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### Improving superficial hyperthermia treatment

*Temperature matters*

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# CHAPTER 3

## POST-OPERATIVE RE-IRRADIATION WITH HYPERTHERMIA IN LOCOREGIONAL BREAST CANCER RECURRENCE: TEMPERATURE MATTERS

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## ABSTRACT

**Purpose:** To investigate the impact of hyperthermia dose on locoregional (LR) control, overall survival (OS) and toxicity in patients with LR recurrent breast cancer treated with post-operative re-irradiation (reRT) and hyperthermia (HT).

**Methods:** A single-center observational cohort study of 112 women with resected LR recurrent breast cancer was performed. Patients were treated with post-operative reRT 8 x 4 Gy (n = 34) or 23 x 2 Gy (n = 78), combined with 4 - 5 weekly HT sessions guided by invasive thermometry, and divided in 'low' (n = 56) and 'high' thermal dose (n = 56) groups by the best session with highest median cumulative equivalent minutes at 43 °C (Best CEM43 T50) <7.2 min and ≥7.2 min, respectively. Actuarial LR control, OS and late toxicity incidence were analyzed. Backward multivariable Cox regression analysis was performed to identify associations between patient and treatment characteristics and clinical outcome.

**Results:** Median follow-up period was 43 months (range 1 - 107 months). Thermal dose was associated with LR control ( $p = 0.0013$ ), but not with OS ( $p = 0.29$ ) or late toxicity ( $p = 0.74$ ). Three-year LR control was 74.0 % vs. 92.3 % in the low and high thermal dose group, respectively ( $p = 0.008$ ). After three years, 25.5 % of the patients had late toxicity grade 3 - 4. Multivariable analysis showed that presence of distant metastasis (HR 17.6; 95 % CI 5.2 - 60.2), lymph node involvement (HR 2.9; 95 % CI 1.2 - 7.2), site of recurrence (chest wall vs. breast; HR 4.6; 95 % CI 1.8 - 11.6) and thermal dose (low vs. high; HR 4.1; 95 % CI 1.4 - 11.5) were associated with LR control.

**Conclusions:** Patients with resected LR recurrent breast cancer receiving state-of-the-art post-operative reRT combined with high dose HT had 18 % higher LR control than patients receiving low dose HT, without augmenting treatment-associated toxicity.

## INTRODUCTION

Advances in diagnostic imaging and treatment have improved locoregional control and survival of patients with breast cancer [1, 2]. The growing number of long-term breast cancer survivors may lead to an increased cumulative incidence of locoregional (LR) recurrence or second ipsilateral primary breast cancer [3]. In the Netherlands, the 10-year risk of local and regional recurrence after treatment of primary non-metastatic breast cancer is 4.3 % and 2.6 %, respectively [4]. The optimal management in LR recurrent breast cancer is dependent on the heterogeneity of prognostic factors and previous treatments of patients, and consequently requires multidisciplinary assessment and treatment to achieve durable LR control and prolong disease-free survival [1, 3]. Only few prospective clinical trials investigated the optimal treatment for patients with LR recurrent or second primary breast cancer after prior radiotherapy [1, 4]. Based on studies conducted in the 1990s, the 5-year LR control and overall survival rates for ipsilateral LR recurrent breast cancer are 60 - 70 % and 40 - 65 %, respectively [5-7]. Re-irradiation combined with hyperthermia (reRT-HT) can be considered for patients with an (isolated) LR recurrence or second ipsilateral primary breast cancer [1, 3]. HT involves elevation of tumor temperature to 40 - 44 °C for approximately one hour and is a proven radiosensitizer [8]. Most randomized and non-randomized studies performed in the 1980s and 1990s exploring (re)RT-HT involved patients with unresectable LR recurrence. A meta-analysis of these studies showed better complete response rates for patients treated with (re)RT-HT (n = 1792) than for those receiving RT alone (n = 318); 62 % versus 38 %, respectively [8]. Evidence regarding clinical outcome in patients with resected LR recurrence treated with post-operative reRT-HT is limited to five single-arm observational studies (n = 445) with inhomogeneous patient populations and inconsistent toxicity reporting [9-13]. These studies suggest that post-operative reRT-HT offers good 3-year LR control (68 - 83 %) compared to surgery alone. Based on these studies reRT-HT is reimbursed for resected LR recurrent or second ipsilateral primary breast cancer in regions where HT is available, such as the Netherlands. However, strong clinical evidence is lacking.

Establishing the HT dose-effect relationship may help assessing the effectiveness of adding HT to reRT in patients with LR recurrent breast cancer treated with post-operative reRT-HT. Unfortunately, the delivered HT dose was poorly monitored and documented in many of the aforementioned breast cancer studies [8, 11-14]. This is a major problem as the effects of HT such as direct cell death [15-18], inhibition of DNA damage repair [19-22], tumor re-oxygenation [23-26] and stimulation of the immune response [27, 28] are temperature dependent. Clinical evidence for a thermal dose-effect relationship has been demonstrated in patients treated with RT-HT for primary locally advanced cervical cancer [29] and in patients with unresectable LR recurrent breast cancer [14], where higher

intratumoral temperature was associated with improved complete response rate and LR control. However, the temperature is often poorly recorded in HT delivery for breast cancer, and generally limited to superficial skin temperatures. This is a problem as the skin temperatures are to a lesser extent associated with clinical outcome than invasively measured temperatures [14].

In the present observational study, we investigated the influence of invasively measured HT temperature levels on locoregional (LR) control, overall survival (OS) and toxicity in a cohort of patients with resected LR recurrent or second ipsilateral primary breast cancer treated with post-operative reRT-HT. All patients received surgery with intent of tumor-free margins and all had invasive thermometry during HT allowing thermal dose analysis.

## METHODS

One-hundred-and-twelve patients with surgically removed locoregional recurrent or second ipsilateral primary breast cancer with the intention to achieve tumor-free margins, were included in this observational study at the Amsterdam UMC, location AMC. Surgery was performed in different hospitals. Patients were treated with post-operative re-irradiation combined with superficial HT guided with invasive thermometry between 2010 and 2017. We conducted the study in accordance with the Declaration of Helsinki. Due to the large cohort and anonymous inclusion of patients in the database, individual informed consent was not deemed necessary, waived by the local Ethics Committee on Nov 9, 2019; W19\_425 # 19.492.

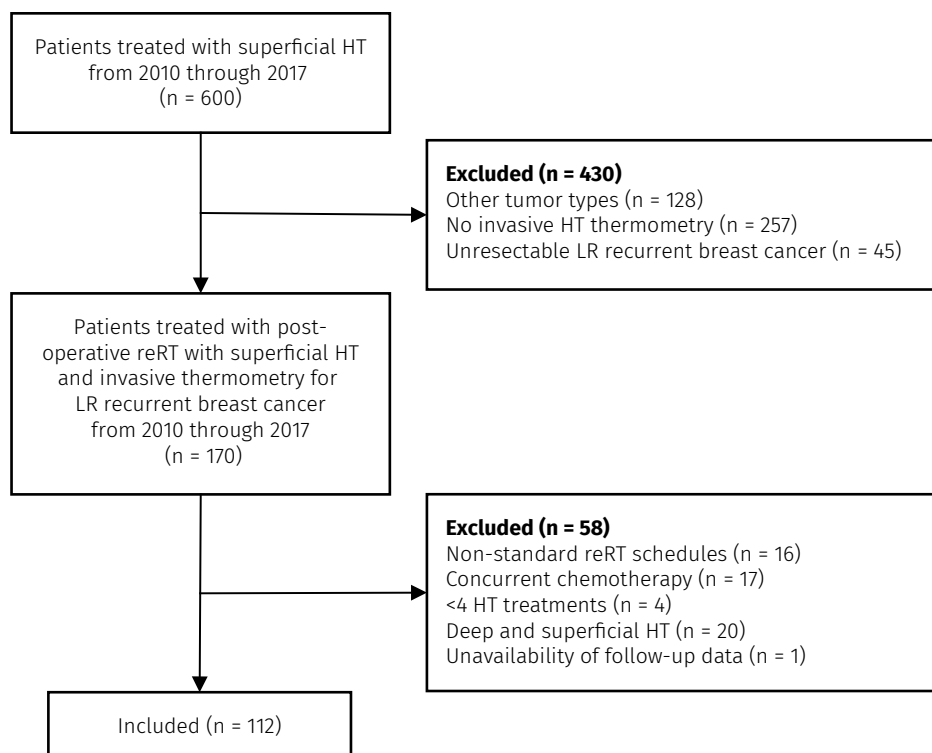
In total, 600 patients were treated with superficial HT from 2010 through 2017 (Figure 1). Exclusion criteria were other tumor types (n = 128), absence of invasive HT thermometry (n = 257), unresectable LR recurrent breast cancer (n = 45), non-standard reRT schedules (n = 16), concurrent chemotherapy (n = 17), < 4 HT treatment sessions (n = 4), patients treated with both deep and superficial HT (n = 20) or no follow-up data available (n = 1). Six patients received reRT-HT treatment twice in the period 2010 - 2017; of these patients the first reRT-HT treatment series where invasive temperature was measured was included.

### Data collection

Available data were collected from the RT and HT patient charts by one investigator (PTV). Follow-up during reRT-HT consisted of weekly consultation by the treating radiation oncologist. Follow-up after reRT-HT consisted of a telephone consultation after two weeks after the last reRT fraction, followed by a physical consultation after four to eight weeks. Thereafter, patients either had regular follow-up consults at the Amsterdam UMC or in their referring hospital. In the event of incomplete follow-up data in the patient charts at the Amsterdam UMC, a request for missing data was sent to referring specialists and general practitioners.

### Treatment

The present locoregional recurrence was surgically removed in all 112 patients, by mastectomy (n = 70), local excision (n = 41) after previous mastectomy, or local excision after previous breast conserving treatment (n = 1). Most patients previously received one or more lines of systemic therapy (n = 95), either as adjuvant treatment for their primary breast cancer (n = 61), or as treatment for previous recurrent disease (n = 32) or distant metastasis (n = 2). In total, 71 patients received neo-adjuvant or adjuvant systemic treatment for the present recurrence.



**Figure 1.** Study flowchart. *Abbreviations:* HT= hyperthermia; LR= locoregional; reRT= re-irradiation.

### Radiotherapy

All patients received post-operative radiotherapy (median dose 64.0 Gy; range 42.6 - 73.8 Gy) for their initial breast cancer (n = 105) and/or a previous recurrence (n = 15). Eight patients received reRT, either local (n = 7) or regional (n = 1) for a previous locoregional recurrence. The overlap between reRT and re-reRT fields was minimized in these patients.

Radiotherapy treatment of the present locoregional recurrence or second primary ipsilateral breast cancer consisted of reRT to a total dose of 32 Gy in 8 fractions of 4 Gy (n = 34) or 46 Gy in fractions of 2 Gy (n = 78) combined with HT. From 2010 through 2014, 8 fractions of 4 Gy were given twice a week [12], thereafter 23 daily fractions of 2 Gy were given five times a week. An exception was made in five patients for whom the 8 x 4 Gy schedule was chosen due to frailty or long travel distance. ReRT-planning was performed using three different techniques. From 2010 to halfway through 2014 the lateral chest wall and/or regional lymph nodes areas were irradiated using two opposing anterior-posterior photon fields (AP-PA) and the anterior chest wall with electrons, the breast was treated

with two tangential fields. From mid-2014 onward intensity-modulated radiation therapy (IMRT) was applied using 5 - 7 beam angles, and from early 2016 volumetric-modulated arc therapy (VMAT) was implemented using two (counter)clockwise partial arcs.

One-hundred-and-ten patients finished the combined treatment according to protocol. Two patients did not finish the scheduled 23 x 2 Gy RT treatment due to personal reasons and received 22 fractions. In general, the chest wall alone (n = 71), chest wall with regional lymph nodes (n = 40) or breast with regional lymph nodes (n = 1) were target volumes. Besides the present local re-irradiated site, patients were also re-irradiated on the axillary lymph node regions alone (n = 15), periclavicular +/- axillary lymph node regions (n = 14) and/or the internal mammary +/- periclavicular +/- axillary lymph nodes regions (n = 12). Seventeen patients were irradiated on contralateral lymph node regions. Sixteen patients received a sequential RT boost on the lymph node areas that were not previously irradiated using 2 fractions of 4 Gy or 2 fractions of 2 Gy, to a total dose of 40 Gy in 10 fractions or 50 Gy in 25 fractions, respectively. Two patients received a simultaneous boost to a total dose of 61.18 Gy in 23 fractions on the lymph node areas that had or was suspect for a macroscopic lymph node metastasis that was not previously irradiated.

### *Hyperthermia*

ReRT was combined with a weekly superficial HT session of the previously irradiated target volume within one hour after reRT. HT treatment aimed at elevating invasive temperatures to a minimum of 41 °C for one hour while maintaining maximum normal tissue (skin) temperatures below 44 °C. Conformal contact (flexible) microstrip microwave applicators (Istok, Fryazino, Russia; Medlogix, Rome, Italy) operating at 434 MHz were used that can heat up to a depth of 4 cm from the skin [30]. A water bag containing temperature-controlled circulating deionized water (39 - 42 °C) was positioned between the applicator and skin [30–32]. Depending on the depth of the target area, the water bag temperature settings and applicator power setting of the applicator were adjusted according to protocol to achieve the desired therapeutic invasive and skin surface temperatures and penetration depth [33, 34].

Extensive temperature monitoring was performed during treatment, exceeding present HT quality assurance (QA) guidelines [33]. Temperature monitoring was performed with multisensory seven-sensor copper-constantan thermocouple probes (Volnec RD Inc., Hradec Králové, Czech Republic) placed invasively ( $8 \pm 5$  sensors) to improve monitoring of the thermal dose in the target area and on the skin surface ( $80 \pm 30$  sensors) to prevent temperature hotspots that might potentially result in thermal toxicity [35]. Temperature measurements were performed every 30 seconds [35]. Catheters for invasive temperature monitoring were routinely placed when the subcutaneous tissue of the target area had a thickness  $\geq 1$  cm.

For each patient, we calculated invasive temperature and skin surface temperature and thermal dose variables of the target area (see supplementary materials for details). The thermal dose parameter generally used to quantify the overall HT dose is the median cumulative equivalent minutes at 43 °C (CEM43 T50), which incorporates both treatment duration and temperature [36, 37]. In 29 patients the invasive temperature was not registered during all HT treatment sessions because the catheter was removed prematurely (n = 25), placed after the first HT session (n = 2) or there was an error in registering invasive temperature during one of the HT sessions (n = 2). Since invasive measurements were not performed in all sessions in 26 % of the included patients, we divided the population into equal-sized ‘low’ and ‘high’ thermal dose groups by the best HT session, i.e. the session that had the highest CEM43 T50. Best CEM43 T50 was < 7.2 min and  $\geq$  7.2 min for the low and high thermal dose group, respectively.

Table 1 summarizes the patient characteristics and Table 2 summarizes the treatment characteristics stratified by low and high thermal dose.

### **Study endpoints**

Actuarial LR control was calculated from the date of the first reRT fraction until the first infield local and/or regional recurrence. Patients without infield LR recurrence at death or last follow-up were censored. Actuarial OS was calculated from the date of the first reRT fraction until death. Death of any cause was an event. Patients alive at last follow-up were censored.

Toxicity was defined according to the Common Terminology Criteria of Adverse Events (CTCAE) version 5.0 [38]. Toxicity was considered acute when occurring within three months after the first reRT fraction and late when it occurred more than three months after the first reRT fraction. Actuarial late toxicity was calculated from the date of the first reRT fraction until the first grade 3 - 5 late toxicity. Patients without grade 3 - 5 late toxicity at death or last follow-up were censored. Acute and late toxicity grade 1 - 5 is also presented in tables.

### **Statistical analysis**

Differences between the two thermal dose groups in terms of patient-, tumor- and treatment characteristics were tested using Fisher’s exact test, the independent samples t-test and the Mann-Whitney U test depending on the type of data.

The following HT treatment variables were used: Since multiple reRT-HT sessions were delivered, the treatment of an individual patient cannot be characterized by a single thermal dose or time interval between reRT-HT. For each patient, the median time interval between the end of reRT fraction and start steady state of the four or five HT fractions was calculated. Pre-heating time was defined as the time from power on to start steady state of the HT treatment.

Duration of LR control, survival and late toxicity were analyzed by the actuarial method of Kaplan and Meier [39]. Groups were compared by the log-rank test.

Multivariable analysis of LR control, OS and late toxicity was performed by (backwards) stepwise Cox regression. In the multivariable analysis, we started with nine factors that were closest to  $p = 0$  in univariate analysis (see Supplementary Table 2); where nQuery Advanced (version 8.0) was used to determine the number of factors based on a sample size of 112 patients. Associations between patient, tumor and treatment characteristics and LR control, OS and late toxicity were investigated. Fisher's exact test was used to test for differences in the incidence of acute or late toxicity between thermal dose groups. Furthermore, multicollinearity was detected using the variance inflation factor and we tested for interactions between variables.

All analyses were performed using R (version 3.6.3) with packages survival (version 3.2-7) and survminer (version 0.4.8), the tests were two-sided and  $p < 0.05$  was considered significant. Accuracy of statistical estimates is reported using 95 % Wald confidence intervals.

**Table 1.** Tumor and patient characteristics, stratified by low and high thermal dose (TD).

Best CEM43 T50 (minutes)	
Age (years)	
<b>Initial breast cancer</b>	
Pathological tumor stage <sup>a</sup>	(y)pT0-T2 (y)pT3-T4
Pathological lymph nodes stage <sup>a</sup>	(y)pN0 (y)pN+
<b>Present recurrent breast cancer</b>	
Time interval initial diagnosis – present recurrence (years)	
Time interval previous breast cancer – present recurrence (years) <sup>b</sup>	
Pathological tumor stage	(y)pT0 (y)pT1-T2 (y)pT3-T4
Pathological lymph nodes stage	(y)pN0 (y)pN+
Contralateral lymph nodes	
Distant metastasis <sup>c</sup>	
Lymphovascular invasion	
Histological type	Invasive carcinoma NST ILC DCIS Other
BR differentiation grade <sup>d</sup>	Well-differentiated (G1) Moderately differentiated (G2) Poorly differentiated (G3)
Estrogen receptor +	
Her2neu + <sup>a</sup>	
Triple negative	

Values are depicted with mean ± standard deviation, median (range) or the number of patients (%).

a. Data missing for 1 patient in low thermal dose group; b. previous breast cancer= initial diagnosis or LR recurrence or second ipsilateral primary breast cancer; c. contralateral lymph nodes are not counted as distant metastasis; d. data missing for 3 patients in the low thermal dose group and 1 patient in the high thermal dose group.

Low TD (n = 56)	High TD (n = 56)	p
3.4 (0.1 - 7.1)	15.9 (7.4 - 101.9)	<0.001
63.2 ± 12.8	64.1 ± 8.8	n.s.
52 (94.6 %)	55 (98.2 %)	n.s.
3 (5.4 %)	1 (1.8 %)	
33 (59.8 %)	37 (66.1 %)	n.s.
22 (40.2 %)	19 (33.9 %)	
10.1 (0.1 - 27.5)	9.8 (2.1 - 28.8)	n.s.
8.3 (0.1 - 27.0)	7.4 (1.4 - 28.8)	n.s.
0 (0 %)	0 (0 %)	n.s.
42 (75.0 %)	44 (78.5 %)	
14 (25.0 %)	12 (21.5 %)	
37 (66.1 %)	47 (83.9 %)	n.s.
19 (33.9 %)	9 (16.1 %)	
13 (23.3 %)	7 (12.5 %)	n.s.
5 (8.9 %)	2 (3.6 %)	n.s.
31 (55.4 %)	22 (39.3 %)	n.s.
46 (82.1 %)	39 (69.6 %)	n.s.
8 (14.3 %)	15 (26.8 %)	
1 (1.8 %)	2 (3.6 %)	
1 (1.8 %)	0 (0 %)	
2 (3.8 %)	4 (7.3 %)	n.s.
26 (49.1 %)	30 (54.5 %)	
25 (47.2 %)	21 (38.2 %)	
37 (66.1 %)	41 (73.2 %)	n.s.
8 (14.5 %)	7 (12.5 %)	n.s.
15 (26.8 %)	13 (23.2 %)	n.s.

*Abbreviations:* TD= thermal dose; Best CEM43 T50= the median invasive thermal dose of the best session; NST= No Special Type; ILC= Invasive Lobular Cancer; DCIS= Ductal Carcinoma In Situ; BR= Bloom Richardson; G=grade; n.s.= not significant.

**Table 2.** Treatment characteristics of the included patients, stratified by low and high thermal dose (TD).

<b>Previous treatment</b>	
Previous locoregional recurrences	1 2
Chemotherapy	
Endocrine therapy	
HER2-targeted therapy	
Median initial total RT dose (Gy; incl. boost) <sup>a</sup>	
<b>Present treatment</b>	
Surgery	Breast conservation Mastectomy Local resection
Chemotherapy	
Endocrine therapy	
HER2-targeted therapy	
RT scheme	8 x 4 Gy 23 x 2 Gy
RT boost	Sequential (2 x 4 Gy) Sequential (2 x 2 Gy) Simultaneous (23 x 0.66 Gy)
RT target	Local Locoregional
Median time interval reRT-HT (min)	
Hyperthermia treatments	4 5
Invasive temperature	T10 (°C) T50 (°C) T90 (°C) Average CEM43 T0 (min) Average CEM43 T50 (min) Average CEM43 T100 (min)
Skin temperature	Highest T0 (°C) T10 (°C) T50 (°C) T90 (°C)

Values are depicted with mean  $\pm$  standard deviation, median (range) or the number of patients (%).

a. data missing for 4 patients in each group.

*Abbreviations:* TD= thermal dose; RT= radiotherapy; T10, T50, T90= The temperature exceeded by

Low TD (n = 56)	High TD (n = 56)	p
20 (35.7 %)	17 (30.4 %)	n.s.
0 (0.0 %)	2 (3.6 %)	
18 (32.1 %)	17 (30.4 %)	n.s.
16 (28.6 %)	11 (19.6 %)	n.s.
3 (5.4 %)	3 (5.4 %)	n.s.
64.0 (42.6 - 73.4)	64.0 (42.6 - 73.8)	n.s.
0 (0.0 %)	1 (1.8 %)	n.s.
31 (55.4 %)	39 (69.6 %)	
25 (44.6 %)	16 (28.6 %)	
30 (53.6 %)	25 (44.7 %)	n.s.
31 (55.4 %)	35 (62.5 %)	n.s.
6 (10.7 %)	3 (5.4 %)	n.s.
17 (30.4 %)	17 (30.4 %)	n.s.
39 (69.6 %)	39 (69.6 %)	
1 (1.8 %)	3 (5.4 %)	n.s.
9 (16.1 %)	3 (5.4 %)	
0 (0.0 %)	2 (3.4 %)	
33	38	n.s.
23	18	
60.1 ± 13.1	62.9 ± 15.3	n.s.
19 (33.9 %)	19 (33.9 %)	n.s.
37 (66.1 %)	37 (66.1 %)	
41.2 ± 0.9	42.2 ± 0.7	<0.001
40.0 ± 0.8	41.2 ± 0.6	<0.001
39.0 ± 0.9	40.1 ± 0.7	<0.001
9.4 (0.1 - 86.6)	26.7 (3.0 - 144.0)	<0.001
1.7 (0.0 - 6.6)	9.0 (2.3 - 49.2)	<0.001
0.4 (0.0 - 2.8)	2.5 (0.1 - 24.1)	<0.001
44.0 ± 0.8	43.8 ± 1.0	n.s.
41.8 ± 0.4	41.9 ± 0.3	n.s.
40.5 ± 0.4	40.7 ± 0.4	n.s.
39.3 ± 0.5	39.5 ± 0.5	n.s.

10 %, 50 % or 90 % of the measurements, respectively; average CEM43 T0, average CEM43 T50, average CEM43 T100= the average of the maximum, median and minimum thermal dose of all sessions, respectively; n.s.= not significant.

## RESULTS

In this cohort of 112 patients treated with postoperative reRT plus superficial HT, the median follow-up period was 43 months (range 1 - 107 months). There were no significant differences in patient and treatment characteristics between the low and high thermal dose groups (Table 1, 2).

### Locoregional control and overall survival

Twenty-four patients developed an in-field recurrence (21.4 %). The 3-year actuarial LR control rate was 83.2 %. LR control was significantly different for the low and high thermal dose group ( $p = 0.0013$ ; Figure 2A). Three-year LR control rates for the low and high thermal dose group were 74.0 % vs. 92.3 %, respectively ( $p = 0.008$ ). Associations of other thermal dose parameters with LR control are available in the supplementary materials. For patients treated with 23 x 2 Gy ( $n = 78$ ), three-year LR control rates for the low and high thermal dose group were 97.3 % and 81.6 %, respectively ( $p = 0.013$ ).

Twenty-five patients died resulting in a 3-year overall survival of 85.4 %. Overall survival was not significantly different for the low and high thermal dose group ( $p = 0.29$ ; Figure 2B). Six out of 25 patients died of causes other than breast cancer and two of unknown cause.

Several prognostic factors were evaluated for an association with LR control and OS both in univariate (Supplementary Table 2) and in multivariable analysis (Table 3). In univariate analysis LR control was significantly associated with the time interval from the previous tumor (either primary tumor or previous recurrence) to the present recurrence, present recurrence site (chest wall/ breast), presence of lymph node metastases or distant metastases and the achieved thermal dose (Best CEM43 T50). In the backward multivariable analysis four factors remained associated with LR control (Table 3). The presence of distant metastases (HR 17.6; 95 % CI 5.2 - 60.2) and lymph node involvement (HR 2.9; 95 % CI 1.2 - 7.2) and chest wall recurrence (as opposed to breast recurrence) (HR 4.6; 95 % CI 1.8 - 11.6) impaired LR control. A higher thermal dose (Best CEM43 T50) improved LR control (HR 4.1; 95 % CI 1.4 - 11.5).

For overall survival, nine tumor related variables were significantly associated in univariate analysis (Supplementary Table 2), i.e. the time interval between the initial diagnosis and the present recurrence, the time interval between previous breast cancer (either primary tumor or previous recurrence) and the present recurrence, the primary pathological tumor stage, triple-negative breast cancer, the tumor size, absence of contralateral breast cancer growth, positive lymph node status, distant metastasis and positive estrogen receptor. Three factors remained associated with OS in backward multivariable analysis. Patients

with smaller recurrences ( $\leq 5$  cm; HR 0.3; 95 % CI 0.1 - 0.8), patients with absence of contralateral breast cancer growth (HR 3.4; 95 % CI 1.3 - 8.6) and patients with positive estrogen receptor (HR 0.2; 95 % CI 0.1 - 0.5) had longer OS.

**Table 3.** Prognostic factors for locoregional control and overall survival after backwards stepwise multivariable Cox regression.

	HR (95 % CI)	p
<b>Locoregional control</b>		
Distant metastases (yes/ no) <sup>a</sup>	17.6 (5.2 – 60.2)	<0.001
Location (chest wall/ breast)	4.6 (1.8 – 11.6)	0.001
Best CEM43 T50 (low/ high)	4.1 (1.4 – 11.5)	0.009
Tumor positive lymph nodes (yes/ no)	2.9 (1.2 – 7.2)	0.019
<b>Overall survival</b>		
Estrogen receptor (+/ -)	0.2 (0.1 – 0.5)	<0.001
Contralateral breast cancer growth (yes/ no)	3.4 (1.3 – 8.6)	0.011
Tumor size ( $\leq 5$ cm/ $> 5$ cm)	0.3 (0.1 – 0.8)	0.016

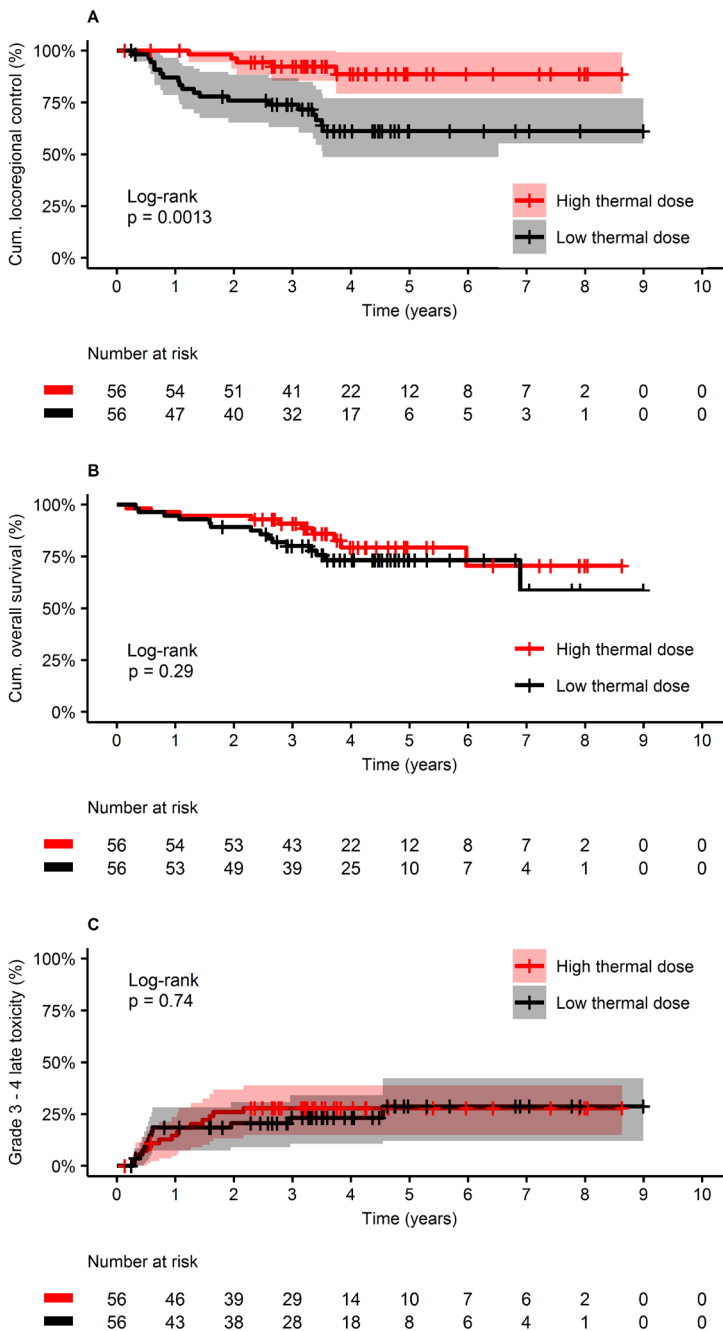
a. Contralateral lymph nodes are not counted as distant metastasis.

Abbreviations: HR= Hazard ratio; CI= 95 % Wald confidence interval; Best CEM43 T50= the best median invasive thermal dose of all hyperthermia sessions.

## Toxicity

There was no significant difference in acute toxicities between the thermal dose groups ( $p = 0.24$ ; Supplementary Table 3). During reRT-HT treatment 14.3 % of the patients experienced mild symptoms, varying from pain to discomfort (grade 1). Transient skin ulceration occurred in 8 patients and was a consequence of radiation dermatitis. Twenty patients (17.9 %) developed burns due to HT treatment (grade 1 - 2).

Late toxicity grade 2, 3 and 4 after reRT-HT was observed in 56.3 % ( $n = 63$ ), 25.0 % ( $n = 28$ ) and 0.9 % ( $n = 1$ ) of the patients, respectively, no grade 5 toxicity was reported. The actuarial risk of grade 3 and 4 late toxicity after one and three year was 16.5 % and 25.5 %, respectively. There was no difference in grade 3 - 4 late toxicity between the low and high thermal dose group ( $p = 0.74$ ; Figure 2C). Six months after treatment, one grade 4 skin ulceration occurred in the high thermal dose group (Table 4). The most frequently reported grade  $\geq 3$  late toxicity was fibrosis. One patient developed a grade 3 burn as a result of a temperature hotspot (44.3 °C) on the mastectomy scar during the first HT treatment. The reRT dose fractionation schedule only was associated with late toxicity grade 3 - 4 in univariate ( $p = 0.036$ ) and backward multivariable analyses (HR 3.1; 95 % CI 1.1 - 9.2), where patients treated with 23 x 2 Gy had more late toxicity grade 3 - 4 compared to 8 x 4 Gy.



**Figure 2.** Kaplan-Meier survival analysis for A) locoregional control, B) overall survival and C) grade 3 - 4 late toxicity for the high thermal dose group (red) and the low thermal dose group (black). Shaded area represents the 95 % confidence intervals.

**Table 4.** Number and type of late toxicities according to CTCAE v5.0, stratified by thermal dose group. Patients can have multiple late toxicities, 59.8 % of the patients (n = 67) had more than one type of late toxicity.

CTC-AE score	Low thermal dose (n = 56)		High thermal dose (n = 56)	
	Toxicity	n (%)	Toxicity	n (%)
1-2	Lymphedema	20 (35.7 %)	Lymphedema	19 (33.9 %)
	Chest(wall) pain	13 (23.2 %)	Chest(wall) pain	17 (30.4 %)
	Fibrosis	13 (23.2 %)	Fibrosis	17 (30.4 %)
	Telangiectasia	12 (21.4 %)	Telangiectasia	15 (26.8 %)
	Rib fracture	9 (16.1 %)	Rib fracture	17 (30.4 %)
	Hyperpigmentation	7 (12.5 %)	Hyperpigmentation	8 (14.3 %)
	Joint range of motion decreased	10 (17.9 %)	Joint range of motion decreased	4 (7.1 %)
	Pneumonitis	3 (5.4 %)	Pneumonitis	2 (3.6 %)
	Skin ulceration	2 (3.6 %)	Skin ulceration	3 (5.4 %)
	Burn	3 (5.4 %)	Burn	1 (1.8 %)
	Brachial plexopathy	1 (1.8 %)	Brachial plexopathy	1 (1.8 %)
	Arrhythmia	1 (1.8 %)		
	3	Fibrosis	13 (23.2 %)	Fibrosis
			Chest wall pain	3 (5.4 %)
			Burn consequences	1 (1.8 %)
			Rib fracture	1 (1.8 %)
4	-		Skin ulceration	1 (1.8 %)
5	-		-	

## DISCUSSION

This paper presents the results of post-operative re-irradiation and superficial HT of 112 patients with surgically removed locoregional recurrent breast cancer or second ipsilateral primary breast cancer treated between 2010 through 2017 with emphasis on the effect of the achieved thermal dose on the outcome. The 3-year actuarial LR control rate was 83.2 % and 3-year OS was 85.4 %. Grade 3 late toxicity consisted predominantly of fibrosis of the chest wall ( $n = 25$ ), actuarial 3-year late grade 3 and 4 toxicity rate was 24.6 % and 0.9 %, respectively.

Patients with high thermal dose had significantly better LR control than patients with lower thermal dose ( $p = 0.0013$ ). No significant effect of thermal dose on overall survival or toxicity was observed. Three-year LR control rates for the low and high thermal dose group were 74.0 % vs. 92.3 %, respectively. Similar thermal dose-effect relationships were found in univariate or multivariable analysis for locally advanced cervical cancer [29, 40] and for patients with unresectable LR recurrent breast cancer [14]. The dose-effect relationship found in this study indirectly suggests that post-operative reRT-HT is effective after surgery for locoregional recurrent breast cancer, and that HT is of additional value to post-operative RT. Randomized studies are warranted to confirm these results. This observational study found a strong HT dose-effect relationship in patients with surgically removed LR recurrent breast cancer, which underlines the need to measure the temperature invasively (when possible) during HT treatment and the necessity to achieve high thermal dose.

Adequate thermal dose during superficial HT was our goal in all patients. International quality assurance guidelines for superficial HT advise a T90 of 40 °C for 60 min and a T50 exceeding 41 °C for one hour [33], which corresponds well with the invasive T90 and T50 found in the high thermal dose group in our study;  $40.0 \pm 0.7$  °C and  $41.2 \pm 0.6$  °C, respectively. However, the T90 and T50 in the low thermal dose group were approximately 1 °C lower. The importance of achieving a T50  $\geq 41$  °C is in accordance with preclinical studies which found strong dose-effect relationships for several biological effects of heating [18, 20], including inhibition of DNA-damage repair [19, 21, 22] and direct cell kill of hypoxic tumor cells [20], mechanisms likely to be effective for T50 > 41 °C and a < 60 min reRT-HT time interval [40, 41].

In approximately 50 % of the patients in our study the targeted invasive temperature T50 of 41 °C could not be reached due to treatment-limiting hotspots on the skin; the low thermal dose group. The occurrence and severity of hotspots is largely determined by local anatomical features, such as scar tissue, that can result in reduced local perfusion, which can lead to local hot spots. All patients had scars from previous surgeries, but only scars or similar anatomical features in the low dose group led to treatment-limiting hotspots.

Currently, studies are developed that focus on achieving a higher invasive thermal dose by introducing technical solutions to suppress occurrence of treatment-limiting hotspots. The difference in impact of hot spot incidence in the low and high dose group could possibly indicate a form of patient selection. However, patient and treatment related factors were well balanced between the low and high thermal dose groups (Table 1, 2). Thermal dose was an independent prognostic variable in multivariable analysis, which included other prognostic factors for LR control (Table 3). Thus insufficient thermal dose appears to be the sole explanation for worse tumor response in the low thermal dose group.

Both the 83.2 % 3-year LR control rate found in our study for all patients and the 3-year LR control rate for the low thermal dose group (74.0 %) were comparable to the 3-year LR control rates between 68 - 83 % reported in five observational studies investigating clinical outcome after reRT-HT in patients with surgically removed LR recurrent breast cancer [9–13]. Kapp et al., analyzing results of 262 treatment fields in 89 patients, reported that in multivariable analysis patients treated with a higher minimum invasive temperature had slightly better LR control [9]. Unfortunately, the other four studies [10–13] either did not take the achieved invasive thermal dose into account or had insufficient invasive temperature data during HT treatment, preventing analysis of the prognostic value of thermal dose.

The 3-year OS rate found in our study was higher compared to OS rates reported by others, 85.4 % vs. 66 - 75 % [10, 12], respectively. This difference likely reflects the continuous improvements in earlier breast cancer diagnosis and treatment over time, including increased use of diagnostic breast MRI and PET-CT, more effective systemic treatment and improved RT techniques. To illustrate the latter, in this study patients treated from 2015 through 2017 (n = 78) with state-of-the-art post-operative reRT (23 x 2 Gy) and HT achieved excellent LR control. However, also in this subgroup, locoregional control was 16 % higher for patients treated with a high thermal dose vs. low thermal dose ( $p = 0.013$ ). This suggests that post-operative reRT-HT after surgical resection of locoregional recurrent breast cancer remains valuable in combination with modern systemic therapy, surgery and RT treatment.

Besides thermal dose, all prognostic factors associated with LR control or OS found in multivariable analysis in the present study are in agreement with results from previous reports [1, 9, 12, 42, 43]. Estrogen receptor positivity reflects a favorable biology and distant metastases, tumor positive lymph nodes, larger LR recurrence size and contralateral breast cancer growth reflect a higher disease burden and thus a poorer prognosis [1, 9, 12, 42, 43]. For patients with an isolated LR recurrence, often a more aggressive strategy is used to aim for cure [1]. The poor prognosis for a LR recurrence on the chest wall compared to a local recurrence in the breast is in line with the findings of Buchholz et al. [1].

Our observational study has some limitations. Due to its retrospective nature the occurrence of a locoregional recurrence, survival and late toxicity might be underreported or reported at a later date. In addition, invasive temperature monitoring was not possible during all HT sessions in all patients. Since the baseline toxicity before reRT-HT was not consistently evaluated, it was not possible to discriminate between late toxicity already induced by previous breast cancer treatments, or induced by the present treatment. Nevertheless, we expect that increased late toxicity from double treatments may influence the quality of life of breast cancer survivors.

The reported late grade 3 and 4 toxicity in this study reflects the cumulative effect of previous and present treatments [44]. Late grade 3 and 4 toxicity rates after post-operative reRT-HT in this study were 26.8 % and 0.9 %, respectively. This is lower than the 43 % late grade 3 - 4 toxicity rate reported by Oldenborg et al. in a similar cohort of breast cancer patients treated with post-operative reRT-HT [12].

## CONCLUSION

Post-operative re-irradiation and hyperthermia in locoregional recurrent breast cancer showed a 3-year locoregional control rate of 83 %, 3-year overall survival of 85 % and 3-year late toxicity grade 3 and 4 of 25.5 %. Patients who were re-irradiated combined with a high thermal dose had 18 % higher locoregional control without additional toxicity compared to patients receiving low thermal dose, 3-year locoregional control was 92 % vs. 74 %, respectively. Since better heating significantly improved the locoregional control these results indirectly suggest the efficacy of post-operative re-irradiation with hyperthermia for locoregional recurrent breast cancer.

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## SUPPLEMENTARY MATERIALS

### Methods: Thermal dose

For each patient, we calculated temperature and thermal dose variables of the skin surface and invasive target area. The average maximum temperature and the temperatures exceeded by 10 %, 50 %, and 90 % of all sensors (T10, T50, T90) during the steady state period of the session, respectively, and the minimum temperature were calculated as well as the highest maximum and lowest minimum temperature. The thermal dose was defined as the cumulative equivalent minutes at 43 °C (CEM43) and was calculated per sensor using:

$$\text{CEM43} = \sum_{t=0}^{t=\text{total}} R^{(43-T)} \Delta t$$

where  $\Delta t$  = time interval (minutes),  $T$  = average temperature during time interval  $\Delta t$  (°C), and  $R$  represents the sensitivity to temperature change.  $R$  is commonly set to 0.25 for  $T < 43$  °C and 0.5 for  $T \geq 43$  °C [35, 36, 45]. The maximum (CEM43 T0), CEM43 T10, CEM43 T50, CEM43 T90 and minimum CEM43 (CEM43 T100) were calculated of all HT sessions.

The thermal dose parameter generally used to quantify the overall hyperthermia dose is the CEM43 T50 [14], either as the average, total or highest CEM43 T50 of all HT treatment sessions. In 29 patients the invasive temperature was not registered during all HT treatment sessions. Since invasive measurements were not performed in all sessions in 26 % of the included patients, we divided the population into an equal-sized ‘low’ and ‘high’ thermal dose group by the best HT session with highest CEM43 T50  $< 7.2$  min and  $\geq 7.2$  min, respectively. Furthermore, we determined the average CEM43, the total CEM43 of all sessions and the total imputed CEM43, which was corrected for the number of HT treatments with invasive thermometry.

### Results: Thermal dose vs. locoregional control

The invasive thermal dose parameters that were associated with locoregional (LR) control, were besides the CEM43 T50 of the best session, also the average CEM43 T50 ( $p = 0.0058$ ), the total CEM43 T50 ( $p = 0.022$ ) and others (Supplementary Table 1). High invasive thermal dose resulted in improved locoregional control compared with lower invasive thermal dose.

The only skin surface thermal dose parameter associated with LR control was the CEM43 T0, both the average of all sessions ( $p = 0.012$ ) and the total CEM43 T0 ( $p = 0.0097$ ). In contradiction to the invasive thermal dose relationships with locoregional control, here a higher thermal dose on the skin surface was associated with worse LR control.

**Supplementary Table 1.** P-values of the log-rank tests for the relationship between thermal dose parameters and locoregional control. Groups were divided by the median of the respective thermal dose parameter. Statistically significant relationships are printed in bold.

	Average	Best session	Total	Imputed total (n = 112)
<b>Invasive</b>			<b>(n = 83)<sup>1</sup></b>	
T0	<b>0.0038</b>			
T10	<b>0.0058</b>			
T50	<b>0.03</b>			
T90	0.22			
T100	0.31			
CEM43 T0	0.054	<b>0.0015</b>	0.17	<b>0.016</b>
CEM43 T10	0.074	<b>0.0064</b>	0.25	<b>0.025</b>
CEM43 T50	<b>0.0058</b>	<b>0.0013</b>	<b>0.022</b>	<b>0.021</b>
CEM43 T90	<b>0.041</b>	<b>0.02</b>	0.31	0.12
CEM43 T100	0.036	0.14	0.49	0.18
<b>Skin surface</b>			<b>(n = 112)<sup>1</sup></b>	
T0	0.073			
T10	0.12			
T50	0.6			
T90	0.55			
T100	0.36			
CEM43 T0	<b>0.012</b>	0.087	<b>0.0097</b>	
CEM43 T10	0.058	0.21	0.058	
CEM43 T50	0.39	0.17	0.33	
CEM43 T90	0.3	0.41	0.12	
CEM43 T100	0.64	0.77	0.18	

<sup>1</sup> In 83 patients (74 %) the invasive temperature was measured during all HT sessions, in the other patients invasive temperature was measured for at least one HT session. Skin surface temperature was measured during all HT sessions in all 112 patients.

*Abbreviations:* T0= maximum temperature; T10, T50, T90= the temperature exceeded by 10 %, 50 % and 90 % of the temperature measurements during the steady state of hyperthermia treatment, respectively; T100= minimum temperature; CEM43= cumulative equivalent minutes at 43 °C; CEM43 T0= maximum CEM43; CEM43 T10, CEM43 T90= the thermal dose exceeded by 10 % and 90 % of the sensors during the steady state of hyperthermia treatment, respectively; CEM43 T50; median CEM43; CEM43 T00= minimum CEM43.

**Supplementary Table 2.** Results of univariate Cox regression analyses for locoregional control and overall survival.

	<b>Locoregional control</b>		<b>Overall survival</b>	
	HR (95 % CI)	<i>p</i>	HR (95 % CI)	<i>p</i>
<b>Patient characteristics</b>				
Age ( $\leq 50$ / $> 50$ )	1.6 (0.6 - 4.2)	n.s.	1.1 (0.4 - 3.1)	n.s.
<b>Initial breast cancer</b>				
Time interval primary tumor - present recurrence (years)	0.95 (0.89 - 1.0)	n.s.	0.9 (0.84 - 0.97)	0.005
Time interval previous breast cancer - present recurrence (years) <sup>a</sup>	0.91 (0.85 - 0.98)	0.013	0.91 (0.85 - 0.98)	0.012
Primary pathological stage (T1 - T4)	1.4 (1.0 - 1.9)	n.s.	1.5 (1.0 - 1.2)	0.009
Previous local recurrences (yes/ no)	2.0 (0.9 - 4.5)	n.s.	1.0 (0.4 - 2.3)	n.s.
<b>Present recurrent breast cancer</b>				
Histological type (NST, ILC, DCIS, other)	1.1 (0.5 - 2.3)	n.s.	1.1 (0.5 - 2.2)	n.s.
Triple-negative breast cancer (yes/ no)	1.9 (0.8 - 4.4)	n.s.	4.3 (1.9 - 9.6)	<0.001
Differentiation grade (G1 - G3)	1.1 (0.5 - 2.1)	n.s.	2.0 (1.0 - 3.9)	n.s.
Lymphovascular invasion (yes/ no)	1.3 (0.6 - 2.9)	n.s.	1.8 (0.8 - 4.0)	n.s.
Tumor size ( $\leq 5$ cm / $> 5$ cm)	0.6 (0.2 - 1.5)	n.s.	0.3 (0.1 - 0.8)	0.015
Location (chest wall/ breast)	2.9 (1.3 - 6.5)	0.011	1.6 (0.7 - 3.6)	n.s.
Contralateral breast cancer growth (yes/ no)	2.8 (1.0 - 8.4)	n.s.	4.3 (1.7 - 12.0)	0.002
Lymph node status (yes/ no)	2.3 (1.0 - 5.2)	0.045	3.1 (1.4 - 7.1)	0.007
Distant metastases (yes/ no) <sup>b</sup>	12.0 (4.1 - 33.0)	<0.001	4.9 (1.6 - 15.0)	0.004
Estrogen receptor + (yes/ no)	0.5 (0.2 - 1.1)	n.s.	0.2 (0.1 - 0.5)	<0.001
<b>Present treatment</b>				
Best CEM43 T50 (low/ high)	4.4 (1.6 - 12.0)	0.003	1.5 (0.7 - 3.4)	n.s.
Median time interval group (short/ long)	1.3 (0.6 - 2.9)	n.s.	0.9 (0.4 - 1.9)	n.s.
Pre-heating time	0.9 (0.8 - 1.0)	n.s.	0.9 (0.9 - 1.0)	n.s.
Number of hyperthermia treatments (4/ 5)	0.5 (0.2 - 1.1)	n.s.	0.5 (0.2 - 1.1)	n.s.
Re-irradiation dose (8 x 4 Gy/ 23 x 2 Gy)	0.6 (0.3 - 1.4)	n.s.	0.6 (0.3 - 1.4)	n.s.
Chemotherapy (yes/ no)	0.8 (0.3 - 1.7)	n.s.	1.6 (0.7 - 3.5)	n.s.

a. Previous breast cancer= initial diagnosis or locoregional recurrence or second ipsilateral primary breast cancer; b. Contralateral lymph nodes are not counted as distant metastasis.

*Abbreviations:* HR= Hazard ratio; CI= 95 % Wald confidence interval; NST= invasive breast cancer of no special type; ILC= invasive lobular cancer; DCIS= ductal carcinoma in situ; Best CEM43 T50= the best median invasive thermal dose of all hyperthermia sessions; n.s. = not significant.

**Supplementary Table 3.** Number and type of acute toxicities according to CTC-AE v5.0 stratified by thermal dose group. Patients can have multiple types of acute toxicities, 68.8% of the patients (n = 77) had more than one type of acute toxicity.

CTC-AE score	Low thermal dose		High thermal dose	
	Toxicity	Patients (%)	Toxicity	Patients (%)
1-2	Radiation dermatitis	39 (69.6 %)	Radiation dermatitis	41 (73.2 %)
	Chest(wall) pain	20 (35.7 %)	Chest(wall) pain	22 (39.3 %)
	Hyperpigmentation	17 (30.4 %)	Hyperpigmentation	18 (32.1 %)
	Burn	8 (14.3 %)	Burn	12 (21.5 %)
	Lymphedema	6 (10.7 %)	Lymphedema	7 (12.5 %)
	Skin ulceration	5 (8.9 %)	Skin ulceration	3 (5.4 %)
	Pneumonitis	1 (1.8 %)	Pneumonitis	1 (1.8 %)
	Telangiectasia	2 (3.6 %)	Catheter related infection	2 (3.6 %)
3	Radiation dermatitis	6 (10.7 %)	Radiation dermatitis	7 (12.5 %)
	Chest(wall) pain	2 (3.6 %)		
4	-		-	
5	-		-	