



Contents lists available at ScienceDirect

## Advanced Drug Delivery Reviews

journal homepage: [www.elsevier.com/locate/addr](http://www.elsevier.com/locate/addr)

# A moderate thermal dose is sufficient for effective free and TSL based thermochemotherapy

G.C. van Rhoon<sup>a,\*</sup>, M. Franckena<sup>a</sup>, T.L.M. ten Hagen<sup>b</sup><sup>a</sup> Department of Radiation Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, the Netherlands<sup>b</sup> Department of Pathology, Erasmus MC, the Netherlands

## ARTICLE INFO

## Article history:

Received 31 October 2019

Received in revised form 26 March 2020

Accepted 30 March 2020

Available online xxxx

## Keywords:

Thermal dose

Thermochemotherapy

Thermosensitive liposomes

Extravasation

Tumor blood flow

Hyperthermia

## ABSTRACT

Hyperthermia, i.e. heating the tumor to a temperature of 40–43 °C is considered by many a valuable treatment to sensitize tumor cells to radiotherapy and chemotherapy. In recent randomized trials the great potential of adding hyperthermia to chemotherapy was demonstrated for treatment of high risk soft tissue sarcoma: +11.4% 5 yrs. overall survival (OS) and for ovarian cancer with peritoneal involvement nearly +12 months OS gain. As a result interest in combining chemotherapy with hyperthermia, i.e. thermochemotherapy, is growing. Extensive biological research has revealed that hyperthermia causes multiple effects, from direct cell kill to improved oxygenation, whereby each effect has a specific temperature range. Thermal sensitization of the tumor cell for chemotherapy occurs for many drugs at temperatures ranging from 40 to 42 °C with little additional increase of sensitization at higher temperatures. Increasing perfusion/oxygenation and increased extravasation are two other important hyperthermia induced mechanisms. The combination of free drug and hyperthermia has not been found to increase tumor drug concentration. Hence, enhanced effectiveness of free drug will depend on the thermal sensitization of the tumor cells for the applied drug. In contrast to free drugs, experimental animal studies combining hyperthermia and thermo-sensitive liposomal (TSL) drugs delivery have demonstrated to result in a substantial increase of the drug concentration in the tumor. For TSL based chemotherapy, hyperthermia is critical to both increase perfusion and extravasation as well as to trigger TSL drug release, whereby the temperature controlled induction of a local high drug concentration in a highly permeable vessel is driving the enhanced drug uptake in the tumor. Increased drug concentrations up to 26 times have been reported in rodents. Good control of the tissue temperature is required to keep temperatures below 43 °C to prevent vascular stasis. Further, careful timing of the drug application relative to the start of heating is required to benefit optimal from the combined treatment. From the available experimental data it follows that irrespective whether chemotherapy is applied as free drug or using a thermal sensitive liposomal carrier, the optimal thermal dose for thermochemotherapy should be 40–42 °C for 30–60 min, i.e. equivalent to a CEM43 of 1–15 min. Timing is critical: most free drug should be applied simultaneous with heating, whereas TSL drugs should be applied 20–30 min after the start of hyperthermia.

© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The publication of Cavaliere et al. [1] in 1967 was the first clinical study to demonstrate the clinical potential of hyperthermia to boost the effectiveness of chemotherapy. In their study Cavaliere et al. showed that hyperthermic (41.5–43.5 °C) regional perfusion of isolated limbs resulted in complete disappearance of the tumor in 10 of 22 patients. The subsequently general interest raised in the combined application of heat and drugs is believed to be the fundament of today's interest in the application of hyperthermia as sensitizing agent specific for chemotherapy but also for its combination with radiotherapy.

During the last decades extensive biological research has been performed to identify the biological mechanisms induced by hyperthermia alone as well as in combination with radiotherapy or chemotherapy. As a result of this research hyperthermia is considered by many a strong if not the strongest biological sensitizer for radiotherapy and chemotherapy [2–5]. In early years the general strategy was that independent whether hyperthermia was combined with radiotherapy or chemotherapy the aim should be to apply hyperthermia with the minimum temperature in the tumor exceeding 42 °C to maximize the effect of induced direct cell kill and direct sensitization of radiotherapy or chemotherapy. However, the experience obtained from early clinical studies showed that with the hyperthermia equipment available in the late 1980's applying hyperthermia at a minimum tumor temperature of 42 °C was not realistic [6–8]. In contrast, the numerous positive

\* Corresponding author.

E-mail address: [g.c.vanrhoon@erasmusmc.nl](mailto:g.c.vanrhoon@erasmusmc.nl) (G.C. van Rhoon).

randomized trials combining hyperthermia with radiotherapy published in the 1990's and early 2000's reported that the impressive and statistically significant improved treatment outcome (local-control and survival) were obtained with average target temperatures in the range of 40–42 °C [9–18]. Hence, indicating that the ability of hyperthermia to improve tumor oxygenation and reduce repair of DNA damage are dominating factors explaining the heat induced enhancement of radiotherapy or chemotherapy effectiveness.

### 1.1. Phase III trials for chemotherapy plus hyperthermia

The current revival of clinical interest in combining chemotherapy plus hyperthermia follows publication of several phase III trials [19–37] in the last two decades with encouraging results for different tumor entities. Table 1 provides a list of phase III studies comparing chemotherapy vs chemotherapy plus hyperthermia for which a full paper has been published in a peer reviewed journal. The 2003 publication of the results of the phase III study of Hyperthermic Intravesical Chemotherapy (HIVEC) in high-risk non-muscle invasive bladder cancer by Colombo et al. reporting an increase of 17 to 57% in the 2-yr recurrence free survival marks a growing interest in HIVEC. Long term results were published in 2011 showing a three-fold increase in the 10 yrs. DFS for the HIVEC group (53% MMC + hyperthermia vs 17% MMC-alone) and confirmed the encouraging early results [19,20]. Other randomized controlled trials (RCTs) showed the impact of doubling the MMC dose on treatment outcome [25,26]. Arends et al. [27] compared for the same patient group the efficiency of standard therapy with BCG with MMC + hyperthermia and found in the group of patient analyzed according to the treatment delivered per protocol (pp) a statistical significant improved recurrence free survival at 2 yr, increase from 65% for BCG to 82% for MMC + hyperthermia. Lammers et al. [38] conclude in their systemic review that MMC based thermochemotherapy reduces the risk of NMIBC recurrence by 59% when compared with MMC alone. Overall bladder preservation after thermochemotherapy is 87.6%. However, due to the limited number of randomized trials and different study designs, definitive conclusions cannot be drawn with respect to time to recurrence and time to progression [38].

The application of hyperthermic intraperitoneal chemotherapy (HIPEC) has been investigated as 1) an adjuvant treatment in close connection to primary surgery of patients with gastric cancer and colon cancer to reduce the risk for a peritoneal recurrence/metastases or 2) as a follow-up treatment for patients that already present with intraperitoneal tumor growth after earlier treatment. Five RCTs addressed the potential of HIPEC to prevent peritoneal carcinomatosis [28–32]. Three of the five studies report a benefit on treatment outcome for the treatment including HIPEC [29–31]. However, the most recent and largest RCT by Klaver et al. [32] finds that adding HIPEC to standard treatment (surgery plus adjuvant systemic chemotherapy) does not translate in a higher probability of patients with peritoneal free survival at 18 months after treatment. The use of HIPEC as treatment for patients already presenting with peritoneal carcinomatosis of colorectal, gastric and ovarium cancer has been published in four RCTs [22,33–36]. All four RCTs show that adding HIPEC to cytoreductive surgery (CRS) results in an improved overall survival. The study by Van Driel et al. [22] on the use of HIPEC in ovarian cancer reports longer median overall survival for the surgery plus HIPEC arm by nearly 12 months. However, following the results of the PRODIGE 7 trial [39]<sup>1</sup> investigating HIPEC with oxaliplatin for 30 min in a well-defined cohort of patients who are meeting precise inclusion and exclusion criteria, there is ongoing discussion whether HIPEC should remain first line treatment for patients with peritoneal carcinomatosis of colorectal origin [40,41]. The use of chemotherapy plus hyperthermia for the treatment of solid tumors has been reported in two RCTs [23,24,37] with the most recent study

by Issels et al. [23,24] showing an impressive increase in median overall survival from 6.2 yrs. for neoadjuvant chemotherapy (EIA) alone to 15.4 yrs. when hyperthermia is added. Within Germany the positive outcome of this phase III study translated in the requirement that any Soft Tissue Sarcoma center must provide hyperthermia.

Notably, in all these level I evidence thermochemotherapy studies the average temperatures applied ranged from 40 to 42 °C, i.e. temperatures that can be achieved realistically in clinical practice with current hyperthermia technology. The promising clinical results, together with highly innovative approaches for advanced drug delivery using smart temperature triggered drug carriers [42,43] has invoked a new interest from the medical oncology community in hyperthermia. To address the question on what thermal dose should be best applied in the combined treatment of chemotherapy plus hyperthermia this review identifies three strategies of interaction along which hyperthermia may act to enhance the effectiveness of chemotherapy: thermal sensitization, thermal enhanced perfusion and thermal enhanced extravasation. An additional consideration for the applied thermal dose follows from the temperature required to trigger thermal drug release using smart drug delivery carriers. For each strategy a potential relation with thermal dose is discussed. A brief discussion is included on thermal dose effect relationship in their clinical data. The review ends with summarizing the required thermal dose for thermochemotherapy and how this translate in demands for current and future hyperthermia technology.

## 2. Chemotherapy and tumor microenvironment

Characteristic for solid tumors is their chaotic vasculature. As a result tumor growth is highly controlled by the poor microenvironmental conditions. At the same time the microenvironment is highly determinative for the efficacy of the chemotherapy. In general the vasculature network of the tumor is primitive and chaotically developed resulting in inadequate supply of oxygen and nutrients as well as inadequate removal of waste products. As a consequence, tumors generally contain regions of low oxygenation (hypoxia) that are associated with elevated interstitial fluid pressure, glycolysis, low pH and reduced bioenergetic status. Tumor cells under these conditions are still viable but demonstrate substantial resistance to conventional radiotherapy and chemotherapy [44–49]. Although, there is growing understanding that poor microenvironmental conditions also influences the tumors aggressiveness and metastatic spread by modifying intracellular pathways [48], hypoxia is still considered a major critical factor for treatment resistance [45,50]. A secondary effect of the poor vascular network of tumor is that vascular based drug delivery is restricted with the lowest drug concentration at the hypoxic regions. In addition with enlarged distances between tumor vessels, drugs have to diffuse over larger distances, further compromising drug concentration at tumors cells located away of functional vessels.

Conventional chemotherapy is mostly effective against rapidly dividing cells, whereby the agents are non-selective. Although, aimed to kill preferentially tumor cells the chemotherapy will also damage the rapidly dividing normal healthy tissue cells, causing severe and unintended and undesirable side effects. The above explained tumor characteristics causes the bio-accessibility of the drug to the tumors cells to be poor, for which higher systemic drug doses are required to improve outcome. However, higher doses also lead to increased toxicity and incidence of multi-drug resistance [51,52]. Therefore it is desirable to combine chemotherapy with other therapies to enhance drug delivery at the site of the tumor, targeting to enhance drug selectivity and increase sensitivity, i.e. sensitization, of the tumor cells for the drug applied.

## 3. Hyperthermia: biological effects

Research on the biological effects of hyperthermia during the last decades focused on the so-called mild hyperthermia, i.e. temperature

<sup>1</sup> data only available as abstract

**Table 1**  
Randomized Trials of chemotherapy and hyperthermia.

Authors	Tumor	N	Control arm	Study arm	Temperature [°C] and duration [min]	Outcome	Control arm	Study arm
HIVEC								
Colombo et al. 2003, 2011. [19,20]	NMIBC: Intermediate or high risk	83	MMC-alone: 20 mg/50 ml	MMC + HT	40–44 °C; 40–60 min.	Recurrence 10 yrs. DFS (91m <sup>a</sup> )	80% 15%	<b>40%<sup>b</sup></b> <b>53%</b>
Gofrit et al. 2004. [25]	NMIBC: Ta/1 G2–3 high-risk	52	MMC + HT 20 mg/50 ml	MMC + HT 40 mg/50 ml	40–44 °C; 40 min.	Recurrence (15.2m <sup>a</sup> )	37.5% mean 10 m.	19% after 13.7 m.
Moskovitz et al. 2005. [26]	NIMBC: Intermediate and high-risk	47	MMC + HT 20 mg/50 ml	MMC + HT 40 mg/50 ml	40–44 °C; 60 min.	Recurrence FU > 10 m	9% after 10 m.	20% after 9 m.
Arends et al. 2016. [27]	NIMBC: Intermediate and high-risk	190	BCG (full dose)	MMC + HT 20 mg/50 ml MMC	42 ± 2 °C; 60 min.	RFS at 24 m ITT analysis PP analysis	65% 65%	<b>78%</b> <b>82%</b>
HIPEC in adjuvant setting to prevent peritoneal recurrence								
Hamazoe et al. 1994. [28]	gastric cancer	82	surgery alone	Surgery+HIPEC MMC (10 mg/l)	~43 °C; 50–60 min.	5 yrs. OS	52.5%	64.3%
Fujimoto et al. 1999. [29]	Advanced gastric cancer	141	surgery alone	Surgery+ HIPEC MMC 10 mg/l	~43 °C; 120 min.	OS 2 yrs OS 4 yrs OS 8 yrs	77% 58% 49%	88% 76% <b>62%</b>
Yonemura et al. 2001. [30]	T2–4 gastric cancer	139	#1 - CRS	#2 -CRS + HIPEC 30 mg MMC+ 300 mg cisp/6–8lt #3 -CRS + normotherm HIPEC 30 mg MMC+ 300 mg cisp/6–8lt	#2: 42–43 °C #3: 37 °C	5 yr OS	#1–42%	<b>#2–61%</b> (#1 vs #2) <b>#3–43%</b> (#2 vs #3)
Cui et al. 2014. [31]	Advanced gastric cancer	192	#1 Control: surgery alone	#2 -Preop chemo-Surgery #3 -Surgery+HIPEC #4 -Preop. Chemo-Surgery-HIPEC	41–43 °C; 90 min.	3 yrs. OS	35.4%	#2–62.5% #3–58.3% <b>#4–75.0%</b>
Klaver et al. 2019. [32]	Colon Cancer	204	Resection primary tumor adjuvant syst. Chemotherapy	Standard plus HIPEC iv 5FU (400 mg/m <sup>2</sup> ) + leucovorin (20 mg/m <sup>2</sup> ) plus peritoneal oxaliplatin (460 mg/m <sup>2</sup> )	42–43 °C; 30 min.	Peritoneal free survival at 18 m.	76.2%	80.9%
HIPEC for treatment of peritoneal metastasis								
Verwaal et al. 2003, 2008. [33,34]	peritoneal carcinomatosis of colorectal cancer	105	Systemic chemotherapy (5FU leucovorin)	CRS + HIPEC (MMC 70 mg max) + syst. Chemotherapy	40–42 °C; 90 min.	median PFS median DSS (94m <sup>a</sup> )	7.7 m. 12.6 m.	<b>12.6 m.</b> <b>22.2 m.</b>
Yang et al. 2011. [35]	Gastric cancer	68	CRS-alone	CRS + HIPEC (Cisp. 120 mg + MMC 30 mg in 6lt)	43 ± 0.5 °C; 60–90 min.	Median OS	6.5 m.	<b>11.0 m.</b>
Spillotis et al. 2015. [36]	Recurrent Ovarian cancer after initial surgery and systemic chemo	120	CRS followed by systemic chemo	CRS + HIPEC (Cisp+Paclitaxel or doxorubicin+paclitaxel/MMC) + systemic chemo	42.5 °C; 60 min.	Mean OS OS at 3 yrs	13.4 m. 18%	<b>26.7 m.</b> <b>75%</b>
Van Driel et al. 2018. [22]	Ovarian Cancer	245	CRS	CRS + HIPEC (100 mg/m <sup>2</sup> Cispl)	40 °C; 90 min.	median OS	33.9 m.	<b>45.7 m.</b>
Conventional chemo plus hyperthermia								
Sugimachi et al. 1994. [37]	Oesophageal Ca	40	Chemotherapy (30 mg Bleo +50 mg Cisp) + surgery	Chemotherapy +HT (intraluminal)	42–45 °C; 30 min.	Pathological effectiveness	14%	<b>58%</b>
Issels et al. 2010, 2018. [23,24]	High risk soft tissue sarcoma	341	neo-adjuvant chemotherapy (EIA)	EIA plus regional hyperthermia	40–42 °C; 60 min.; 7–8 heat fractions	median OS OS at 10 yrs. (135m <sup>a</sup> )	6.2 yrs 42.7%	<b>15.4 yrs</b> <b>51.3%</b>

HIVEC: Hyperthermie intravesical chemotherapy; HIPEC: Hyperthermic Intraperitoneal Chemotherapy.

BCG: bacillus Calmette–Guérin therapy; MMC: mitomycin C; HT: hyperthermia.

RFS: recurrence free survival; PFS: progression free survival; DSS: disease specific survival.

EIA: etoposide, ifosfamide, and Adriamycin.

CRS: cytoreductive surgery.

<sup>a</sup> Median follow-up.

<sup>b</sup> Bold is statistical significant.

ranging of 39–42 °C, and revealed that the biological and physiological effects induced by mild hyperthermia are capable of boosting the effectiveness of chemotherapy and radiotherapy to kill tumor cells [2–5,47,53–56]. Excellent reviews by Issels [56], van den Tempel et al. [55], Oei et al. [5,57], Dewhirst et al. [2] provide clear and detailed overviews of the various macroscopic (perfusion, tumor microenvironment) and microscopic (evading DNA repair, blocking cell survival, mechanistic sensitization, cellular thermal stress response) biological effects induced by mild hyperthermia.

Independent of any synergistic action between chemotherapy and hyperthermia is the ability of hyperthermia to selectively kill tumor cells under hypoxic and acidic conditions at temperatures of 42 °C and up. A direct consequence of the poor blood flow in the hypoxic regions is that the limited heat dissipation by perfusion makes them easy to heat, i.e. higher temperatures are more easily achieved. It is widely accepted that tumor cells under hypoxic and acidic conditions are selectively killed by hyperthermia as they are much more sensitive to heat than cells in a well-oxygenated environment [58,59]. In addition, at

temperatures between 41 and 43 °C hyperthermia increases membrane permeability (thermal sensitization) and reduces repair of DNA damage, both increasing the effectiveness of drugs to kill tumor cells [4,55]. Finally, already at relative low temperatures perfusion and extravagation is enhanced resulting in an increased drug delivery and availability at the tumor [46,60–62]. The temperature ranges for the different effects are illustrated in Fig. 1.

Together the above illustrates that mild hyperthermia is an ideal complementary treatment to combine with chemotherapy as it provides all the required benefits in a single treatment to enhance killing of the hypoxic tumor cells, i.e. the cells relatively insensitive for chemotherapy and to boost drug effectiveness. The plethora of thermal effects can be used to enhance the effectiveness of chemotherapy following different objectives (Fig. 2). The first and simplest one aims only at direct thermal sensitization of the applied drug. The second objective aims only at temperature modulated enhanced perfusion and extravasation to increase tumor drug concentration, hyperthermia is given prior to the administration of the chemotherapy. In the third objective the aim is to combine the increase in perfusion and extravasation with a temperature triggered drug release specific at the site of the target in order to maximize drug concentration in the tumor. Often it will be difficult to apply the treatment with a clear separation between the effects of objective one and two, with time interval between drug and hyperthermia administration, absolute temperature and duration of heating as discriminating factors between both objectives. This third objective is more exclusive as it requires the use of chemotherapy delivered by thermal sensitive liposomes. This approach is currently by many considered to be the most potent one to improve tumor specific drug absorption.

#### 4. Current use of thermal dose in thermochemotherapy

Essential for the development of optimal treatment strategies for thermochemotherapy is that besides the dose prescription of the chemotherapy, the quality of the applied hyperthermia treatment is quantified using an appropriate thermal dose parameter. The answer to what is the appropriate thermal dose parameter is not easily given. The most important feature of a thermal dose parameter is that it should be able to reflect the dominant mechanisms of interaction between the drug used and the level of heating achieved [63].

Studies investigating the thermal dose effect relationship in thermochemotherapy are extremely rare [64–66]. In contrast, finding a thermal dose effect relationship for the combined radiotherapy and

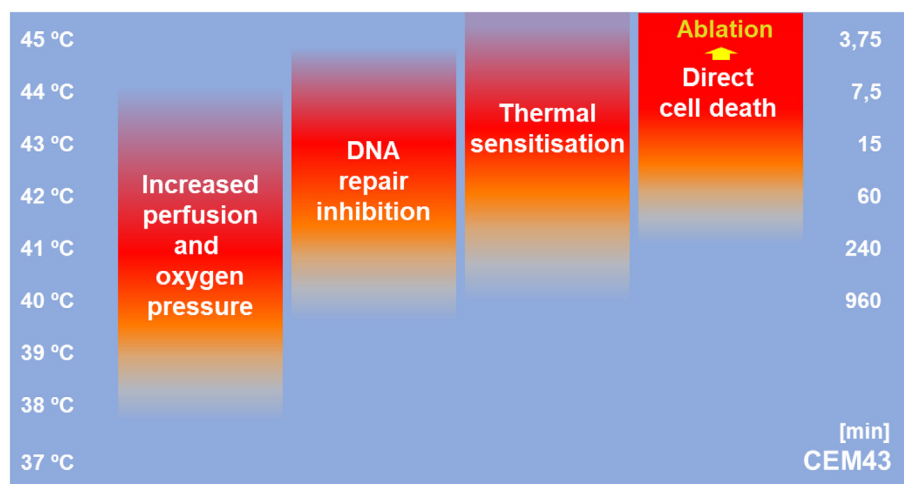
hyperthermia treatment has received interest from early clinical application. Over the past decades many clinical studies have been published demonstrating thermal dose effect relations using various expression of temperature alone (average-, minimum temperature) or as parameter integrating the combined effect of time and temperature [67–73]. In current daily practice, the cumulative equivalent minutes at 43 °C (CEM43) model is the most common used model to report the quality or thermal dose of the hyperthermia treatment applied [2,74,75]. The CEM43 model provides a normalizing tool to convert various time-temperature exposures to an equivalent exposure time in minutes at a reference temperature, commonly 43 °C, using the formula:

$$\text{CEM43} = \int_0^t R^{(43-T)} dt \text{ [min]}$$

In this formula T represents the actual applied temperature of the target tissue and R the factor to compensate for a 1 °C temperature change. R is experimental determined and has been set at a value of 0.5 for  $T > 43$  °C, i.e. the equivalent time doubles per degree temperature increase, and 0.25 for  $T \leq 43$  °C, i.e. the equivalent time decrease by a factor of four per degree temperature decrease.

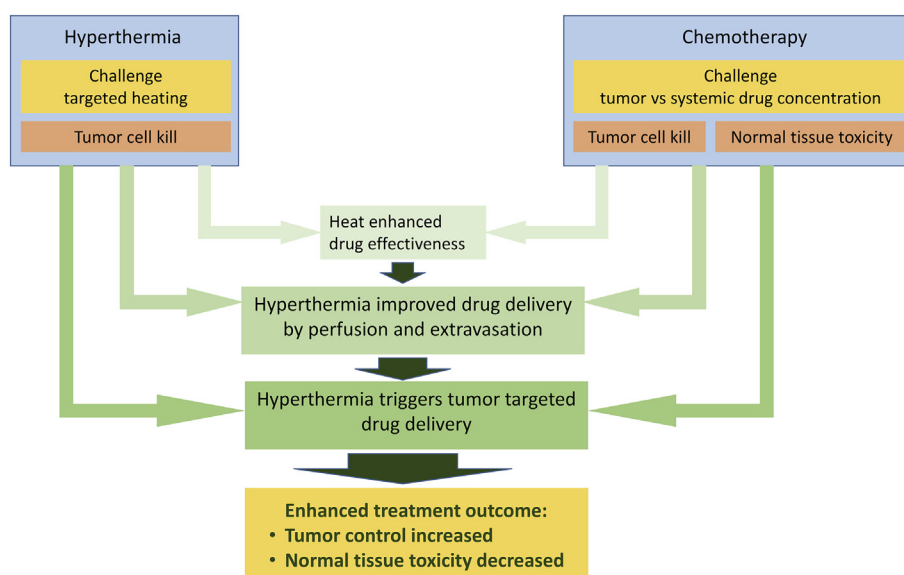
Although the model is widely used in studies searching for thermal dose effect relationships, the ability of the CEM43 concept to predict thermal initiated cell kill has a serious number of limitations as explained in detail elsewhere [75]. For patients with locally advanced cervical cancer (LACC) that were treated by thermoradiotherapy, with hyperthermia applied using a fixed exposure time and number of treatments, CEM43 and Trise has been shown to correlate with treatment outcome in a large study ( $n = 420$ ), independently of the radiotherapy dose parameters [68]. TRISE is a thermal dose parameter based on the temperature exceeded by 50% of measurement sites and duration of heating [68]. Most recent, the predictive value of Trise has been replicated in an independent study involving 227 patients diagnosed with LACC [76]. Besides these clinical studies, there is still ongoing research in developing multiparameter mathematical models to capture the complex interaction of hyperthermia with tumor and normal tissue cells in a single formula [77–80].

In their excellent review Isels et al. [81] identify six hallmarks of hyperthermia as targeted therapy that should be considered when designing a clinical trial combining chemotherapy and hyperthermia. For four of the six hallmarks (blocking cell survival, inducing cellular stress response, evading DNA repair, sensitization to radiotherapy and



**Fig. 1.** The graph shows for the different biological mechanisms of hyperthermia the threshold temperature to initiate the effect and if applicable a maximum temperature at which the effect ceases or transfer in a next higher one. As example perfusion start to increase from 39 °C and around 42 °C vascular stasis will develop; DNA repair inhibition starts around 41.5 °C and transfer in direct cell death at 44 °C or higher [75].





**Fig. 2.** Schematic representation of the different synergistic combinations of chemotherapy and hyperthermia, with as challenges to increase drug concentration and focus heating to the tumor. Enhanced treatment outcome might follow by heat: enhancing the effectiveness of the chemotherapy, improved drug delivery by perfusion and extravasation, boosting local drug release at the tumor by using thermo-sensitive liposome drug carriers.

chemotherapy) the relation of the magnitude of the biological effect of the hallmark with thermal dose, i.e. temperature and duration of exposure, is exponential and in general following the CEM43 model [4,5,53,58,82,83]. The thermal dose effect relationship for the hallmark 'modulating immune response' is less clear, still being subject of research [17]. For the last hallmark 'changing tumor environment' the dominant biological mechanism is the ability of hyperthermia to change the tumor blood perfusion and vascular permeability [84,85], with associated physiological improvements. The relation of thermal dose with increased perfusion is complex but can also be covered with the CEM43 model. In summary, application of the CEM43 model to integrate the history of the applied temperature time profile has a rational from the biological and physiological effects and is to be considered as a practical solution even though it is based on direct cell kill. For a more extensive discussion see recent reviews on thermal dose parameters [2,53,75,86].

#### 4.1. Direct sensitization of chemotherapy by hyperthermia

Although in general the combined treatment of chemotherapy with hyperthermia will increase treatment effectiveness the underlying mechanism for the thermal enhancement is complex and varies with the type of drug. Earlier Issels has classified the interaction of heat and chemotherapy in three categories [56,87]:

1. Independent, hyperthermia and the drug appear to act by independent mechanisms.

2. Additive, hyperthermia results in additional damage: with increasing temperature the effectiveness of the cytotoxic mechanism is enhanced.

3. Synergistic, little or no thermal sensitization occurs at low temperatures, but marked sensitization occurs at temperatures above 42 °C.

According to Issels [56,88] antimetabolites (e. g. 5-fluorouracil, methotrexate) and vinca-alkaloids or taxanes (paclitaxel and docetaxel) do not show any significant thermal enhancement of cytotoxicity, i.e. the combined effect of heat and drug exposure upon cell survival is equal with the effect of each individual treatment alone (=independent action). The efficacy of alkylating agents (e.g. cyclophosphamide, ifosfamide and melphalan) and nitrosoureas (e.g. BCNU) was enhanced by hyperthermia, both in vitro and in vivo (additive). For these agents timing of drug and heat delivery was shown to be important with, in

general, maximum effect in vivo when the agents were applied immediately before hyperthermia. Gemcitabine (2',2'-difluorodesocytidine) is an exception: simultaneous application led to decreased cytotoxicity, while an interval of 24 h led to an enhanced cell killing [89]. Some drugs are only enhanced, e.g. bleomycin, above a threshold temperature of 42.5 °C, while platinum drugs and alkylating agents show a gradual increase of the effect with increasing temperatures. For cisplatin and analogues (e.g. carboplatin) a potentiating effect of hyperthermia on cytotoxicity has been demonstrated already at relatively low temperatures of 40.5 °C. Table 2 shows in vivo TER-values as reported by Urano et al. for common drugs and various tumor types as measured in animal experiments. In general, in vivo TER varies between drugs and tumors, but if a TER exists a common value is between 1.5 and 2.5 in the temperature range of 40–42.5 °C with only a relative small increase at higher temperatures. However, for some specific combinations of drugs and tumor types the TER may reach higher values, whereby one should note that for temperatures above 43 °C a part of the increase in the TER was reported to be a contribution of direct thermal cell kill. Interesting for Ifosfamide is that increasing the treatment time from 30 to 90 min at 41.5 °C increased the TER from 1.52 to 3.60 [90].

**Table 2**

Thermal enhancement ratio for various chemotherapeutics and as function of temperature for a F5a-II and mammary tumor.

Temperature [°C]	Thermal enhancement ratio (TER)			
	40–42	42.5–44	40–42	42.5–44
Tumortype	F5a-II		Mammary Ca	
Drug				
Cisplatin	1.5 ± 0.2	1.6 ± 0.2	2.9	3.6
Cyclophosphamide	2.3 ± 0.3	2.7 ± 0.4		1.6 ± 0.1
Ifosfamide (30 min)	1.5 ± 0.3			
Ifosfamide (90 min)	3.6 ± 0.5			
Melphalan	3.6 ± 0.5			
BCNU	2.3 ± 0.2	2.7 ± 0.2		
Bleomycin	1.2 ± 0.4	1.7 ± 0.3		
Mytomyacin C	1.0			2.8 ± 0.5
5-Fluorouracil	1.0	1.0		
Doxorubicin	1.0	1.0	1.0	1.0

Data taken from Urano et al. [90].

In addition to the use of thermochemotherapy as a first line treatment approach, experimental research has shown that chemoresistance could be reversed, at least partially, by the addition of heat for several anticancer drugs (e.g. CDDP, mitomycin C, anthracyclines, BCNU, melphalan) including CDDP especially for CDDP-resistant cells [91,92]. The experimental results are recently supported by Wessalowski et al.. In a phase 2 study, involving 44 children and adolescents with refractory or recurrent malignant germ-cell tumors, they report encouraging long term results for local tumor control after retreating them using the same chemotherapy in combination hyperthermia as a salvage protocol [156].

Clearly, for designing the strategy to combine chemotherapy and hyperthermia in primary or recurrent setting a good understanding of the action mechanism between the two agents is crucial to achieve maximal clinical effect and to define realistic demands on sequence of and interval time between each agent, overall treatment time as well as the required optimal tumor temperature range (40–44 °C).

#### 4.2. Hyperthermia and perfusion

The first physiological reaction of human tissue on an increase of tissue temperature is an increase in blood perfusion. This physiological response has however, a complex character and depending on the exposure time and height of the temperature, opposite effects on tumor perfusion [62,93–98].

Within the hyperthermia field consensus exist that the changes in perfusion are paralleled by similar changes in tumor oxygenation. Heat induced perfusion effects and presumably also the proportionality between perfusion and oxygenation changes, varies between species, tumor types, the applied thermal dose, the time of measurement (directly after heat or 24 h later), number of heat fractions and also the rate of heating [60,93–96]. The relation between improved perfusion and oxygenation is important as many anticancer drugs require molecular oxygen to be maximally cytotoxic (i.e. hypoxia can be a direct cause of therapeutic resistance) and tumor cell resistance is caused by hypoxia induced alterations in the cellular genome and proteome [48,99,100]. In animal studies, improvement in tumor oxygenation has been shown to increase tumor cell killing by cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea, Doxorubicin, and Taxol [101–103]. As reoxygenation occurs at relatively low thermal doses this should reflect in the thermal goals of hyperthermia [104].

Literature has shown that already a slight elevation of tissue temperature to 39–40 °C results in an increase of tumor blood flow and improved microcirculation [60,62,95,105]. When tumor temperature is higher and the exposure time is longer, tumor perfusion will increase further until a threshold thermal dose is reached from where tumor blood flow will decrease. In general heating to temperatures between 40 and 42 °C for 30–60 min are found to increase perfusion and oxygenation, although this will vary between tumor types and species. For tumors vascular stasis is expected to occur after heating for 60 to 120 min at temperatures around 43 °C, while for normal tissue this is reported to occur after 60 min heating at 45–47 °C [97,106]. Whereas for tumors the reported increase in perfusion is a factor two, the increase in perfusion for normal tissues like muscle and skin can reach a factor 10. Further, it has been shown that the resting perfusion levels and the ability to increase perfusion changes when multiple hyperthermia treatments are administered with 1–2 days interval, with the highest increase after the first heat exposure and stabilizing values for subsequent exposures [96]. Nearly all studies investigating the response of tumor and normal tissue perfusion on the thermal dose of the heat exposure have been obtained in rodents. Despite the general consensus that hyperthermia improves blood perfusion, studies in humans to assess tumor perfusion during or shortly after hyperthermia are still scarce and provide inconclusive data reporting increasing as well as decreasing tumor blood flow [107–113].

In contrast, the great potential of hyperthermia to increase drug delivery to a tumor with high interstitial fluid pressure (IFP) was recently demonstrated by Stapleton et al. [49]. They applied hyperthermia to a MDA-MB-231 human breast adenocarcinoma tumor model located in the lower abdominal mammary fat pad of female SCID mice. Heating the tumor for 20 min at 42 °C resulted in a rapid decrease of IFP to normal tissue levels, whereby the authors indicate that the initial bulk decrease in IFP following hyperthermia appeared to be driven by a significant increase in perfusion and vascular volume during the heat application. Applying a single dose of nanotherapeutics (Doxil) combined with heat resulted in an up to 2.2 fold increase in the enhanced volume fraction. Radial analysis also revealed greater concentration at the center of the tumor for heated vs the non-heated control animals. In a low IFP tumor type (4 T1) adding heat did not enhance drug delivery.

In summary, hyperthermia at 40–42 °C for 30–60 min results in an increased perfusion of the tumor during and immediately after heating, that is mirrored by an improved pO<sub>2</sub> level. A temperature of 43 °C can be used but provides an increased risk of vascular stasis to occur after 60 min of heating. There is still an ongoing discussion on the duration of the increased perfusion and pO<sub>2</sub> level. However, when carefully selecting the temperature and duration of heating, it seems reasonable to assume that the improved conditions are indeed present for the duration of drug circulation in the body as was shown by the Stapleton et al. study [49].

#### 4.3. Hyperthermia and extravasation

In a recent review Dewhirst and Secomb [114] have extensively discussed current understanding of the complex path of transporting drugs from the intravenous injection to the tumor cells to be killed. Once the drug has arrived at the microvascular structure of the tumor, the drugs have to cross the layer of endothelial cells, which is considered the main transport barrier. The ability of drugs to cross this barrier is high for small lipophilic solutes (e.g. O<sub>2</sub>). Larger and hydrophilic solutes can only pass through gaps between the endothelial cells [114,115]. As explained above characteristic for tumors is their chaotic vasculature. The fast proliferation of the endothelial cells in the tumor vessel wall causes less tight junctions between the cells. In animal models it is commonly found that tumor vasculature is leaky (10× more than normal vessels [114]) and that the large pores allow under normal conditions passage of macromolecules or nanoparticles up to a diameter around 100 nm. Whether the same effect occurs also in humans is still debated [116–119]. When mild hyperthermia is applied to the tumor the higher temperature causes an increase of perfusion and a shrinkage of the endothelial cells (disaggregation of the endothelial cell cytoskeleton). This process translates in enhanced extravasation though the thermally induced larger pores between the endothelial cells. The potential impact of the thermally induced enhanced extravasation on drug concentration in the tumor has been investigated in multiple studies and the level of extravasation was found to vary between tumors and to depend on the intrinsic permeability of the tumor vasculature [106,120–129].

In-vivo experimental studies using the dorsal skin flap window chamber model have shown the existence of a temperature threshold for enhancing extravasation. Kong et al. [106] reports a threshold of 39 °C for extravasation in human SKOV-3 ovarian carcinoma, while for temperatures from 40 to 42 °C nanoparticle extravasation increases with temperature. Maximum extravasation was noted at the end of the 60 min heat exposure at 42 °C. In the SKOV-3 tumor model hemorrhaging and collapsing of vessels occurs after several minutes of exposure at 43 °C. After heating the enhanced extravasation returned to normal at 6 h post heating. Interestingly they report the existence of vascular thermotolerance: reheating of the tumor at 42 °C for 1 h following 8 h after the first heating does not result in any increase of extravasation. Li et al. [85,130] also used the window chamber model to investigate thermal dose dependence of extravasation in four

different tumors, i.e. murine B16 melanoma, BFS-1 sarcoma, Lewis Lung Carcinoma (LLC) and human BLM melanoma cells. They found that minimum exposure time at 41 °C to initiate liposome extravasation differed between tumors: murine BFS-1 sarcoma and LLC carcinoma required 20 min exposure while murine B16 melanoma and human BLM melanoma required 30 min exposure. In all tumors longer exposure time resulted in higher extravasation levels and also diffusion from the perivascular regions to deeper extravascular extracellular space (up to 27.5  $\mu\text{m}$  from the vessels). Like Kong et al., Li et al. [85,106] reports that tumor vasculature permeability was preserved after heating. In their study Li et al. [85] found that permeability was preserved up to 8 h post-hyperthermia, though at a lesser extent and was completely lost at 24 h post-hyperthermia. They also observed that extravasation was heterogeneous within the tumor and also between different tumor models with extravasation in LLC carcinoma > murine B16 melanoma > murine BFS-1 sarcoma and human BLM melanoma. In all studies hyperthermia at 42 °C did not enable extravasation in normal vasculature. In Fig. 3 the findings of Kong et al. and Li et al. are presented graphically to illustrate the variability in the time needed to reach maximum extravasation and the duration of enhanced extravasation as reported by these authors.

#### 4.4. Hyperthermia and tumor drug concentration

Several studies investigated whether hyperthermia may increase the concentration of free, i.e. non-encapsulated or non-liposomal, drugs in tumor tissue [84,119,128,130,131]. Kong et al., and Manzoor et al. reported no effect of hyperthermia on the tumor Dox concentration, when free DOX (5 mg/kg) was applied during heating at normal temperature (34 °C) or at 41–42 °C [84,130]. In contrast Ponce et al. reported a 2-fold increase of tumor DOX concentration when rats were given free DOX (5 mg/kg) during hyperthermia [128]. In this study interstitial tumor heating was used with a temperature gradient of 45–46 °C at the center to 39.0 °C at the tumor border. For other free drugs (CDDP) or combinations (DOX + Alvespyimycin) application during hyperthermia was not found to affect tumor drug concentration [119,131]. The common opinion is that hyperthermia has little influence on the absorption of free drugs by the tumor. In general a small molecular weight chemotherapeutic, like DOX, has a short plasma half-live in systemic circulation. The rapidly declining vascular drug concentration will diminish the potential increase in drug absorption facilitated by hyperthermia driven enhanced vascular permeability.

In contrast to free drug the experimental animal studies combining hyperthermia and thermo-sensitive liposomal (TSL) drugs delivery as discussed above have demonstrated that this results in a substantial

increase of the drug concentration in the tumor. Hereby, hyperthermia is the component that controls the initiation of enhanced drug extravasation, via increased tumor perfusion and permeability of the tumor vessels, as well as to trigger TSL drug release at the tumor location. The dominant factor in the process that drives the enhanced drug uptake in the tumor is the ability to nearly instantly create a very high drug concentration in the 'leaky' tumor vasculature [131,132]. In a recent overview Hijnen et al. [133] analyzed 13 studies investigating thermosensitive liposomal drug delivery mediated by hyperthermia using high intensity focused ultrasound (HIFU) with or without MR-guidance for temperature control. The benefit of MR-guided HIFU heating is the ability to deliver a precise controlled, spatial and temporal, temperature distribution of  $\pm 1$  °C to tumors with a diameter up to 4 cm. In their analyses, Hijnen et al. [133], found the doxorubicin concentration to be up to 26 times higher in heated vs non heated tumors. Thermal dose reported varied from 15 to 40 min and 40–43 °C.

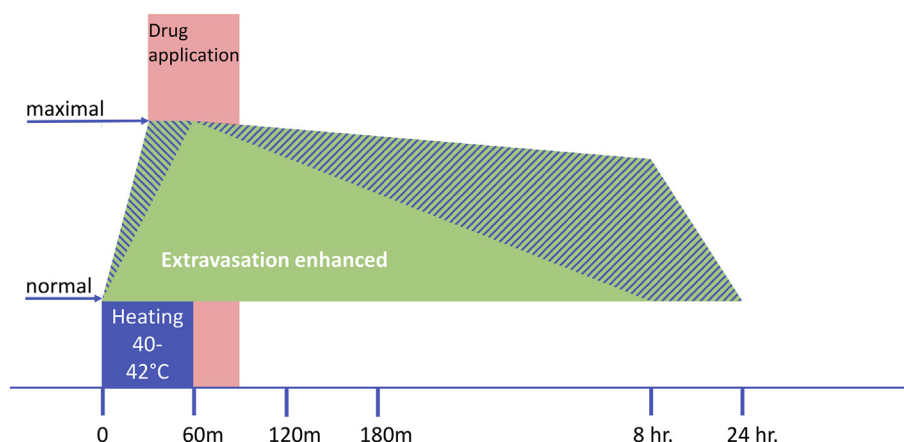
In summary, hyperthermia at 40–42 °C for 30–60 min is effective to realize sensitization of the tumor cells for the applied chemotherapy (TER 1.5–2.5) and to enhance tumor perfusion as well as extravasation that lasts for several hours. Hyperthermia also triggers the site specific TSL drug release that is mandatory to realize a high drug concentration in the permeable tumor vasculature, i.e. the driving force that ultimately results in the enhanced drug concentration in the tumor. Administration of chemotherapy and hyperthermia is preferably at the same time or as closely together as possible. If multiple administrations of thermochemotherapy are planned then these should be separated by approximately 72 h to avoid vascular thermotolerance for heat.

#### 5. Role of thermal dose in heat mediated clinical drug delivery

When defining the strategy for an optimal thermal dose to be applied in thermochemotherapy it is important to consider the thermal dose constraints from:

- the drug perspective, i.e. thermal sensitization and improved drug uptake
- the perspective of patient tolerance, i.e. what thermal dose is safe and tolerated,
- the technology perspective, what thermal dose can be applied under good quality control and the ability for tumor specific delivery of the hyperthermia.

In the end the balance between these three perspectives will be made by the field, i.e. clinicians, patients and physicists. From an economical perspective also manufacturers will contribute by providing adequate equipment to easily apply thermochemotherapy, which can be seamless integrated in existing treatment protocols, like for instance



**Fig. 3.** Schematic representation of the induction of enhanced extravasation induced by 60 min of heating at 40–42 °C following the data presented in [85,106]. The figure shows that hyperthermia at 40–42 °C for 30–60 min enhances extravasation quickly (30–60 min to maximum) followed by a decay over time and can last up to 24 h.

HIPEC and HIVEC. Clearly, clinical treatment outcome will be decisive of this quest to the optimal thermal dose and ultimately for acceptance of thermochemotherapy.

### 5.1. Control of thermal dose

Many systems exist to apply hyperthermia. Each system has its own benefits with regard to its ability to preferentially heat the target volume, temperature monitoring, ease of use and total costs.

The most user friendly devices are the systems to apply hyperthermia in combination with drug perfusion as in HIPEC or HIVEC. In these systems the perfusion liquid is heated to 42 °C and circulated in the peritoneum or bladder. The homogeneity of heating is dictated by quality of perfusing the chemotherapeutic fluid through the organ. Clearly this is easier to control for the bladder than for the peritoneum [134–138].

For heating of tumors at all other places in the body a large variety of devices using either non-ionizing electromagnetic fields (EMF) or ultrasound (US) are available, with historical EMF systems being used more commonly. With EMF systems large tumor sizes can be heated at any location, superficial or deep, in the body. However, due to the long wavelength of the EMF - frequency range 10–915 MHz - the spatial resolution to control the heating pattern is limited to several centimeters. US systems use ultrasound energy to heat tissue operate in a frequency range of 0.5–8 MHz. The benefit of US is the short wavelength in tissue resulting in a tight heating focus of mm dimension and a much larger penetration depth in muscle tissue compared to EMF. A disadvantage of US is the high reflection at air to soft tissue transitions as well as the high absorption of US energy by bone. The small heating focus of US makes it ideal for small tumors but for heating large tumors advanced scanning technology is required. So far heating tumors to 4–5 cm diameter has been demonstrated in anesthetized animals. EMF systems have demonstrated in many clinical trials the ability to heat large volume tumors with sizes in any direction to above 10 cm.

The ability to preferentially heat the target volume depends besides the heating characteristics of the hyperthermia device also on the definition of the target volume. In HIVEC the target volume is the non-muscle invasive bladder cancer (NMIBC). NMIBC is typically limited to the bladder wall in which small tumor nodules at multiple locations need to be heated [136]. A similar target definition applies for HIPEC tumor locations [137]. As for both indications tumor depth is limited to a few mm, heating by thermal conduction from the circulating and heated drug fluid is a simple, efficient and effective method to heat the tumor to 40–42 °C. However, for the larger solid tumors that are heated by EMF or US techniques it is advisable to define the volume to be heated larger than the gross tumor volume. For solid tumors the inflowing blood will be at 37 °C and need some distance in order to reach a temperature of 40–42 °C. When considering only the microenvironment the target volume to be heated should be extended by 2–3 cm, this should be sufficient to reach temperature equilibrium between the smaller vessels and tumor tissue [139–142].

### 5.2. Measuring thermal dose

In general every hyperthermia system is equipped with multi-sensor temperature probes to monitor the tissue temperature increase at either (minimal-) invasive or superficial location. Fiber-optic or Bowman (thermistor probe with high resistance carbon wires for read-out) temperature probes are immune for EMF and hence provide reliable temperature measurement. Fiber-optics probes can hold up to 6 sensors and hence can measure a temperature profile along the thermometry catheter track. Bowman probes are single sensor devices and use mechanical thermal mapping to measure a temperature profile along the thermometry catheter. Thermocouple probes consist of two metal (copper, constantan) wires and are sensitive to pick-up the RF-signal, and affect as well the EMF energy distribution pattern. Special RF-filtering and read-out procedures have been introduced to minimize these effects

[143,144]. An advantage of thermocouples is that they are available with up to 14 sensors in a single probe [145,146]. Accurate temperature measurement using any of the invasive probes described are known to have high absorption of the acoustic signal by the plastic tubing encasing the fibers, thermistors and thermocouples. Rivens et al. [147] report that very fine-wire thermocouples are often the method of choice, though even these devices need special attention to avoid movement of the probe by the US and correction for thermal conductivity errors in the high temperature gradient fields. A major short coming of invasive thermometry is that it provides only information of the temperature at the location of the probe. As both the clinician and the patient are not enthusiastic to place invasive thermometry catheters knowledge about the 3D temperature distribution is extremely limited. So it is no surprise that in the hyperthermia community great interest exists for non-invasive thermometry using magnetic resonance imaging, i.e. MR-thermometry. In ultrasound based hyperthermia the introduction of non-invasive MR thermometry has enabled the ability of real-time feedback control to carefully adapt the spatial ultrasound energy deposition pattern to obtain an optimal thermal dose delivery [148]. Also hybrid hyperthermia devices have been developed integrating MR-compatible, high energy EMF applicator to realize MR-thermometry guided hyperthermia [149–154].

### 5.3. Defining thermal dose

From the above it follows that adding hyperthermia to chemotherapy may result in:

- Thermal sensitization of tumor cells for chemotherapy by a factor 1.5–2.5.
- At best a doubling of tumor drug concentration when using standard chemotherapy or non-temperature sensitive liposomal chemotherapy.
- A 2 to 26 fold increase of tumor drug concentration and an improved homogeneity drug absorption between tumor periphery and center, when using temperature sensitive liposomal chemotherapy [133,155].

As explained for many drugs in vivo TER is reported to be maximal for temperatures between 40.0 and 42.5 °C and a heat duration of 30–90 min. This TER temperature range overlaps very well with the required temperature range to enhance perfusion/oxygenation and extravasation for many tumors. The only restriction is to avoid long duration heating at 43 °C to prevent the occurrence of vascular stasis.

For a few drugs a threshold temperature of 42.5 °C is mentioned for thermal enhancement. Doxorubicin is one the drugs for which this 42.5 °C threshold applies, however, doxorubicin is also the drug mostly studied using a thermo-sensitive liposome as a smart drug carrier. Here, the great potential of TSL-encapsulated drugs to enhance tumor drug concentration outbalances the benefit of TER and priority should be given to maximally benefit of improved perfusion and extravasation.

When considering the potential contributions via TER versus that of increased drug concentration using TSL chemotherapy, current biological knowledge indicates that the optimal thermal dose should be 40–42 °C for a duration of 30–60 min to achieve maximal treatment outcome. This thermal dose guarantees an effective TER for most drugs and also the highest enhancement of drug concentration via TSL drug delivery. Timing of the drug and hyperthermia application should be simultaneous for free drugs and while TSL drug application should start 20–30 min after start of the hyperthermia treatment to achieve maximum drug delivery. Expressed as CEM43 values the proposed thermal dose translates in a CEM43 of 1 to 15 min.

## 6. Conclusion

Clinical experience shows that the therapeutic application of hyperthermia in large tumors is characterized by a heterogeneous temperature distribution over the target volume. In general patients tolerated



temperatures in the range of 40–42 °C quite well, whereas higher temperatures are associated with discomfort and pain complaints. Furthermore, temperatures around 43 °C have been associated with an increased probability of acute toxicity and in experimental studies with vascular collapse. Most importantly, the results reported in randomized studies confirm that this realistic thermal dose target results in a significant and relevant improved treatment outcome for the combined treatment arm. Therefore, in current clinical practice consensus exists that the objective of clinical hyperthermia is to aim for target temperatures of 40–42 °C. Extensive research is ongoing to optimize the thermal dose distribution for the individual patient using new advanced hyperthermia technology supported by hyperthermia treatment planning.

When combining chemotherapy and hyperthermia the potential benefits of each of the biological mechanisms and the optimal temperature at which these mechanisms are triggered and its duration have to be taken into account to define the best strategy for thermochemotherapy. Hence, considering the potential benefits of thermal enhancement versus enhanced drug concentration when adding hyperthermia to chemotherapy either applied as free drug or using a thermal sensitive liposomal carrier the optimal thermal dose for thermochemotherapy should be 40–42 °C for 30–60 min or a CEM43 of 1–15 min with the chemotherapy simultaneous or 30 min after starting hyperthermia.

## Acknowledgement

This work was in part funded by the Dutch Cancer Society (KWF), The Netherlands: grants KWF DDHK 2013-6072 and 11368.

## References

- [1] R. Cavaliere, E.C. Ciocatto, B.C. Giovannella, C. Heidelberger, R.O. Johnson, M. Margottini, B. Mondovi, G. Moricca, A. Rossi-Fanelli, Selective heat sensitivity of cancer cells. *Biochemical and clinical studies*, *Cancer* 20 (1967) 1351–1381.
- [2] M.W. Dewhirst, Z. Vujaskovic, E. Jones, D. Thrall, Re-setting the biologic rationale for thermal therapy, *Int. J. Hyperth.* 21 (2005) 779–790.
- [3] H.H. Kampinga, Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field, *Int. J. Hyperth.* 22 (2006) 191–196.
- [4] P.M. Krawczyk, B. Eppink, J. Essers, J. Stap, H. Rodermond, H. Odijk, A. Zelensky, C. van Bree, L.J. Stalpers, M.R. Buist, T. Soullie, J. Rens, H.J. Verhagen, M.J. O'Connor, N.A. Franken, T.L. Ten Hagen, R. Kanaar, J.A. Aten, Mild hyperthermia inhibits homologous recombination, induces BRCA2 degradation, and sensitizes cancer cells to poly (ADP-ribose) polymerase-1 inhibition, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 9851–9856.
- [5] A.L. Oei, L.E. Vriend, J. Crezee, N.A. Franken, P.M. Krawczyk, Effects of hyperthermia on DNA repair pathways: one treatment to inhibit them all, *Radiat. Oncol.* 10 (2015) 165.
- [6] B. Emami, C. Scott, C.A. Perez, S. Asbell, P. Swift, G. Grigsby, A. Montesano, P. Rubin, W. Curran, J. Delrowe, H. Arastu, K. Fu, E. Moros, Phase III study of interstitial thermoradiotherapy compared with interstitial radiotherapy alone in the treatment of recurrent or persistent human tumors. A prospectively controlled randomized study by the radiation therapy group, *Int. J. Radiat. Oncol. Biol. Phys.* 34 (1996) 1097–1104.
- [7] C.A. Perez, B. Gillespie, T. Pajak, N.B. Hornback, B. Emami, P. Rubin, Quality assurance problems in clinical hyperthermia and their impact on therapeutic outcome: a report by the radiation therapy oncology group, *Int. J. Radiat. Oncol. Biol. Phys.* 16 (1989) 551–558.
- [8] C.A. Perez, T. Pajak, B. Emami, N.B. Hornback, L. Tupchong, P. Rubin, Randomized phase III study comparing irradiation and hyperthermia with irradiation alone in superficial measurable tumors. Final report by the radiation therapy oncology group, *Am. J. Clin. Oncol.* 14 (1991) 133–141.
- [9] J. van der Zee, D. Gonzalez Gonzalez, G.C. van Rhoon, J.D. van Dijk, W.L. van Putten, A.A. Hart, Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumours: a prospective, randomised, multicentre trial. Dutch Deep Hyperthermia Group, *Lancet* 355 (2000) 1119–1125.
- [10] C.C. Vernon, J.W. Hand, S.B. Field, D. Machin, J.B. Whaley, J. van der Zee, W.L. van Putten, G.C. van Rhoon, J.D. van Dijk, D. Gonzalez Gonzalez, F.F. Liu, P. Goodman, M. Sherar, Radiotherapy with or without hyperthermia in the treatment of superficial localized breast cancer: results from five randomized controlled trials. International collaborative hyperthermia group, *Int. J. Radiat. Oncol. Biol. Phys.* 35 (1996) 731–744.
- [11] M. Franckena, L.J. Stalpers, P.C. Koper, R.G. Wiggendaad, W.J. Hoogendaad, J.D. van Dijk, C.C. Warlam-Rodenhuis, J.J. Jobsen, G.C. van Rhoon, J. van der Zee, Long-term improvement in treatment outcome after radiotherapy and hyperthermia in locoregionally advanced cervix cancer: an update of the Dutch deep hyperthermia trial, *Int. J. Radiat. Oncol. Biol. Phys.* 70 (2008) 1176–1182.
- [12] M.S. Chi, K.L. Yang, Y.C. Chang, H.L. Ko, Y.H. Lin, S.C. Huang, Y.Y. Huang, K.W. Liao, M. Kondo, K.H. Chi, Comparing the effectiveness of combined external beam radiation and hyperthermia versus external beam radiation alone in treating patients with painful bony metastases: a phase 3 prospective, randomized, controlled trial, *Int. J. Radiat. Oncol. Biol. Phys.* 100 (2018) 78–87.
- [13] Y. Harima, K. Nagata, K. Harima, V.V. Ostapenko, Y. Tanaka, S. Sawada, A randomized clinical trial of radiation therapy versus thermoradiotherapy in stage IIIB cervical carcinoma, *Int. J. Hyperth.* 17 (2001) 97–105.
- [14] Y. Harima, T. Ohguri, H. Imada, H. Sakurai, T. Ohno, Y. Hiraki, K. Tuji, M. Tanaka, H. Terashima, A multicentre randomised clinical trial of chemoradiotherapy plus hyperthermia versus chemoradiotherapy alone in patients with locally advanced cervical cancer, *Int. J. Hyperth.* 32 (2016) 801–808.
- [15] N.G. Huilgol, S. Gupta, C.R. Sridhar, Hyperthermia with radiation in the treatment of locally advanced head and neck cancer: a report of randomized trial, *J. Cancer Res. Ther.* 6 (2010) 492–496.
- [16] M. Kang, W.Q. Liu, Y.T. Qin, Z.X. Wei, R.S. Wang, Long-term efficacy of microwave hyperthermia combined with chemoradiotherapy in treatment of nasopharyngeal carcinoma with cervical lymph node metastases, *Asian Pac. J. Cancer Prev.* 14 (2013) 7395–7400.
- [17] N.R. Datta, S.G. Ordenez, U.S. Gaipil, M.M. Paulides, H. Crezee, J. Gellermann, D. Marder, E. Puric, S. Bodis, Local hyperthermia combined with radiotherapy and –/or chemotherapy: recent advances and promises for the future, *Cancer Treat. Rev.* 41 (2015) 742–753.
- [18] N.R. Datta, S. Rogers, S.G. Ordenez, E. Puric, S. Bodis, Hyperthermia and radiotherapy in the management of head and neck cancers: a systematic review and meta-analysis, *Int. J. Hyperth.* 32 (2016) 31–40.
- [19] R. Colombo, L.F. Da Pozzo, A. Salonia, P. Rigatti, Z. Leib, J. Baniel, E. Caldarella, M. Pavone-Macaluso, Multicentric study comparing intravesical chemotherapy alone and with local microwave hyperthermia for prophylaxis of recurrence of superficial transitional cell carcinoma, *J. Clin. Oncol.* 21 (2003) 4270–4276.
- [20] R. Colombo, A. Salonia, Z. Leib, M. Pavone-Macaluso, D. Engelstein, Long-term outcomes of a randomized controlled trial comparing thermochemotherapy with mitomycin-C alone as adjuvant treatment for non-muscle-invasive bladder cancer (NMIBC), *BJU Int.* 107 (2011) 912–918.
- [21] V.J. Verwaal, Long-term results of cytoreduction and HIPEC followed by systemic chemotherapy, *Cancer J.* 15 (2009) 212–215.
- [22] W.J. van Driel, S.N. Koole, K. Sikorska, J.H. Schagen van Leeuwen, H.W.R. Schreuder, R.H.M. Hermans, I. de Hingh, J. van der Velden, H.J. Arts, L. Massuger, A.G.J. Aalbers, V.J. Verwaal, J.M. Kieffer, K.K. Van de Vijver, H. van Tinteren, N.K. Aaronson, G.S. Sonke, Hyperthermic Intraperitoneal chemotherapy in ovarian cancer, *N. Engl. J. Med.* 378 (2018) 230–240.
- [23] R.D. Issels, L.H. Lindner, J. Verweij, P. Wust, P. Reichardt, B.C. Schem, S. Abdel-Rahman, S. Daugaard, C. Salat, C.M. Wendtner, Z. Vujaskovic, R. Wessalowski, K.W. Jauch, H.R. Durr, F. Ploner, A. Baur-Melnyk, U. Mansmann, W. Hiddemann, J.Y. Blay, P. Hohenberger, R. European Organisation for, T. Treatment of Cancer Soft, G. Bone Sarcoma, O. European Society for Hyperthermic, Neo-adjuvant chemotherapy alone or with regional hyperthermia for localised high-risk soft-tissue sarcoma: a randomised phase 3 multicentre study, *Lancet Oncol.* 11 (2010) 561–570.
- [24] R.D. Issels, L.H. Lindner, J. Verweij, R. Wessalowski, P. Reichardt, P. Wust, P. Ghadjar, P. Hohenberger, M. Angele, C. Salat, Z. Vujaskovic, S. Daugaard, O. Mella, U. Mansmann, H.R. Durr, T. Knosel, S. Abdel-Rahman, M. Schmidt, W. Hiddemann, K.W. Jauch, C. Belka, A. Gronchi, R. European Organization for the, T. Treatment of Cancer-Soft, G. Bone Sarcoma, O. the European Society for Hyperthermic, Effect of neoadjuvant chemotherapy plus regional hyperthermia on long-term outcomes among patients with localized high-risk soft tissue sarcoma: the EORTC 62961-ESHO 95 randomized clinical trial, *JAMA Oncol.* 4 (2018) 483–492.
- [25] O.N. Gofrit, A. Shapiro, D. Podes, A. Sidi, O. Nativ, Z. Leib, J.A. Witjes, A.G. van der Heijden, R. Naspro, R. Colombo, Combined local bladder hyperthermia and intravesical chemotherapy for the treatment of high-grade superficial bladder cancer, *Urology* 63 (2004) 466–471.
- [26] B. Moskovitz, G. Meyer, A. Kravtsov, M. Gross, A. Kastin, K. Biton, O. Nativ, Thermochemotherapy for intermediate or high-risk recurrent superficial bladder cancer patients, *Ann. Oncol.* 16 (2005) 585–589.
- [27] T.J. Arends, O. Nativ, M. Maffezzini, O. de Cobelli, G. Canepa, F. Verweij, B. Moskovitz, A.G. van der Heijden, J.A. Witjes, Results of a randomised controlled trial comparing intravesical chemohyperthermia with mitomycin C versus bacillus calmette-guerin for adjuvant treatment of patients with intermediate- and high-risk non-muscle-invasive bladder cancer, *Eur. Urol.* 69 (2016) 1046–1052.
- [28] R. Hamazoe, M. Maeta, N. Kaibara, Intraperitoneal thermochemotherapy for prevention of peritoneal recurrence of gastric cancer. Final results of a randomized controlled study, *Cancer* 73 (1994) 2048–2052.
- [29] S. Fujimoto, M. Takahashi, T. Mutou, K. Kobayashi, T. Toyosawa, Successful intraperitoneal hyperthermic chemoperfusion for the prevention of postoperative peritoneal recurrence in patients with advanced gastric carcinoma, *Cancer* 85 (1999) 529–534.
- [30] Y. Yonemura, X. de Aretxabala, T. Fujimura, S. Fushida, K. Katayama, E. Bandou, K. Sugiyama, T. Kawamura, K. Kinoshita, Y. Endou, T. Sasaki, Intraoperative chemohyperthermic peritoneal perfusion as an adjuvant to gastric cancer: final results of a randomized controlled study, *Hepatogastroenterology* 48 (2001) 1776–1782.
- [31] H.B. Cui, H.E. Ge, X.Y. Bai, W. Zhang, Y.Y. Zhang, J. Wang, X. Li, L.P. Xing, S.H. Guo, Z.Y. Wang, Effect of neoadjuvant chemotherapy combined with hyperthermic

- intraperitoneal perfusion chemotherapy on advanced gastric cancer, *Exp. Ther. Med.* 7 (2014) 1083–1088.
- [32] C.E.L. Klaver, D.D. Wisselink, C.J.A. Punt, P. Snaebjornsson, J. Crezee, A.G.J. Aalbers, A. Brandt, A.J.A. Bremers, J.W.A. Burger, H.F.J. Fabry, F. Ferenschild, S. Festen, W.M.U. van Grevenstein, P.H.J. Hemmer, I. de Hingh, N.F.M. Kok, G.D. Musters, L. Schoonderwoerd, J.B. Tuynman, A.W.H. van de Ven, H.L. van Westreenen, M.J. Wiersema, D.D.E. Zimmerman, A.A. van Zweenen, M.G.W. Dijkgraaf, P.J. Tanis, C.c. group, Adjuvant hyperthermic intraperitoneal chemotherapy in patients with locally advanced colon cancer (COLOPEC): a multicentre, open-label, randomised trial, *Lancet Gastroenterol. Hepatol.* 4 (2019) 761–770.
- [33] V.J. Verwaal, S. van Ruth, E. de Bree, G.W. van Slooten, H. van Tinteren, H. Boot, F.A. Zoetmulder, Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer, *J. Clin. Oncol.* 21 (2003) 3737–3743.
- [34] V.J. Verwaal, S. Bruin, H. Boot, G. van Slooten, H. van Tinteren, 8-year follow-up of randomized trial: cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy in patients with peritoneal carcinomatosis of colorectal cancer, *Ann. Surg. Oncol.* 15 (2008) 2426–2432.
- [35] X.J. Yang, C.Q. Huang, T. Suo, L.J. Mei, G.L. Yang, F.L. Cheng, Y.F. Zhou, B. Xiong, Y. Yonemura, Y. Li, Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy improves survival of patients with peritoneal Carcinomatosis from gastric cancer: final results of a phase III randomized clinical trial, *Ann. Surg. Oncol.* 18 (2011) 1575–1581.
- [36] J. Spiliotis, E. Halkia, E. Lianos, N. Kalantzi, A. Grivas, E. Efstathiou, S. Giassas, Cytoreductive surgery and HIPEC in recurrent epithelial ovarian cancer: a prospective randomized phase III study, *Ann. Surg. Oncol.* 22 (2015) 1570–1575.
- [37] K. Sugimachi, H. Kuwano, H. Ide, T. Toge, M. Saku, Y. Oshiumi, Chemotherapy combined with or without hyperthermia for patients with Esophageal-carcinoma - a prospective randomized trial, *Int. J. Hyperth.* 10 (1994) 485–493.
- [38] R.J. Lammers, J.A. Witjes, B.A. Inman, I. Leibovitch, M. Laufer, O. Nativ, R. Colombo, The role of a combined regimen with intravesical chemotherapy and hyperthermia in the management of non-muscle-invasive bladder cancer: a systematic review, *Eur. Urol.* 60 (2011) 81–93.
- [39] F. Quenet, D. Elias, L. Roca, D. Goere, L. Ghouti, M. Pocard, O. Facy, C. Arvieux, G. Lorimier, D. Pezet, F. Marchal, V. Loi, P. Meeus, H. De Forges, T. Stanbury, J. Paineau, O. Glehen, A UNICANCER phase III trial of hyperthermic intra-peritoneal chemotherapy (HIPEC) for colorectal peritoneal carcinomatosis (PC): PRODIGE 7, *J. Clin. Oncol.* 36 (2018).
- [40] A. Konigsrainer, B. Rau, Cytoreductive Surgery (CRS) and Hyperthermic IntraPeritoneal Chemotherapy (HIPEC): don't throw the baby out with the bathwater, *Pleura Peritoneum* 3 (2018).
- [41] T.E. Grotz, K.F. Fournier, J.C. Boughey, Cytoreduction with or without HIPEC: Where Do we Go from Here? *American College of Surgeons*, 2019 5 Feb.
- [42] H. Grull, S. Langereis, Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using MRI-guided high intensity focused ultrasound, *J. Control. Release* 161 (2012) 317–327.
- [43] G.A. Koning, A.M. Eggermont, L.H. Lindner, T.L. ten Hagen, Hyperthermia and thermosensitive liposomes for improved delivery of chemotherapeutic drugs to solid tumors, *Pharm. Res.* 27 (2010) 1750–1754.
- [44] M.R. Horsman, J. Overgaard, Overcoming tumour radiation resistance resulting from acute hypoxia, *Eur. J. Cancer* 28A (1992) 717–718.
- [45] M.R. Horsman, J. Overgaard, The impact of hypoxia and its modification of the outcome of radiotherapy, *J. Radiat. Res.* 57 (Suppl. 1) (2016) i90–i98.
- [46] M.R. Horsman, P. Vaupel, Pathophysiological basis for the formation of the tumor microenvironment, *Front. Oncol.* 6 (2016) 66.
- [47] J. Overgaard, C. Grau, J.C. Lindegaard, M.R. Horsman, The potential of using hyperthermia to eliminate radioresistant hypoxic cells, *Radiother. Oncol.* 20 (Suppl. 1) (1991) 113–116.
- [48] A. Wouters, B. Pauwels, F. Lardon, J.B. Vermorken, Review: implications of in vitro research on the effect of radiotherapy and chemotherapy under hypoxic conditions, *Oncologist* 12 (2007) 690–712.
- [49] S. Stapleton, M. Dunne, M. Milosevic, C.W. Tran, M.J. Gold, A. Vedadi, T.D. McKee, P.S. Ohashi, C. Allen, D.A. Jaffray, Radiation and heat improve the delivery and efficacy of Nanotherapeutics by modulating Intratumoral fluid dynamics, *ACS Nano* 12 (2018) 7583–7600.
- [50] P.B. Elming, B.S. Sorensen, A.L. Oei, N.A.P. Franken, J. Crezee, J. Overgaard, M.R. Horsman, Hyperthermia: the optimal treatment to overcome radiation resistant hypoxia, *Cancers (Basel)* 11 (2019).
- [51] M. Chidambaram, R. Manavalan, K. Kathiresan, Nanotherapeutics to overcome conventional cancer chemotherapy limitations, *J. Pharm. Pharm. Sci.* 14 (2011) 67–77.
- [52] S. Senapati, A.K. Mahanta, S. Kumar, P. Maiti, Controlled drug delivery vehicles for cancer treatment and their performance, *Signal. Transduct. Target Ther.* 3 (2018) 7.
- [53] M.W. Dewhirst, C.T. Lee, K.A. Ashcraft, The future of biology in driving the field of hyperthermia, *Int. J. Hyperth.* 32 (2016) 4–13.
- [54] A.L. Oei, C.M. van Leeuwen, V.R. Ahire, H.M. Rodermond, R. Ten Cate, A.M. Westermann, L.J.A. Stalpers, J. Crezee, H.P. Kok, P.M. Krawczyk, R. Kanaar, N.A.P. Franken, Enhancing synthetic lethality of PARP-inhibitor and cisplatin in BRCA-proficient tumour cells with hyperthermia, *Oncotarget* 8 (2017) 28116–28124.
- [55] N. van den Tempel, M.R. Horsman, R. Kanaar, Improving efficacy of hyperthermia in oncology by exploiting biological mechanisms, *Int. J. Hyperth.* 32 (2016) 446–454.
- [56] R. Issels, Hyperthermia combined with chemotherapy – biological rationale, clinical application, and treatment results, *Onkologie* 22 (1999) 7.
- [57] A.L. Oei, L.E.M. Vriend, P.M. Krawczyk, M.R. Horsman, N.A.P. Franken, J. Crezee, Targeting therapy-resistant cancer stem cells by hyperthermia, *Int. J. Hyperth.* 33 (2017) 419–427.
- [58] J.R. Lepock, Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage, *Int. J. Hyperth.* 19 (2003) 252–266.
- [59] J.L. Roti Roti, Cellular responses to hyperthermia (40–46 degrees C): cell killing and molecular events, *Int. J. Hyperth.* 24 (2008) 3–15.
- [60] R.J. Griffin, R.P. Dings, A. Jamshidi-Parsian, C.W. Song, Mild temperature hyperthermia and radiation therapy: role of tumour vascular thermotolerance and relevant physiological factors, *Int. J. Hyperth.* 26 (2010) 256–263.
- [61] R.J. Griffin, A. Ogawa, B.W. Williams, C.W. Song, Hyperthermic enhancement of tumor radiosensitization strategies, *Immunol. Investig.* 34 (2005) 343–359.
- [62] C.W. Song, H. Park, R.J. Griffin, Improvement of tumor oxygenation by mild hyperthermia, *Radiat. Res.* 155 (2001) 515–528.
- [63] S.B. Field, G.P. Raaphorst, Thermal dose, in: S.B. Field, J.W. Hand (Eds.), *An Introduction to the Practical Aspects of Clinical Hyperthermia*, Taylor & Francis, London, N. Y 1990, pp. 69–76.
- [64] R. Stahl, T. Wang, L.H. Lindner, S. Abdel-Rahman, M. Santl, M.F. Reiser, R.D. Issels, Comparison of radiological and pathohistological response to neoadjuvant chemotherapy combined with regional hyperthermia (RHT) and study of response dependence on the applied thermal parameters in patients with soft tissue sarcomas (STS), *Int. J. Hyperth.* 25 (2009) 289–298.
- [65] R.D. Issels, S.W. Preeninger, A. Nagele, E. Boehm, H. Sauer, K.W. Jauch, H. Denecke, H. Berger, K. Peter, W. Wilmanns, Ifosfamide plus etoposide combined with regional hyperthermia in patients with locally advanced sarcomas: a phase II study, *J. Clin. Oncol.* 8 (1990) 1818–1829.
- [66] T. Ohguri, Y. Harima, H. Imada, H. Sakurai, T. Ohno, Y. Hiraki, K. Tuji, M. Tanaka, H. Terashima, Relationships between thermal dose parameters and the efficacy of definitive chemoradiotherapy plus regional hyperthermia in the treatment of locally advanced cervical cancer: data from a multicentre randomised clinical trial, *Int. J. Hyperth.* 34 (2018) 461–468.
- [67] M. de Bruijne, B. van der Holt, G.C. van Rhoon, J. van der Zee, Evaluation of CEM43 degrees CT90 thermal dose in superficial hyperthermia: a retrospective analysis, *Strahlenther. Onkol.* 186 (2010) 436–443.
- [68] M. Franckena, D. Fatehi, M. de Bruijne, R.A. Canters, Y. van Norden, J.W. Mens, G.C. van Rhoon, J. van der Zee, Hyperthermia dose-effect relationship in 420 patients with cervical cancer treated with combined radiotherapy and hyperthermia, *Eur. J. Cancer* 45 (2009) 1969–1978.
- [69] E.L. Jones, J.R. Oleson, L.R. Prosnitz, T.V. Samulski, Z. Vujaskovic, D. Yu, L.L. Sanders, M.W. Dewhirst, Randomized trial of hyperthermia and radiation for superficial tumors, *J. Clin. Oncol.* 23 (2005) 3079–3085.
- [70] J.R. Oleson, T.V. Samulski, K.A. Leopold, S.T. Clegg, M.W. Dewhirst, R.K. Dodge, S.L. George, Sensitivity of hyperthermia trial outcomes to temperature and time: implications for thermal goals of treatment, *Int. J. Radiat. Oncol. Biol. Phys.* 25 (1993) 289–297.
- [71] M. Sherar, F.F. Liu, M. Pintilie, W. Levin, J. Hunt, R. Hill, J. Hand, C. Vernon, G. van Rhoon, J. van der Zee, D.G. Gonzalez, J. van Dijk, J. Whaley, D. Machin, Relationship between thermal dose and outcome in thermoradiotherapy treatments for superficial recurrences of breast cancer: data from a phase III trial, *Int. J. Radiat. Oncol. Biol. Phys.* 39 (1997) 371–380.
- [72] D.S. Kapp, R.S. Cox, Thermal treatment parameters are most predictive of outcome in patients with single tumor nodules per treatment field in recurrent adenocarcinoma of the breast, *Int. J. Radiat. Oncol. Biol. Phys.* 33 (1995) 887–899.
- [73] B. Rau, P. Wust, W. Tilly, J. Gellermann, C. Harder, H. Riess, V. Budach, R. Felix, P.M. Schlag, Preoperative radiochemotherapy in locally advanced or recurrent rectal cancer: regional radiofrequency hyperthermia correlates with clinical parameters, *Int. J. Radiat. Oncol. Biol. Phys.* 48 (2000) 381–391.
- [74] W.C. Dewey, Arrhenius relationships from the molecule and cell to the clinic, *Int. J. Hyperth.* 10 (1994) 457–483.
- [75] G.C. van Rhoon, Is CEM43 still a relevant thermal dose parameter for hyperthermia treatment monitoring? *Int. J. Hyperth.* 32 (2016) 50–62.
- [76] M. Kroesen, H.T. Mulder, J.M.L. van Holthe, A.A. Aangeenbrug, J.W.M. Mens, H.C. van Doorn, M.M. Paulides, E. Oomen-de Hoop, R.M. Vernhout, L.C. Lutgens, G.C. van Rhoon, M. Franckena, Confirmation of thermal dose as a predictor of local control in cervical carcinoma patients treated with state-of-the-art radiation therapy and hyperthermia, *Radiother. Oncol.* 140 (2019) 150–158.
- [77] S.C. Brunningk, J. Ijaz, I. Rivens, S. Nill, G. Ter Haar, U. Oelfke, A comprehensive model for heat-induced radio-sensitisation, *Int. J. Hyperth.* 34 (2018) 392–402.
- [78] J. Pearce, Mathematical models of laser-induced tissue thermal damage, *Int. J. Hyperth.* 27 (2011) 741–750.
- [79] J.A. Pearce, Models for thermal damage in tissues: processes and applications, *Crit. Rev. Biomed. Eng.* 38 (2010) 1–20.
- [80] J.A. Pearce, Comparative analysis of mathematical models of cell death and thermal damage processes, *Int. J. Hyperth.* 29 (2013) 262–280.
- [81] R. Issels, E. Kampmann, R. Kanaar, L.H. Lindner, Hallmarks of hyperthermia in driving the future of clinical hyperthermia as targeted therapy: translation into clinical application, *Int. J. Hyperth.* 32 (2016) 89–95.
- [82] B. Eppink, P.M. Krawczyk, J. Stap, R. Kanaar, Hyperthermia-induced DNA repair deficiency suggests novel therapeutic anti-cancer strategies, *Int. J. Hyperth.* 28 (2012) 509–517.
- [83] S.B. Field, C.C. Morris, The relationship between heating time and temperature: its relevance to clinical hyperthermia, *Radiother. Oncol.* 1 (1983) 179–186.
- [84] G. Kong, G. Anyarambhatla, W.P. Petros, R.D. Braun, O.M. Colvin, D. Needham, M.W. Dewhirst, Efficacy of liposomes and hyperthermia in a human tumor xenograft model: importance of triggered drug release, *Cancer Res.* 60 (2000) 6950–6957.



- [85] L. Li, T.L. ten Hagen, M. Bolkestein, A. Gasselhuber, J. Yatvin, G.C. van Rhoon, A.M. Eggermont, D. Haemmerich, G.A. Koning, Improved intratumoral nanoparticle extravasation and penetration by mild hyperthermia, *J. Control. Release* 167 (2013) 130–137.
- [86] M.W. Dewhirst, B.L. Viglianti, M. Lora-Michiels, M. Hanson, P.J. Hoopes, Basic principles of thermal dosimetry and thermal thresholds for tissue damage from hyperthermia, *Int. J. Hyperth.* 19 (2003) 267–294.
- [87] O. Dahl, Mechanisms of thermal enhancement of chemotherapeutic cytotoxicity, in: M. Urano, E. Douple (Eds.), *Chemopotential by Hyperthermia*, VSP, Utrecht 1994, pp. 9–28.
- [88] R.D. Issels, Hyperthermia adds to chemotherapy, *Eur. J. Cancer* 44 (2008) 2546–2554.
- [89] J. Havenan, R.C. Rietbroek, A. Geerdink, J. Van Rijn, P.J. Bakker, Effect of hyperthermia on the cytotoxicity of 2',2'-difluorodeoxycytidine (gemcitabine) in cultured SW1573 cells, *Int. J. Cancer* 62 (1995) 627–630.
- [90] M. Urano, M. Kuroda, Y. Nishimura, For the clinical application of thermochemotherapy given at mild temperatures, *Int. J. Hyperth.* 15 (1999) 79–107.
- [91] A. Mansouri, K.J. Henle, A.M. Benson, A.J. Moss, W.A. Nagle, Characterization of a cisplatin-resistant subline of murine RIF-1 cells and reversal of drug resistance by hyperthermia, *Cancer Res.* 49 (1989) 2674–2678.
- [92] L.R. Towle, Hyperthermia and drug resistance, in: M. Urano, E. Douple (Eds.), *Chemopotential by Hyperthermia*, VSP, Utrecht 1994, pp. 91–113.
- [93] M.R. Horsman, Tissue physiology and the response to heat, *Int. J. Hyperth.* 22 (2006) 197–203.
- [94] A.M. Lokshina, C.W. Song, J.G. Rhee, S.H. Levitt, Effect of fractionated heating on the blood flow in normal tissues, *Int. J. Hyperth.* 1 (1985) 117–129.
- [95] Z. Vujaskovic, C.W. Song, Physiological mechanisms underlying heat-induced radiosensitization, *Int. J. Hyperth.* 20 (2004) 163–174.
- [96] C.W. Song, M.S. Patten, L.M. Chelstrom, J.G. Rhee, S.H. Levitt, Effect of multiple heatings on the blood flow in RIF-1 tumours, skin and muscle of C3H mice, *Int. J. Hyperth.* 3 (1987) 535–545.
- [97] H.S. Reinhold, A.P.V.D. Berg, Effects of hyperthermia on blood flow and metabolism, in: S.B. Field, J.W. Hand (Eds.), *An Introduction to the Practical Aspects of Clinical Hyperthermia*, Taylor & Francis, London, N. Y 1990, pp. 77–106.
- [98] C.W. Song, I.B. Choi, B.S. Nah, S.K. Sahu, J.L. Osborn, Microvasculature and perfusion in normal tissue and tumors, in: M.H. Seegenschmiedt, P. Fessenden, C.C. Vernon (Eds.), *Thermoradiotherapy and Thermochemotherapy: Biology, Physiology, Physics*, Springer Berlin Heidelberg, Berlin, Heidelberg 1995, pp. 139–156.
- [99] D.M. Brizel, S.P. Scully, J.M. Harrelson, L.J. Layfield, R.K. Dodge, H.C. Charles, T.V. Samulski, L.R. Prosnitz, M.W. Dewhirst, Radiation therapy and hyperthermia improve the oxygenation of human soft tissue sarcomas, *Cancer Res.* 56 (1996) 5347–5350.
- [100] B.A. Teicher, Hypoxia and drug resistance, *Cancer Metastasis Rev.* 13 (1994) 139–168.
- [101] L. Milas, N. Hunter, K.A. Mason, C. Milross, L.J. Peters, Tumor reoxygenation as a mechanism of taxol-induced enhancement of tumor radioresponse, *Acta Oncol.* 34 (1995) 409–412.
- [102] L. Milas, N.R. Hunter, K.A. Mason, C.G. Milross, Y. Saito, L.J. Peters, Role of reoxygenation in induction of enhancement of tumor radioresponse by paclitaxel, *Cancer Res.* 55 (1995) 3564–3568.
- [103] B. Teicher, N. Dupuis, T. Kusumoto, M. Liu, F. Liu, K. Menon, G. Schwartz, E. Frei, Decreased tumor oxygenation after cyclophosphamide, reoxygenation and therapeutic enhancement with a perflubron emulsion carbogen breathing, *Int. J. Oncol.* 3 (1993) 197–203.
- [104] Y. Tang, A.J. McGoron, Increasing the rate of heating: a potential therapeutic approach for achieving synergistic tumour killing in combined hyperthermia and chemotherapy, *Int. J. Hyperth.* 29 (2013) 145–155.
- [105] A. Shakil, J.L. Osborn, C.W. Song, Changes in oxygenation status and blood flow in a rat tumor model by mild temperature hyperthermia, *Int. J. Radiat. Oncol. Biol. Phys.* 43 (1999) 859–865.
- [106] G. Kong, R.D. Braun, M.W. Dewhirst, Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature, *Cancer Res.* 61 (2001) 3027–3032.
- [107] C.H. Cho, G. Sreenivasa, M. Plotkin, H. Pietsch, P. Wust, L. Ludemann, Tumour perfusion assessment during regional hyperthermia treatment: comparison of temperature probe measurement with H(2)(15)O-PET perfusion, *Int. J. Hyperth.* 26 (2010) 404–411.
- [108] L. Ludemann, G. Sreenivasa, H. Amthauer, R. Michel, J. Gellermann, P. Wust, Use of H(2) (15)O-PET for investigating perfusion changes in pelvic tumors due to regional hyperthermia, *Int. J. Hyperth.* 25 (2009) 299–308.
- [109] F.M. Waterman, L. Komarnicky, D.B. Leeper, The response of human tumour blood flow to a fractionated course of thermoradiotherapy, *Int. J. Hyperth.* 14 (1998) 421–434.
- [110] F.M. Waterman, R.E. Nerlinger, D.J. Moylan 3rd, D.B. Leeper, Response of human tumor blood flow to local hyperthermia, *Int. J. Radiat. Oncol. Biol. Phys.* 13 (1987) 75–82.
- [111] F.M. Waterman, L. Tupchong, C.R. Liu, Modified thermal clearance technique for determination of blood flow during local hyperthermia, *Int. J. Hyperth.* 7 (1991) 719–733.
- [112] F.M. Waterman, L. Tupchong, R.E. Nerlinger, J. Matthews, Blood flow in human tumors during local hyperthermia, *Int. J. Radiat. Oncol. Biol. Phys.* 20 (1991) 1255–1262.
- [113] J.C. Acker, M.W. Dewhirst, G.M. Honore, T.V. Samulski, J.A. Tucker, J.R. Oleson, Blood perfusion measurements in human tumours: evaluation of laser Doppler methods, *Int. J. Hyperth.* 6 (1990) 287–304.
- [114] M.W. Dewhirst, T.W. Secomb, Transport of drugs from blood vessels to tumour tissue, *Nat. Rev. Cancer* 17 (2017) 738–750.
- [115] M.B. Schaaf, A.D. Garg, P. Agostinis, Defining the role of the tumor vasculature in antitumor immunity and immunotherapy, *Cell Death Dis.* 9 (2018) 115.
- [116] Y. Dou, K. Hynynen, C. Allen, To heat or not to heat: challenges with clinical translation of thermosensitive liposomes, *J. Control. Release* 249 (2017) 63–73.
- [117] P.S. Yarmolenko, Y. Zhao, C. Landon, I. Spasojevic, F. Yuan, D. Needham, B.L. Viglianti, M.W. Dewhirst, Comparative effects of thermosensitive doxorubicin-containing liposomes and hyperthermia in human and murine tumours, *Int. J. Hyperth.* 26 (2010) 485–498.
- [118] A.L.B. Seynhaeve, M. Amin, D. Haemmerich, G.C. van Rhoon, T.L.M.T. Hagen, Hyperthermia and smart drug delivery systems for solid tumor therapy, *Adv. Drug Deliv. Rev.* (2020 Feb 21) <https://doi.org/10.1016/j.addr.2020.02.004> accepted, Epub ahead of print (pii:S0169-409X(20)30010-7).
- [119] M. Dunne, B. Epp-Ducharme, A.M. Sofias, M. Regenold, D.N. Dubins, C. Allen, Heat-activated drug delivery increases tumor accumulation of synergistic chemotherapies, *J. Control. Release* 308 (2019) 197–208.
- [120] M.H. Gaber, N.Z. Wu, K. Hong, S.K. Huang, M.W. Dewhirst, D. Papahadjopoulos, Thermosensitive liposomes: extravasation and release of contents in tumor microvascular networks, *Int. J. Radiat. Oncol. Biol. Phys.* 36 (1996) 1177–1187.
- [121] A.T. Lefor, S. Makohon, N.B. Ackerman, The effects of hyperthermia on vascular permeability in experimental liver metastasis, *J. Surg. Oncol.* 28 (1985) 297–300.
- [122] G. Kong, R.D. Braun, M.W. Dewhirst, Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size, *Cancer Res.* 60 (2000) 4440–4445.
- [123] G. Kong, M.W. Dewhirst, Hyperthermia and liposomes, *Int. J. Hyperth.* 15 (1999) 345–370.
- [124] A.M. Ponce, Z. Vujaskovic, F. Yuan, D. Needham, M.W. Dewhirst, Hyperthermia mediated liposomal drug delivery, *Int. J. Hyperth.* 22 (2006) 205–213.
- [125] L. Willerdling, S. Limmer, M. Hossann, A. Zengerle, K. Wachholz, T.L. Ten Hagen, G.A. Koning, R. Sroka, L.H. Lindner, M. Peller, Method of hyperthermia and tumor size influence effectiveness of doxorubicin release from thermosensitive liposomes in experimental tumors, *J. Control. Release* 222 (2016) 47–55.
- [126] A. Gasselhuber, M.R. Dreher, F. Rattay, B.J. Wood, D. Haemmerich, Comparison of conventional chemotherapy, stealth liposomes and temperature-sensitive liposomes in a mathematical model, *PLoS One* 7 (2012), e47453.
- [127] A. Gabizon, R. Catane, B. Uziely, B. Kaufman, T. Safra, R. Cohen, F. Martin, A. Huang, Y. Barenholz, Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes, *Cancer Res.* 54 (1994) 987–992.
- [128] A.M. Ponce, B.L. Viglianti, D. Yu, P.S. Yarmolenko, C.R. Michelich, J. Woo, M.B. Bally, M.W. Dewhirst, Magnetic resonance imaging of temperature-sensitive liposome release: drug dose painting and antitumor effects, *J. Natl. Cancer Inst.* 99 (2007) 53–63.
- [129] Q. Chen, A. Krol, A. Wright, D. Needham, M.W. Dewhirst, F. Yuan, Tumor microvascular permeability is a key determinant for antitumor effects of doxorubicin encapsulated in a temperature sensitive liposome, *Int. J. Hyperth.* 24 (2008) 475–482.
- [130] L. Li, T.L. ten Hagen, M. Hossann, R. Suss, G.C. van Rhoon, A.M. Eggermont, D. Haemmerich, G.A. Koning, Mild hyperthermia triggered doxorubicin release from optimized stealth thermosensitive liposomes improves intratumoral drug delivery and efficacy, *J. Control. Release* 168 (2013) 142–150.
- [131] A.A. Manzoor, L.H. Lindner, C.D. Landon, J.Y. Park, A.J. Simnick, M.R. Dreher, S. Das, G. Hanna, W. Park, A. Chilkoti, G.A. Koning, T.L. ten Hagen, D. Needham, M.W. Dewhirst, Overcoming limitations in nanoparticle drug delivery: triggered, intravascular release to improve drug penetration into tumors, *Cancer Res.* 72 (2012) 5566–5575.
- [132] L. Li, T.L. ten Hagen, D. Schipper, T.M. Wijnberg, G.C. van Rhoon, A.M. Eggermont, L.H. Lindner, G.A. Koning, Triggered content release from optimized stealth thermosensitive liposomes using mild hyperthermia, *J. Control. Release* 143 (2010) 274–279.
- [133] N. Hijnzen, S. Langereis, H. Grull, Magnetic resonance guided high-intensity focused ultrasound for image-guided temperature-induced drug delivery, *Adv. Drug Deliv. Rev.* 72 (2014) 65–81.
- [134] G. Schooneveldt, A. Bakker, E. Balidemaj, R. Chopra, J. Crezee, E.D. Geijsen, J. Hartmann, M.C. Hulshof, H.P. Kok, M.M. Paulides, A. Sousa-Escandon, P.R. Stauffer, P.F. Maccarini, Thermal dosimetry for bladder hyperthermia treatment. An overview, *Int. J. Hyperth.* 32 (2016) 417–433.
- [135] G. Schooneveldt, H.P. Kok, E. Balidemaj, E.D. Geijsen, F. van Ommen, J. Sijbrands, A. Bakker, J.J. de la Rosette, M.C. Hulshof, T.M. de Reijke, J. Crezee, Improving hyperthermia treatment planning for the pelvis by accurate fluid modeling, *Med. Phys.* 43 (2016) 5442.
- [136] P.R. Stauffer, G.C. van Rhoon, Overview of bladder heating technology: matching capabilities with clinical requirements, *Int. J. Hyperth.* 32 (2016) 407–416.
- [137] R. Helderma, D.R. Loke, H.P. Kok, A.L. Oei, P.J. Tanis, N. Franken, J. Crezee, Variation in clinical application of hyperthermic intraperitoneal chemotherapy: a review, *Cancers (Basel)* 11 (2019).
- [138] M.J. Furman, R.J. Picotte, M.J. Wante, B.R. Rajeshkumar, G.F. Whalen, L.A. Lambert, Higher flow rates improve heating during hyperthermic intraperitoneal chemoperfusion, *J. Surg. Oncol.* 110 (2014) 970–975.
- [139] H.P. Kok, P. Wust, P.R. Stauffer, F. Bardati, G.C. van Rhoon, J. Crezee, Current state of the art of regional hyperthermia treatment planning: a review, *Radiat. Oncol.* 10 (2015) 196.
- [140] M.M. Paulides, P.R. Stauffer, E. Neufeld, P.F. Maccarini, A. Kyriakou, R.A. Canters, C.J. Diederich, J.F. Bakker, G.C. Van Rhoon, Simulation techniques in hyperthermia treatment planning, *Int. J. Hyperth.* 29 (2013) 346–357.
- [141] J.J. Lagendijk, The influence of bloodflow in large vessels on the temperature distribution in hyperthermia, *Phys. Med. Biol.* 27 (1982) 17–23.

- [142] K. Sumser, E. Neufeld, R.F. Verhaart, V. Fortunati, G.M. Verduijn, T. Drizdal, T. van Walsum, J.F. Veenland, M.M. Paulides, Feasibility and relevance of discrete vasculature modeling in routine hyperthermia treatment planning, *Int. J. Hyperth.* 36 (2019) 801–811.
- [143] A.A. De Leeuw, J. Crezee, J.J. Lagendijk, Temperature and SAR measurements in deep-body hyperthermia with thermocouple thermometry, *Int. J. Hyperth.* 9 (1993) 685–697.
- [144] J.J. Lagendijk, A.A. De Leeuw, Temperature errors using multi-sensor thermocouple probes with a common constantan wire, *Int. J. Hyperth.* 9 (1993) 763–764.
- [145] A. Bakker, R. Holman, D.B. Rodrigues, H. Dobsicek Trefna, P.R. Stauffer, G. van Tienhoven, C.R.N. Rasch, H. Crezee, Analysis of clinical data to determine the minimum number of sensors required for adequate skin temperature monitoring of superficial hyperthermia treatments, *Int. J. Hyperth.* 34 (2018) 910–917.
- [146] H.P. Kok, L. Korshuize-van Straten, A. Bakker, R. de Kroon-Oldenhof, G.H. Westerveld, E. Versteijne, L.J.A. Stalpers, J. Crezee, Feasibility of on-line temperature-based hyperthermia treatment planning to improve tumour temperatures during locoregional hyperthermia, *Int. J. Hyperth.* 34 (2018) 1082–1091.
- [147] I. Rivens, A. Shaw, J. Civalé, H. Morris, Treatment monitoring and thermometry for therapeutic focused ultrasound, *Int. J. Hyperth.* 23 (2007) 121–139.
- [148] N.M. Hijnen, E. Heijman, M.O. Kohler, M. Ylihautala, G.J. Ehnholm, A.W. Simonetti, H. Grull, Tumour hyperthermia and ablation in rats using a clinical MR-HIFU system equipped with a dedicated small animal set-up, *Int. J. Hyperth.* 28 (2012) 141–155.
- [149] P. Wust, B. Hildebrandt, G. Sreenivasa, B. Rau, J. Gellermann, H. Riess, R. Felix, P.M. Schlag, Hyperthermia in combined treatment of cancer, *Lancet Oncol.* 3 (2002) 487–497.
- [150] J. Gellermann, B. Hildebrandt, R. Issels, H. Ganter, W. Włodarczyk, V. Budach, R. Felix, P.U. Tunn, P. Reichardt, P. Wust, Noninvasive magnetic resonance thermography of soft tissue sarcomas during regional hyperthermia: correlation with response and direct thermometry, *Cancer* 107 (2006) 1373–1382.
- [151] K. Kuroda, K. Oshio, A.H. Chung, K. Hynynen, F.A. Jolesz, Temperature mapping using the water proton chemical shift: a chemical shift selective phase mapping method, *Magn. Reson. Med.* 38 (1997) 845–851.
- [152] P. Wust, J. Gellermann, C. Harder, W. Tilly, B. Rau, S. Dinges, P. Schlag, V. Budach, R. Felix, Rationale for using invasive thermometry for regional hyperthermia of pelvic tumors, *Int. J. Radiat. Oncol. Biol. Phys.* 41 (1998) 1129–1137.
- [153] G.C. van Rhoon, P. Wust, Introduction: non-invasive thermometry for radiotherapy, *Int. J. Hyperth.* 21 (2005) 489–495.
- [154] F. Adibzadeh, K. Sumser, S. Curto, D.T.B. Yeo, A.A. Shishegar, M.M. Paulides, Systematic review of pre-clinical and clinical devices for magnetic resonance-guided radiofrequency hyperthermia, *Int. J. Hyperth.* 37 (2020) 15–27.
- [155] D. Haemmerich, A. Motammar, Thermosensitive liposomes for image-guided drug delivery, *Adv. Cancer Res.* 139 (2018) 121–146.
- [156] Rudiger Wessalowski, Dominik Schneider, Oliver Mills, Verena Friemann, Olga Kyrilopoulou, Jorg Schaper, Christiane Matuschek, Karin Rothe, Ivo Leuschner, Reinhart Willert, Stefan Schonberger, Ulrich Gobel, Gabriele Calaminus, for the MAKEI study group, Regional deep hyperthermia for salvage treatment of children and adolescents with refractory or recurrent non-testicular malignant germ-cell tumours: an open-label, non-randomised, single-institution, phase 2 study, *Lancet Oncology* 14 (2013) 843–852, [https://doi.org/10.1016/S1470-2045\(13\)70271-793](https://doi.org/10.1016/S1470-2045(13)70271-793).