Liposomal Doxorubicin in Conjunction with Reirradiation and Local Hyperthermia Treatment in Recurrent Breast Cancer: A Phase I/II Trial


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ABSTRACT

Purpose: This is the first study to evaluate the tolerability and activity of liposomal doxorubicin (Caelyx; Schering-Plough Pharmaceuticals) ≤60 mg/m² in patients with locally recurrent breast cancer, when administered in conjunction with reirradiation and local hyperthermia treatment.

Experimental Design: Fifteen female patients, who had undergone a radical mastectomy and conventional radiotherapy (60 Gy) in the front chest wall, were entered on a multimodal protocol consisting of initial treatment with radiotherapy and a monthly infusion of liposomal doxorubicin ≤60 mg/m² in conjunction with local hyperthermia treatment. All patients received reirradiation up to a total dose of 30.6 Gy (1.8 Gy/fraction, 5 days a week). To evaluate the drug’s safety, the first 5 patients initially received a dose of 40 mg/m² liposomal doxorubicin, which was then escalated to 60 mg/m². The other 10 patients received 60 mg/m² for all six cycles of chemotherapy. Hyperthermia (HT) was produced in the region of interest (ROI) using waveguides at a frequency of 433 MHz. The RSS was obtained from the curves representing the change in the ROI’s surface with time for each patient, as fitted by linear regression. Linear regression analysis was used to study the relationship between the time interval from liposomal doxorubicin infusion to HT and the RSS.

Results: At doses of ≤60 mg/m², liposomal doxorubicin was well tolerated, with only mild hematological and non-hematological toxicity. All patients showed an objective measurable response, with 3 patients (20%) demonstrating a clinically complete response. There was a significant correlation between the duration of response and Avg Min T90 > 44°C (r ≤ 0.917, P < 0.0001) and the Mean[Tmin] (r ≤ 0.909, P < 0.0001). The RSS was significantly correlated with the interval between liposomal doxorubicin infusion and HT, as the smaller the time interval, the greater the clinical benefit (r = 0.76, P = 0.001).

Conclusions: The multimodal treatment was effective and well tolerated, producing an objective measurable response in all patients. Local HT had a significant effect on patients’ response to the drug. The relationship between thermal dose and liposomal action requires further investigation.

INTRODUCTION

Recurrence of breast cancer in the front chest wall after mastectomy and RT² poses a major problem, as limited therapeutic options remain for clinical application. The current and potential role of HT treatment for cancer has already been documented (1). The ESHO has monitored five protocols running in Europe. An odds ratio of ≤1.37 for survival in favor of combined treatment has been reported for protocol ESHO-I/ Medical Research Council, which examined the role of RT plus HT treatment versus RT alone for the treatment of advanced breast cancer (2). Protocol ESHO-5/Medical Research Council for chest wall recurrence has given an odds ratio of ≤3.43 for combined treatment (RT + HT; Ref. 2). This role of HT as an adjuvant treatment to RT was also documented by Vernon et al. (3), who reported the results of a large randomized multicenter trial. Among 306 patients, the overall complete response rate for RT alone was 41%, and for combined treatment, it was 59%, giving, after stratification by trial, an odds ratio of ≤2.3. The greatest effect was observed in patients with recurrent lesions in areas irradiated previously, where further irradiation was limited to low doses.

PEGylated liposomal doxorubicin hydrochloride (Caelyx; Schering-Plough Pharmaceuticals), based on the Stealth liposome delivery system, is a novel drug formulation with a prolonged circulation time and preferential extravasation at tumor sites that improves their clinical activity.

REFERENCES

1. The abbreviations used are: RT, radiotherapy; HT, hyperthermia; ESHO, European Society of Hyperthermic Oncology; ROI, region of interest; CCR, clinically complete response; PR, partial response; DFILR, disease-free interval to local relapse; RSS, region of interest’s surface slope; PPE, palmar-plantar erythrodysesthesia.
sites because of their size and structure (4, 5). Although anthraacyclines are radiosensitizers, the use of these agents concurrently with RT often increases toxicity in normal surrounding tissues (6, 7). Therefore, selective drug localization to tumors could be of great importance, because the drug remains tumor localized during RT (8). Ranson et al. (9) reported encouraging results with Caelyx treatment for metastatic breast cancer, whereas Symon et al. (10) concluded that Stealth liposomal doxorubicin accumulates selectively in metastatic breast carcinoma cells instead of normal skeletal muscle tissues. Furthermore, the behavior of liposomes in heated conditions has already been reported by Gaber et al. (11) in 1996.

In this study, we evaluate the safety and feasibility of Stealth liposomal doxorubicin in the treatment of locally recurrent breast cancer in combination with HT treatment and reirradiation in relapses from mastectomy in areas irradiated previously. The major rationale for this study was to investigate the possible clinical benefit of HT treatment and RT administered in conjunction with liposomal doxorubicin. The mechanisms of action of Caelyx in targeting tumors through abnormal vasculature and its prolonged circulation time are enhanced by heating, which affects the pH, thereby facilitating break-up of the entrapped liposome (11). RT also has a synergistic effect because of the radiosensitization of high intratumoral levels of doxorubicin (7, 8).

PATIENTS AND METHODS

From October 1998 to November 1999, 15 female patients with advanced breast cancer and local recurrence were enrolled in the study. All patients had undergone a radical mastectomy and RT in the front chest wall, with the initial RT schedule constituting a total dose of $\leq 60$ Gy with 2 Gy/fraction (5 days a week for 6 weeks). Local recurrence was observed between 37 and 48 months (median 43 months) after the completion of front chest wall irradiation. The recurrence presented as nonhealing, and 48 months (median 43 months) after the completion of front chest wall irradiation. The recurrence presented as nonhealing, affecting the pH, thereby facilitating break-up of the entrapped liposome (11). RT also has a synergistic effect because of the radiosensitization of high intratumoral levels of doxorubicin (7, 8).

Table 1

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Caelyx</th>
<th>Caelyx</th>
<th>Caelyx</th>
<th>Caelyx</th>
<th>Caelyx</th>
<th>Caelyx</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT</td>
<td>Baseline</td>
<td>2nd</td>
<td>3rd</td>
<td>4th</td>
<td>5th</td>
<td>6th (sessions of treatment)</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>4 weeks</td>
<td>4 weeks</td>
<td>4 weeks</td>
<td>4 weeks</td>
<td>(interval between sessions)</td>
</tr>
</tbody>
</table>

- Urea and serum creatinine lower than upper limit of laboratory normal
- Total and direct bilirubin lower than upper limit of laboratory normal
- Serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and alkaline phosphatase lower than upper limit of laboratory normal.

All patients were referred to the Radiotherapy Department of the Areteion University Hospital and officially enrolled in the study when the initial diagnosis of relapse was made. Registration for this Phase I/II trial stopped at 15 patients, as subsequent patients referred to the department were randomized to combined treatment of either reirradiation plus Caelyx plus HT or reirradiation plus HT, as part of an ongoing Phase III trial for this protocol.

Trial Design. According to the protocol, the first i.v. infusion of Caelyx was given concurrently with RT, which was administered with an X-ray beam from a 6-MV linear accelerator (Philips SL75) up to a total dose of 30.6 Gy (1.8 Gy/fraction, 5 days a week). Caelyx infusions were then repeated every 4 weeks over a period of 6 months. HT treatment was administered every 4 weeks for a period of 6 months after every infusion of Caelyx. The time interval between Caelyx infusion and HT treatment ranged from 3 to 40 h (median = 32, mean = 27.1 \pm 11.3), depending on the patients’ transport arrangements from other hospitals to the Radiotherapy Department of Areteion University Hospital, the referral center for HT treatment and RT.

The multimodal treatment was therefore sequenced as follows: the baseline consisted of concurrent initiation of reirradiation and Caelyx plus the first HT treatment; the 2nd-6th month of combined treatment consisted of Caelyx infusion plus HT treatment. The total treatment schedule continued for 5 months, as shown in Table 1.

The RT was carried out after three-dimensional treatment planning using two tangential fields. Two patients who presented secondary skin lesions in anatomical areas outside the region irradiated previously. Patients ranged from 49 to 58 years of age (median 52 years).

Inclusion Criteria. Patients had to meet the following criteria according to the study protocol:

- Relapse from breast cancer in the front chest wall (superficial tumors, no $\geq 3$ cm in depth)
- Contrast-enhanced computed tomography scan performed before treatment
- Normal electrocardiogram and cardiolipin levels in normal range
- Age 18–75 years, Karnofsky performance status $>70\%$, and life expectancy $>2$ months
- Laboratory values (performed within 1 week before study)
  - Absolute neutrophil count $> 3,000$/mm$^3$
  - Platelet count $> 100,000$/mm$^3$
  - Hemoglobin $>10$ grams/dl
Table 2  Response criteria based on a modification of criteria established by the AIDS Clinical Trials Group (ACTG)

Changes in the front chest wall secondary lesions (ROI) were measured from the baseline as stated in “Patients and Methods” once a month during treatment, every 10 days in the 1st month of follow-up and once a month during the rest of the follow-up period. At the end of the follow-up period, these changes were expressed as an objective measurable response (CCR, PR, SD, NR, or PD), as defined by the ACTG criteria given below.

<table>
<thead>
<tr>
<th>Definition</th>
<th>Objective measurable response</th>
<th>Corresponding definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>No evidence of tumor</td>
<td>CCR</td>
<td>Absence of any detectable residual disease, including tumor-associated oedema</td>
</tr>
<tr>
<td>Definitely better</td>
<td>PR</td>
<td>(a) A decrease in the surface of the secondary front chest wall lesion, ranging from 50 to 99%</td>
</tr>
<tr>
<td>Possibly better</td>
<td>SD</td>
<td>(b) CCR criteria met except for tumor-associated oedema</td>
</tr>
<tr>
<td>Unchanged</td>
<td>NR</td>
<td>A decrease in the surface of the secondary front chest wall lesion, ranging from 1 to 49%</td>
</tr>
<tr>
<td>Possibly worse</td>
<td>NR</td>
<td>An increase in the surface of the secondary front chest wall lesion, ranging from 1 to 24%</td>
</tr>
<tr>
<td>Definitely worse</td>
<td>PD</td>
<td>(a) An increase in the surface of the secondary front chest wall lesion of &gt;25%</td>
</tr>
<tr>
<td>(b) The development of new secondary lesions or an increase in tumor-associated oedema</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* SD, stable disease; NR, no response; PD, progressive disease.

Board at Aretaion University Hospital in Athens. All patients gave written informed consent before enrolment in the study.

Pretreatment and Treatment Evaluation. Baseline studies included a physical examination, complete biochemical profile, bone scan, evaluation of an electrocardiogram and cardiolipin levels, and computed tomography scan of the chest and upper abdomen. Whole blood cell counts were performed once a week during the RT period and every 4 weeks thereafter during the chemotherapy and HT treatment. Serum urea and creatinine, as well as electrocardiograms and cardiolipin levels, were assessed every 3 weeks during the treatment schedule. Acute radiation toxicity was documented twice weekly, and chemotherapy-induced toxicity was documented every week. Radiation toxicity was assessed using the grading scale of the Radiation Therapy Oncology Group and the European Organization for Research and Treatment of Cancer (12). Overall chemotherapeutic toxicity was assessed using the WHO scale (13). Any specific chemotherapy-associated toxicity, such as PPE (2), was also evaluated (14).

Clinical response to treatment was assessed by the study investigators, who measured changes in front chest wall secondary lesions from the baseline once a month during treatment, every 10 days during the 1st month of follow-up, and once a month during the rest of the follow-up period until local failure. Every patient carried out self-examination in the interval between these follow-up measurements and reported changes in the ROI to the authors; therefore, treatment response could be continuously evaluated and updated. Changes in the ROI were assessed using modified criteria established by the AIDS Clinical Trials Group (15), as defined in Table 2. The major end points were the lesion surface and the lesion morphology in terms of keratinization of ulcerative regions.

To ensure objective measurement of the lesion surface, a new, practical method was established as follows: at the baseline, at every session of Caelyx chemotherapy and during follow-up, a thin, transparent paper was placed on the front chest wall over the secondary lesion, and the outer limits of the lesion were recorded. To aid accurate and consistent registration of the ROI, the ensiform appendix of the sternum and the jugular fossa were used as anatomical markers for every measurement. The paper was then digitized using an eight-bit resolution scanner, and the surface of the ROI was measured automatically using AutoCAD software (Release 12 for Windows; Autodesk, Inc.). A color photograph was also taken of the ROI, and comparative (but subjective) evaluations were made during the assessments of response to treatment. During follow-up, any increase in the surface of the front chest wall lesion, local redevelopment of new secondary lesions, or an increase in tumor-associated edema in a region in which a CCR had been monitored were recorded as local failure. Because all patients presented a clinical response (CCR or PR) to combined treatment, the duration of response was registered as the time from the development of a clinical response to the time of appearance of local failure. The duration of response was referred to the DFILR. All relapsed patients were referred to the Palliative Care Unit at Aretaion University Hospital for pain relief and palliation of symptoms. Follow-up continued until death.

Caelyx Administration and Dose Escalation. Caelyx was diluted in 250 ml of dextrose/water solution and infused over a 45-min period. Ondansetron (8 mg i.v.) was given as antiemetic treatment. Blood pressure and symptoms were monitored every 5 min during the Caelyx infusion and every 15 min during the following hour. To evaluate the safety of Caelyx administration, the first 5 patients to enter the protocol were put on a dose escalation pilot study. They received an initial dose of 40 mg/m², which was increased in 10 mg/m² increments for every following session of Caelyx administration. The target dose was 60 mg/m², as opposed to the 50 mg/m² dose level established in previous monotherapy studies (16). The other 10 patients received Caelyx 60 mg/m² for all six cycles of chemotherapy. The first session of Caelyx was administered concurrently with the reirradiation treatment.

HT Treatment. All patients received HT treatment every 4 weeks, 3–40 h (median 32 h) after the Caelyx infusion. The heat was applied, using waveguides, with a rectangular or circular aperture (National Technical University of Athens), working at a frequency of 433 MHz (17). As the maximum effective diameter of the applicator was ~7 cm, more than one field of HT treatment was applied sequentially in all patients to cover the entire relapsed region in the front chest wall. The applicators were water loaded and had an extra chamber with circulating cooling water to avoid any adverse events, such as skin burning or blisters resulting from the contact of high microwave power with the skin surface. A thermocouple placed adjacently on the
skin at the footprint of the antenna confirmed that the temperature at the skin surface did not exceed the therapeutic range. The temperatures derived from this thermocouple were not recorded; they were monitored for safety reasons to ensure that the temperature did not exceed 45°C in the epidermis. The emitted power ranged from 25 to 40 W. This whole procedure was administered for 60 min, with a target temperature of 44°C. A catheter was inserted interstitially into the can-44°C was administered for 60 min, with a target temperature of 44°C. A catheter was inserted interstitially into the cancerous mass to a depth of 1.5–2 cm after a local anesthetic. A thermocouple, with three points of measurement along its axis every 0.8 cm, was placed into the catheter to monitor the interstitial temperature throughout the HT treatment, and the data were stored in a PC using a compatible data acquisition system, producing ASCI files in tab-delimited format. These files were then entered onto a worksheet (SPSS 6.0 for Windows) for further analysis. The average maximum (Mean[T-max]) and minimum (Mean[Tmin]) temperatures were calculated, as well as the average time (in min) during which 90% of the recorded temperatures exceeded 44°C (Avg Min T90 > 44°C; Refs. 18 and 19). An example of the area of HT treatment and use of the applicator and thermocouple are shown in Fig. 1. The patient shown in Fig. 1 presented at the start of the study with multinodular and ulcerative desquamation in the front chest wall and in the left arm.

Statistical Analysis. The Kaplan-Meier method of life table analysis (20) and Log-rank test (21) were used for the comparative assessment of DFILR for patients with Avg Min T90 > 44°C <16 min versus those with Avg Min T90 > 44°C ≥ 16 min. The possible affection of response to the DFILR was assessed using the Log-rank test. Thermal parameters concerning Mean[Tmin], Mean[Tmax], and Avg Min T90 > 44°C were correlated with DFILR using the nonparametric Spearman correlation coefficient, r_s (22). Likewise, the extend of disease in terms of surface of ROI at baseline was correlated with DFILR using the Spearman correlation coefficient. The RSS was obtained from the curves representing the change of the ROI’s surface with time for each patient, fitted by linear regression. Linear regression analysis was used to study the relationship between the RSS and the time interval between Caelyx infusion and HT treatment.

RESULTS

Toxicity. The main toxicities observed during Caelyx infusion are shown in Table 3. Two patients (2 of 15; 13.3%) presented with acute back pain and mild dyspnea during the first 3–4 min of the first Caelyx infusion (dose 60 mg/m²). In these two cases, the Caelyx infusion was stopped, and 500 mg i.v. of methylprednisolone was administered. After 10 min, the symptoms improved, and the patients received subsequent Caelyx cycles without any further incidence of acute dyspnea. Three patients (20%) presented with grade I vomiting after two to three cycles (Table 3). Five patients (33.3%) experienced hot flushes lasting ~3 days after Caelyx infusion (dose 60 mg/m²). Grade I PPE was observed in 3 patients (20%) at 60 mg/m². Two patients (13.3%) reported asthenia related to chemotherapy. Three patients (20%) presented with grade I oral mucositis. All hematological toxicities were mild (up to grade II), as shown in Table 3. Cardiotoxicity was not observed during the combined treatment. Three patients (20%) presented with grade I (Radiation Therapy Oncology Group/Eastern Cooperative Oncology Group) radiation-induced dermatitis, which was mild (dry desquamation) and easily manageable with symptomatic treatment. One patient (6.7%), after combined RT and HT, reported moist desquamation with blisters and skin burns. However, pain sensation was decreased at these sites, and they did not cause much inconvenience. Neither interstitial nor surface temperature measurements exceeded 45°C in this or any other patient. This patient had a CCR but presented a deformation in the front chest wall with fibrosis 4 months later, as shown in Fig. 2.

Response. The change in the ROI’s surface from the baseline through to 1 month’s follow-up after the end of the combined treatment is shown in Fig. 3. Twelve patients (80%) presented a PR, and 3 (20%) had a CR during the 1st month of follow-up. The DFILR, along with the HT treatment characteristics and Caelyx dosages, are shown in Table 4. Linear regression analysis for the average time interval between Caelyx infusion and HT treatment and the RSS, obtained by linear curve estimation from the lines representing the change in the ROI’s surface, are shown in Fig. 4. The interval between Caelyx infusion and HT treatment was significantly correlated with the
RSS ($r^2 = 0.76$, $P = 0.001$), indicating that a quicker response was obtained when the time interval between HT treatment and liposomal administration was shorter. According to the monitored temperatures, there was a significant correlation between the DFILR and the Avg Min $T_{90}$ ($r_s^2 = 0.917$, $P < 0.0001$) and the Mean[Tmin] ($r_s = 0.909$, $P < 0.0001$), whereas no correlation was found between the DFILR and Mean[Tmax]. Splitting the population into two groups revealed that $T_{90} > 44^\circ C$ had a significant impact on patients’ DFILR. The DFILR was much better in the group of patients with $T_{90} > 44^\circ C$ of $\geq 16$ min compared with those for whom $T_{90} > 44^\circ C$ was $< 16$ min ($P = 0.005$, Log-rank test), as indicated by the Kaplan-Meier distribution for response rate shown in Fig. 5. The surface or ROI at baseline (initial extend of local disease) was negatively but poorly correlated with DFILR ($r_s^2 = -0.37$, $P = 0.17$). Nevertheless, a patient who presented CCR had significantly better DFILR ($P = 0.0047$, Log-rank test). During follow-up, patients with local failure received palliative care, with administration of transdermal fentanyl for pain relief and local cutaneous infusions of antibiotics for infections in the open wounds of secondary lesions. There was a median survival of 10.9 months (SE = 0.9; 95% confidence interval = 9.2–12.6). Death occurred as a result of liver metastases.

**DISCUSSION**

Recurrent malignant tumors, especially in the chest wall, are mainly perfused by small abnormal vessels, the so-called “Phase III” vessels (23). The majority of Stealth liposomes enter the interstitium of the tumor through gaps in the endothelium of the newly and abnormally formed vessels that feed the tumor (24–26). A smaller proportion of liposomes may actually pass directly through the thin walls of the defective endothelial cell lining of the new vessels via a process called transcytosis (27). HT treatment can interact with PEGylated doxorubicin in two ways: (a) at low temperatures ($< 41^\circ C$), the blood flow increases, and the probability of liposomal doxorubicin passing through the tumor increases proportionally; and (b) at high temperatures ($> 41^\circ C$), microcirculation is corrupted, and pH decreases; the corruption of microvessels accommodates the intratumoral insertion of Stealth liposomes, and the decrease in pH results in physiochemical destabilization and breakdown of the liposomal envelope (28–30). Indeed, according to a study by Gaber et al. (11), HT selectively enhances the delivery and the rate of release of doxorubicin inside targeted tumors by the extravasation of liposomes. Moreover, in a recent study, Kong et al. (31) reported that over normothermic conditions ($> 39^\circ C$), nanoparticles extravasation (such as liposomes) into the tumor interstitium increased with temperature. Additionally, HT has a direct effect on the liposomal envelope of Caelyx at temperatures close to the transition temperature ($42^\circ C$ and $45^\circ C$; Ref. 32), resulting in increased leakage of doxorubicin, especially from cholesterol-rich liposomes (such as Stealth macromolecules). HT treatment, as an adjuvant therapeutic modality to RT, is therefore an effective means of overcoming hypoxic radioresistance in the treatment of human tumors (33, 34). Furthermore,
the increase in blood flow at low thermal doses, resulting in an increase of pO₂, enhances the efficiency of irradiation (35).

The actions of lipases released from dying neoplastic cells (36) and of various synthases, enzymes, and radicals released by inflammatory or phagocytic cells inside tumors (37–39) are also responsible for the breakdown of the liposomal surface. The cytotoxic effect of RT, producing oxidizing molecules and radicals (40), may further contribute to the establishment of intratumoral conditions that rapidly break down the extravasated liposomes. Moreover, radiation enhances nitric oxide production, resulting in vascular permeability and, consequently, an increase in the extravasation of liposomes (41, 42).

Stealth liposomal doxorubicin (Caelyx; Schering-Plough Pharmaceuticals) is a novel way of delivering chemotherapeutic drugs because Stealth formulation effectively escapes macrophage-mediated phagocytosis of the reticuloendothelial system (43–45). The effect of PEGylated liposomal doxorubicin on solid tumors may be summarized as follows (4, 15, 38): a change in the toxicity profile reduces acute adverse effects, such as nausea and vomiting, and reduces the incidence of alopecia. It also has greater activity in highly angiogenic tumors (such as Kaposi’s sarcoma) and is effective treatment of tumors moderately sensitive to doxorubicin (such as breast and ovarian carcinomas), with the possibility of increased tumor response because of enhanced drug accumulation. Harrington et al. (46) showed that the remainder of the injected Caelyx had an increasingly higher intratumoral accumulation versus the blood pool. Koukourakis et al. (47) reported similar results with radiolabeled [99mTc]Te-diethylenetriaminepentaacetic acid-Caelyx in cancerous lung tissues, where the Caelyx accumulation was two to four times greater than in normal tissues. Gabizon et al.

**Table 4** Hyperthermia treatment characteristics, dosage of Caelyx, and response for each patient

All patients received HT treatment, as detailed in “Patients and Methods.” The mean minimum and maximum temperatures at the tumor site, as well as the average time during which 90% of the recorded temperatures exceeded 44°C, were calculated from readings taken at three points along a catheter inserted into the tumor to a depth of 1.5–2 cm. To assess the safety of Caelyx administration, patients 1–5 received Caelyx at an initial dose of 40 mg/m², escalating to 60 mg/m². All patients presented a clinical response (CCR or PR) to treatment, and the DFILR was registered as the time from development of clinical response to the time of local relapse.

<table>
<thead>
<tr>
<th>No.</th>
<th>Mean (Tmin) (°C)</th>
<th>Mean (Tmax) (°C)</th>
<th>Average T₉₀ &gt; 44°C (°C)</th>
<th>Response</th>
<th>DFILR (days)</th>
<th>Dose of Caelyx (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41.3</td>
<td>44.8</td>
<td>5.2</td>
<td>PR</td>
<td>45</td>
<td>40–60</td>
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<tr>
<td>2</td>
<td>42.7</td>
<td>45.0</td>
<td>17.4</td>
<td>PR</td>
<td>98</td>
<td>40–60</td>
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<tr>
<td>3</td>
<td>43.0</td>
<td>45.2</td>
<td>12.1</td>
<td>PR</td>
<td>87</td>
<td>40–60</td>
</tr>
<tr>
<td>4</td>
<td>41.6</td>
<td>45.7</td>
<td>6.5</td>
<td>PR</td>
<td>60</td>
<td>40–60</td>
</tr>
<tr>
<td>5</td>
<td>42.6</td>
<td>45.6</td>
<td>10.3</td>
<td>PR</td>
<td>80</td>
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<td>6</td>
<td>43.4</td>
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<tr>
<td>7</td>
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<td>60</td>
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<td>CCR</td>
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<td>9</td>
<td>42.2</td>
<td>44.4</td>
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<tr>
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<td>13.3</td>
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<td>7.1</td>
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<tr>
<td>15</td>
<td>42.2</td>
<td>44.2</td>
<td>6.8</td>
<td>PR</td>
<td>67</td>
<td>60</td>
</tr>
</tbody>
</table>

* Dose escalation in patients 1–5; + continued follow-up.

**Fig. 4** Linear regression analysis for the average time interval between Caelyx infusion and HT treatment and the RSS obtained by linear curve estimation from the lines representing the change in the ROI’s surface (see Fig. 3).

**Fig. 5** Kaplan-Meier curve as calculated when the DFILR is plotted for patients with Avg Min T₉₀ > 44°C <16 min versus Avg Min T₉₀ > 44°C ≥ 16 min (P = 0.005, Log-rank test). +3 patients with continued follow-up.
(4) reported a peaking of drug levels in malignant effusions between 3 to 7 days after injection. Khalīfa et al. (48) reported excellent tumor accumulation 72 h after Caelyx injection in recurrent high-grade gliomas. Similar results were also reported by Northfelt et al. (49), who found greater accumulation of the drug in Kaposi’s sarcoma lesions 72 h after injection. In this study, the intermediate time between Caelyx infusion and HT ranged from 3 to 40 h. However, statistical analysis showed that the best results concerning both response and DFILR were obtained when the intermediate time between the two treatment modalities did not exceed 12 h, especially when HT treatment was administered 3–4 h after PEGylated doxorubicin injection. Indeed, the RSS was significantly correlated with the interval between Caelyx infusion and HT; the smaller the time interval, the better the results obtained ($r = 0.76$, $P = 0.001$). This observation may be related to the first phase of Caelyx pharmacokinetics, as one-third of the injected dose clears from the plasma within 3.2–4.9 h (4). By increasing the blood flow or the extravasation of liposomes, HT may enhance the intratumoral concentration of the drug during this period. Kong et al. (31) reported that enhanced nanoparticle extravasation was observed 4 h after heating, decaying back to baseline at 6 h postheating. A similar phenomenon was observed by Koukourakis et al. (47), who recorded that liposomal drugs accumulated better in highly angiogenic tumors. Moreover, Gaber et al. (11) reported a dramatic extravasation of liposomes when heated for 1 h at 42°C and 45°C, 1 h after Caelyx injection, resulting in a slight increase in doxorubicin release and continued extravasation of the liposomes after the HT session. As a general conclusion, it seems that there may be two “therapeutic windows” for combined treatment with HT: (a) one for the acute phase of Caelyx pharmacokinetics (50) right after the infusion; and (b) another during the 72 h after Caelyx infusion (4, 11, 31, 48, 49). These observations may also suggest that patients should receive two sessions of HT treatment: (a) the first session within 1–2 h of Caelyx infusion to take advantage of the first acute phase (plasma concentration) of liposomes (50), and (b) the second 72 h after infusion or initial hyperthermic treatment to exploit the intratumoral phase of Caelyx pharmacokinetics (49–51) and to prevent thermo-tolerance (52). This latter effect was demonstrated by Huang et al. (53), who reported that HT treatment of tumors in mice 1 h after i.v. injection of encapsulated doxorubicin was 51% more effective in terms of life span compared with mice that did not receive HT treatment and that two sessions of HT treatment were superior to a single session. Thermotolerance effect in terms of resistance in the extravasation of nanoparticles was also reported by Kong et al. (31).

Studies of HT treatment in combination with liposomal drug administration have shown an enhanced therapeutic effect compared with either treatment modality alone, both in terms of free drug uptake and tumor growth delay (53, 54). In preclinical studies, a 5- to 15-fold increase has been seen in the doxorubicin concentration in tumor cells using HT and the liposomal drug delivery method, compared with free doxorubicin and heat (53). The efficacy of combined RT with Stealth liposomal doxorubicin on patients with metastatic or relapsed breast cancer has already been reported (9, 55, 56). Moreover, Dvorak et al. (57) reported a case of complete remission in skin metastases after the combination of PEGylated doxorubicin with local HT. However, this is the first study to report the feasibility and response of high doses of Caelyx, $\leq 60$ mg/m$^2$, administered concurrently with RT and local HT in patients with relapses from mastectomy and previously irradiated front chest wall. Patients who presented CCR had a significantly better DFILR. However, the extend of local relapse was negatively but nonsignificantly correlated with the DFILR. This result might be because of the poor statistical power related to the small number of patients. Patients with the more extended local relapse in the front chest wall were presented in our department with a considerable delay. This fact obviously raises the argument that the sooner the patient with relapses underwent the treatment, the better the response was monitored.

Caelyx, administered at a dose of $\leq 60$ mg/m$^2$ every 4 weeks, was well tolerated, with only mild hematological and nonhematological toxicity in all patients. At doses of 60 mg/m$^2$, Ranson et al. (9) reported grade 3 skin toxicity, mucositis, and neutropenia, but the time interval between treatment sessions was 3 weeks versus the 4-week interval used in this study. The low toxicity profile of Caelyx reported in the literature (4, 15, 38) was also confirmed in this study, as no alopecia was observed, and nausea and vomiting were mild (grade 1). Reirradiation of the front chest wall with 30.6 of Gy (1.8 of Gy, 5 days a week) was also well tolerated by the patients. In the present study, the average minimum temperature had a significant impact on the DFILR, and according to the Log-rank test, $T_{\text{min}} > 44°C$ had a particularly significant ($P = 0.005$) impact on the DFILR. These results agree with those of Engin et al. (58), who reported that thermal parameters associated with the duration of local control in superficial malignant tumors include Mean[T-min] and $T_{\text{min}} > 43°C-44°C$. However, Kong et al. (31) reported that the optimum heating range for conveying the greatest benefit in drug delivery inside cancerous tissues is 40°C–42°C and not $>43°C$, which is the normal hyperthermic range. Our results regarding temperatures $>44°C$ may be correlated mostly with the interaction of HT with RT (1) and secondarily on the exposure of liposomes to HT (31). Taking into account the inhomogeneity of temperature of most HT treatments, it appears that the combination of HT, RT, and liposomes would result in various degrees of clinical benefit, depending on the temperature at the local region of tumor. Mild heating of a tumor ($40°C$–42°C) conveys the greatest benefit regarding the drug delivery with liposomes (31); higher temperatures ($>43°C$) would result in better results concerning the interaction of HT with irradiation (1–3). Future work needs to focus on optimizing liposomal administration with HT treatment and understanding the effect of thermal dose on liposomal drug delivery. Moreover, special attention should be given to the combination of HT treatment and the new generation of thermosensitive liposomes, because the preliminary results from preclinical studies are encouraging (11).

The combination of the three therapeutic modalities discussed in this study has not been used previously by clinicians. Although care should be taken when drawing long-term conclusions from the statistics of a small patient population, the triple-modality treatment used in this study has produced a trend of clinical benefit, with significant responses to treatment observed even in patients with a poor prognosis. The fact that 3 patients presented complete remission concerning the skin metastases in
the front chest wall should not be underestimated. Although this combined treatment seems to hold great promise for the improvement of current therapeutic regimens for cancer, it is not possible to conclude from this Phase II trial that it is Caelyx, which makes all of the difference, as the RT may have interacted with the HT treatment to produce the effect. The combination of HT with RT is a well-documented multimodal treatment (2, 3). To separate out the effects of the various modalities and to evaluate the clinical benefit of Caelyx itself, a randomized Phase III trial of RT + HT + Caelyx versus RT + HT for patients with locally recurrent and previously irradiated breast cancer has been approved by the Ethical Committee of Areteion University Hospital and is now in progress. In the meantime, the authors conclude that Caelyx ≤60 mg/m² every 4 weeks is a tolerable dosage and recommend its use in future trials involving multimodal protocols. Future studies need also to focus on the interval between HT treatment and liposomal administration, as in this study, a quicker response was observed when the time interval was shorter. The authors also recommend, in agreement with the current literature (31, 48–53), that two sessions of HT should be performed, the first at 1 h and the second 72 h after i.v. infusion of Caelyx; this would take advantage from the interaction of HT with RT (1, 52) and liposomes (31, 49–52) as well. This schedule is being administered in the randomized Phase III trial discussed above, the results of which will be reported on completion of the study.

In conclusion, despite the small number of patients, the promising but preliminary results presented here should serve as the stimulus and foundation for the continued study of multimodal therapy in the treatment of locally recurrent, previously irradiated breast cancer, especially as the current treatment of choice for these poor prognosis patients is almost always symptomatic relief and palliative care only.

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