomes preferentially accumulate within the tumor over typically 24–48 hours. Tumors can be actively targeted by adding antibodies to the liposome surface, which are specific to either the cancer cells themselves (**Figure 1C**), or specific to the endothelial cells of the tumor vasculature (**Figure 1D**). One limitation of this antibody-based approach is that due to the heterogeneity of tumors, not all tumors or cancer cells have the unique antigen for the targeting antibody to bind. Another targeting method includes the use of an external trigger to release the drug either within the interstitium (i.e., after letting the liposomes accumulate via EPR effect) (**Figure 1E1**) or by releasing the drug within the vasculature of the tumor (**Figure 1E2**). The latter method requires liposomes specifically designed to respond to the specific trigger. Depending on the liposome, various external energy sources or biological signals may trigger drug release; these include heat, light, pH, and ultrasound, among others. In this chapter, we will focus on using heat as trigger, i.e., we will discuss in detail the evolution and current status of thermosensitive (or temperature sensitive) liposomes (TSLs).
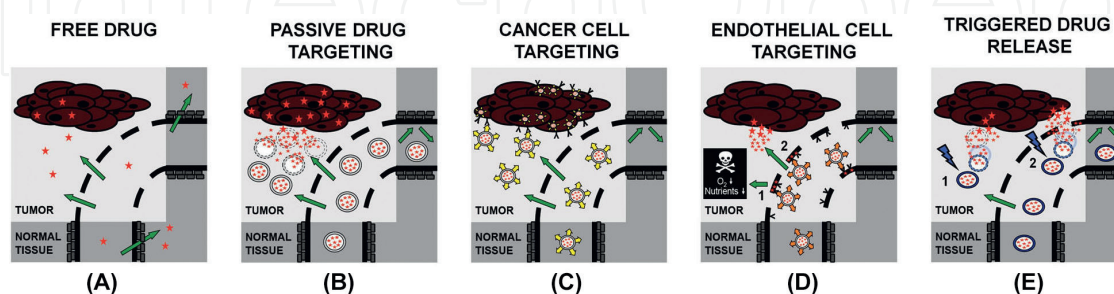
Figure 1. Current drug targeting strategies. (A) Conventional therapy or free drug infusion. (B) Passive targeting by liposomes utilizing EPR effect. (C) Active targeting of liposomes labeled with tumor-specific antibody. (D) Active targeting of liposomes with endothelial cell-specific antibody. (E) Triggered drug release either (1) within the tumor interstitium or (2) intravascular release. TSL fall into this last category reproduced with permission from Ref. [1]. Copyright (2015) American Chemical Society.

The strategy is that TSLs are administered systemically, followed by local hyperthermia (>40–42°C). The local hyperthermia triggers drug release within the targeted region, by which the drug becomes bioavailable and can exhibit the intended cytotoxic effect. Thus, the combination of TSL with a localized heating modality allows for localized drug delivery.

Note, however, that TSLs may have additional clinical applications outside cancer therapy, as there are various other clinical indications where it is necessary to deliver a drug targeted to a specific region within the body.

2. Background: evolution of thermosensitive liposomes (TSLs)

Liposomes as carriers of therapeutic drugs have been long investigated as a tool for improving the therapeutic index (i.e., decreasing the toxicity associated with drug delivery, while improving delivery to tumor). In 1995, Doxil [3] became the first nanoliposomal drug to be approved by Food and Drug Administration (FDA). However, with liposomes, a major limitation was directing the liposomes to the tumor. Initial liposomal formulations such as Doxil depend on preferential passive liposome accumulation based on the enhanced permeability of the tumor blood vessels, together with the lack of lymph drainage (EPR effect). However, it takes a considerable time (about 1–2 days) for the liposomal drug to accumulate within tumor. Moreover, the drug accumulated within the tumor is not bioavailable as it is still encapsulated within the liposomes [4]. The result was that Doxil achieved reduced toxicity while efficacy was in general not better than unencapsulated drug.

In 1978, Yatvin et al. [5] suggested for the first time the use of temperature sensitive liposomes (TSLs) (i.e., liposomes that release the encapsulated drug in response to heat) combined with hyperthermia for targeting the drug to tumors or local infections. The basic idea was to administer this liposomal drug systemically, and then expose only the tissue region where drug delivery is intended to hyperthermia. They proposed to use slightly higher temperature (42–44°C) than normal body temperature (37°C) to target drug delivery. This first TSL formulation used the two lipids dipalmitoyl phosphatidylcholine (DPPC) and distearoyl phosphatidylcholine (DSPC) to make liposomes sensitive to heat. DPPC and DSPC have "liquid-crystalline transition temperatures (Tm)" of 41 and 54°C, respectively; here Tm is the temperature at which the lipids undergoe a transformation from a solid gel-like structure (i.e., highly impermeable to hydrophilic substances) to a highly permeable liquid structure. These liposomes are now often termed as traditional thermosensitive liposomes (TTSLs) [6]. The reason that two lipids were used is because TSLs were too leaky when only a single lipid was used. Hence, a combination of DPPC and DSPC (ratio 3:1) was used, and these first TSLs encapsulated the antibiotic neomycin with the aim of treating bacterial infections.

Use of TTSLs for cancer treatment was first tested by Weinstein et al. [7] in 1979 in mice-carrying lung cancer. They showed that there was a fourfold increase in the amount of methotrexate delivered to heated tumors using the initial TTSL composition with slightly varied ratios (DPPC:DSPC = 7:3). A major limitation of this initial formulation was the quick elimination of the liposomes within 1 hour of the infusion.

In the following decades, various modified compositions were proposed based on the original formulation above. The primary goal was to increase the liposome stability and reduce leakage of the contained drug when exposed to serum [8]. This search led to the incorporation of cholesterol to the composition of liposomes [8, 9]. Gaber et al. showed that by using cholesterol, the phase transition can be avoided as the lipids are in liquid-ordered phase [10]. However, the incorporation of cholesterol delayed the complete release of the encapsulated drug doxorubicin to about 30 minutes at 42°C. Also around the same time, strategies were developed for circumventing the reticuloendothelial system and the immune system [11], which was addressed by the incorporation of polyethylene glycol (PEG) in the 1990s. Some studies showed that clearance of TSL was size dependent [12]. Larger liposomes were cleared quickly whereas smaller liposomes took a longer time to be cleared. However, small liposomes are less stable at normal body temperature (37°C). Hence, liposomes in the size range of 50–200 nm were recommended [12]. Around the same time mid-1990s, Kono et al. [13, 14] proposed the incorporation of thermosensitive polymers into liposomes to make them temperature sensitive. TSLs carrying polymers such as poly (N-isopropylacrylamide) were being evaluated [14]. However, a major setback for using these polymers was that they were not biodegradable. The next major breakthrough occurred in 2000 when Needham et al. [15] reported the successful incorporation of lysolipids and PEG into the liposomal lipid composition (DPPC:MPPC:DSPE-PEG2000 in the ratio of 90:10:4). Lysolipids are derivatives of lipid in which acyl derivatives are removed by hydrolysis making them more hydrophilic. The incorporation of lysolipids caused the rapid release of the encapsulated doxorubicin at hyperthermia temperatures (42°C). These have been called as lysolipid temperature-sensitive liposomes (LTSL). LTSL released much more rapidly (seconds) than prior formulations [16]. LTSLs were found to substantially improve delivery efficacy, with 3.5 times enhanced tumor drug delivery compared to TTSL, and ~17 times higher than unencapsulated drug [17]. A formulation similar to the one proposed by Needham is so far the only TSL formulation that made it into human clinical trials. However, the plasma half-life of LTSL is still not ideal with median initial plasma half-life of about 1 hour in humans and 1.5 hours in dogs [18, 19].

In 2004, Lindner et al. proposed a novel formulation based on the new lipid 1.2-dipalmitoyl-sn-glycero-3-phosphoglyceroglycerol (DPPG$_2$) with prolonged plasma half-life and similarly short release rates as LTSL [20, 21]. Initial studies with DPPG$_2$-TSL filled with carboxyfluorescein (CF) demonstrated initial plasma half-life 5 hours in rats [20]. More recent studies with doxorubicin-filled DPPG$_2$-TSL in cats showed plasma half-life of around 1 hour [22], similar to doxorubicin-LTSL.

As naturally occurring lipids were used for making TSL, they are usually considered safe.

2.1. Extravascular release versus intravascular triggered release

Three key requirements must be fulfilled by TSLs to be effective:

- Encapsulation of therapeutically relevant drug payload with minimum leakage.

- Avoidance of mononuclear phagocyte system (MPS) to prolonged circulation.

- Release of the payload (encapsulated drug) at target location (e.g., tumor).

The delivery strategy of initial liposome formulations (nonthermosensitive Stealth liposome, e.g., Doxil) was based on passive accumulation in tumor interstitial space (**Figure 1B**), followed by slow release within the interstitium (extravascular release). Since TSL release is actively triggered, TSL-based delivery may be used based on either of two delivery approaches: extravascular and intravascular triggered release (indicated by (1) and (2) in **Figure 1E**), based on whether release occurs inside the vasculature/blood or in the tumor interstitium. For extravascular triggered release, the targeting is passive and mostly relies on the EPR effect. For intravascular triggered delivery, there is no explicit-targeting mechanism, but rather targeting is based by location where heating is induced.

Since extravascular triggered release requires passive accumulation of TSL in the tumor interstitium before trigger of release, there is a necessary time delay between TSL administration and hyperthermia (typically several hours). This also means that TSLs of adequate plasma stability are required, with a plasma half-life exceeding many hours. Computer models suggest that the optimal release rate for extravascular triggered release is in the order of many minutes to hours [16, 23].

For intravascular triggered release, hyperthermia occurs ideally immediately after, or even during TSL administration [24]. This is because any leakage of drug after delivery is detrimental during intravascular triggered delivery, as it reduces the amount available for release. Thus, plasma stability requirements are less stringent than for extravascular triggered delivery. Release occurs while TSLs transit the heated tumor region; this transit time is in the range of a few seconds for most tumors, and thus ideally TSL should release very rapidly (within seconds).

Both intravascular and extravascular triggered approaches are the subject of ongoing preclinical studies as described in the previous section [25, 26]. It is interesting to note that, while the benefit of faster releasing TSL has been demonstrated in 2000 [17], it was only elucidated recently that intravascular triggered delivery was the dominant delivery mechanism, and responsible for improved delivery with fast-release TSL [16, 27].

Table 1 summarizes the differences between intravascular and extravascular triggered release.

Mathematical models are an effective tool to evaluate different TSL delivery strategies and drug transport kinetics. Such models have several advantages which include: ability to utilize a large body of physiological and physiochemical data, prediction of pharmacokinetics and target tissue dose, and extrapolation of results both across species and routes of administration [28, 29]. The latter point is of relevance since results from experimental animal studies often do not translate into human patients, and models can thus help explain such deviations [30].

Drug delivery system	Tumor targeting	Initiation of heating	Typical TSL leakage rate	Ideal TSL release rate
Extravascular triggered TSL (TSL-e)	Passive (EPR)	Hours after TSL infusion	hours-days	hours
Intravascular triggered TSL (TSL-i)	Active via heat source	During, or immediately after TSL infusion	minutes	seconds

Table 1. Comparison of TSL for intravascular and extravascular triggered release.

In 2012, extravascular and intravascular triggered release approaches were compared using a mathematical model [16]. Specifically, the following drug delivery strategies were compared based on the chemotherapy agent doxorubicin: (1) unencapsulated drug; (2) nonthermosensitive stealth liposomes; (3) intravascular triggered TSL (TSL-i); and (4) extravascular triggered TSL (TSL-e). The models predict that intravascular triggered release results in considerably higher drug uptake by cancer cells (i.e., efficacy) compared to the other delivery modalities (**Figure 2**). During intravascular triggered delivery, new TSLs enter the tumor vasculature and release drug as long as hyperthermia is present. The systemic blood volume thus serves as reservoir of nonbioavailable drug that becomes bioavailable when entering the target tissue region.

2.2. Release kinetics

As described above, the intravascular triggered delivery strategy is most effective when TSLs have very rapid release, within seconds. This is the reason why the later, fast-release formulations that release within seconds greatly improved drug accumulation in tumors compared to early formulations that required many minutes to release (**Figure 3**). Unfortunately, plasma
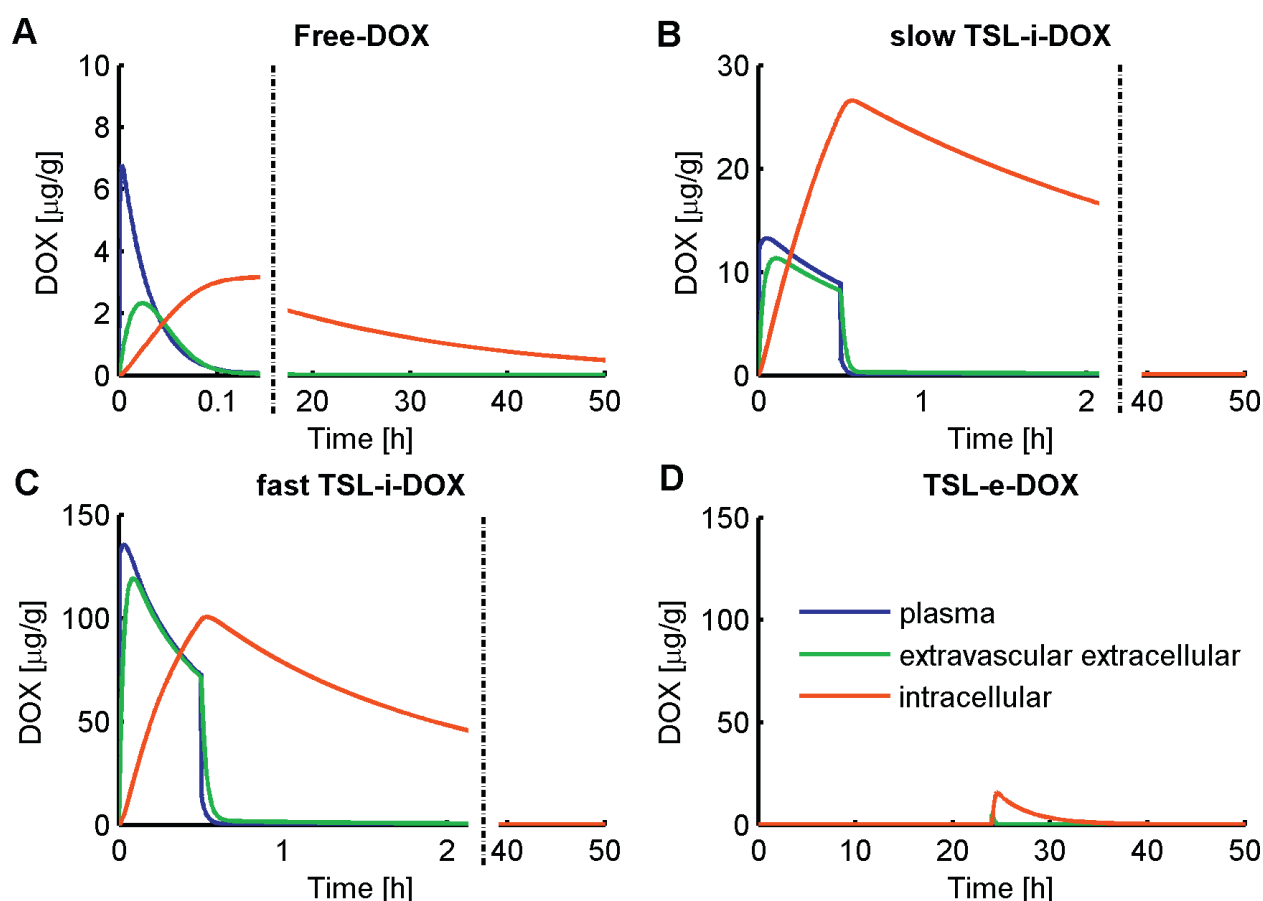
Figure 2. Doxorubicin concentrations (unencapsulated drug) in plasma, extravascular-extracellular space (EES), and inside cells are plotted over time for different delivery systems: (A) free-DOX (unencapsulated drug), (B) slow-release TSL-i-DOX (release rate ~min), (C) fast-release TSL-i-DOX (release rate ~seconds), and (D) TSL-e-DOX [7]. Hyperthermia for 30 minutes was applied immediately for TSL-i, and after 24 hours for TSL-e (to allow for TSL accumulation in EES). Reproduced from Ref. [16].
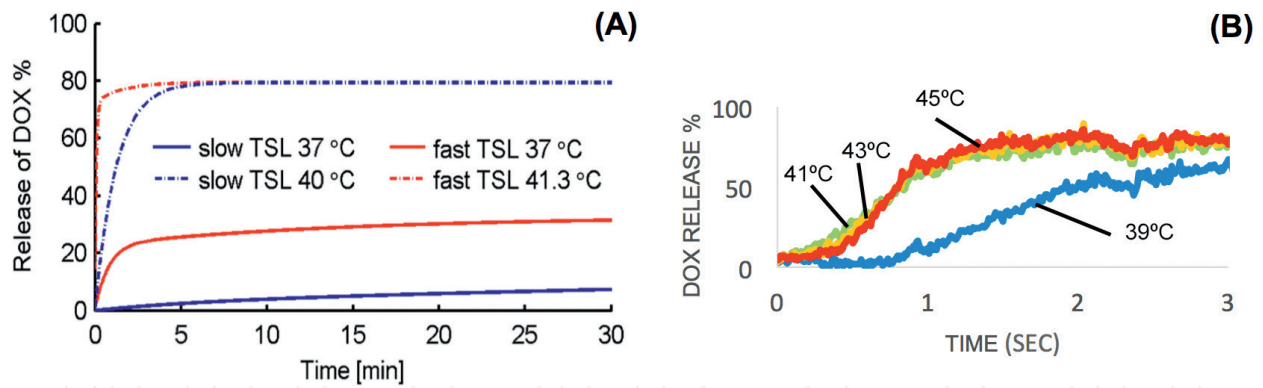
Figure 3. (A) Graph shows release rate during hyperthermia (40–41°C), as TSL stability at body temperature (37°C), comparing a slow-release formulation and a newer fast-release formulation (reproduced from Ref. [16]). (B) Graph shows a release rate within first few seconds between 39 and 45°C of a fast-release formulation in fetal bovine serum (FBS) (unpublished data). TSLs were prepared according to Needham et al. [15] with slight modifications. (DPPC: MSPC: DSPE-PEG2000 85.3:9.7:5), and loaded with Dox using a citrate-based pH gradient.

stability is directly tied to release during hyperthermia, i.e., typically the faster a liposome releases when heated, the more this liposome leaks at body temperature (**Figure 3A**) [26].

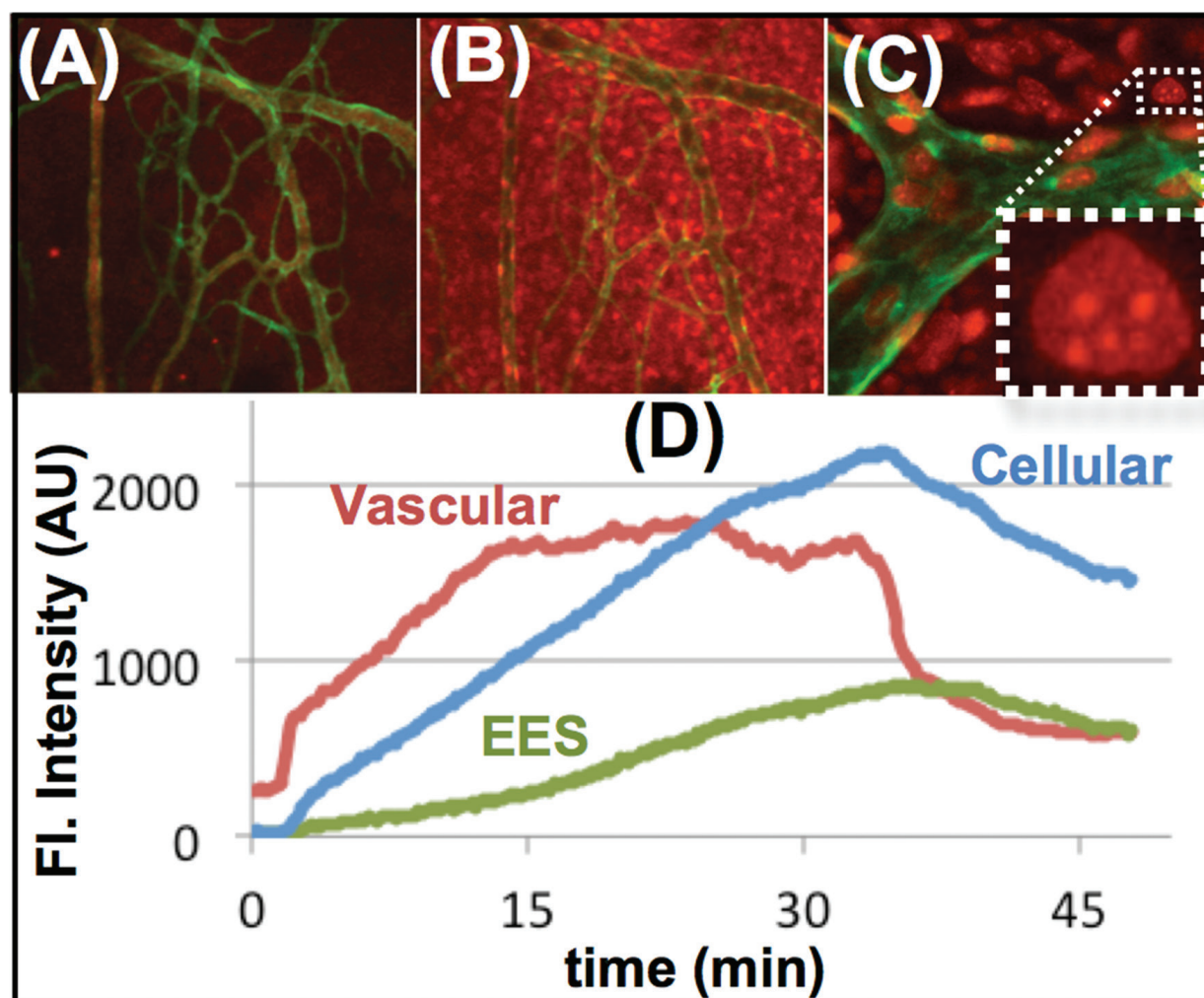## 2.3. Intravital microscopy

Intravital microscopy is an important technology that enables visualization of drug release and uptake at microscopic scales. This enables better understanding of how the drug is taken up by the tumor once it is released from the TSL. Using fluorescent compounds such as CF or doxorubicin, it is possible to observe the drug in different compartments (e.g., inside vasculature and cells). This imaging methodology requires visual access to tumors, which is typically provided by windows (**Figure 4**).
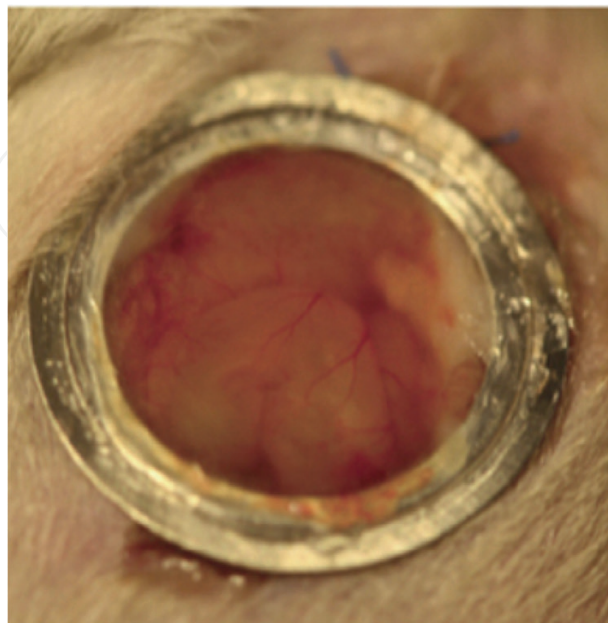
Figure 4. Window chamber in a mouse. Reproduced with permission from Ref. [32]. Copyright (2013) Nature Publishing.

A detailed procedure of implantation of a window chamber was explained by Ritsma et al. [31]. A small viable piece of tumor (~1–3 mm^3) is transplanted into the fascia of the dorsal skin flap which is placed within a window chamber of the recipient mice [32]. To allow visualization during hyperthermia, the tumor needs to be heated. For this purpose, a special heating coil has been developed that allows the uniform heating of these window chambers carrying tumors. The imaging takes place while animals are under anesthesia, after TSLs have been administered. While tumors are exposed to hyperthermia, tumors are imaged using confocal fluorescence microscopes using appropriate excitation and emission filters depending on fluorescence properties of the molecule. **Figure 5** demonstrates the uptake of doxorubicin released from the TSL within the blood during hyperthermia, and drug uptake by cancer cell nuclei.

Figure 5. Intravital fluorescence microscopy demonstrates intravascular triggered release. Images show labeled endothelial cells (green) and doxorubicin fluorescence (red). Tissue accumulation and cell uptake are demonstrated during hyperthermia-induced release from TSL (30 minutes, 42°C), (A) after 5 minutes and (B) after 20 minutes (field of view (FOV) 500 × 500 μm). (C) Subcellular doxorubicin localization is observed at higher magnification (inlet). (D) Aggregate fluorescence within vessels, interstitium (EES), and intracellular regions extracted from image data in (A) and (B). These data demonstrate triggered initial intravascular release, followed by tissue uptake within EES and cells. Unpublished data courtesy of Dr. Timo ten Hagen.

2.4. Targeted thermosensitive liposomes

Various targeting moieties such as antibodies are nowadays widely used for targeted delivery of liposomes and other nanoparticles and have also been investigated for TSL. The idea of attaching an antibody to a TSL was reported by Sullivan and Huang [33] as early as 1985. Sullivan and Huang [33] used covalently attached antiH2Kk antibody to a palmitic acid derivative to make heat sensitive immunoliposomes (with DPPC) carrying carboxyfluorescein. They used a similar approach to successfully deliver uridine inhibitors to lymphoma cells *in vitro*. However, *in vivo* evaluation in mice carrying human ovarian cancers did not yield encouraging results. This was attributed to the leakiness of the liposomes, which used egg phosphatidylcholine, egg phosphatidylglycerol, cholesterol, and phosphatidylethanolamine in the ratio 38.1:4:32:1.9 [34].

With the development of newer TSL formulations (e.g., LTSL), there has been an increased interest in conjugating targeting molecules to TSL, particularly to those carrying the chemotherapeutic drug doxorubicin. Antibodies, peptides, and aptamers have been successfully added to TSLs. **Table 2** summarizes the targeting molecules that have been used.

Antibodies targeting common receptors found in cancers such as human epidermal growth factor receptor 2 (HER-2) [35] and epidermal growth factor receptor (EGFR) [36] have been conjugated to LTSL carrying doxorubicin that are being evaluated in animal models. Kullberg et al. [37] showed that adding listeriolysin O along with HER-2 antibody enhanced cytoplasmic delivery of the cargo.

Similarly, Na et al. added elastin-like peptide (ELP), which significantly improved the drug uptake within cells [38].

References	Type	Targeting molecule	Target	Payload encapsulated
[33]	Antibody	Anti H2Kk	H2Kk	Carboxyfluorescein
[35]	Antibody	Anti Her-2	Her-2	Calcein
[36]	Antibody	Anti EGRF	EGFR	Calcein, Dox
[38]	Peptide	Val-Pro-Gly-Val-Gly	Intracelluar delivery	Dox
[39]	Peptide	Cys-Arg-Glu-Lys-Ala	Clotted plasma proteins in tumor vessels	Dox
[40]	Peptide	Arg-Cys-D-Phe-Asp-Gly	tumor and angiogenic endothelial cells	Dox
[41]	Peptide	CCRGDKGPDC	$\alpha v\beta 3$-positive cells	Dox
[42]	Aptamer	AS1411	nucleolin receptors	Contrast agent (Gd-DTPA)
[43]	Peptide cargo		Bone regeneration	107–111 pentapeptide of the parathyroid hormone-related protein

Table 2. Targeted thermosensitive liposomes.

Moreover, peptides that target tumors have also been added to TSLs. Wang et al. added the tumor homing pentapeptide (Cys-Arg-Glu-Lys-Ala) (CREKA) to TSLs and evaluated their efficacy in MCF-7 bearing nude mice [39]. Dicheva et al. [40] added a cyclic pentapeptide to TSL-doxorubicin improving the drug uptake and delivery. Deng et al. [41] improved the anti-tumor efficacy by adding the iRGD peptide.

Most recently, Zhang et al. [42] used an aptamer conjugated TSLs loaded with contrast agent that targeted the nucleoporin receptors. Besides displaying excellent biocompatibility, they showed promise in the early detection of cancers.

In a somewhat different application, Lopez et al. developed a collagen-based scaffold to which TSLs were covalently attached via targeting molecule to slowly release a peptide cargo with proosteogenic effect from the scaffold [43].

3. Payloads

Ever since the initial studies where neomycin was encapsulated in TSL [5], several other drugs and reporter molecules have been encapsulated by various TSL formulations and evaluated. Several combinations have successfully made it to various stages of preclinical and clinical trials. A brief overview of the compounds that have been successfully encapsulated will be reviewed here.

The fluorescent reporter carboxyfluorescein (CF) has been the molecule of choice for studying the release kinetics of TSLs. Starting from the initial studies until today, CF has been used in the study of various TSL combinations. Other fluorescent molecules such as calcein have also been used to study TSL release.

Yatvin et al. encapsulated cisplatin (cis-dichlorodiammineplatinum(II)) in 1981 [44] and evaluated against mice tumor sarcoma. This suggested that a whole array of different compounds could be encapsulated by TSLs. However, until the late 1980s, only water soluble compounds were being encapsulated into TSLs.

Doxorubicin is an amphiphilic compound that was encapsulated into the TSL toward the end of 1980s. Doxorubicin is a highly cytotoxic chemotherapeutic drug belonging to the group of anthracyclines, with several off target effects such as cardiotoxicity and myelo-suppression. Tomita et al. encapsulated doxorubicin in DPPC: cholesterol-based TSL to improve stability [45]. Other formulations further attempted to improve TSL stability [10, 46]. Unezaki et al. reported the active loading of doxorubicin against a pH gradient into TSLs [47], which resulted in more than 90% encapsulation efficacy. The TSL composition developed by Needham et al. [15] is the formulation that progressed furthest toward clinical use, with ongoing clinical trials that will be discussed later. One of the significant developments that occurred more recently for TSL-Dox was the incorporation of contrast agents. Several researchers encapsulated doxorubicin along with gadolinium-based contrast agents [48–50]. This provided the ability of monitoring the release of a contrast agent from TSL

and subsequent tissue uptake by magnetic resonance imaging (MRI), indicating the tissue regions where doxorubicin may be delivered to.

Following doxorubicin, several groups encapsulated other drugs belonging to the same family of anthracycline drugs in TSL, including daunorubicin, idarubicin, and epirubicin. The initial studies with daunorubicin in the mid-1990s in mice models of sarcoma were disappointing [51]. However, more recent studies with newer formulations of idarubicin-TSL showed superior survival rate and tumor growth inhibition as compared to free idarubicin [52]. Similar results were demonstrated with epirubicin-TSL in animals [53].

The successful encapsulation of anthracyclines with high efficiency prompted the search for other molecules with high encapsulation efficiency. Liu et al. [54] reported that using metal ions such as Zn or Cu could lead to high efficacy in encapsulation of cisplatin. Moreover, the presence of metal bound liposomes increased the cytotoxicity.

Apart from anthracyclines [65], the drugs bleomycin [55], melphalan [56], placitaxel [57], docetaxel [58], and gemcitabine [59] have been encapsulated into TSL and delivered to tumors, while reducing systemic drug toxicities.

Another fluorescent compound that was successfully encapsulated in TSL is the cancer drug 5-fluorouracil. Sabbagh et al. used a lipid combination containing DPPC, cholesterol, and PEG to encapsulate 5-fluorouracil. They further found that complexing 5-fluorouracil with copper-polyethylenimine improved the stability of the liposomes with a higher encapsulation efficacy [60].

Recently, vinorelbine was encapsulated into a TSL formulation [61]. Vinorelbine is a wide spectrum chemotherapeutic agent used in treatment of breast, lung, and liver cancers. However, free vinorelbine is associated with venous toxicity causing blisters when infused directly into the blood. The authors reported that combining vinorelbine-TSL with hyperthermia resulted in enhanced antitumor activity. In another study, Wang et al. showed that [62] vinorelbine-TSL in combination with radiofrequency ablation (RFA) improved the survival of micecarrying liver tumors.

Another interesting recent application of TSL is the targeted delivery of the antibiotic ciprofloxacin to aid wound healing. Wardlow et al. [63] demonstrated the encapsulation of ciprofloxacin in TSL, and used these for delivery to hyperthermic areas using a rat model. They suggested that this formulation could be used for chronic wound healing. However, work still remains to evaluate these TSLs in an animal model of chronic would healing.

Most recently, it was reported that chemotherapeutic drugs vincristine and doxorubicin were coloaded into TSL in combination. Li et al. showed that multiple drug loading could be achieved to exploit the synergy between drugs [64].

It should be noted that each drug has to be individually tested, i.e., there is no single TSL formulation that would work for all drugs. In addition, the release rate and leakage will vary for different drugs, and it may not always be possible to achieve rapid release within seconds as is ideal for intravascular triggered release. **Table 3** summarizes the drugs that have been encapsulated in a TSL formulation and the liposomal composition.

References	Composition	Ratio	Cargo/payload
[5]	DPPC, DSPC	3:1	Neomycin
[7]	DPPC, DSPC	7:3	Methotrexate
[44]	DPPC, DSPC	7:1	Cisplatin
[45]	DPPC, Cho		Doxorubicin
[10]	DPPC, HSPC, Cho, PEG	100:50:30:6	Doxorubicin
[51]	DSPC:Cho		Daunorubicin
[52]	DPPC:DSPC:DSPE-PEG	6:3.5:0.5	Idarubicin
[53]	DPPC:MSPC:DSPG:DSPE-mPEG2000	82:8:10:4	Epirubicin
[55]	DPPC: DSPC	9:1	Bleomycin
[56]	Phosphatidyl choline, cho		Melphalan
[57]	DPPC:MSPC:DSPE-PEG2000:DSPG	83:3:10:4	Paclitaxel
[58]	DPPC:DSPE:PEG2000:EPC:MSPC: DTX	82:11:4:3:4	Docetaxel
[59]	DPPC:DSPC:DPPG$_2$	50:20:30	Gemcitabine
[60]	DPPC:CHO:DSPE-PEG	90:5:5	5-Fluorouracil
[61]	DPPC:MPPC:DSPE-PEG2000	86:5:4	Vinorelbine
[63]	DPPC:MSPC:DSPE-PEG	85.3:9.7:5.0	Ciprofloxacin
[64]	DPPC: DSPE-PEG2000: MSPC	75:17:8	Doxorubicin & Vincristine
[48]	DPPC:MSPC:DSPE-PEG2000	85.3:9.7:5.0	Doxorubicin & ProHance®

Table 3. Thermosensitive liposomes composition and payloads.

4. Heating modalities

TSLs have been used successfully in combination with various heating modalities in both animal models and in human clinical trials. Some of these heating modalities include devices already in clinical use; others have only been used in animals. Ideally, only the targeted tissue region is exposed to temperatures within the range where TSL release (typically above ~40°C). In addition, higher temperatures (>43–50°C) may result in reduced blood perfusion [66] and should be avoided since without perfusion TSLs are not transported to the target site. Thus, in an ideal case, the targeted tissue region should be exposed to a quite narrow temperature range (~40–43°C). For larger tumors—particularly as can be the case in humans or large animals—achieving this temperature uniformity in large tissue volumes is challenging, and the hyperthermia device becomes an important element affecting TSL-based drug delivery efficacy.

Since deep-seated tumors are typically identified based on medical imaging (e.g., computed tomography (CT), magnetic resonance imaging (MRI), or ultrasound imaging), TSLs may be used for image-guided drug delivery. Here, the intent is to deliver drug to a specific region of the body identified by medical imaging. Since TSLs are administered systemically, image-guided drug delivery is realized by exposing the targeted tissue volume to hyperthermic temperatures by image-guided heating devices. Thus, one important aspect that should guide

the selection of the heating modality is ability to expose only the targeted tumor region to uniformly hyperthermic temperatures.

4.1. Animal hyperthermia systems

4.1.1. Water bath

Water bath as a heat source has been used extensively in preclinical studies, especially involving small animals. Usually the animal is anesthetized, hair removed if necessary and the region surrounding the tumor is immersed in a water bath, which is preheated to the required temperature (usually >40°C). It is essential to make sure that the skin distant from the targeted tumor is not exposed to the heat from the water bath. For example, some researchers used a water bath cover made of material that does not conduct heat, with the other parts of the body covered by a polystyrene cover [50]. Other studies used a plastic syringe to hold the leg of mice in place to expose only the tumor to heated water while protecting the other leg from heat [32]. Ultrasound gel or vaseline has also been applied to protect the tissues surrounding the tumor from heat exposure.

4.1.2. Light sources

Various light sources have been employed to induce hyperthermia, usually for surface heating of subcutaneous tumors. Several groups used a cold lamp, which emits visible light (350–700 nm wavelength) [50, 59]. By adjusting the power of the lamp, a target temperature of ~41–43°C was achieved. White cotton wool was placed around the area surrounding the tumor to avoid heating and drug delivery.

Near infrared (NIR) lasers (~800–1000 nm wavelength) have also been used as a heating sources in nanoparticle-based drug delivery systems, which can penetrate tissue to depths in the range of ~0.5 cm [51, 67, 68]. The diameter of the laser spot can be adjusted by optical lenses to correspond to the targeted area.

4.1.3. Microwave hyperthermia

Microwave devices have a long history for use in hyperthermia studies [69] and have been used in combination with TSLs, for example by the first *in vivo* TSL study by Weinstein et al. in 1979 [7]. They used a system specifically designed to expose subcutaneous rodent tumors to hyperthermia through microwave antennas placed on the skin. Three other studies also used surface microwave applicators: one trial in dogs [19], one in cats [22], and a phase I trial in humans for breast cancer; the latter two used a FDA-approved microwave hyperthermia system [70]. While there are also interstitial microwave antennas for heating deep tissue regions [69], to our knowledge these have not yet been used in combination with TSL.

4.2. Clinically used hyperthermia and thermal ablation devices

4.2.1. Radiofrequency ablation

Thermal tumor ablation is a heat-based cancer therapy, where the cancer is killed by heat alone, by heating above ~50°C. Most widely used is radiofrequency ablation (RFA), where

radiofrequency electric current is applied to tissue via electrode inserted into the tumor under image guidance [71]. The electric current results in localized tissue heating (**Figure 6**). In the clinic, RFA is used guided by medical imaging techniques such as MRI, ultrasound, or CT, and is currently in use for liver, lung, kidney, and other types of cancer.

Since local tumor recurrence often occurs at the margin of the tissue regions killed by heat, TSLs have been combined with RFA to preferentially deliver chemotherapy to these margins that are exposed to sublethal, hyperthermic temperatures (**Figure 7**). This combination is also being examined in clinical trials for treatment of primary liver cancer.

There are other technologies for tumor ablation similar to RFA used clinically, such as microwave ablation and laser. While these could also be combined with TSL, studies on such combinations are not yet available.

### 4.2.2. High-intensity focused ultrasound (HIFU)

High-intensity focused ultrasound (HIFU) in combination with magnetic resonance imaging (MRgHIFU) is in clinical use for treatment of tumors by heating them to >50°C (i.e., thermal
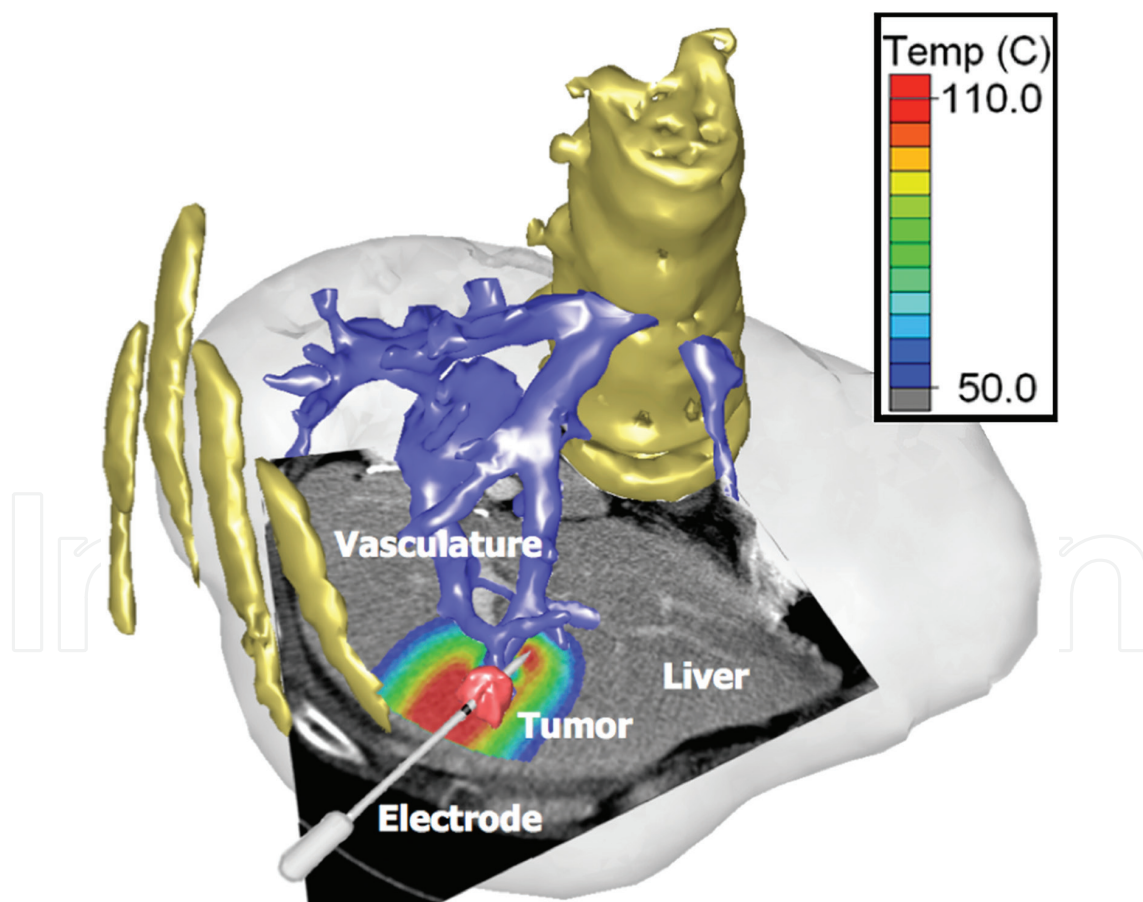
Figure 6. Overview of liver radiofrequency tumor ablation procedure as clinically performed. An RFA electrode is inserted into the tumor under imaging guidance, and the tumor is killed by heat. Tumor recurrences following ablation occur primarily in the margin of the ablation zone, where inadequate temperatures were obtained (<50°C). The combination with TSL delivers drug at high dose to this ablation margin, potentially reducing recurrences. Adapted from Ref. [72].
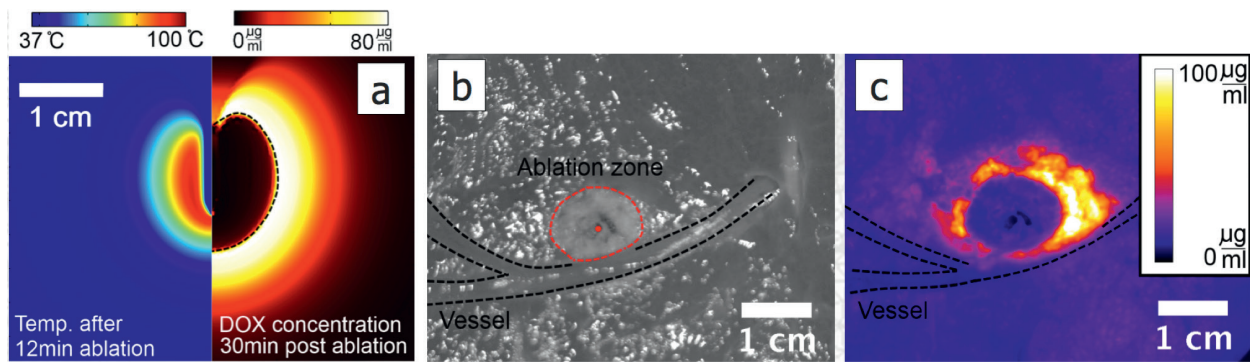
Figure 7. Combination of tumor ablation with TSL. (a) Two-dimensional (2-D) computer simulation of temperature (left), and of drug delivery (right), showing drug uptake in the margin of the ablation zone. Results are based on a prior computer model [73]. (b) Results of a recent porcine animal study (normal liver) demonstrating visible ablation zone, and (c) drug delivery in the margin of the ablation zone visualized by fluorescence imaging (unpublished results), qualitatively similar to computer model prediction in (a).
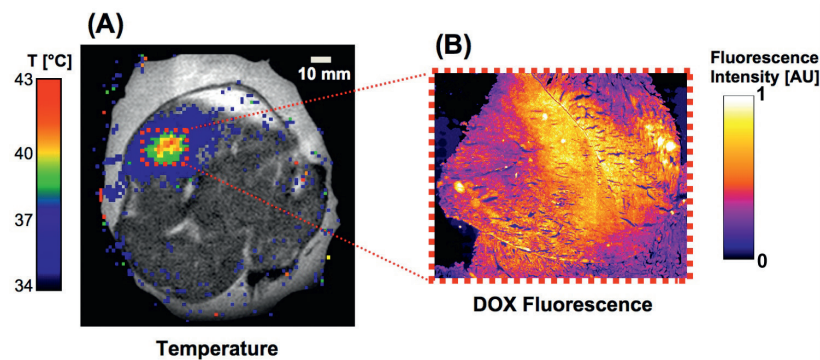
Figure 8. (A) Temperature map during MRgHIFU-hyperthermia measured via MR thermometry, overlaid on preprocedural proton density-weighted image of rabbit thigh muscle. The targeted tissue region is heated to ∼40–42°C after administration of TSL-Dox. (B) Doxorubicin distribution visualized via fluorescence microscopy in extracted tissue sample demonstrating localized, image-guided drug delivery. Figure adapted from Ref. [75].

ablation). HIFU employs focusing of ultrasound emitted from external ultrasound transducers into deep tissue regions, resulting in highly localized tissue heating (∼mm range diameter of focal spot). The focal spot can be electronically steered, allowing precise spatial targeting with mm accuracy. A technique named magnetic resonance (MR) thermometry allows real-time noninvasive imaging of tissue temperature and is ideally suited to monitor and control HIFU heating (**Figure 8A**) [73]. MRgHIFU thus allows noninvasive targeted heating of deep tissue regions while monitoring and controlling desired temperature, thus being ideally suited for TSL-based drug delivery (**Figure 8B**) [74–76]

5. Clinical trials

TSL formulations have been evaluated in both veterinary trials as well as in human clinical trials as cancer therapy. TSL formulations using doxorubicin have been evaluated in

the veterinary clinic for various cancers. A TSL-doxorubicin formulation (ThermoDox® by Celsion), which is based on the LTSL formulation by Needham et al., has been actively evaluated in the treatment of hepatocellular carcinoma and recurrent breast wall cancers. These clinical trials are briefly discussed below.

5.1. Animal trials

A phase I clinical trial was conducted in companion dogs with solid tumors (carcinomas and sarcomas). Of the 20 dogs that were enrolled in the study, from those that were treated at least twice with TSL-Dox, 12 dogs had stable disease and 6 had partial response. The toxicities observed were manageable [19].

Similarly, TSL-doxorubicin was evaluated in a pilot trial in the veterinary clinic for the treatment of feline soft tissue sarcoma [22]. Eleven cats with advanced sarcoma were divided into three treatment groups with increasing dosage of TSL-doxorubicin. Up to six treatments were delivered every alternate week with a radiofrequency applicator. Two cats in the highest dosage group showed partial response. Dosage was well tolerated in all the cats showing potential for larger studies.

5.2. Human trials

There have been several human clinical trials with TSL-Dox, all with the formulation ThermoDox® (Celsion Corp.), which is based on the LTSL formulation [15].

The furthest progress has been combining TSL with radiofrequency ablation (RFA) in primary liver cancer (i.e., hepatocellular carcinoma). The motivation was delivery of high doses of doxorubicin to the margin of the zone killed by heat, as shown in **Figure 6**. As there was a significant proportion of patients that had local tumor recurrence just outside the ablation zone, there was a strong premise for this approach. Wood et al. [18] reported results at the conclusion of a phase I study involving RFA and TSL-Dox in liver cancer patients. This safety trial showed that TSL-Dox was well tolerated with manageable side effects up to a maximum tolerated dose (MTD) of 50 mg/m^2 (this is in the same range as the MTD for unencapsulated doxorubicin). With the successful completion of this phase I trial, TSL-doxorubicin in combination with RFA was fast tracked to a phase III trial for primary/metastatic liver tumors in the "HEAT trial" (NCT00617981) [77], which unfortunately failed. There have been several possible explanations that have been attributed to this failure, which have been described in detail by Dou et al. [78]. However, a retrospective analysis showed that TSL-Dox in combination with RFA performed better in those patients where the RFA duration was at least 45 minutes [79], which was supported by further animal studies [80]. As a result, a follow-up phase III trial ("OPTIMA trial," NCT0211265) was initiated where required RFA duration was increased, and this trial is ongoing.

Another trial recently initiated in England also focuses on liver cancer (both primary and metastatic cancer) and combines TSL-Dox with HIFU ("TARDOX trial," NCT02181075).

In addition, there have been a few phase I and phase I/II trials where TSL-Dox was combined with microwave hyperthermia for recurrent chest breast wall cancer ("DIGNITY trial,"

NCT00826085). Zagar et al. reported the results of a phase I study using TSL-Dox in recurrent breast wall cancer [70]. Patients who had exhausted all other therapies were enrolled in this trial. Almost 17% of the enrolled patients showed complete remission and another 31% showed partial response. Based on the promising results of these prior phase I/II trials, a follow-up trial has been initiated in Europe ("EURO-DIGNITY," NCT02850419).

Finally, a phase I study has been recently announced in the United States, where TSL-Dox will be combined with MRgHIFU for treating childhood sarcomas (NCT02536183). Thus, at least four different ongoing clinical trials are in various stages of recruiting patients.

1. A phase I study of lyso-thermosensitive liposomal doxorubicin and MR-HIFU for pediatric refractory solid tumors (NCT02536183).

2. Targeted chemotherapy using focused ultrasound for liver tumors (TARDOX) (NCT02181075).

3. Study of ThermoDox with standardized radiofrequency ablation (RFA) for treatment of hepatocellular carcinoma (HCC) (OPTIMA) (NCT0211265).

4. Heat-activated target therapy of local-regional relapse in breast cancer patients (EURO-DIGNITY) (NCT02850419).

6. Conclusion

While TSLs have been first proposed almost 40 years ago, only within the last decade have first results from clinical trials in humans become available. Animal studies have shown that in ideal conditions, up to 20–30 times of bioavailable drug can be delivered to the tumor tissue (measured within a few hours of infusion) as compared to administration of the same dosage of free drug. TSL benefit from reduced off-target toxicity effects, similar to nontemperature sensitive liposomes already in clinical use. The efficacy of TSL depends both on the specific liposomal formulation (e.g., release rate, plasma stability), the encapsulated drug, and on the specific heating modality. Several such heating modalities are clinically available, with MRgHIFU being one of the most attractive methods. HIFU is noninvasive, allows exquisite spatial control of heating with mm accuracy, and combined with MR-thermometry tissue temperature can be monitored and controlled in real time.

Contrary to most other nanoparticle approaches, TSLs can be employed for image-guided drug delivery where the goal is to deliver drug to a region identified by medical imaging. Such an approach may find additional clinical applications apart from cancer. One limitation of many current TSL formulations is the still relatively short plasma half-life (~1 hour), which limits the duration available for delivery, reduces the quantity of encapsulated drug available for release, and also negatively impacts systemic toxicities.

In summary, TSLs represent a highly promising drug delivery approach that has the potential for considerable clinical impact in the near future.

Abbreviations

CF	Carboxyfluorescein
Dox	Doxorubicin
DPPC	Dipalmitoyl phosphatidylcholine
DPPG$_2$	1,2-dipalmitoyl-sn-glycero3-phosphodiglycerol
DSPC	Distearoyl phosphatidylcholine
DSPE-mPEG2000	Distearoyl glycero phosphoethanolamine
DSPE-PEG-2000	1,2-distearoyl-snglycero-3-phosphoethanolamine-N-polyethylene glycol 2000
DSPG	Distearoyl glycero phosphoglycerol
EPR	Enhanced permeability and retention
HIFU	High-intensity focused ultrasound
HSPC	Hydrogenated soy phosphatidylcholine
LTSL	Lysolipid temperature sensitive liposomes
MPPC	Monopalmitoyl phosphatidylcholine
MRgHIFU	Magnetic resonance-guided high-intensity focused ultrasound
MSPC	Myristoyl lyso glycero phosphocholine
MTD	Maximum tolerated dose
PEG	Poly ethylene glycol
RFA	Radiofrequency ablation
TSL	Thermosensitive liposomes
TTSL	Traditional thermosensitive liposomes

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References

[1] Kunjachan S, Ehling J, Storm G, Kiessling F, Lammers T. Noninvasive imaging of nanomedicines and nanotheranostics: Principles, progress, and prospects. Chemical Reviews. 2015;**115**:10907-10937. DOI: 10.1021/cr500314d

[2]   Maeda H. The enhanced permeability and retention (epr) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. Advances in Enzyme Regulation. 2001;**41**:189-207

[3]   Barenholz Y. Doxil(r)—the first fda-approved nano-drug: Lessons learned. Journal of Controlled Release. 2012;**160**:117-134. DOI: 10.1016/j.jconrel.2012.03.020

[4]   Park K. Facing the truth about nanotechnology in drug delivery. ACS Nano. 2013;**7**:7442-7447. DOI: 10.1021/nn404501g

[5]   Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. Science. 1978;**202**:1290-1293

[6]   Ta T, Porter TM. Thermosensitive liposomes for localized delivery and triggered release of chemotherapy. Journal of Controlled Release. 2013;**169**:112-125. DOI: 10.1016/j.jconrel.2013.03.036

[7]   Weinstein JN, Magin RL, Yatvin MB, Zaharko DS. Liposomes and local hyperthermia: Selective delivery of methotrexate to heated tumors. Science. 1979;**204**:188-191

[8]   Allen TM, Cleland LG. Serum-induced leakage of liposome contents. Biochimica et Biophysica Acta. 1980;**597**:418-426

[9]   Lelkes PI, Friedmann P. Stabilization of large multilamellar liposomes by human serum in vitro. Biochimica et Biophysica Acta. 1984;**775**:395-401

[10] 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. Pharmaceutical Research. 1995;**12**:1407-1416

[11] Mumtaz S, Ghosh PC, Bachhawat BK. Design of liposomes for circumventing the reticuloendothelial cells. Glycobiology. 1991;**1**:505-510

[12] Magin RL, Hunter JM, Niesman MR, Bark GA. Effect of vesicle size on the clearance, distribution, and tumor uptake of temperature-sensitive liposomes. Cancer Drug Delivery. 1986;**3**:223-237

[13] Kono K. Thermosensitive polymer-modified liposomes. Advanced Drug Delivery Reviews. 2001;**53**:307-319

[14] Kono K, Zenitani K, Takagishi T. Novel ph-sensitive liposomes: Liposomes bearing a poly(ethylene glycol) derivative with carboxyl groups. Biochimica et Biophysica Acta. 1994;**1193**:1-9

[15] 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 Research. 2000;**60**:1197-1201

[16] Gasselhuber A, Dreher MR, Rattay F, Wood BJ, Haemmerich D. Comparison of conventional chemotherapy, stealth liposomes and temperature-sensitive liposomes in a mathematical model. PLoS One. 2012;**7**:e47453. DOI: 10.1371/journal.pone.0047453

[17] Kong G, Anyarambhatla G, Petros WP, Braun RD, Colvin OM, Needham D, Dewhirst MW. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: Importance of triggered drug release. Cancer Research. 2000;**60**:6950-6957

[18] Wood BJ, Poon RT, Locklin JK, Dreher MR, Ng KK, Eugeni M, Seidel G, Dromi S, Neeman Z, Kolf M, Black CD, Prabhakar R, Libutti SK. Phase I study of heat-deployed liposomal doxorubicin during radiofrequency ablation for hepatic malignancies. Journal of Vascular and Interventional Radiology. 2012;**23**:248-255.e7. DOI: 10.1016/j.jvir.2011.10.018

[19] Hauck ML, LaRue SM, Petros WP, Poulson JM, Yu D, Spasojevic I, Pruitt AF, Klein A, Case B, Thrall DE, Needham D, Dewhirst MW. Phase I trial of doxorubicin-containing low temperature sensitive liposomes in spontaneous canine tumors. Clinical Cancer Research. 2006;**12**:4004-4010. DOI: 10.1158/1078-0432.CCR-06-0226

[20] Lindner LH, Eichhorn ME, Eibl H, Teichert N, Schmitt-Sody M, Issels RD, Dellian M. Novel temperature-sensitive liposomes with prolonged circulation time. Clinical Cancer Research. 2004;**10**:2168-2178

[21] Hossann M, Wiggenhorn M, Schwerdt A, Wachholz K, Teichert N, Eibl H, Issels RD, Lindner LH. In vitro stability and content release properties of phosphatidylglyceroglycerol containing thermosensitive liposomes. Biochimica et Biophysica Acta. 2007;**1768**:2491-2499. DOI: 10.1016/j.bbamem.2007.05.021

[22] Zimmermann K, Hossann M, Hirschberger J, Troedson K, Peller M, Schneider M, Bruhschwein A, Meyer-Lindenberg A, Wess G, Wergin M, Dorfelt R, Knosel T, Schwaiger M, Baumgartner C, Brandl J, Schwamberger S, Lindner LH. A pilot trial of doxorubicin containing phosphatidyldiglycerol based thermosensitive liposomes in spontaneous feline soft tissue sarcoma. International Journal of Hyperthermia. 2016;**33**(2):178-190. DOI: 10.1080/02656736.2016.1230233

[23] El-Kareh AW, Secomb TW. A mathematical model for comparison of bolus injection, continuous infusion, and liposomal delivery of doxorubicin to tumor cells. Neoplasia. 2000;**2**:325-338

[24] Chen Q, Tong S, Dewhirst MW, Yuan F. Targeting tumor microvessels using doxorubicin encapsulated in a novel thermosensitive liposome. Molecular Cancer Therapeutics. 2004;**3**:1311-1317

[25] Kneidl B, Peller M, Winter G, Lindner LH, Hossann M. Thermosensitive liposomal drug delivery systems: State of the art review. International Journal of Nanomedicine. 2014;**9**:4387-4398. DOI: 10.2147/IJN.S49297

[26] Lokerse WJ, Kneepkens EC, Ten Hagen TL, Eggermont AM, Grull H, Koning GA. In depth study on thermosensitive liposomes: Optimizing formulations for tumor specific therapy and in vitro to in vivo relations. Biomaterials. 2016;**82**:138-150. DOI: 10.1016/j.biomaterials.2015.12.023

[27] Manzoor AA, Lindner LH, Landon CD, Park JY, Simnick AJ, Dreher MR, Das S, Hanna G, Park W, Chilkoti A, Koning GA, ten Hagen TL, Needham D, Dewhirst MW.

Overcoming limitations in nanoparticle drug delivery: Triggered, intravascular release to improve drug penetration into tumors. Cancer Research. 2012;**72**:5566-5575. DOI: 10.1158/0008-5472.can-12-1683

[28] Gustafson DL, Rastatter JC, Colombo T, long ME. Doxorubicin pharmacokinetics: Macromolecule binding, metabolism, and excretion in the context of a physiologic model. Journal of Pharmaceutical Sciences. 2002;**91**:1488-1501

[29] Yang RS, Andersen ME, Pharmacokinetics, Norwalk: Appleton and Lange; 1994. pp. 49-73

[30] Park K. Lessons learned from thermosensitive liposomes for improved chemotherapy. Journal of Controlled Release. 2014;**174**:219

[31] Ritsma L, Steller EJA, Ellenbroek SIJ, Kranenburg O, Borel Rinkes IHM, van Rheenen J. Surgical implantation of an abdominal imaging window for intravital microscopy. Nature Protocols. 2013;**8**:583-594

[32] Li L, ten Hagen TL, Hossann M, Suss R, van Rhoon GC, Eggermont AM, Haemmerich D, Koning GA. Mild hyperthermia triggered doxorubicin release from optimized stealth thermosensitive liposomes improves intratumoral drug delivery and efficacy. Journal of Controlled Release. 2013;**168**:142-150. DOI: 10.1016/j.jconrel.2013.03.011

[33] Sullivan SM, Huang L. Preparation and characterization of heat-sensitive immunoliposomes. Biochimica et Biophysica Acta. 1985;**812**:116-126

[34] Vingerhoeds MH, Steerenberg PA, Hendriks JJ, Dekker LC, Van Hoesel QG, Crommelin DJ, Storm G. Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma in vitro and in vivo. British Journal of Cancer. 1996;**74**:1023-1029

[35] Kullberg M, Mann K, Owens JL. A two-component drug delivery system using her-2-targeting thermosensitive liposomes. Journal of Drug Targeting. 2009;**17**:98-107. DOI: 10.1080/10611860802471562

[36] Haeri A, Zalba S, Ten Hagen TL, Dadashzadeh S, Koning GA. Egfr targeted thermosensitive liposomes: A novel multifunctional platform for simultaneous tumor targeted and stimulus responsive drug delivery. Colloids and Surfaces B Biointerfaces. 2016;**146**:657-669. DOI: 10.1016/j.colsurfb.2016.06.012

[37] Kullberg M, Owens JL, Mann K. Listeriolysin o enhances cytoplasmic delivery by her-2 targeting liposomes. Journal of Drug Targeting. 2010;**18**:313-320. DOI: 10.3109/10611861003663549

[38] Na K, Lee SA, Jung SH, Hyun J, Shin BC. Elastin-like polypeptide modified liposomes for enhancing cellular uptake into tumor cells. Colloids and Surfaces B Biointerfaces. 2012;**91**:130-136. DOI: 10.1016/j.colsurfb.2011.10.051

[39] Wang C, Wang X, Zhong T, Zhao Y, Zhang WQ, Ren W, Huang D, Zhang S, Guo Y, Yao X, Tang YQ, Zhang X, Zhang Q. The antitumor activity of tumor-homing peptide-modified thermosensitive liposomes containing doxorubicin on mcf-7/adr: In vitro and in vivo. International Journal of Nanomedicine. 2015;**10**:2229-2248. DOI: 10.2147/ijn.s79840

[40] Dicheva BM, ten Hagen TL, Seynhaeve AL, Amin M, Eggermont AM, Koning GA. Enhanced specificity and drug delivery in tumors by crgd-anchoring thermosensitive liposomes. Pharmaceutical Research. 2015;**32**:3862-3876. DOI: 10.1007/s11095-015-1746-7

[41] Deng Z, Xiao Y, Pan M, Li F, Duan W, Meng L, Liu X, Yan F, Zheng H. Hyperthermia-triggered drug delivery from irgd-modified temperature-sensitive liposomes enhances the anti-tumor efficacy using high intensity focused ultrasound. Journal of Controlled Release. 2016;**243**:333-341. DOI: 10.1016/j.jconrel.2016.10.030

[42] Zhang K, Liu M, Tong X, Sun N, Zhou L, Cao Y, Wang J, Zhang H, Pei R. Aptamer-modified temperature-sensitive liposomal contrast agent for magnetic resonance imaging. Biomacromolecules. 2015;**16**:2618-2623. DOI: 10.1021/acs.biomac.5b00250

[43] Lopez-Noriega A, Ruiz-Hernandez E, Quinlan E, Storm G, Hennink WE, O'Brien FJ. Thermally triggered release of a pro-osteogenic peptide from a functionalized collagen-based scaffold using thermosensitive liposomes. Journal of Controlled Release. 2014;**187**:158-166. DOI: 10.1016/j.jconrel.2014.05.043

[44] Yatvin MB, Muhlensiepen H, Porschen W, Weinstein JN, Feinendegen LE. Selective delivery of liposome-associated cis-dichlorodiammineplatinum(ii) by heat and its influence on tumor drug uptake and growth. Cancer Research. 1981;**41**:1602-1607

[45] Tomita T, Watanabe M, Takahashi T, Kumai K, Tadakuma T, Yasuda T. Temperature-sensitive release of adriamycin, an amphiphilic antitumor agent, from dipalmitoylphosphatidylcholine-cholesterol liposomes. Biochimica et Biophysica Acta. 1989;**978**:185-190

[46] Merlin JL. Encapsulation of doxorubicin in thermosensitive small unilamellar vesicle liposomes. European Journal of Cancer. 1991;**27**:1026-1030

[47] Unezaki S, Maruyama K, Takahashi N, Koyama M, Yuda T, Suginaka A, Iwatsuru M. Enhanced delivery and antitumor activity of doxorubicin using long-circulating thermosensitive liposomes containing amphipathic polyethylene glycol in combination with local hyperthermia. Pharmaceutical Research. 1994;**11**:1180-1185

[48] Negussie AH, Yarmolenko PS, Partanen A, Ranjan A, Jacobs G, Woods D, Bryant H, Thomasson D, Dewhirst MW, Wood BJ, Dreher MR. Formulation and characterization of magnetic resonance imageable thermally sensitive liposomes for use with magnetic resonance-guided high intensity focused ultrasound. International Journal of Hyperthermia. 2011;**27**:140-155. DOI: 10.3109/02656736.2010.528140

[49] de Smet M, Heijman E, Langereis S, Hijnen NM, Grull H. Magnetic resonance imaging of high intensity focused ultrasound mediated drug delivery from temperature-sensitive liposomes: An in vivo proof-of-concept study. Journal of Controlled Release. 2011;**150**:102-110. DOI: 10.1016/j.jconrel.2010.10.036

[50] Willerding L, Limmer S, Hossann M, Zengerle A, Wachholz K, Ten Hagen TL, Koning GA, Sroka R, Lindner LH, Peller M. Method of hyperthermia and tumor size influence effectiveness of doxorubicin release from thermosensitive liposomes in experimental tumors. Journal of Controlled Release. 2016;**222**:47-55. DOI: 10.1016/j.jconrel.2015.12.004

[51] van Bree C, Krooshoop JJ, Rietbroek RC, Kipp JB, Bakker PJ. Hyperthermia enhances tumor uptake and antitumor efficacy of thermostable liposomal daunorubicin in a rat solid tumor. Cancer Research. 1996;**56**:563-568

[52] Lu T, Lokerse WJ, Seynhaeve AL, Koning GA, ten Hagen TL. Formulation and optimization of idarubicin thermosensitive liposomes provides ultrafast triggered release at mild hyperthermia and improves tumor response. Journal of Controlled Release. 2015;**220**:425-437. DOI: 10.1016/j.jconrel.2015.10.056

[53] Wu Y, Yang Y, Zhang FC, Wu C, Lu WL, Mei XG. Epirubicin-encapsulated long-circulating thermosensitive liposome improves pharmacokinetics and antitumor therapeutic efficacy in animals. Journal of Liposome Research. 2011;**21**:221-228. DOI: 10.3109/08982104.2010.520273

[54] Liu H, Zhang Y, Han Y, Zhao S, Wang L, Zhang Z, Wang J, Cheng J. Characterization and cytotoxicity studies of dppc:M(2+) novel delivery system for cisplatin thermosensitivity liposome with improving loading efficiency. Colloids and Surfaces B Biointerfaces. 2015;**131**:12-20. DOI: 10.1016/j.colsurfb.2015.04.029

[55] Tiwari SB, Udupa VN, Rao S, Devi PU. Thermochemotherapy: Synergism between hyperthermia and liposomal bleomycin in mice bearing melanoma b16f1. Pharmacy and Pharmacology Communications. 2000;**6**:19-23. DOI: 10.1211/146080800128735421

[56] Chelvi TP, Ralhan R. Hyperthermia potentiates antitumor effect of thermosensitive-liposome-encapsulated melphalan and radiation in murine melanoma. Tumour Biology. 1997;**18**:250-260

[57] Wang ZY, Zhang H, Yang Y, Xie XY, Yang YF, Li Z, Li Y, Gong W, Yu FL, Yang Z, Li MY, Mei XG. Preparation, characterization, and efficacy of thermosensitive liposomes containing paclitaxel. Drug Delivery. 2016;**23**:1222-1231. DOI: 10.3109/10717544.2015.1122674

[58] Zhang H, Gong W, Wang ZY, Yuan SJ, Xie XY, Yang YF, Yang Y, Wang SS, Yang DX, Xuan ZX, Mei XG. Preparation, characterization, and pharmacodynamics of thermosensitive liposomes containing docetaxel. Journal of Pharmaceutical Sciences. 2014;**103**:2177-2183. DOI: 10.1002/jps.24019

[59] Limmer S, Hahn J, Schmidt R, Wachholz K, Zengerle A, Lechner K, Eibl H, Issels RD, Hossann M, Lindner LH. Gemcitabine treatment of rat soft tissue sarcoma with phosphatidyldiglycerol-based thermosensitive liposomes. Pharmaceutical Research. 2014;**31**:2276-2286. DOI: 10.1007/s11095-014-1322-6

[60] Al Sabbagh C, Tsapis N, Novell A, Calleja-Gonzalez P, Escoffre JM, Bouakaz A, Chacun H, Denis S, Vergnaud J, Gueutin C, Fattal E. Formulation and pharmacokinetics of thermosensitive stealth(r) liposomes encapsulating 5-fluorouracil. Pharmaceutical Research. 2015;**32**:1585-1603. DOI: 10.1007/s11095-014-1559-0

[61] Zhang H, Wang ZY, Gong W, Li ZP, Mei XG, Lv WL. Development and characteristics of temperature-sensitive liposomes for vinorelbine bitartrate. International Journal of Pharmaceutics. 2011;**414**:56-62. DOI: 10.1016/j.ijpharm.2011.05.013

[62] Wang S, Mei XG, Goldberg SN, Ahmed M, Lee JC, Gong W, Han HB, Yan K, Yang W. Does thermosensitive liposomal vinorelbine improve end-point survival after percutaneous radiofrequency ablation of liver tumors in a mouse model? Radiology. 2016;**279**:762-772. DOI: 10.1148/radiol.2015150787

[63] Wardlow R, Bing C, VanOsdol J, Maples D, Ladouceur-Wodzak M, Harbeson M, Nofiele J, Staruch R, Ramachandran A, Malayer J, Chopra R, Ranjan A. Targeted antibiotic delivery using low temperature-sensitive liposomes and magnetic resonance-guided high-intensity focused ultrasound hyperthermia. International Journal of Hyperthermia. 2016;**32**:254-264. DOI: 10.3109/02656736.2015.1134818

[64] Li M, Li Z, Yang Y, Wang Z, Yang Z, Li B, Xie X, Song J, Zhang H, Li Y, Gao G, Yang J, Mei X, Gong W. Thermo-sensitive liposome co-loaded of vincristine and doxorubicin based on their similar physicochemical properties had synergism on tumor treatment. Pharmaceutical Research. 2016;**33**:1881-1898. DOI: 10.1007/s11095-016-1924-2

[65] AllenTM, Martin FJ. Advantages of liposomal delivery systems for anthracyclines. Seminars in Oncology. 2004;**31**:5-15

[66] Rossmann C, Haemmerich D. Review of temperature dependence of thermal properties, dielectric properties, and perfusion of biological tissues at hyperthermic and ablation temperatures. Critical Reviews in Biomedical Engineering. 2014;**42**:467-492

[67] You J, Shao R, Wei X, Gupta S, Li C. Near-infrared light triggers release of paclitaxel from biodegradable microspheres: Photothermal effect and enhanced antitumor activity. Small. 2010;**6**:1022-1031. DOI: 10.1002/smll.201000028

[68] You J, Zhang P, Hu F, Du Y, Yuan H, Zhu J, Wang Z, Zhou J, Li C. Near-infrared light-sensitive liposomes for the enhanced photothermal tumor treatment by the combination with chemotherapy. Pharmaceutical Research. 2014;**31**:554-565. DOI: 10.1007/s11095-013-1180-7

[69] Ryan TP, Brace CL. Interstitial microwave treatment for cancer: Historical basis and current techniques in antenna design and performance. International Journal of Hyperthermia. 2017;**33**:3-14. DOI: 10.1080/02656736.2016.1214884

[70] Zagar TM, Vujaskovic Z, Formenti S, Rugo H, Muggia F, O'Connor B, Myerson R, Stauffer P, Hsu IC, Diederich C, Straube W, Boss MK, Boico A, Craciunescu O, Maccarini P, Needham D, Borys N, Blackwell KL, Dewhirst MW. Two phase I dose-escalation/pharmacokinetics studies of low temperature liposomal doxorubicin (ltld) and mild local hyperthermia in heavily pretreated patients with local regionally recurrent breast cancer. International Journal of Hyperthermia. 2014;**30**:285-294. DOI: 10.3109/02656736.2014.936049

[71] Rossmann C, Rattay F, Haemmerich D. Platform for patient-specific finite-element modeling and application for radiofrequency ablation. 2012;**1**:0. DOI:10.1615/VisualizImageProcComputatBiomed.2012004898

[72] Gasselhuber A, Dreher MR, Negussie A, Wood BJ, Rattay F, Haemmerich D. Mathematical spatio-temporal model of drug delivery from low temperature sensitive liposomes during radiofrequency tumour ablation. International Journal of Hyperthermia. 2010;**26**:499-513. DOI: 10.3109/02656731003623590

[73] Rieke V, Butts Pauly K. Mr thermometry. Journal of Magnetic Resonance Imaging. 2008; **27**:376-390. DOI: 10.1002/jmri.21265

[74] Gasselhuber A, Dreher MR, Partanen A, Yarmolenko PS, Woods D, Wood BJ, Haemmerich D. Targeted drug delivery by high intensity focused ultrasound mediated hyperthermia combined with temperature-sensitive liposomes: Computational modeling and preliminary in vivo validation. International Journal of Hyperthermia. 2012;**28**:337-348. DOI: 10.3109/02656736.2012.677930

[75] Staruch RM, Hynynen K, Chopra R. Hyperthermia-mediated doxorubicin release from thermosensitive liposomes using mr-hifu: Therapeutic effect in rabbit vx2 tumours. International Journal of Hyperthermia. 2015;**31**:118-133. DOI: 10.3109/02656736.2014.992483

[76] Grull H, Langereis S. Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using mri-guided high intensity focused ultrasound. Journal of Controlled Release. 2012;**161**:317-327. DOI: 10.1016/j.jconrel.2012.04.041

[77] Celsion's phase iii thermodox(r) heat study recommended for continuation by data monitoring committee. 2017. Available from: http://investor.celsion.com/releasedetail.cfm?releaseid=469443 (Accessed: February 2, 2017)

[78] Dou Y, Hynynen K, Allen C. To heat or not to heat: Challenges with clinical translation of thermosensitive liposomes. Journal of Controlled Release. 2017;**249**:63-73. DOI: 10.1016/j.jconrel.2017.01.025

[79] Lencioni R, Tak W.-Y, Chen M. H, Finn R. S, Sherman M, Makris L, O'Neal M, Simonich W, Haemmerich D, Reed R, Borys N, Poon R. T. P, Abou-Alfa G. K: Standardized radio-frequency ablation (srfa) ≥ 45 minutes (m) plus lyso-thermosensitive liposomal doxorubicin (ltld) for solitary hepatocellular carcinoma (hcc) lesions 3-7 cm: A retrospective analysis of phase iii heat study. Journal of Clinical Oncology. 2014; 32:e15143–e15143. DOI:10.1200/jco.2014.32.15_suppl.e15143

[80] Swenson CE, Haemmerich D, Maul DH, Knox B, Ehrhart N, Reed RA. Increased duration of heating boosts local drug deposition during radiofrequency ablation in combination with thermally sensitive liposomes (thermodox) in a porcine model. PLoS One. 2015;**10**:e0139752. DOI: 10.1371/journal.pone.0139752