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# Image-guided targeted doxorubicin delivery using thermosensitive liposomes and hyperthermia to optimize loco-regional control in breast cancer; study protocol of the phase I i-GO feasibility study

# Phase I Feasibility Study of High-Intensity Focused Ultrasound-Induced Hyperthermia, Lyso-Thermosensitive Liposomal Doxorubicin, and Cyclophosphamide in Metastatic Breast Cancer



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**Title**

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Phase I Feasibility Study of High-Intensity Focused Ultrasound-Induced Hyperthermia, Lyso-Thermosensitive Liposomal Doxorubicin, and Cyclophosphamide in Metastatic Breast Cancer

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## Abstract

### Introduction

In breast cancer, local tumour control is thought to be optimized by administering higher local levels of cytotoxic chemotherapy, in particular doxorubicin. However, systemic administration of higher dosages of doxorubicin is hampered by its toxic side effects. In this study, we aim to increase doxorubicin deposition in the primary tumour without changing systemic doxorubicin concentration and thus without interfering with systemic efficacy and toxicity. This is to be achieved by combining lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®, Celsion Corporation, Lawrenceville, NJ, USA) with mild local hyperthermia, induced by Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU). When heated above 39.5 °C, LTLD releases a high concentration of doxorubicin intravascularly within seconds. In absence of hyperthermia, LTLD leads to a similar biodistribution and antitumour efficacy compared to conventional doxorubicin.

### Methods and analysis

This is a single-arm phase I study in 12 chemotherapy-naïve patients with *de novo* stage IV HER2-negative breast cancer. Previous endocrine treatment is allowed. Study treatment consists of up to 6 cycles of LTLD at 21-day intervals, administered during MR-HIFU induced hyperthermia to the primary tumour. We will aim for 60 minutes of hyperthermia at 40-42 °C using a dedicated MR-HIFU breast system (Profound Medical, Mississauga, Canada). Afterwards, intravenous cyclophosphamide will be

administered. Primary endpoints are safety, tolerability and feasibility. The secondary endpoint is efficacy, assessed by radiological response.

**Future impact**

This approach could lead to optimal loco-regional control with less extensive or even no surgery, in *de novo* stage IV patients and in stage II/III patients allocated to receive neo-adjuvant chemotherapy.

**Ethics and dissemination**

This study has obtained ethical approval by the Medical Research Ethics Committee UMC Utrecht (Protocol NL67422.041.18, METC number 18-702). Informed consent will be obtained from all patients before study participation. Results will be published in an academic journal.

**Trial registration number**

NCT03749850, EudraCT 2015-005582-23.

**Keywords**

High Intensity Focused Ultrasound,

MR-HIFU

ThermoDox

Lyso-thermosensitive liposomal doxorubicin (LTLD)

Temperature sensitive liposome

Targeted drug delivery

Hyperthermia

Image-guided therapy

De novo stage IV breast cancer

Synchronous stage IV breast cancer

Metastatic breast cancer

**Strengths and limitations**

- This first in human clinical trial investigates the combination of Lyso-Thermosensitive Liposomal Doxorubicin and Magnetic Resonance guided High Intensity Focused Ultrasound induced hyperthermia in breast cancer patients.
- A dedicated MR-HIFU breast system with real-time MR temperature feedback will be used for safe non-invasive local hyperthermia treatment of breast tumours.
- Because the study population consists of patients with *de novo* stage IV breast cancer, both local and systemic response to the treatment can be monitored.
- A survival benefit of treating the primary tumour in patients with metastatic breast cancer has not been proven, therefore study participants will participate altruistically in the interest of future patients.
- This approach could lead to improved local control during palliative chemotherapy in *de novo* stage IV breast cancer or neoadjuvant chemotherapy in stage II/III disease, with less extensive or even no surgery.

## Introduction

Both neo-adjuvant and adjuvant chemotherapy of breast cancer aim to improve survival by eradicating microscopic distant metastases. In addition, neo-adjuvant treatment offers the opportunity to observe the biological behaviour of the primary tumour and increase the likelihood of less extensive radical (breast conserving) surgery. Given the fact that pathological complete response (pCR) is achieved at best in 68% of patients [1], efforts should be focused on improving primary tumour response. This may be achieved by increasing the dose of chemotherapy at the site of the tumour. In pre-clinical data, a higher concentration of chemotherapy in the tumour is correlated with increased tumour response, in particular for doxorubicin, one of the most frequently applied cytostatics in breast cancer treatment [2, 3]. However, the administration of higher doses of doxorubicin is hampered by its systemic side effects. In the i-GO study we aim to increase doxorubicin levels in the primary tumour, without interfering with systemic efficacy and toxicity, by combining lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®; Celsion Corporation, Lawrenceville, NJ, USA) with mild local hyperthermia, induced by Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU). This will be followed by the intravenous administration of a second cytostatic agent, cyclophosphamide. The combined administration of doxorubicin and cyclophosphamide (AC) is a well-known regimen in the standard of care treatment in both the (neo-) adjuvant setting as in the treatment of metastatic breast cancer.

The i-GO study will be a phase I feasibility study in stage IV breast cancer patients who present with distant metastases and a primary tumour in situ (*de novo* stage IV patients). Several studies suggest that by obtaining loco-regional control in metastatic breast cancer, overall survival in advanced disease will be improved [4, 5]. However, randomized controlled trials have shown conflicting results [6, 7]. As such it remains a subject of continuous research.

Lyso-thermosensitive liposomal doxorubicin

LTLD is a temperature-sensitive liposomal encapsulation of doxorubicin. Doxorubicin is a cytotoxic (chemotherapy) agent that is approved and frequently used for the treatment of a wide range of cancers, including breast cancer. When heated to 40-42 °C, LTLD releases the encapsulated doxorubicin intravascularly within seconds [8-10]. (Figure 1.) In small animal tumour models, LTLD combined with hyperthermia results in a 3-25 fold higher tumour concentration than conventional doxorubicin [2, 11-15] and increased antitumour efficacy [2, 9, 11]. In the absence of hyperthermia, doxorubicin leaks slowly from the liposome, and after two hours all of the doxorubicin is released [8]. Furthermore, LTLD without hyperthermia leads to a similar biodistribution [12, 13] and antitumour efficacy [9, 11] compared to conventional doxorubicin.

Magnetic resonance-guided high intensity focused ultrasound

MR-HIFU is a truly non-invasive treatment modality, that combines magnetic resonance imaging (MRI) and high intensity focused ultrasound to perform image-guided thermal tissue ablation (55-70 °C) [16-18] or mild local hyperthermia (40-43

°C) [19-21]. Unlike other heating methods, using microwaves, radiofrequency or non-focused ultrasound, HIFU allows for non-invasive localized heating of deep-seated tumours [22]. In addition to treatment planning based on anatomical MRI, MR-guidance can provide temperature feedback and control during hyperthermia treatment, through real-time MR-thermometry. For this study we will use a dedicated MR-HIFU breast system: the Sonalleve MR-HIFU breast tumour therapy system (hereafter referred to as 'MR-HIFU breast system', Profound Medical, Mississauga, Canada), integrated with a clinical 1.5 Tesla MR scanner (Achieva, Philips Healthcare, Best, The Netherlands). This system has a lateral sonication approach, which enables specific heating of the breast tumour, while reducing the risk of heating the skin or other organs to a minimum [23]. A phase I study in our hospital with MR-HIFU ablation of breast tumours showed that the MR-HIFU breast system allows for safe, accurate and precise thermal ablation [24, 25].

Previous clinical studies

This will be the first-in-human study to evaluate LTLD with MR-HIFU hyperthermia in breast cancer patients. LTLD has been studied previously in combination with superficial hyperthermia in patients with chest wall recurrences of breast cancer [26]. This phase I/II study showed that LTLD at 40 mg/m$^2$ with superficial hyperthermia was safe and the 48% overall response (14/29, 95% CI:30–66%) was promising in this heavily pre-treated population. A large randomized phase III study in 701 patients with hepatocellular carcinoma compared LTLD at 50 mg/m$^2$ with radiofrequency ablation (RFA) to RFA alone (the HEAT study) [27, 28]. In that study the primary

endpoint of 33% improvement in progression free survival was not met. However, a post-hoc analysis in the subgroup of 285 patients with solitary lesions that were treated with ≥45 min of RFA showed a significant overall survival benefit for the combination treatment (Hazard Ratio for Overall Survival 0.63 (95% CI, 0.41–0.96; P < 0.05), in favour of RFA+LTLD with ≥45 minutes heating). Systemic adverse events increased in the RFA+LTLD arm (83% vs 35% with RFA alone) as expected, with a similar profile to that of conventional doxorubicin [28].

Furthermore, the combination of LTLD and ultrasound guided HIFU hyperthermia has been evaluated in a phase I proof-of-concept study in ten patients with incurable primary or metastatic liver tumours (the TARDOX study) [29, 30]. Adverse events did not differ from those associated with doxorubicin alone and in the group of patients that underwent invasive thermometry sufficient mean tumour temperatures were measured. In seven out of ten patients, the intratumoural doxorubicin concentration doubled after HIFU, although a within-patient comparison was not possible for all patients. We aim to take advantage of the same principle to treat the primary tumour in patients presenting with metastatic breast cancer. Monitoring the treatment by MR-thermometry may further enhance safety, efficacy and feasibility. Using multiple cycles of LTLD + MR-HIFU hyperthermia is expected to increase treatment efficacy and mimics the standard of care treatment.

## Methods and analysis

This single-arm phase I feasibility study aims to determine the safety, tolerability and feasibility of the combination of LTLD, MR-HIFU induced mild local hyperthermia, and

cyclophosphamide, for the enhanced local treatment of the primary tumour in patients presenting with metastatic breast cancer. All eligible participants will receive up to 6 cycles of LTLD at 21-day intervals, administered during MR-HIFU induced hyperthermia to the primary tumour and cyclophosphamide administered afterwards.

Patient population

We will include 6 or 12 adult female patients with *de novo* stage IV (distant metastases at the time of diagnosis, with the primary tumour in situ) HER2-negative breast cancer, who have not received previous chemotherapy for their disease. Previous endocrine treatment in those with hormone-receptor positive disease is allowed. Potentially eligible patients will be referred to the department of Medical Oncology at the University Medical Center Utrecht, The Netherlands.

Inclusion criteria

Patients must meet all of the following inclusion criteria:

- Histologically confirmed adenocarcinoma of the breast and planned for palliative chemotherapy with doxorubicin and cyclophosphamide.
- Biopsy-proven stage T1-2AnyNM1 at diagnosis of breast cancer.
- Measurable disease according to either RECIST 1.1 or PERCIST 1.0 at baseline.
- Non-pregnant, non-lactating female at least 18 years of age. If the patient is of child-bearing age, she must have a negative serum pregnancy test prior to enrolment and must agree to practice an acceptable form of birth control while on study.
- The tumour is located within the reach of the HIFU beam (based on pre-treatment dynamic contrast-enhanced (DCE-) MRI findings).
- The distance of the tumour from the skin, nipple, and pectoral wall is at least 1.0 cm (based on pre-treatment DCE-MRI findings).

- The target breast is expected to fit in the cup of the MR-HIFU breast system (based on pre-treatment MRI findings).
- The patient is able to provide written informed consent and willing to comply with protocol requirements.

Exclusion criteria

Patients will be excluded if any of the following conditions are observed:

- HER2-positive disease or classic invasive lobular carcinoma (ILC).
- A treatment plan with curative intent is available.
- Any prior chemotherapy treatment for invasive breast cancer (previous anti-hormonal therapy is allowed).
- Any prior therapy with anthracyclines.
- The patient weighs ≥90 kg (restriction of the HIFU table top).
- Any concomitant malignancy or previous malignancy in the last 5 years, except basal cell or squamous cell cancer of the skin or in situ carcinoma of the cervix. Subjects with a prior contralateral breast malignancy more than 5 years ago can be included if they did not receive any chemotherapy.
- Any previous malignancy in the unilateral breast (even if more than 5 years ago)
- Prior sensitivity (including rash, dyspnoea, wheezing, urticarial, or other symptoms) attributed to any liposomal-encapsulated drug.
- Baseline laboratory values:

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| Absolute Neutrophil Count (ANC) | < 1.5 x 10^9/L |
| Platelets | < 75 x 10^9/L |
| Haemoglobin | < 5.6 mmol/L (transfusion is allowed) |
| Total Bilirubin | > 1.5 times upper limit of normal |
| Alanine Transaminase (ALAT) and Aspartate Transaminase (ASAT) | > 2.5 times upper limit of normal |

>5 times upper limit of normal in case of liver metastases

Estimated Glomerular Filtration Rate < 30 ml/min/1.73m$^2$

- World Health Organization Performance Status (WHO-PS) >2.
- Left Ventricular Ejection Fraction <50% (validated by baseline scan).
- History of: acute coronary syndrome in the last year, cerebral vascular accident in the last year, abnormal cardiac stress testing within the last six months, symptomatic coronary artery disease, uncontrolled hypertension or cardiomyopathy, cardiac valvular surgery or open-heart surgery in the last year or known structural heart disease.
- Any condition which may interfere with the hyperthermia portion of the trial such as: functioning cardiac pacemaker; metal plates, rods or prosthesis of the chest wall, breast prosthesis in the treated breast, severe numbness and/or tingling of the chest wall or breast, skin grafts and/or flaps on the breast or chest wall, scar tissue or surgical clips in the HIFU beam path.
- Active infection.
- Body temperature > 38.0 degrees Celsius on the day of a MR-HIFU treatment.
- Concurrent use of any of the following prohibited medications within a reasonable wash-out time: protease inhibitors, cyclosporine, carbamazepine, phenytoin, valproic acid, paclitaxel, trastuzumab and other liposomal drugs (AbelectTM, Ambisome™, NyotranTM, etc.) or lipid-complexed drugs. Caution will be exercised with medications, dietary components and herbal supplements that affect CYP2A4, CYP2D6 or P-gp or have been described to interact with doxorubicin in other ways.
- Contraindications to MR imaging (e.g., pacemaker in situ, severe claustrophobia, metal implants incompatible with the MRI-scan, body size incompatible with MR bore).
- Contraindications to gadolinium-based contrast agents, including prior allergic reaction to gadolinium-based contrast agent, and/or renal failure.

- Contraindications to sedation and analgesia with Propofol and Remifentanil, including history of Chronic Obstructive Pulmonary Disease (COPD) that results in the inability to perform a physical activity corresponding with a Metabolic Equivalent (MET(57)) of 4; dependence on artificial ventilation at home; sleep apnoea or an American Society of Anaesthesiologists (ASA) classification ≥4.
- Inability to lie in prone position.
- A medical or psychiatric condition or other circumstances which would significantly decrease the chances of understanding the informed consent process, obtaining reliable data, achieving study objectives, or completing the study treatment and/or examinations.

Endpoints

Primary endpoints are safety, tolerability and feasibility. These will be evaluated by the following assessments.

Safety and tolerability:

- Incidence and severity of Adverse Events and Severe Adverse Events
- Incidence of Dose Limiting Toxicity (DLT, systemic and loco-regional)
- Necessity for dose adjustments, delay and early cessation
- Incidence and severity of post-procedural pain
- Patient reported tolerability (questionnaires)
- Cardiotoxicity: Left Ventricular Ejection Fraction measurement and electrocardiogram abnormalities.

Feasibility:

- The number of cycles in which hyperthermia treatment was sufficient: at least 30 minutes at the target temperature of 40-42 °C.

- The number of completed cycles with MR-HIFU induced hyperthermia, LTLD and cyclophosphamide
- Quality of MR thermometry data acquired during the MR-HIFU treatment
- Spatiotemporal temperature distribution in the tumour
- Total duration of the study procedures on a treatment day.

Secondary endpoints consist of efficacy parameters:

- Assessment of distant radiological objective response rates
- Assessment of local radiological objective response rates

Study procedures

The study design (Figure 2) was based on the AC regimen, a well-known chemotherapeutic regimen that consists of doxorubicin and cyclophosphamide. This regimen is used in the (neo-)adjuvant setting as well as in the first-line chemotherapy treatment of metastatic breast cancer. Standard of care for our study population consists of 6 cycles at 21-days intervals. In this study we will replace doxorubicin in this regimen with the combination of LTLD and MR-HIFU induced hyperthermia. All participants will receive procedural sedation and analgesia with propofol and remifentanil to limit patient movement during the treatment and to establish a regular breathing pattern that will facilitate respiratory gated MR-thermometry [31]. To prevent any hypersensitivity reactions to LTLD, the participants will also receive a premedication regimen of steroids, H1- and H2- histamine antagonists.

MR-HIFU hyperthermia will be performed on the MR-HIFU breast system, with the patient in prone position. We will aim for 60 minutes of hyperthermia at 40-42 °C to

the breast tumour, in four blocks of 15 minutes. After each block the MR thermometry is restarted to minimize the possible influence of magnetic field drift or patient displacement. When MR thermometry indicates that the target temperature is reached, 50 mg/m$^2$ of LTLD will be administered intravenously over 30 minutes, via a peripherally inserted central catheter (PICC), while the patient is on the MR-HIFU breast system. Temperature will be monitored by respiratory navigator-gated MR-thermometry, using the proton resonance frequency shift method [32, 33]. In case the target temperature is not reached, conventional doxorubicin (60 mg/m$^2$) will be administered instead of LTLD. Shortly after MR-HIFU, 600 mg/m$^2$ of cyclophosphamide will be administered intravenously according to standard of care in the AC regimen.

Participants will receive up to six treatment cycles. Feasibility will be evaluated after each MR-HIFU treatment and during the course of the cycles. Safety and tolerability will be assessed three hours after MR-HIFU treatment, during telephone contact on day +1 and +7 and during a hospital visit on day +14 and +21 of each cycle, by monitoring of adverse events, laboratory measurements and evaluation of pain. Cardiotoxicity evaluations (LVEF and ECG) will be performed at baseline, after cycle 3 and after cycle 6. The participants will be asked to fill out the Dutch version of the Functional Assessment of Cancer Therapy – Breast (FACT-B, version 4, FACIT)[34] at baseline and after each treatment cycle, combined with a selection of questions adapted from the Dutch version of the Cancer Therapy Satisfaction Questionnaire (CTSQ, Pfizer 2007, modified with permission from Pfizer)[35, 36] in cycles 3 and 6.

Before starting the next cycle, any toxicities will be evaluated and if necessary, dose adjustments will be made. DLT will be categorized in systemic or loco-regional toxicity (Table 1). Thus, we aim to distinguish systemic chemotherapy effects from local effects of MR-HIFU hyperthermia and/or the high local doxorubicin concentration. Planned dose adjustments for these categories have been established. In case of a systemic DLT the LTLD dosage will be decreased, while for loco-regional DLT the duration of hyperthermia will be decreased. No dose increases will be performed. Depending on the severity and nature of the toxicity, study treatment can be delayed or even ceased. In case of solely loco-regional DLT, technical issues or other feasibility issues that restrict the use of MR-HIFU treatment, the participant will receive the standard of care AC regimen. If hyperthermia is insufficient (i.e. the target temperature of 40-42 °C is not reached or was only maintained for less than 30 minutes) in two separate cycles, the treatment is not considered feasible for that patient and study participation will end.

For the secondary endpoint of efficacy, MRI of the breast will be performed using a 3 Tesla MRI scanner with a dedicated breast coil, at baseline and after cycle 2 and 6 to determine local radiological objective response. In addition, MRI of the breast will be performed during each MR-HIFU treatment. However, the receiver coil in the MR-HIFU breast system is not suited for clinical imaging. In case a complete radiological response of the breast tumour is obtained after less than 6 cycles, the patient will continue with the conventional AC regimen. $^{18}$F -fluorodeoxyglucose (FDG-) Positron Emission Tomography combined with Computed Tomography (PET/CT) of the thorax

and abdomen will be performed at baseline and CT or PET/CT after cycle 2 and cycle 6, to determine the distant objective response according to RECIST 1.1 [37] or PERCIST 1.0 [38]. PET/CT will be performed for response evaluation in patients with only PERCIST-measurable disease, such as patients with only bone metastases.

The participants will be followed for adverse events from the time of signing informed consent until the end of study visit after six cycles of chemotherapy. Afterwards patients will receive standard of care treatment.

If the patient consents to the biobank study, additional blood samples will be taken from the PICC-line at seven time points (Figure 2) when the patient is already at the hospital. These blood samples will be collected in the UMC Utrecht Biobank for future research. Moreover, in case tissue samples of the breast tumour and/or metastases were obtained in standard care before inclusion or following study participation, we will ask for consent to perform additional analyses on these samples.

Interim analysis

An interim analysis of safety and efficacy will determine whether accrual will continue after six participants (Supplementary materials 1). Safety will be evaluated once the first six patients complete two treatment cycles. If safety is sufficiently proven or is deemed inadequate, the trial will end after six participants. Otherwise accrual will continue until twelve patients have been treated, if necessary after dose adjustments. Systemic efficacy will be evaluated once the first six patients have received the CT scan after cycle 2. If four or more of the first six participants show distant disease progression at that time the trial will be stopped, as this suggests that efficacy against

disease outside the heated treatment field is inadequate. This early stopping rule was based on a phase III trial with liposomal doxorubicin in metastatic breast cancer [39] where 77.5% of the subjects were free of disease progression at two months post-randomization (the 95% confidence interval of 2/6 patients does not contain 0.775). An independent, qualified monitor will monitor the study procedures. An external Data Safety Monitoring Board (DSMB) will review accumulating safety data at regular intervals throughout the study, perform the interim safety and efficacy analyses and monitor trial data integrity.

Data analysis

Descriptive statistics will be used to describe the incidence and severity of adverse events (National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0), the patient reported outcomes in the questionnaires and feasibility parameters including the number of completed study treatment cycles, duration of study procedures and spatiotemporal temperature distribution during MR-HIFU treatment. For the secondary endpoint of efficacy, distant and local radiological objective response rates (RECIST 1.1) will be described.

## Discussion

This is the first clinical trial that investigates the combination of LTLD and MR-HIFU induced hyperthermia in breast cancer. In a small number of patients we will focus primarily on safety, tolerability and feasibility of this procedure. We hypothesize that the combination of LTLD and MR-HIFU hyperthermia leads to improved treatment of

the primary tumour, without changing the systemic doxorubicin concentration and thus without interfering with systemic efficacy and toxicity. A future randomized study with a control group receiving the standard of care AC regimen would be needed to prove this. Including patients with *de novo* stage IV breast cancer provides the unique possibility to monitor both local and systemic disease simultaneously. While in this setting a survival benefit of treating the primary tumour has not been proven, the study treatment (if proven safe and feasible) could in the future improve outcomes in the neoadjuvant setting.

We aim to replace doxorubicin by LTLD plus MR-HIFU hyperthermia in all six cycles of the AC regimen, because we expect this to maximize the local treatment effect. In each cycle, the feasibility to achieve tumour hyperthermia at 40-42 °C for 30 minutes will be verified with MR-thermometry. If hyperthermia treatment is repeatedly insufficient, or if (after any number of cycles) radiological complete response is already obtained, patients will continue on the standard-of-care AC regimen. The number of MR-HIFU hyperthermia plus LTLD cycles that our patients are willing and able to complete could be less than six, which would be an important feasibility finding.

Our goal is to maintain an equivalent systemic efficacy compared to the standard-of-care AC regimen using 60 mg/m$^2$ conventional doxorubicin. Pharmacokinetic studies showed that the area-under the curve (AUC0-∞) of free/unencapsulated doxorubicin in plasma of patients receiving LTLD 50 mg/m$^2$ with local hyperthermia or RFA [26, 40, 41] was higher than the AUC0-∞ of conventional doxorubicin 60 mg/m$^2$ [42-44].

To be able to compare the AUCs we converted the AUC0-∞ of the metabolite doxorubicinol that was measured in the LTLD studies to the AUC0-∞ of doxorubicin [45-47] (Additional explanation in Supplementary materials 2). The 50 mg/m$^2$ LTLD dose was also recommended for and well-tolerated in the phase III trial in combination with RFA [28]. Due to local toxicity, the recommended dose for LTLD combined with local superficial hyperthermia for chest wall recurrences was decreased to 40 mg/m$^2$ [40]. In our study local (skin) toxicity is not expected because a margin of at least 1.0 cm is preserved from the tumour to the skin, therefore the LTLD dose of 50 mg/m$^2$ was chosen. Real time MR-thermometry and the lateral configuration of the MR-HIFU breast system will help mitigate this risk. If however local DLT do occur, the duration of hyperthermia will be decreased while maintaining the LTLD dosage to avoid decreasing systemic efficacy. We will only decrease LTLD dosage in case of systemic DLT. If despite these measures, systemic efficacy seems inadequate, the trial will be halted prematurely based on the interim analysis for efficacy.

Because this is a small phase I feasibility study, the results will only provide a rough indication of local efficacy based on radiological response. To diminish the burden on participants, we will not perform tissue biopsies or breast surgery and therefore cannot describe the number of pathological complete responses or measure the concentration of doxorubicin in the tumour. Proof-of-concept that hyperthermia increases the tumour doxorubicin concentration has already been established in the

Tardox study, although doxorubicin concentrations were not compared between heated and unheated tumours.

**Conclusions**

With this phase I clinical trial, we aim to show that LTLD combined with MR-HIFU induced hyperthermia on a dedicated MR-HIFU breast system can safely replace doxorubicin in the AC regimen. We hypothesize that this combination will result in improved response of the primary tumour without compromising the systemic efficacy on metastatic sites or increasing systemic toxicity. If feasibility and tolerability are adequate, this approach could in the future lead to optimal loco-regional control with less extensive or even no surgery, in stage II or III breast cancer patients allocated to receive neo-adjuvant chemotherapy. Finally, it could also be suitable for other doxorubicin sensitive tumour types that benefit from enhanced local treatment, such as soft tissue sarcoma.

**Word Count**

3768 words

**Ethics and dissemination**

This study has obtained ethical approval by the Medical Research Ethics Committee of the UMC Utrecht (METC Utrecht) on May 29th 2019 (Protocol NL67422.041.18, METC number 18-702). Informed consent will be obtained by all patients before

study participation. The results will be disseminated by publication in an academic peer-reviewed journal.

## Patient and public involvement

Patient experiences have been the starting point for the grant proposal to the Dutch Cancer Foundation and patients were involved in the design of the study and the choice of outcome measures. Patients will not be actively involved in recruitment or dissemination of study results, however information regarding the study can be found by individual patients on the UMC Utrecht website and clinicaltrials.gov.

## Trial status

Patient recruitment was initiated on March 10th 2020. On the submission date of this article, no patients had been enrolled yet. Due to the COVID-19 outbreak, the study has been temporarily discontinued. Recruitment will be resumed as soon as possible.

## Authors' contributions

JdM, BS, MB, SL, CM, EW and RD were all involved in the design of the study and in writing the manuscript.

PvD, HV and AW critically reviewed the design of the study providing additional comments and suggestions.

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**Competing interests statement**

The authors have no competing interest to declare.

**List of abbreviations**

| | |
|---|---|
| AC | Doxorubicin (A) and cyclophosphamide (C) |
| AF | Alkaline Phosphatase |
| ALAT | Alanine Transaminase |
| ANC | Absolute Neutrophil Count |
| ASAT | Aspartate Transaminase |
| CTSQ | Cancer Therapy Satisfaction Questionnaire |

| | | |
|---|---|---|
| DCE | Dynamic contrast-enhanced |
| DLT | Dose Limiting Toxicity |
| FACT-B | Functional Assessment of Cancer Therapy – Breast |
| LTLD | Lyso-Thermosensitive Liposomal Doxorubicin |
| MR-HIFU | Magnetic Resonance guided High Intensity Focused Ultrasound |
| MRI | Magnetic Resonance Imaging |
| (FDG-) PET/CT | $^{18}$F-Fluorodeoxyglucose Positron Emission Tomography combined with Computed Tomography |
| PICC | Peripherally inserted central catheter |
| RFA | Radiofrequency ablation |

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## Figures and tables

Figure 1 LTLD combined with MR-HIFU hyperthermia on the MR-HIFU breast system.

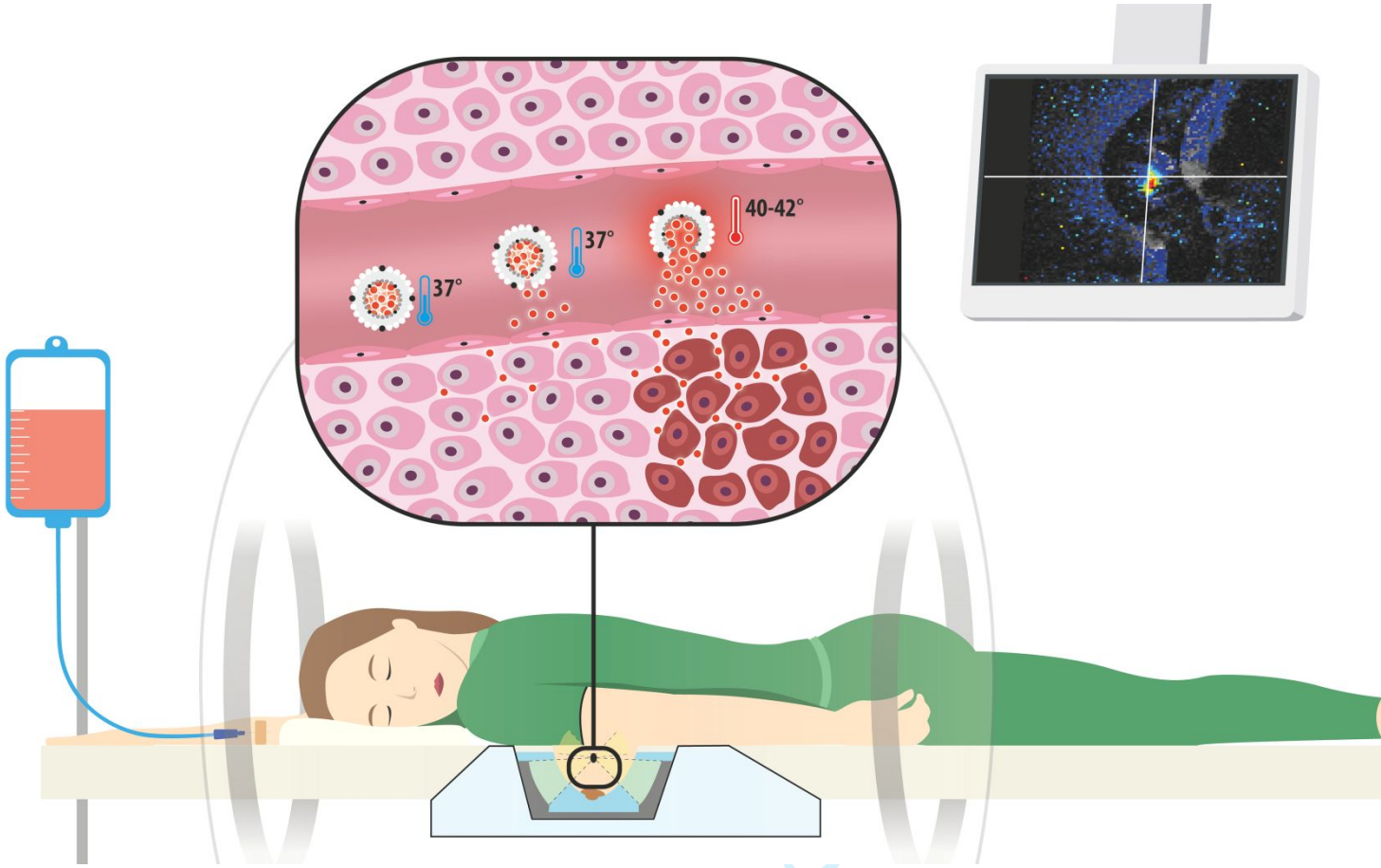
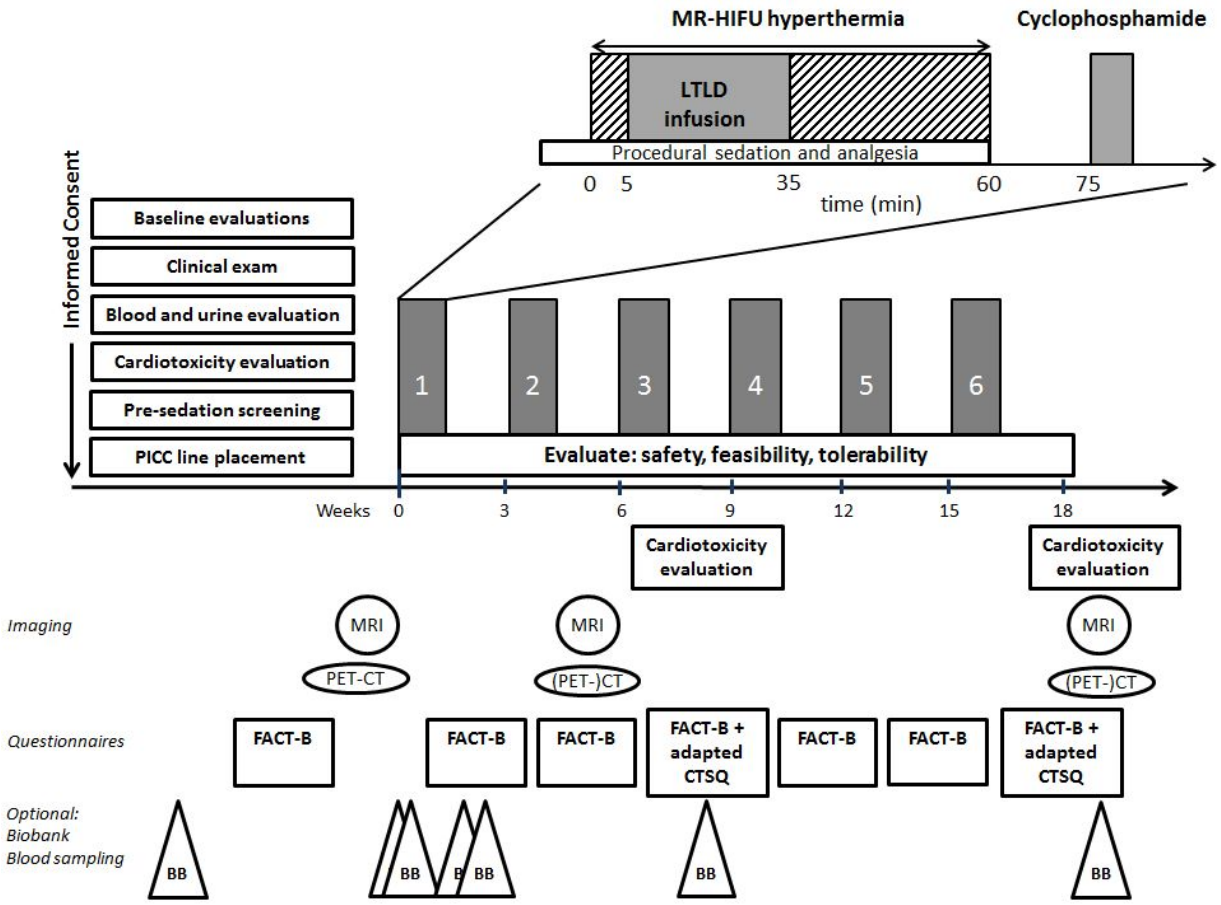


Figure 2 Study procedures



1 Table 1 Definitions of Dose Limiting Toxicity

| | |
|---|---|
| **Dose limiting systemic toxicity** | |
| A | *Hematologic DLT*<br>defined as Grade 3 anaemia, Grade 4 thrombocytopenia, febrile neutropenia, or Grade 4 neutropenia ≥ 7 days in duration. |
| B | *Non-hematologic DLT (non-loco-regional)*<br>defined as Grade 3 or greater toxicity with the exceptions of alopecia, fatigue, nausea or vomiting and loco-regional effects.<br>Including Cardiotoxicity DLT, defined as:<br>• Grade 3 or greater cardiac disorders OR<br>• a decline in LVEF of > 15% while the LVEF remains > 40% OR<br>• a decline to an LVEF of ≤ 40%. |
| **Dose limiting loco-regional toxicity** | |
| C | *Loco-regional DLT*<br>defined as post-procedural effects (e.g. pain or skin effects) on the treated breast warranting dose adjustment or delay. |

2

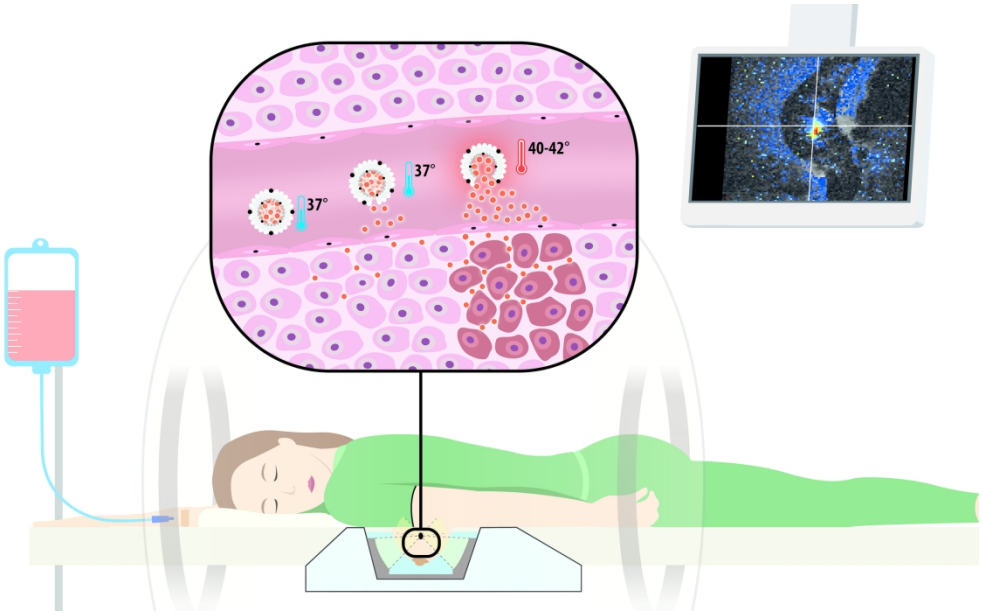



Figure 1 LTLD combined with MR-HIFU hyperthermia on the MR-HIFU breast system.

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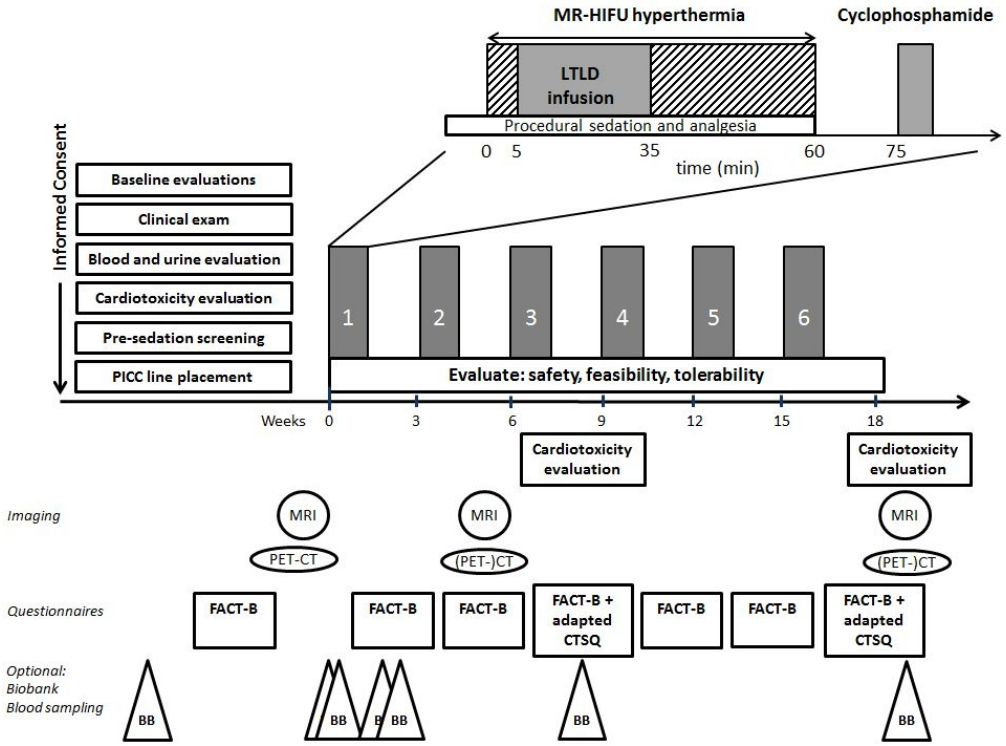


Figure 2 Study procedures

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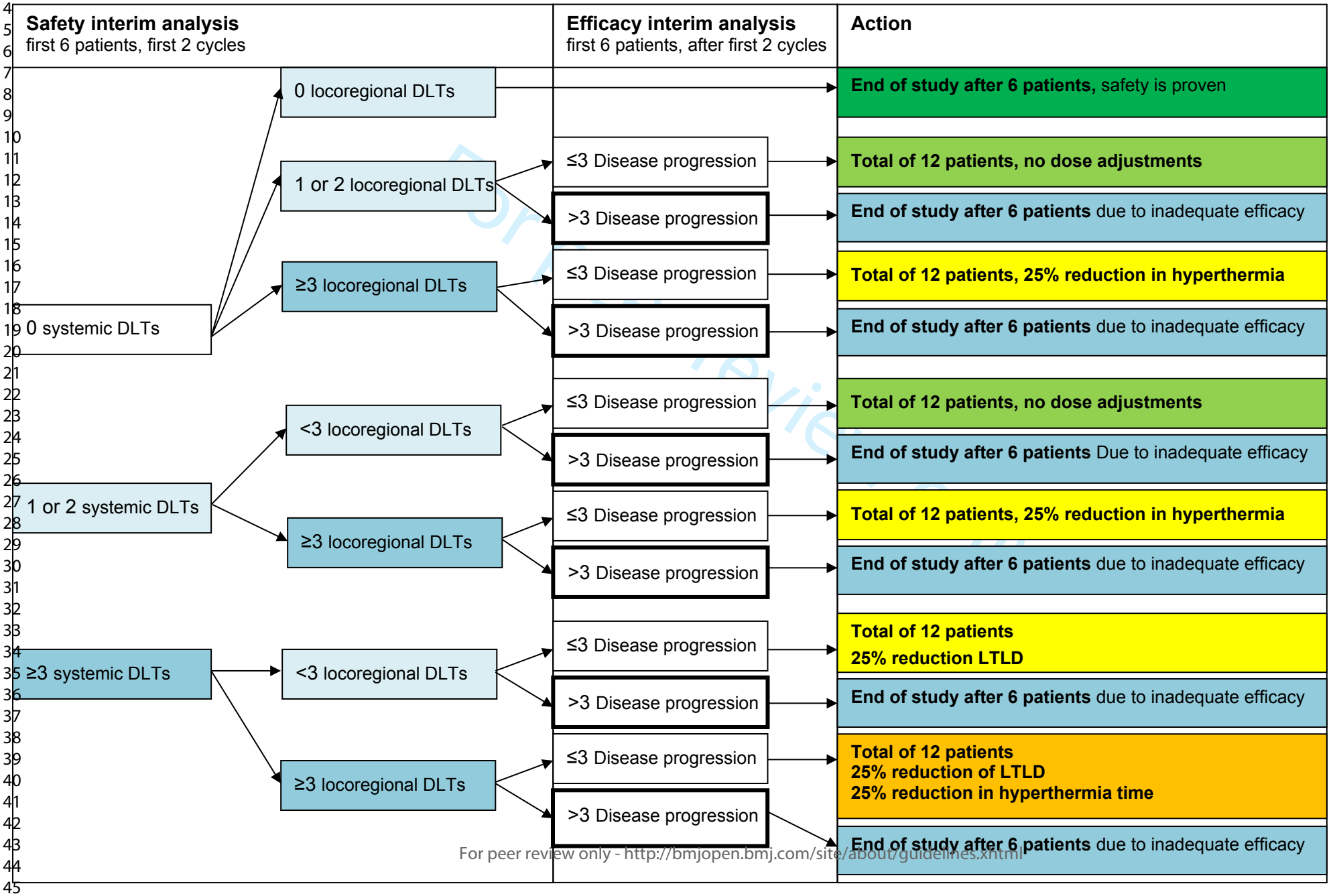
**Supplementary materials:**

**Supplement 1 Flow-chart interim analysis**

**Supplement 2 Comparison of AUC0-∞ of free doxorubicin for LTLD and conventional doxorubicin.**

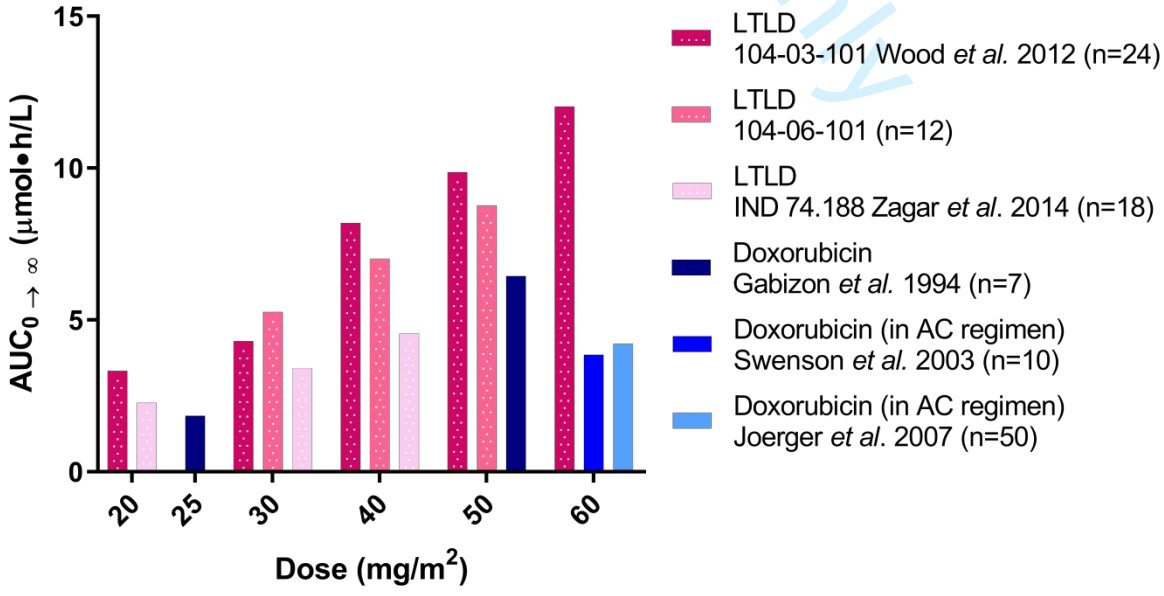
## Supplement 1 Flow-chart interim analysis



**Supplement 2 Comparison of AUC0-∞ of free doxorubicin for LTLD and conventional doxorubicin.**

In order to obtain a systemic dose of free doxorubicin (due to leakage of LTLD at 37 °C) that is as similar to conventional doxorubicin at 60 mg/m$^2$ (which is the standard of care treatment for the patients that will be enrolled in this study) as possible (to avoid undertreatment) we will start at the dose of 50 mg/m$^2$ LTLD, and we will apply dose adjustments when necessary. To compare the systemic dose of free doxorubicin after LTLD plus hyperthermia to the systemic dose of conventional doxorubicin, we summarized the pharmacokinetic data of the three studies with LTLD in which total plasma doxorubicin and the metabolite doxorubicinol were measured with a validated assay (studies 104-03-101 [1], 104-06-101 [2], and IND #174,188 [3]). In these studies the Area Under the Curve from t=0 to infinity (AUC0-∞) of the metabolite doxorubicinol was measured. Note that in these studies LTLD was administered with hyperthermia or RFA treatment. Pharmacokinetic data on LTLD without heating are not available. The mean values were converted to the AUC0-∞ of 'free doxorubicin' based on the mean ratios between doxorubicinol and doxorubicin found in three studies (0.3826, 0.47 and 0.514 respectively, with a mean of 0.456) [4-6]. We compared these AUC0-∞ values of 'free doxorubicin' from the LTLD studies with the AUC0-∞ values of doxorubicin in pharmacokinetic studies of conventional doxorubicin [7-9]. Figure S2 displays the AUC0-∞ of three studies with conventional doxorubicin (actual doxorubicin values are portrayed) and the AUC0-∞ of three studies with LTLD (calculated 'free doxorubicin' values are portrayed). The figure shows that the calculated 'free doxorubicin' after LTLD 50 mg/m$^2$ is at least equal to that of conventional doxorubicin at 60 mg/m$^2$.

**Figure S2: Comparison of the AUC0-∞ of "free" plasma doxorubicin for LTLD + heat (calculated based on doxorubicinol concentration) and conventional doxorubicin.**



## Supplementary References

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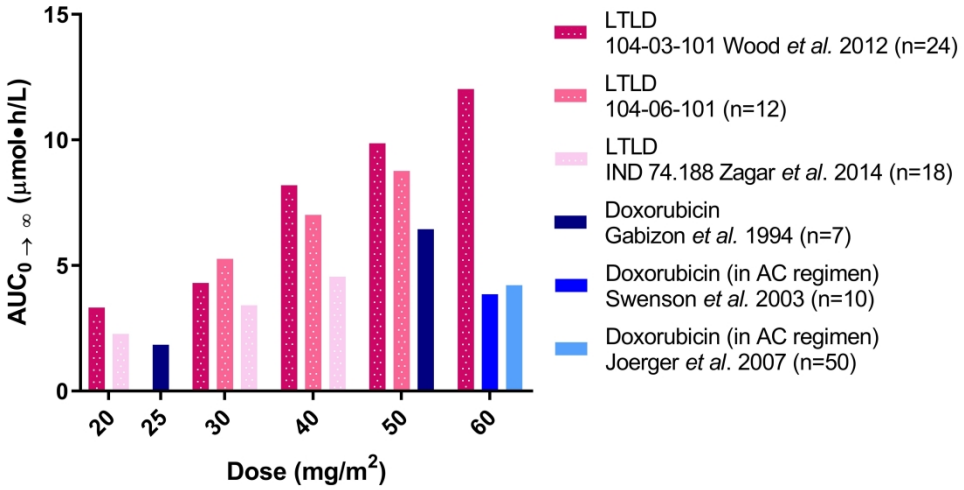


168x90mm (600 x 600 DPI)

BMJ Open

# Study protocol of the i-GO study, a phase I feasibility study of Magnetic Resonance guided High-Intensity Focused Ultrasound-induced hyperthermia, Lyso-Thermosensitive Liposomal Doxorubicin and cyclophosphamide in de novo stage IV breast cancer patients.



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| Secondary Subject Heading: | Radiology and imaging |
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**Title**

Study protocol of the i-GO study, a phase I feasibility study of Magnetic Resonance guided High-Intensity Focused Ultrasound-induced hyperthermia, Lyso-Thermosensitive Liposomal Doxorubicin and cyclophosphamide in de novo stage IV breast cancer patients.

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**Abstract**

**Introduction**

In breast cancer, local tumour control is thought to be optimized by administering higher local levels of cytotoxic chemotherapy, in particular doxorubicin. However, systemic administration of higher dosages of doxorubicin is hampered by its toxic side effects. In this study, we aim to increase doxorubicin deposition in the primary breast tumour without changing systemic doxorubicin concentration and thus without interfering with systemic efficacy and toxicity. This is to be achieved by combining lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®, Celsion Corporation, Lawrenceville, NJ, USA) with mild local hyperthermia, induced by Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU). When heated above 39.5 °C, LTLD releases a high concentration of doxorubicin intravascularly within seconds. In absence of hyperthermia, LTLD leads to a similar biodistribution and antitumour efficacy compared to conventional doxorubicin.

**Methods and analysis**

This is a single-arm phase I study in 12 chemotherapy-naïve patients with *de novo* stage IV HER2-negative breast cancer. Previous endocrine treatment is allowed. Study treatment consists of up to 6 cycles of LTLD at 21-day intervals, administered during MR-HIFU induced hyperthermia to the primary tumour. We will aim for 60 minutes of hyperthermia at 40-42 °C using a dedicated MR-HIFU breast system (Profound Medical, Mississauga, Canada). Afterwards, intravenous cyclophosphamide will be

administered. Primary endpoints are safety, tolerability and feasibility. The secondary endpoint is efficacy, assessed by radiological response.

This approach could lead to optimal loco-regional control with less extensive or even no surgery, in *de novo* stage IV patients and in stage II/III patients allocated to receive neo-adjuvant chemotherapy.

**Ethics and dissemination**

This study has obtained ethical approval by the Medical Research Ethics Committee Utrecht (Protocol NL67422.041.18, METC number 18-702). Informed consent will be obtained from all patients before study participation. Results will be published in an academic peer-reviewed journal.

**Trial registration number**

NCT03749850, EudraCT 2015-005582-23.

**Keywords**

High Intensity Focused Ultrasound,

MR-HIFU

ThermoDox

Lyso-thermosensitive liposomal doxorubicin (LTLD)

Temperature sensitive liposome

Targeted drug delivery

Hyperthermia

Image-guided therapy

De novo stage IV breast cancer

Synchronous stage IV breast cancer

Metastatic breast cancer

**Strengths and limitations**

- This first in human clinical trial investigates the combination of Lyso-Thermosensitive Liposomal Doxorubicin and Magnetic Resonance guided High Intensity Focused Ultrasound induced hyperthermia in breast cancer patients.
- A dedicated MR-HIFU breast system with real-time MR temperature feedback will be used for safe non-invasive local hyperthermia treatment of breast tumours.
- Because the study population consists of patients with *de novo* stage IV breast cancer, both local and systemic response to the treatment can be monitored.
- A survival benefit of treating the primary tumour in patients with metastatic breast cancer has not been proven, therefore study participants will participate altruistically in the interest of future patients.
- This approach could lead to improved local control during palliative chemotherapy in *de novo* stage IV breast cancer or neoadjuvant chemotherapy in stage II/III disease, with less extensive or even no surgery.

## Introduction

Both neo-adjuvant and adjuvant chemotherapy of breast cancer aim to improve survival by eradicating microscopic distant metastases. In addition, neo-adjuvant treatment offers the opportunity to observe the biological behaviour of the primary tumour and increase the likelihood of less extensive radical (breast conserving) surgery. Given the fact that pathological complete response (pCR) is achieved at best in 68% of patients [1], efforts should be focused on improving primary tumour response. This may be achieved by increasing the dose of chemotherapy at the site of the tumour. In pre-clinical data, a higher concentration of chemotherapy in the tumour is correlated with increased tumour response, in particular for doxorubicin, one of the most frequently applied cytostatics in breast cancer treatment [2-4]. Clinically this was confirmed by studies using other chemotherapeutics, i.e. 5-fluorouracil and docetaxel. Higher tumour uptake of radio-active labelled 5-fluorouracil or docetaxel chemotherapy on PET was shown to correlate respectively with longer survival in patients with liver metastasis of colorectal carcinoma [5] and with better tumour response in lung cancer patients [6]. In a study comparing different dose schedules of the adjuvant AC regimen, the highest dosages (60mg/m$^2$ doxorubicin and 600mg/m$^2$ cyclophosphamide) were most effective, and this is currently the standard of care [7]. However, the administration of higher doses of doxorubicin is hampered by its systemic side effects. A randomized study evaluating even higher doxorubicin dosages (60mg/m$^2$ versus 75mg/m$^2$ and 90 mg/m$^2$) did not find a difference in disease-free or overall survival. However, the higher dose levels

did lead to significantly more dose reductions and delays, which could explain why the efficacy did not increase further [8]. In the i-GO study we aim to increase doxorubicin levels in the primary tumour, without interfering with systemic efficacy and toxicity, by combining lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®; Celsion Corporation, Lawrenceville, NJ, USA) with mild local hyperthermia, induced by Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU). This will be followed by the intravenous administration of a second cytostatic agent, cyclophosphamide. The combined administration of doxorubicin and cyclophosphamide (AC) is a well-known regimen in the standard of care treatment in both the (neo-) adjuvant setting as in the treatment of metastatic breast cancer.

The i-GO study will be a phase I feasibility study in stage IV breast cancer patients who present with distant metastases and a primary tumour in situ (*de novo* stage IV patients). Several studies have suggested that by obtaining loco-regional control in metastatic breast cancer, overall survival in advanced disease would be improved [9-11] However, randomized controlled trials have contradicted this [12, 13] A recent presentation at ASCO 2020 [14] confirmed that local treatment in addition to systemic therapy did not improve survival. As such, besides a personal preference of the patient and the possibility of preventing local morbidity, study participation will not have a benefit compared to the standard of care. However, based on pharmacokinetic studies (details outlined in Supplementary materials 1) we do expect at least an equally effective treatment. Study participants will participate altruistically

in the interest of future patients in the neoadjuvant setting. In the future, the combination of LTLD, MR-HIFU hyperthermia, and cyclophosphamide may lead to improved local control during neoadjuvant chemotherapy in stage II/III disease, with less extensive or even no surgery.

Lyso-thermosensitive liposomal doxorubicin

LTLD is a temperature-sensitive liposomal encapsulation of doxorubicin. Doxorubicin is a cytotoxic (chemotherapy) agent that is approved and frequently used for the treatment of a wide range of cancers, including breast cancer. When heated to 40-42 °C, LTLD releases the encapsulated doxorubicin intravascularly within seconds [15-17]. (Figure 1.) In small animal tumour models, LTLD combined with hyperthermia results in a 3-25 fold higher tumour concentration than conventional doxorubicin [2, 18-22] and increased antitumour efficacy [2, 16, 18]. In the absence of hyperthermia, doxorubicin leaks slowly from the liposome, and after two hours all of the doxorubicin is released [15]. Furthermore, LTLD without hyperthermia leads to a similar biodistribution [19, 20] and antitumour efficacy [16, 18] compared to conventional doxorubicin.

Magnetic resonance-guided high intensity focused ultrasound

MR-HIFU is a truly non-invasive treatment modality, that combines magnetic resonance imaging (MRI) and high intensity focused ultrasound to perform image-guided thermal tissue ablation (55-70 °C) [23-25] or mild local hyperthermia (40-43 °C) [26-28]. Unlike other heating methods, using microwaves, radiofrequency or non-focused ultrasound, HIFU allows for non-invasive localized heating of deep-seated

tumours [29]. In addition to treatment planning based on anatomical MRI, MR-guidance can provide temperature feedback and control during hyperthermia treatment, through real-time MR thermometry. For this study we will use a dedicated MR-HIFU breast system: the Sonalleve MR-HIFU breast tumour therapy system (hereafter referred to as 'MR-HIFU breast system', Profound Medical, Mississauga, Canada), integrated with a clinical 1.5 Tesla MR scanner (Achieva, Philips Healthcare, Best, The Netherlands). This system has a lateral sonication approach, which enables specific heating of the breast tumour, while reducing the risk of heating the skin or other organs to a minimum [30]. A phase I study in our hospital with MR-HIFU ablation of breast tumours showed that the MR-HIFU breast system allows for safe, accurate and precise thermal ablation [31, 32].

Previous clinical studies

This will be the first-in-human study to evaluate LTLD with MR-HIFU hyperthermia in breast cancer patients. LTLD has been studied previously in combination with superficial hyperthermia in patients with chest wall recurrences of breast cancer [33]. This phase I/II study showed that LTLD at 40 mg/m$^2$ with superficial hyperthermia was safe and the 48% overall response (14/29, 95% CI:30–66%) was promising in this heavily pre-treated population. A large randomized phase III study in 701 patients with hepatocellular carcinoma compared LTLD at 50 mg/m$^2$ with radiofrequency ablation (RFA) to RFA alone (the HEAT study) [34, 35]. In that study the primary endpoint of 33% improvement in progression free survival was not met. However, a post-hoc analysis in the subgroup of 285 patients with solitary lesions that were

treated with ≥45 min of RFA showed a significant overall survival benefit for the combination treatment (Hazard Ratio for Overall Survival 0.63 (95% CI, 0.41–0.96; P < 0.05), in favour of RFA+LTLD with ≥45 minutes heating). Systemic adverse events increased in the RFA+LTLD arm (83% vs 35% with RFA alone) as expected, with a similar profile to that of conventional doxorubicin [35].

Furthermore, the combination of LTLD and ultrasound guided HIFU hyperthermia has been evaluated in a phase I proof-of-concept study in ten patients with incurable primary or metastatic liver tumours (the TARDOX study) [36, 37]. Adverse events did not differ from those associated with doxorubicin alone and in the group of patients that underwent invasive thermometry sufficient mean tumour temperatures were measured. In seven out of ten patients, the intratumoural doxorubicin concentration doubled after HIFU, although a within-patient comparison was not possible for all patients. We aim to take advantage of the same principle to treat the primary tumour in patients presenting with metastatic breast cancer. Monitoring the treatment by MR thermometry may further enhance safety, efficacy and feasibility. Using multiple cycles of LTLD + MR-HIFU hyperthermia is expected to increase treatment efficacy and mimics the standard of care treatment.

**Methods and analysis**

This single-arm phase I feasibility study aims to determine the safety, tolerability and feasibility of the combination of LTLD, MR-HIFU induced mild local hyperthermia, and cyclophosphamide, for the enhanced local treatment of the primary tumour in patients presenting with metastatic breast cancer. All eligible participants will receive

up to 6 cycles of LTLD at 21-day intervals, administered during MR-HIFU induced hyperthermia to the primary tumour and cyclophosphamide administered afterwards.

Patient population

We will include 6 or 12 adult female patients with *de novo* stage IV (distant metastases at the time of diagnosis, with the primary tumour in situ) HER2-negative breast cancer, who have not received previous chemotherapy for their disease. Previous endocrine treatment in those with hormone-receptor positive disease is allowed. The small samples size was chosen because this is the first study evaluating the combination of MR-HIFU hyperthermia, LTLD and cyclophosphamide. No formal sample size calculation was performed. Potentially eligible patients will be referred to the department of Medical Oncology at the University Medical Center Utrecht, The Netherlands. In order to achieving adequate participant enrolment, medical oncologists in hospitals in the Netherlands will be asked to refer potentially eligible and interested patients.

Inclusion criteria

Patients must meet all of the following inclusion criteria:

- Histologically confirmed adenocarcinoma of the breast and planned for palliative chemotherapy with doxorubicin and cyclophosphamide.
- Biopsy-proven stage T1-2AnyNM1 at diagnosis of breast cancer.
- Measurable disease according to either RECIST 1.1 or PERCIST 1.0 at baseline.
- Non-pregnant, non-lactating female at least 18 years of age. If the patient is of child-bearing age, she must have a negative serum pregnancy test prior to enrolment and must agree to practice an acceptable form of birth control while on study.

- The tumour is located within the reach of the HIFU beam (based on pre-treatment dynamic contrast-enhanced (DCE-) MRI findings).
- The distance of the tumour from the skin, nipple, and pectoral wall is at least 1.0 cm (based on pre-treatment DCE-MRI findings).
- The target breast is expected to fit in the cup of the MR-HIFU breast system (based on pre-treatment MRI findings).
- The patient is able to provide written informed consent and willing to comply with protocol requirements.

Exclusion criteria

Patients will be excluded if any of the following conditions are observed:

- HER2-positive disease or classic invasive lobular carcinoma (ILC).
- A treatment plan with curative intent is available.
- Any prior chemotherapy treatment for invasive breast cancer (previous anti-hormonal therapy is allowed).
- Any prior therapy with anthracyclines.
- The patient weighs ≥90 kg (restriction of the HIFU table top).
- Any concomitant malignancy or previous malignancy in the last 5 years, except basal cell or squamous cell cancer of the skin or in situ carcinoma of the cervix. Subjects with a prior contralateral breast malignancy more than 5 years ago can be included if they did not receive any chemotherapy.
- Any previous malignancy in the unilateral breast (even if more than 5 years ago)
- Prior sensitivity (including rash, dyspnoea, wheezing, urticarial, or other symptoms) attributed to any liposomal-encapsulated drug.
- Baseline laboratory values:

| | |
|---|---|
| Absolute Neutrophil Count (ANC) | < 1.5 x 10^9/L |
| Platelets | < 75 x 10^9/L |

| | |
|---|---|
| Haemoglobin | < 5.6 mmol/L (transfusion is allowed) |
| Total Bilirubin | > 1.5 times upper limit of normal |
| Alanine Transaminase (ALAT) and Aspartate Transaminase (ASAT) | |
| | > 2.5 times upper limit of normal |
| | >5 times upper limit of normal in case of liver metastases |
| Estimated Glomerular Filtration Rate | < 30 ml/min/1.73m$^2$ |

- World Health Organization Performance Status (WHO-PS) >2.
- Left Ventricular Ejection Fraction <50% (validated by baseline scan).
- History of: acute coronary syndrome in the last year, cerebral vascular accident in the last year, abnormal cardiac stress testing within the last six months, symptomatic coronary artery disease, uncontrolled hypertension or cardiomyopathy, cardiac valvular surgery or open-heart surgery in the last year or known structural heart disease.
- Any condition which may interfere with the hyperthermia portion of the trial such as: functioning cardiac pacemaker; metal plates, rods or prosthesis of the chest wall, breast prosthesis in the treated breast, severe numbness and/or tingling of the chest wall or breast, skin grafts and/or flaps on the breast or chest wall, scar tissue or surgical clips in the HIFU beam path.
- Active infection.
- Body temperature > 38.0 degrees Celsius on the day of a MR-HIFU treatment.
- Concurrent use of any of the following prohibited medications within a reasonable wash-out time: protease inhibitors, cyclosporine, carbamazepine, phenytoin, valproic acid, paclitaxel, trastuzumab and other liposomal drugs (AbelectTM, Ambisome™, NyotranTM, etc.) or lipid-complexed drugs. Caution will be exercised with medications, dietary components and herbal supplements that affect CYP2A4, CYP2D6 or P-gp or have been described to interact with doxorubicin in other ways.

- Contraindications to MR imaging (e.g., pacemaker in situ, severe claustrophobia, metal implants incompatible with the MRI-scan, body size incompatible with MR bore).
- Contraindications to gadolinium-based contrast agents and the tumour is not sufficiently visible on MRI without contrast (including prior allergic reaction to gadolinium-based contrast agent, and/or renal failure).
- Contraindications to sedation and analgesia with Propofol and Remifentanil, including history of Chronic Obstructive Pulmonary Disease (COPD) that results in the inability to perform a physical activity corresponding with a Metabolic Equivalent (MET(57)) of 4; dependence on artificial ventilation at home; sleep apnoea or an American Society of Anaesthesiologists (ASA) classification ≥4.
- Inability to lie in prone position.
- A medical or psychiatric condition or other circumstances which would significantly decrease the chances of understanding the informed consent process, obtaining reliable data, achieving study objectives, or completing the study treatment and/or examinations.

Endpoints

Primary endpoints are safety, tolerability and feasibility. These will be evaluated by the following assessments.

Safety and tolerability:

- Incidence and severity of Adverse Events and Severe Adverse Events
- Incidence of Dose Limiting Toxicity (DLT, systemic and loco-regional)
- Necessity for dose adjustments, delay and early cessation
- Incidence and severity of post-procedural pain
- Patient reported tolerability (questionnaires)

- Cardiotoxicity: Left Ventricular Ejection Fraction measurement and electrocardiogram abnormalities.

Feasibility:

- The number of cycles in which hyperthermia treatment was sufficient: at least 30 minutes at the target temperature of 40-42 °C.
- The number of completed cycles with MR-HIFU induced hyperthermia, LTLD and cyclophosphamide
- Quality of MR thermometry data acquired during the MR-HIFU treatment
- Spatiotemporal temperature distribution in the tumour
- Total duration of the study procedures on a treatment day.

Secondary endpoints consist of efficacy parameters:

- Assessment of distant radiological objective response rates
- Assessment of local radiological objective response rates

Study procedures

The study design (Figure 2) was based on the AC regimen, a well-known chemotherapeutic regimen that consists of doxorubicin and cyclophosphamide. This regimen is used in the (neo-)adjuvant setting as well as in the first-line chemotherapy treatment of metastatic breast cancer. Standard of care for our study population consists of 6 cycles at 21-days intervals. In this study we will replace doxorubicin in this regimen with the combination of LTLD and MR-HIFU induced hyperthermia. All participants will receive procedural sedation and analgesia with propofol and remifentanil to limit patient movement during the treatment and to establish a

regular breathing pattern that will facilitate respiratory gated MR thermometry [38]. To prevent any hypersensitivity reactions to LTLD, the participants will also receive a premedication regimen of steroids, H1- and H2- histamine antagonists. Anti-emetics will be administered according to standard-of-care hospital guidelines for the AC regimen.

MR-HIFU hyperthermia will be performed on the MR-HIFU breast system, with the patient in prone position. We will aim for 60 minutes of hyperthermia at 40-42 °C to the breast tumour, in four blocks of 15 minutes. After each block the MR thermometry is restarted to minimize the possible influence of magnetic field drift or patient displacement. When MR thermometry indicates that the target temperature is reached, 50 mg/m$^2$ of LTLD will be administered intravenously over 30 minutes, via a peripherally inserted central catheter (PICC), while the patient is on the MR-HIFU breast system. Temperature will be monitored by respiratory navigator-gated MR thermometry, using the proton resonance frequency shift method [39, 40]. In case the target temperature is not reached, conventional doxorubicin (60 mg/m$^2$) will be administered instead of LTLD. Shortly after MR-HIFU, 600 mg/m$^2$ of cyclophosphamide will be administered intravenously according to standard of care in the AC regimen.

Participants will receive up to six treatment cycles. Feasibility will be evaluated after each MR-HIFU treatment and during the course of the cycles. Safety and tolerability will be assessed three hours after MR-HIFU treatment, during telephone contact on day +1 and +7 and during a hospital visit on day +14 and +21 of each cycle, by

monitoring of adverse events, laboratory measurements and evaluation of pain. Cardiotoxicity evaluations (LVEF and ECG) will be performed at baseline, after cycle 3 and after cycle 6. The participants will be asked to fill out the Dutch version of the Functional Assessment of Cancer Therapy – Breast (FACT-B, version 4, FACIT)[41] at baseline and after each treatment cycle, combined with a selection of questions adapted from the Dutch version of the Cancer Therapy Satisfaction Questionnaire (CTSQ, Pfizer 2007, modified with permission from Pfizer)[42, 43] in cycles 3 and 6. Before starting the next cycle, any toxicities will be evaluated and if necessary, dose reductions will be made. DLT will be categorized in systemic or loco-regional toxicity (Table 1). Thus, we aim to distinguish systemic chemotherapy effects from local effects of MR-HIFU hyperthermia and/or the high local doxorubicin concentration. Planned dose adjustments for these categories have been established (Supplementary materials 2). In case of a systemic DLT the LTLD dosage will be decreased, while for loco-regional DLT the duration of hyperthermia will be decreased. Cyclophosphamide dose will not be reduced. No dose increases will be performed. Depending on the severity and nature of the toxicity, study treatment can be delayed or even ceased. In case of solely loco-regional DLT, technical issues or other feasibility issues that restrict the use of MR-HIFU treatment, the participant will receive the standard of care AC regimen. If hyperthermia is insufficient (i.e. the target temperature of 40-42 °C is not reached or was only maintained for less than 30 minutes) in two separate cycles, the treatment is not considered feasible for that patient and study participation will end.

For the secondary endpoint of efficacy, MRI of the breast will be performed using a 3 Tesla MRI scanner with a dedicated breast coil, at baseline and after cycle 2 and 6 to determine local radiological objective response. In addition, MRI of the breast will be performed during each MR-HIFU treatment. However, the receiver coil in the MR-HIFU breast system is not suited for clinical imaging. In case a complete radiological response of the breast tumour is obtained after less than 6 cycles, the patient will continue with the conventional AC regimen. $^{18}$F -fluorodeoxyglucose (FDG-) Positron Emission Tomography combined with Computed Tomography (PET/CT) of the thorax and abdomen will be performed at baseline and CT or PET/CT after cycle 2 and cycle 6, to determine the distant objective response according to RECIST 1.1 [44] or PERCIST 1.0 [45]. PET/CT will be performed for response evaluation in patients with only PERCIST-measurable disease, such as patients with only bone metastases. If a patient shows distant progression of disease, study participation will end and the patient will be treated according to the standard of care. Additional specific reasons for study withdrawal are dose limiting toxicity that warrants a delay in treatment administration for longer than 14 days or a recurrence of dose limiting toxicity after dose reduction of LTLD (Supplementary materials 2).

The participants will be followed for adverse events from the time of signing informed consent until the end of study visit after six cycles of chemotherapy. Afterwards patients will receive standard of care treatment.

If the patient consents to the biobank study, additional blood samples will be taken from the PICC-line at seven time points (Figure 2) when the patient is already at the

hospital. These blood samples will be collected in the UMC Utrecht Biobank for future research. Moreover, in case tissue samples of the breast tumour and/or metastases were obtained in standard care before inclusion or following study participation, we will ask for consent to perform additional analyses on these samples.

Concomitant care and prohibited interventions

All supportive measures consistent with optimal medical care will be employed, including transfusion of blood and blood products, and treatment with antibiotics, antiemetics, antidiarrheals, and analgesics, as appropriate.

Certain concomitant medications, a number of herbal supplements, food stuffs and nutritions are restricted during the study (Supplementary materials 3). Patients cannot use creams, ointments or lotions on the breast on the MR-HIFU treatment day, to avoid additional risks during the procedure. Patients cannot use methods or treatments that increase the body temperature or skin temperature during the study period (e.g. sauna, hot-water baths, warmth massages), because this could result in increased release of doxorubicin in the warmed areas, possibly causing extra adverse events.

Interim analysis

An interim analysis of safety and efficacy will determine whether accrual will continue after six participants (Supplementary materials 4). Safety will be evaluated once the first six patients complete two treatment cycles. If safety is sufficiently proven or is deemed inadequate, the trial will end after six participants. Otherwise accrual will continue until twelve patients have been treated, if necessary after dose adjustments.

All patients who have signed informed consent will be evaluated for the primary endpoints of safety, feasibility and tolerability. Patients who have been withdrawn from the study because MR-HIFU induced hyperthermia was insufficient in two separate treatment cycles and who did not experience a DLT, will be replaced by another participant for in the interim safety evaluation. If this happens to four patients, the study will be terminated, because of insufficient feasibility.

Systemic efficacy will be evaluated once the first six patients have received the CT scan after cycle 2. If four or more of the first six participants show distant disease progression at that time the trial will be stopped, as this suggests that efficacy against disease outside the heated treatment field is inadequate. This early stopping rule was based on a phase III trial with liposomal doxorubicin in metastatic breast cancer [46] where 77.5% of the subjects were free of disease progression at two months post-randomization (the 95% confidence interval of 2/6 patients does not contain 0.775).

An independent, qualified monitor will monitor the study procedures. An external Data Safety Monitoring Board (DSMB) will review accumulating safety data at regular intervals throughout the study, perform the interim safety and efficacy analyses and monitor trial data integrity (DSMB charter in Supplementary materials 5).

Data analysis

Descriptive statistics will be used to describe the incidence and severity of adverse events (National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0), the patient reported outcomes in the questionnaires and feasibility parameters including the number of completed study treatment cycles, duration of

study procedures and spatiotemporal temperature distribution during MR-HIFU treatment. For the secondary endpoint of efficacy, distant and local radiological objective response rates (RECIST 1.1) will be described.

## Discussion

This is the first clinical trial that investigates the combination of LTLD and MR-HIFU induced hyperthermia in breast cancer. In a small number of patients we will focus primarily on safety, tolerability and feasibility of this procedure. We hypothesize that the combination of LTLD and MR-HIFU hyperthermia leads to improved treatment of the primary tumour, without changing the systemic doxorubicin concentration and thus without interfering with systemic efficacy and toxicity. A future randomized study with a control group receiving the standard of care AC regimen would be needed to prove this. Including patients with *de novo* stage IV breast cancer provides the unique possibility to monitor both local and systemic disease simultaneously. While in this setting a survival benefit of treating the primary tumour has not been proven, the study treatment (if proven safe and feasible) could in the future improve outcomes in the neoadjuvant setting.

We aim to replace doxorubicin by LTLD plus MR-HIFU hyperthermia in all six cycles of the AC regimen, because we expect this to maximize the local treatment effect. In each cycle, the feasibility to achieve tumour hyperthermia at 40-42 °C for 30 minutes will be verified with MR thermometry. If hyperthermia treatment is repeatedly insufficient, or if (after any number of cycles) radiological complete response is

already obtained, patients will continue on the standard-of-care AC regimen. The number of MR-HIFU hyperthermia plus LTLD cycles that our patients are willing and able to complete could be less than six, which would be an important feasibility finding.

Our goal is to maintain an equivalent systemic efficacy compared to the standard-of-care AC regimen using 60 mg/m$^2$ conventional doxorubicin. Pharmacokinetic studies showed that the area-under the curve (AUC0-∞) of free/unencapsulated doxorubicin in plasma of patients receiving LTLD 50 mg/m$^2$ with local hyperthermia or RFA [33, 47, 48] was higher than the AUC0-∞ of conventional doxorubicin 60 mg/m$^2$ [49-51]. To be able to compare the AUCs we converted the AUC0-∞ of the metabolite doxorubicinol that was measured in the LTLD studies to the AUC0-∞ of doxorubicin [52-54] (Additional explanation in Supplementary materials 1). The 50 mg/m$^2$ LTLD dose was also recommended for and well-tolerated in the phase III trial in combination with RFA [35]. Due to local toxicity, the recommended dose for LTLD combined with local superficial hyperthermia for chest wall recurrences was decreased to 40 mg/m$^2$ [47]. In our study local (skin) toxicity is not expected because a margin of at least 1.0 cm is preserved from the tumour to the skin, therefore the LTLD dose of 50 mg/m$^2$ was chosen. Real time MR thermometry and the lateral configuration of the MR-HIFU breast system will help mitigate this risk. If however local DLT do occur, the duration of hyperthermia will be decreased while maintaining the LTLD dosage to avoid decreasing systemic efficacy. We will only decrease LTLD dosage in case of systemic DLT. If despite these measures, systemic efficacy seems

inadequate, the trial will be halted prematurely based on the interim analysis for efficacy.

Because this is a small phase I feasibility study, the results will only provide a rough indication of local efficacy based on radiological response. To diminish the burden on participants, we will not perform tissue biopsies or breast surgery and therefore cannot describe the number of pathological complete responses or measure the concentration of doxorubicin in the tumour. Proof-of-concept that hyperthermia increases the tumour doxorubicin concentration has already been established in the Tardox study, although doxorubicin concentrations were not compared between heated and unheated tumours.

With this phase I clinical trial, we aim to show that LTLD combined with MR-HIFU induced hyperthermia on a dedicated MR-HIFU breast system can safely replace doxorubicin in the AC regimen. We hypothesize that this combination will result in improved response of the primary tumour without compromising the systemic efficacy on metastatic sites or increasing systemic toxicity. If feasibility and tolerability are adequate, this approach could in the future lead to optimal loco-regional control with less extensive or even no surgery, in stage II or III breast cancer patients allocated to receive neo-adjuvant chemotherapy. Finally, it could also be suitable for other doxorubicin sensitive tumour types that benefit from enhanced local treatment, such as soft tissue sarcoma.

**Word Count**

4351 words

## Ethics and dissemination

This study has obtained ethical approval by the Medical Research Ethics Committee of the UMC Utrecht (METC Utrecht) on May 29th 2019 (Protocol NL67422.041.18, METC number 18-702). This paper is based on protocol version 6, dated August 28th 2020. Substantial protocol amendments will also be evaluated by METC Utrecht and communicated to relevant parties by the investigators. Informed consent will be obtained from all patients by an authorized representative of the Principal Investigator before study participation (Informed consent form in Supplementary materials 6). The results of this study will be disseminated by publication in an academic peer-reviewed journal.

## Roles and responsibilities

This is an investigator-driven single-centre clinical trial, with the UMC Utrecht as sponsor and trial site. The UMC Utrecht is responsible for the study design, data collection, data management, analysis, interpretation of data, writing and submission of the report for publication. The Principal Investigator will rapport (serious) adverse (device) events to the METC Utrecht, to the Central Committee on Research Involving Human Subjects (CCMO), and to Celsion Corporation and Profound Medical according to national guidelines. UMC Utrecht has liability insurance which provides cover for damage to research subjects through injury or death caused by the study. Celsion Corporation (manufacturer of the investigational medicinal product) and

Profound Medical (manufacturer of the investigational medical device) will provide technical support during the trial and have provided input on the study protocol. Both manufacturers will be allowed to review and comment on draft publications prior to submission. The investigators at the UMC Utrecht will have ultimate authority over the publication. An external Data Safety Monitoring Board (two clinicians and one statistician) has been established and an independent qualified monitor (Julius Clinical) has been appointed to perform intensive monitoring.

**Data management**

The handling of personal data will comply with the General Data Protection Regulation (GDPR, in Dutch known as AVG). After informed consent is signed, each patient receives a unique subject number. A subject identification code list will be used to link the data to the subject. The key to this pseudonymization code will be available only to the investigators and employees of the research team.

Research data that are relevant for the study will be collected by the investigators on electronical Case Report Forms (eCRFs) in Research Online, in compliance with the Good Clinical Practice (GCP) guidelines for electronic data collection. An audit trail will be available. The completed eCRFs will be reviewed, signed and dated by the Principal Investigator or Co-investigator. Scans, results and registrations of medical imaging will be collected on the Research Imaging Architecture (RIA), which is secured by password-protection and stores pseudonymized images. Data from the MR-HIFU device such as log files and MR images obtained during the MR-HIFU treatment that cannot be stored on the Research Imaging Architecture will be stored

in a secured UMC Utrecht bulk-storage folder. Celsion and Profound will not receive any patient's identifiable (personal) information. UMC Utrecht shall provide pseudonymized data regarding the occurrence and severity of adverse device effects to Profound Medical and regarding the occurrence and severity of adverse events to Celsion Corporation. This cannot be refused by the patient and is obligatory for study participation. If the patient consents (optional), additional pseudonymized data on the study treatment, will also be provided to Profound Medical and Celsion Corporation. Research data will be stored for 15 years after the end of study. Biomaterial is stored in the Central biobank (blood) or at the UMC Utrecht pathology department (tissue samples).

**Patient and public involvement**

Patient experiences have been the starting point for the grant proposal to the Dutch Cancer Foundation and patients were involved in the design of the study and the choice of outcome measures. Patients will not be actively involved in recruitment or dissemination of study results, however information regarding the study can be found by individual patients on the UMC Utrecht website and clinicaltrials.gov.

**Trial status**

Patient recruitment was initiated on March 10th 2020. On the submission date of this article, no patients had been enrolled yet. Due to the COVID-19 outbreak, the study has been temporarily discontinued. Recruitment will be resumed as soon as possible.

**Authors' contributions**

JdM, BS, MB, SL, CM, EW and RD were all involved in the design of the study and in writing the manuscript.

PvD, HV and AW critically reviewed the design of the study providing additional comments and suggestions.

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We thank Celsion Corporation for their support relating the use and safety of ThermoDox and their input during the design of the study.

Finally, we thank Profound Medical for their support relating the use and safety of the MR-HIFU breast system in their role as legal manufacturer of this investigational medical device.

## Competing interests statement

The authors have no competing interest to declare.

## List of abbreviations

| | |
|---|---|
| AC | Doxorubicin (A) and cyclophosphamide (C) |
| AF | Alkaline Phosphatase |
| ALAT | Alanine Transaminase |
| ANC | Absolute Neutrophil Count |
| ASAT | Aspartate Transaminase |
| AUC0-∞ | Area Under the Curve 0-infinity |
| CCMO | Central Committee on Research Involving Human Subjects |
| CTSQ | Cancer Therapy Satisfaction Questionnaire |
| DCE | Dynamic contrast-enhanced |
| DLT | Dose Limiting Toxicity |
| DSMB | Data Safety Monitoring Board |
| eCRF | electronical Case Report Forms |
| FACT-B | Functional Assessment of Cancer Therapy – Breast |
| GCP | Good Clinical Practice |
| GDPR | General Data Protection Regulation |
| LTLD | Lyso-Thermosensitive Liposomal Doxorubicin |
| MR-HIFU | Magnetic Resonance guided High Intensity Focused Ultrasound |
| MRI | Magnetic Resonance Imaging |

| | | |
|---|---|---|
| (FDG-) PET/CT | $^{18}$F-Fluorodeoxyglucose Positron Emission Tomography combined with Computed Tomography |
| PICC | Peripherally inserted central catheter |
| RFA | Radiofrequency ablation |
| RIA | Research Imaging Architecture |

## References


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## Figures and tables

Figure 1 The concept of LTLD combined with MR-HIFU hyperthermia for local drug delivery in the primary breast tumour. The patient is lying in prone position on the dedicated MR-HIFU breast system under procedural sedation and analgesia, with the breast hanging in the water-filled cup. HIFU-induced hyperthermia is administered to the tumour for 60 minutes. Real-time MR thermometry (screen on the right) allows for precise control of the target temperature of 40-42 °C in the tumour. After intravenous infusion, LTLD circulates through the vasculature and releases a small amount of doxorubicin at 37°C. However, when LTLD reaches the heated tumour it releases a high amount of doxorubicin intravascularly within seconds. We hypothesize that the combination of LTLD and MR-HIFU hyperthermia will increase the tumour concentration of doxorubicin without interfering with systemic treatment efficacy and toxicity.

Figure 2 Study procedures. The standard of care palliative AC regimen consists of 6 cycles of doxorubicin and cyclophosphamide at 21-days intervals. In this study we will replace doxorubicin with the combination of LTLD and MR-HIFU induced hyperthermia, in up to six cycles. After informed consent, the baseline procedures will be performed as mentioned. During the cycles, the primary endpoints of safety (adverse events), feasibility and tolerability will be monitored, including cardiotoxicity evaluation and questionnaires on specified time points as indicated in the bottom of the figure. Imaging to determine local (MRI) and systemic ((PET/)CT) response will be

performed at baseline, after cycle two and after cycle six. Optionally, the patient can consent to additional blood sampling for future research, which will be stored in the Biobank.

Table 1 Definitions of Dose Limiting Toxicity

| | |
|---|---|
| **Dose limiting systemic toxicity** | |
| A | *Hematologic DLT*<br>defined as Grade 3 anaemia, Grade 4 thrombocytopenia, febrile neutropenia, or Grade 4 neutropenia ≥ 7 days in duration. |
| B | *Non-hematologic DLT (non-loco-regional)*<br>defined as Grade 3 or greater toxicity with the exceptions of alopecia, fatigue, nausea or vomiting and loco-regional effects.<br>Including Cardiotoxicity DLT, defined as:<br>• Grade 3 or greater cardiac disorders OR<br>• a decline in LVEF of > 15% while the LVEF remains > 40% OR<br>• a decline to an LVEF of ≤ 40%. |
| **Dose limiting loco-regional toxicity** | |
| C | *Loco-regional DLT*<br>defined as post-procedural effects (e.g. pain or skin effects) on the treated breast warranting dose adjustment or delay. |

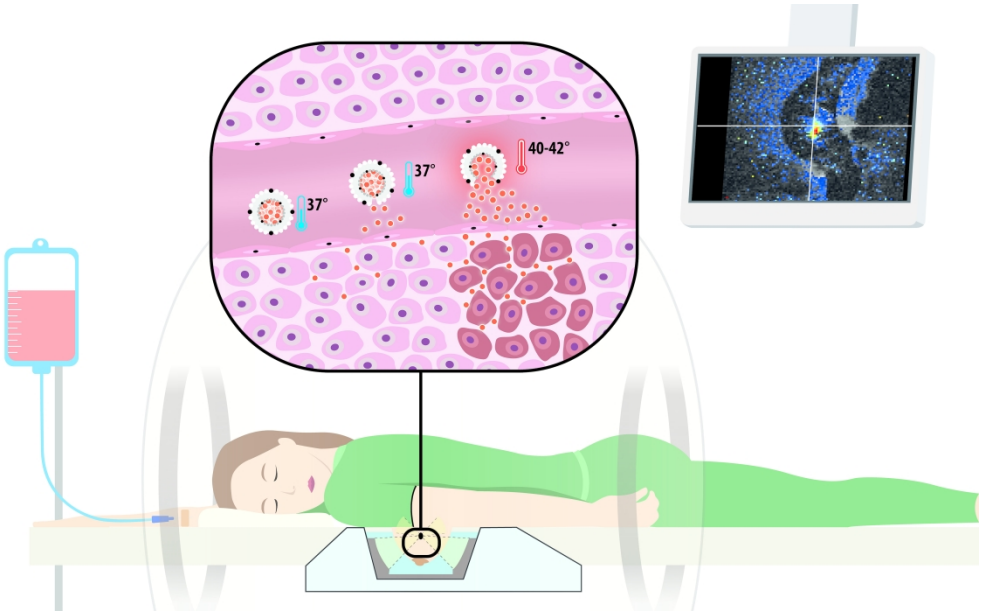


Figure 1 The concept of LTLD combined with MR-HIFU hyperthermia for local drug delivery in the primary breast tumour. The patient is lying in prone position on the dedicated MR-HIFU breast system under procedural sedation and analgesia, with the breast hanging in the water-filled cup. HIFU-induced hyperthermia is administered to the tumour for 60 minutes. Real-time MR thermometry (screen on the right) allows for precise control of the target temperature of 40-42 °C in the tumour. After intravenous infusion, LTLD circulates through the vasculature and releases a small amount of doxorubicin at 37°C. However, when LTLD reaches the heated tumour it releases a high amount of doxorubicin intravascularly within seconds. We hypothesize that the combination of LTLD and MR-HIFU hyperthermia will increase the tumour concentration of doxorubicin without interfering with systemic treatment efficacy and toxicity.

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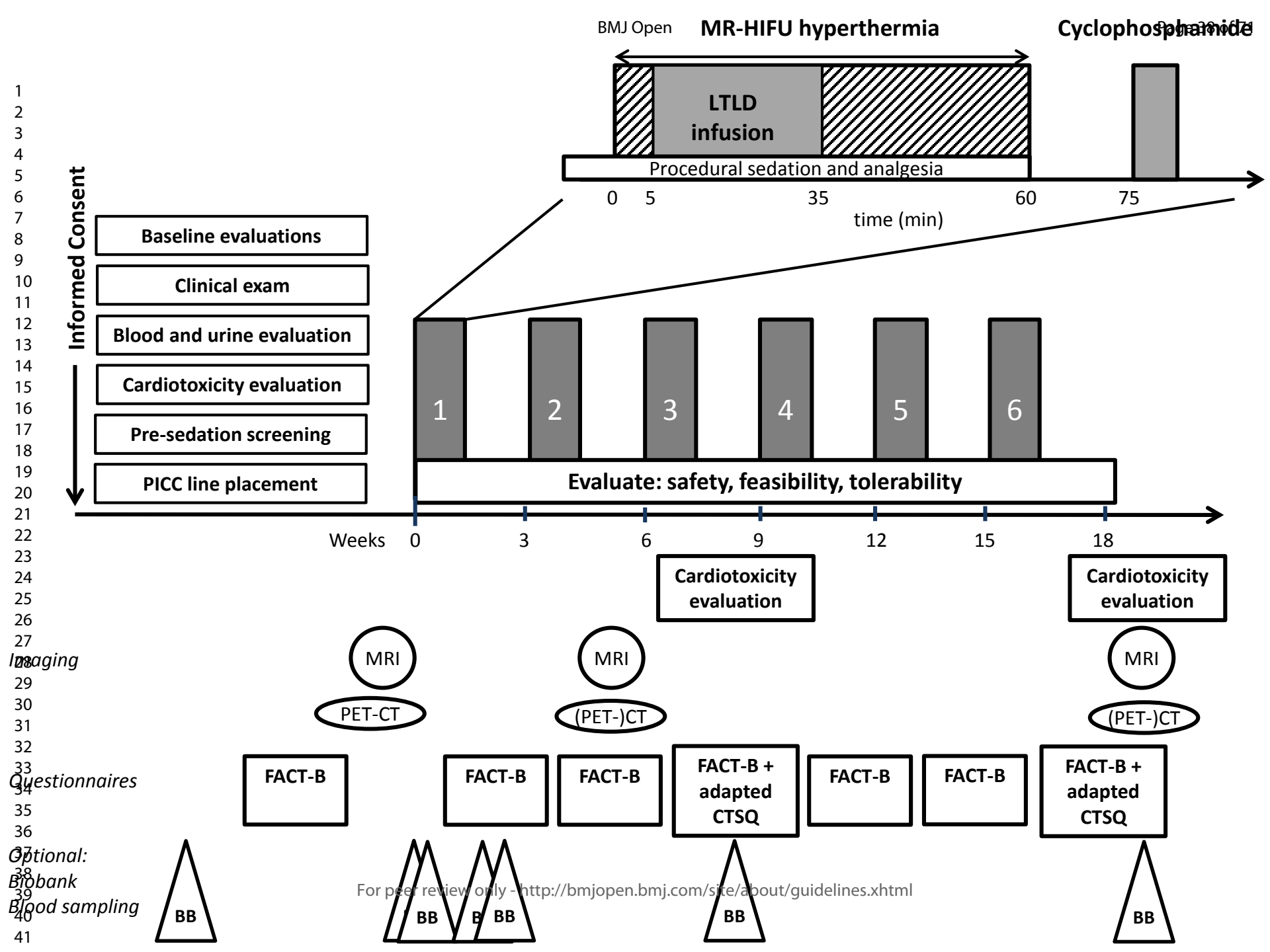
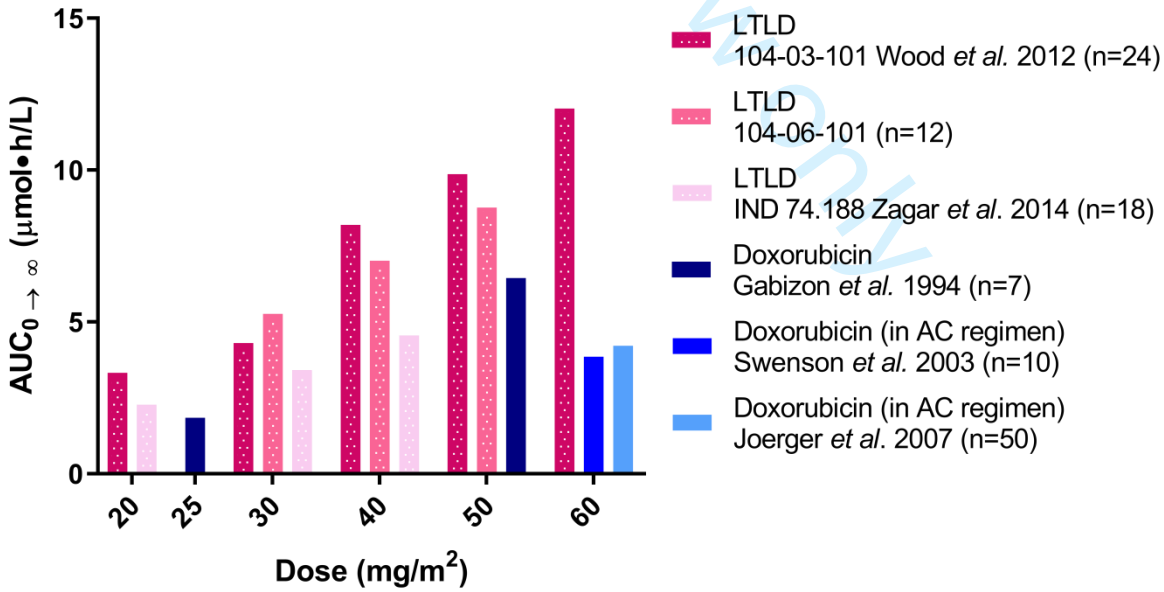


**Supplementary materials:**

**Supplement 1 Comparison of AUC0-∞ of free doxorubicin for LTLD and conventional doxorubicin.**

In order to obtain a systemic dose of free doxorubicin (due to leakage of LTLD at 37 °C) that is as similar to conventional doxorubicin at 60 mg/m$^2$ (which is the standard of care treatment for the patients that will be enrolled in this study) as possible (to avoid undertreatment) we will start at the dose of 50 mg/m$^2$ LTLD, and we will apply dose adjustments when necessary. To compare the systemic dose of free doxorubicin after LTLD plus hyperthermia to the systemic dose of conventional doxorubicin, we summarized the pharmacokinetic data of the three studies with LTLD in which total plasma doxorubicin and the metabolite doxorubicinol were measured with a validated assay (studies 104-03-101 [1], 104-06-101 [2], and IND #174,188 [3]). In these studies the Area Under the Curve from t=0 to infinity (AUC0-∞) of the metabolite doxorubicinol was measured. Note that in these studies LTLD was administered with hyperthermia or RFA treatment. Pharmacokinetic data on LTLD without heating are not available. The mean values were converted to the AUC0-∞ of 'free doxorubicin' based on the mean ratios between doxorubicinol and doxorubicin found in three studies (0.3826, 0.47 and 0.514 respectively, with a mean of 0.456) [4-6]. We compared these AUC0-∞ values of 'free doxorubicin' from the LTLD studies with the AUC0-∞ values of doxorubicin in pharmacokinetic studies of conventional doxorubicin [7-9]. Figure S2 displays the AUC0-∞ of three studies with conventional doxorubicin (actual doxorubicin values are portrayed) and the AUC0-∞ of three studies with LTLD (calculated 'free doxorubicin' values are portrayed). The figure shows that the calculated 'free doxorubicin' after LTLD 50 mg/m$^2$ is at least equal to that of conventional doxorubicin at 60 mg/m$^2$.

**Figure S2: Comparison of the AUC0-∞ of "free" plasma doxorubicin for LTLD + heat (calculated based on doxorubicinol concentration) and conventional doxorubicin.**




## Supplementary References

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**Supplement 2 Dose adjustments in the i-GO study.**

Individual dose adjustments and/or delays may be made based on the emergence of specific adverse events.

Adverse events consist of:

- Systemic toxicity
- Locoregional toxicity

## S2.1 Systemic toxicity

### Myelosuppression

Dose adjustments in case of myelosuppression are summarized in figure S2-1.

If ANC <1.5 x 10^9/L, then the LTLD and cyclophosphamide doses will be held and re-evaluated for treatment in one week. Any second occurrence of ANC <1.5 x10^9/L will require a decrease in LTLD dose to 40 mg/m$^2$. The cyclophosphamide will remain unchanged. LTLD and cyclophosphamide will be administered at day 14 (two weeks after the scheduled dose) if the ANC ≥ 1.5 x 10^9/L. In case of recurrence of ANC <1.5 x10^9/L with the decreased LTLD dose, study participation will end and the patient will continue treatment with the standard of care AC regimen.

If platelets are < 100 x 10^9/L, then the LTLD and cyclophosphamide doses will be held and re-evaluated for treatment in one week. Any second occurrence of platelets < 100 x 10^9/L will require a decrease in LTLD dose to 40 mg/m$^2$. The cyclophosphamide will remain unchanged. These doses will be administered at day 14 (two weeks after the scheduled dose) if the platelets are ≥ 100 x10^9/L. In case of recurrence of platelets < 100 x 10^9/L with the decreased LTLD dose, study participation will end and the patient will continue treatment with the standard of care AC regimen.
If a patient requires drug-withholding for more than 14 days, then the patient will be withdrawn from the study.

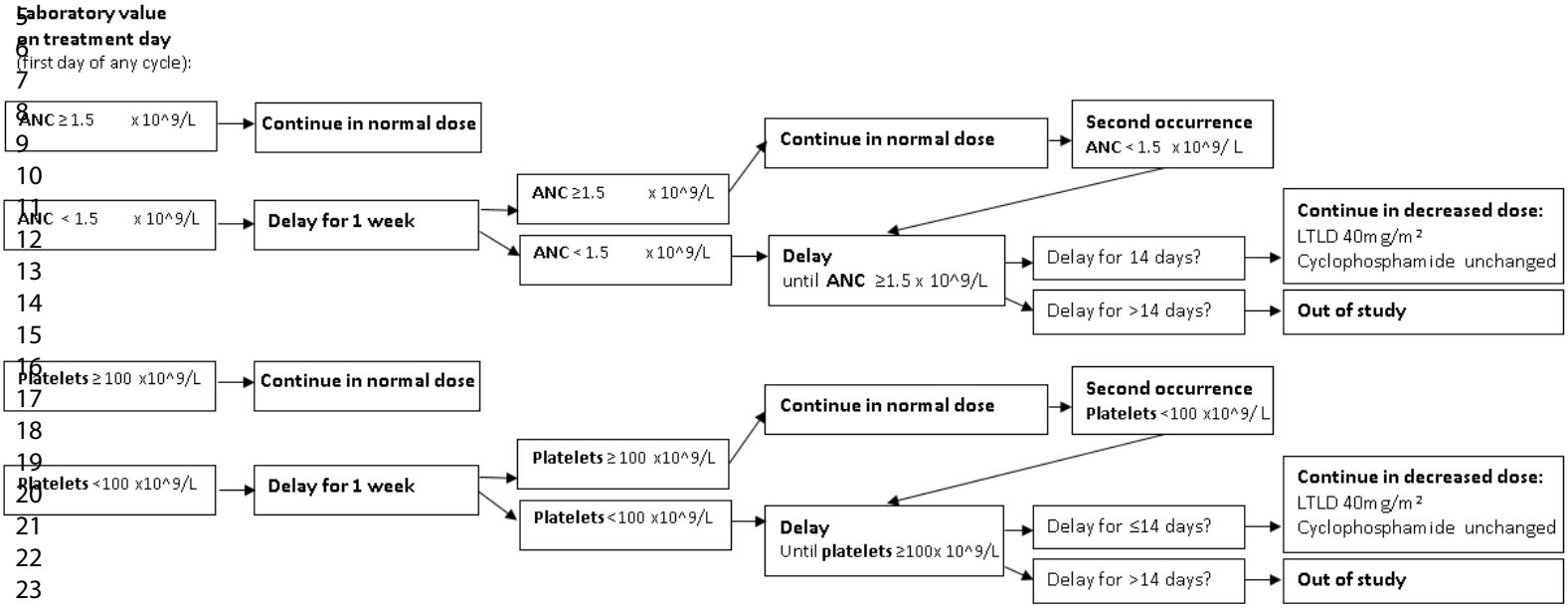


**Figure S2.1: Flow chart of dose adjustments for myelosuppression**

### Hypersensitivity reactions

No dose reductions will be made for hypersensitivity reactions.

**Table S2: Suggested Management for Hypersensitivity Reactions**

| Severity of Symptoms | Treatment Guidelines |
|---|---|
| Mild symptoms: localized cutaneous reactions such as mild pruritus, mild flushing, mild rash (Grades 0-1) | Consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor patient<br>Complete study drug infusion at the initial planned rate |
| Moderate to Severe symptoms: any symptom that is not mild (see list above) such as generalized pruritus, generalized flushing, generalized rash, dyspnea, hypotension with systolic BP < 80 mm Hg, bronchospasm, angioedema and generalized angioedema. (Grades 2-4) | WITHDRAW FROM STUDY |
| Anaphylaxis (Grade 4) | WITHDRAW FROM STUDY |

**Abnormal Liver Tests**

If a patient develops abnormal liver tests, they will be evaluated for causal factors such as bile duct obstruction or liver pathology, with an abdominal ultrasound. If a cause is found, this must be resolved before continuing the treatment. If no other cause than the study treatment is found (or the cause cannot be resolved), patients will have the following dose reductions (summarized in figure S2-2).

If bilirubin ≥25 µmol/L, then the LTLD and cyclophosphamide doses will be held and re-evaluated for treatment in one week. Any second occurrence of bilirubin ≥25 µmol/L will require a dose adjustment to 40mg/m$^2$ LTLD. If the bilirubin is still 25-50 µmol/L after one week, the patient will be treated with a decrease in LTLD dose to 25 mg/m$^2$ (50% of the original dose) and unchanged cyclophosphamide dose. If the bilirubin has normalized < 25 µmol/L after one week, the patient will be treated with a decrease in LTLD dose to 40 mg/m$^2$, the cyclophosphamide dose will remain unchanged. In case bilirubin ≥25 µmol/L recurs after previous LTLD dose reduction, study participation will end and the patient will continue treatment with the standard of care AC regimen.
If the bilirubin ≥ 50 µmol/L, treatment will be delayed until < 50 µmol/L. If a patient requires drug-withholding for more than 14 days, then she will be withdrawn from the study.

If bilirubin <25µmol/L and AF ≤ 600 U/L, but ASAT and ALAT are mildly elevated (1.6-3.5xULN), the patient will be treated with a decrease in LTLD dose to 40 mg/m$^2$ and unchanged cyclophosphamide dose,without delay. In case mildly elevated ASAT or ALAT recur after previous LTLD dose reduction, study participation will end and the patient will continue treatment with the standard of care AC regimen.
If AF > 5xULN (>600U/L) or ASAT > 3,5xULN (>105U/L) or ALAT > 3,5xULN (>123 U/L), treatment will be delayed until liver tests have recovered (bili<25 µmol/L, AF ≤5xULN and ASAT/ALAT≤3.5xULN). Then the patient will be treated with a decrease in LTLD dose to 40 mg/m$^2$ and unchanged cyclophosphamide dose. In case the elevated AF (> 5xULN), ASAT (> 3,5xULN) or ALAT (> 3,5xULN) recur after the LTLD dose reduction, study participation will end and the patient will continue treatment with the standard of care AC regimen.
If a patient requires drug-withholding for more than 14 days, then she will be withdrawn from the study.

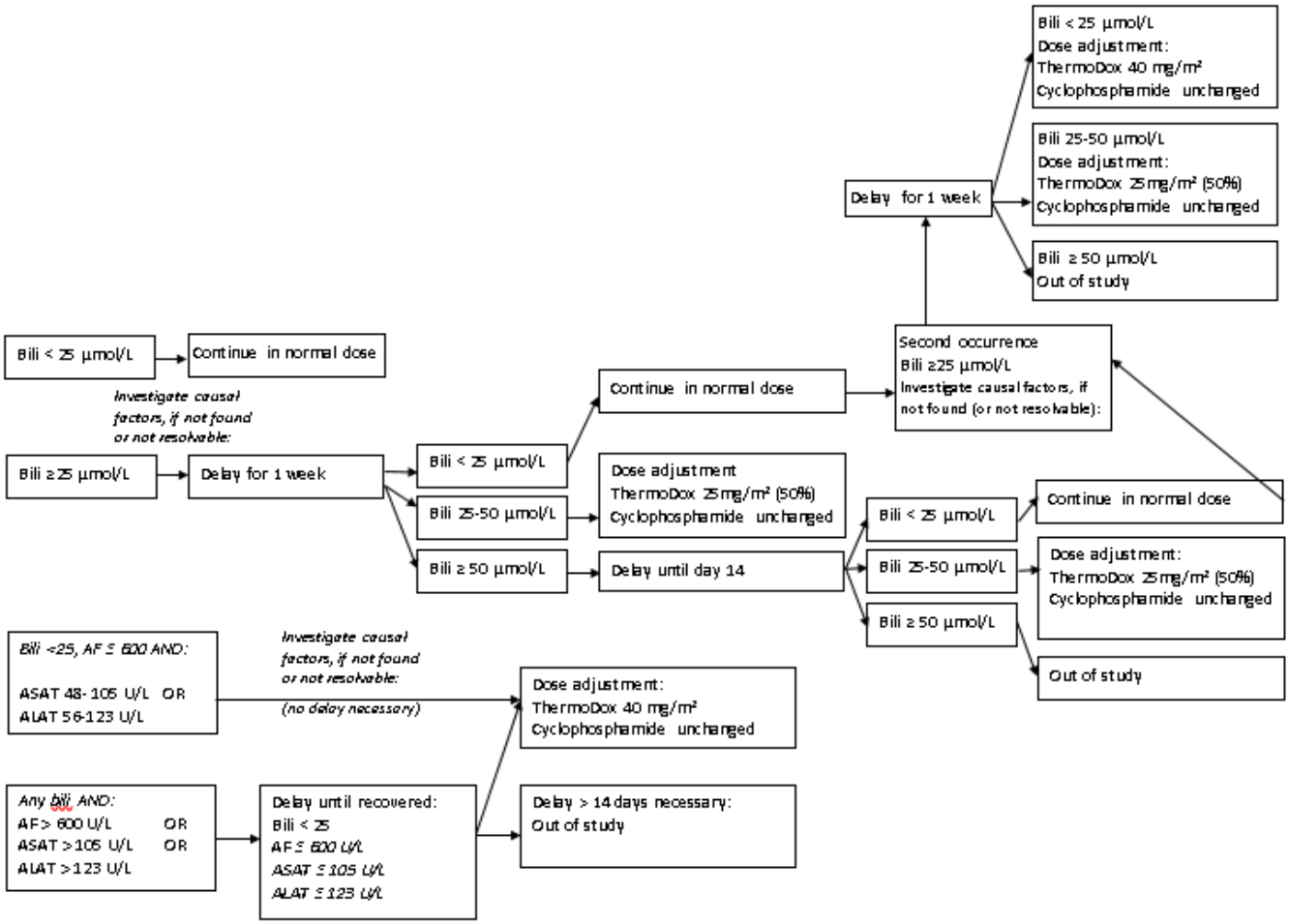


**Figure S2.2: Flow chart of dose adjustments for abnormal liver tests**

## Mucositis

If mucositis is present on any treatment day, then treatment should be held until mucositis has resolved. If mucositis of Grade ≥ 3 occurs, then reduce the dose of LTLD to 40 mg/m$^2$, while the cyclophosphamide dose remains unchanged for subsequent cycles. In case mucositis of grade ≥3 recurs after the LTLD dose reduction, study participation will end and the patient will continue treatment with the standard of care AC regimen. If a patient requires drug-withholding for more than 14 days, then the patient will be withdrawn from the study.

## Ventricular Function

Patients who are receiving protocol therapy will be removed from study treatment under the following conditions:
Signs (tachycardia, S3, elevated jugular venous pressure) AND symptoms of congestive heart failure (edema, dyspnea, paroxysmal nocturnal dyspnea, orthopnea) OR
a decline in LVEF of > 15% while the LVEF remains > 40% OR
a decline to an LVEF of ≤ 40%.

Patients in this category should be followed with an ejection fraction assessment every three months until stable.

**Other adverse events**

For other non-hematologic toxicity ≥ grade 3, no dose modification is required. Instead, such subjects will not be re-treated until the severity of the non-hematologic toxicity drops to ≤ grade 1. If a patient requires drug-withholding for more than 14 days, then the patient will be removed from the trial.

**S2.2 Locoregional toxicity**

**Post-procedural pain**

If a patient experiences post-procedural pain in the treated breast with:

- a Numeric Rate Scale (NRS) of 7 or higher (severe pain) for more than 60 minutes within 24u hours without pain medication, OR
- a NRS of 5 or higher (moderate to severe pain) for more than 60 minutes within 24u hours that does not respond to adequate pain medication,
- Any pain that the patients finds unacceptable or unbearable

then in the next cycle the hyperthermia time will be reduced by 25%: 45 minutes of MR-HIFU treatment. This level of pain is also considered a dose limiting loco-regional toxicity (loco-regional DLT).

If the patient experiences the above specified level of pain again after the hyperthermia time reduction, de time will be reduced further to 30 minutes of MR-HIFU treatment.

If the above specified level of pain still persists/recurs the patient will be withdrawn from the trial.

**Skin effects**

If a grade 1 (CTCAE) skin burn is occurs on the treated breast on any treatment day, then treatment should be held until the skin burn has resolved.

If a patient requires treatment withholding for more than 14 days, then the patient will be removed from the trial.

If the skin burn is resolved in ≤ 14 days the hyperthermia time in the next cycle will be reduced by 25%: 45 minutes of MR-HIFU treatment. If a grade 1 skin burn recurs after dose reduction the hyperthermia time will be further reduced to 30 minutes of MR-HIFU treatment. If the burn recurs after that, the patient will be withdrawn from the study.

If a grade 2 burn occurs on the treated breast the patient will immediately be withdrawn from the study.

For other adverse effects of the skin of the treated breast, that are suspected to be related to the study treatment, treatment will be delayed until the severity of the skin toxicity drops to ≤ grade 1. The hyperthermia time will be reduced by 25% in the next cycle. If a patient requires treatment withholding for more than 14 days, then the patient will be removed from the trial.

Skin burns and other adverse effects of the skin of the treated breast of grade 1 or higher are considered dose limiting loco-regional toxicities (loco-regional DLTs).

**S2.3 Dose adjustments, dose delay or withdrawal from study, based on technical difficulties**

In the study design we specified that we aim to perform 60 minutes of hyperthermia to the primary tumor at a temperature of 40ºC-42ºC, however, the ability to achieve this is also a feasibility parameter. It is possible that in certain patients, the aim will not be achieved, which will lead to an individual (unintended) adjustment of hyperthermia dose in that case. Furthermore, if MR-thermometry is insufficiently accurate to provide a safe MR-HIFU treatment, that treatment is stopped for safety reasons and the patient will receive the standard treatment of doxorubicin and cyclophosphamide.
If we experience technical difficulties during the MR-HIFU treatment (such as dysfunction of the MR-HIFU method, loss of power, mechanical difficulties) and we cannot guarantee the safety and feasibility of an individual patient's MR-HIFU treatment, the patient will receive the standard treatment of doxorubicin and cyclophosphamide.

After the technical difficulties have been resolved, the patient can still receive MR-HIFU and LTLD in the next treatment cycle or cycles.
If for one patient, hyperthermia treatment was for any reason insufficient (i.e. the target temperature 40-42°C was not reached or was only maintained for less than 30 minutes), in two separate treatment cycles, the patient will be excluded from the study, because the treatment is not considered feasible for that patient.

If the target temperature of 40-42°C is not reached, LTLD will not be administered (paragraph 8.3.15). Instead, conventional doxorubicin will be administered. However, if the temperature is initially reached, LTLD infusion is started and shortly afterwards the temperature becomes and remains insufficient, LTLD infusion will be continued as planned. In this case it is no longer possible to replace LTLD with conventional doxorubicin, as this would lead to an unreliable dose. If this scenario occurs twice the patient will be excluded from the study, as described above.

## Supplement 3 Restrictions to concomitant medications and products

Concurrent use of any of the following medications is strictly prohibited: protease inhibitors, cyclosporine, carbamazepine, phenytoin, valproic acid, paclitaxel, trastuzumab and other liposomal drugs (AbelectTM, Ambisome™, NyotranTM, etc.) or lipid-complexed drugs

Doxorubicin is a substrate of CYP3A4, CYP2D6 and P-glycoprotein (P-gp). As detailed in in table S3-1, inducers and inhibitors of these enzymes, as well as medication that acts with doxorubicin via other pathways could result in drug interactions. Caution will be exercised with regard to all the medications mentioned in table S3-1, for interactions are theoretically possible. If deemed necessary, clinically safe and feasible, these medications will be withheld or substituted before participation in the study.

Pre-specified exceptions were made for cyclophosphamide, dexamethasone, propofol, aprepitant and clemastine. These medications will be used as explained in appendix C.

Liposomal drugs (AbelectTM, Ambisome™, NyotranTM, etc.), or lipid-complexed drugs, or intravenous fat emulsions could change the pharmacokinetic profile of LTLD and should not be administered to study subjects while on the trial.

Subjects may take any medication that is not restricted by the protocol and would not be expected to interfere with the intent and conduct of the study. Chronic medications should be dosed on a stable regimen, if possible. In case of medications restricted by the protocol, adequate washout times must be observed. All medications at the time of screening and within 30 days prior to study treatment and other treatments taken by the subject during the study, including those treatments initiated prior to enrollment (ICF signing), must be recorded.

## Table S3.1 Concomitant medications and products with possible interactions

| Sources: (1-6) | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| **Antineoplastic therapy** | | | | | |
| bortezomib | | inhibitor | | | |
| **cyclophosphamide**[1] | Cardiotoxicity / hemorrhagic cystitis | inhibitor | | | |
| cytarabine | Miscellaneous[2] | | | | |
| dasatinib | | inhibitor | | | |
| docetaxel | | inhibitor | | | |
| etoposide | | inhibitor | | | |
| 5-fluorouracil | Cardiotoxicity | | | | |
| ifosfamide | | inhibitor | | | |
| imatinib | | inhibitor | | | |
| lapatinib | | | | inhibitor | |
| lomustine | | inhibitor | | | |
| 6-mercaptopurin: 6-MP / purinethol | Hepatotoxicity | | | | |
| methotrexate | Hepatotoxicity | | | | |
| methoxsalen | | inhibitor | | | |
| mitoxantrone | | inhibitor | | | |
| nafcillin | | inducer | | | |
| paclitaxel | Cardiotoxicity | | | | |
| plicamycin*** | Hematologic | | | | |
| rifamycin agents (all) | | inducer | | | 14 days |
| rifabutin | | inducer** | | | 14 days |
| rifampicin | | inducer** | | inducer** | 14 days |
| rifapentine | | inducer | | | 14 days |

[1] Pre-specified exceptions are described below

[2] Necrotizing colitis manifested by typhlitis (caecal inflammation), bloody stools, and severe and sometimes fatal infections have been associated with a combination of doxorubicin given by intravenous push daily for 3 days and cytarabine given by continuous infusion daily for 7 or more days. Source: Pfizer 2010.

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html

** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html

*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| sorafenib[3] | Possible dose modification | | | | |
| | | | | | |
| streptozocin*** | Hematologic | | | | |
| teniposide | | inhibitor | | | |
| trastuzumab | Cardiotoxicity | | | | 24 weeks |
| vinblastine | | inhibitor | | | |
| vincristine | | inhibitor | | | |
| vinorelbine | | inhibitor | | | |
| | | | | | |
| **(anti-) Hormonal medication** | | | | | |
| abirateron | | | inhibitor | | |
| anastrozole | | inhibitor | | | |
| danazol | | inhibitor | | | |
| drospirenone | | inhibitor | | | |
| ethinyl estradiol | | inhibitor | | | |
| mestranol | | inhibitor | | | |
| mifepristone | | inhibitor | | | |
| progesterone[4] | Hematologic | inhibitor | | | |
| tamoxifen | | inhibitor | | | |
| testosterone | | inhibitor | | | |
| | | | | | |
| **Calcium channel blockers** | | | | | |
| amlodipine | | inhibitor | | | |
| diltiazem | Cardiotoxicity | inhibitor | | | 7 days |
| felodipine | | inhibitor | | | |
| nicardipine (cardene) | | inhibitor | | | |
| nifedipine | | inhibitor | | | |
| nisoldipine | | inhibitor | | | |
| verapamil | Cardiotoxicity | inhibitor | | inhibitor** | 7 days |
| | *Hospital pharmacist's advice: in case of this interaction, no action is needed* | | | | |
| | | | | | |
| **Bètablockers** | | | | | |
| propranolol | Cardiotoxicity | | | | |
| carvedilol | | | | inhibitor | |
| | | | | | |
| **Angiotensin receptor blockers** | | | | | |
| irbesartan | | inhibitor | | | |
| losartan | | inhibitor | | | |
| | | | | | |

[3] In clinical studies, both an increase of 21% and 47%, and no change in the AUC of doxorubicin were observed with concomitant treatment with sorafenib 400 mg twice daily. The clinical significance of these findings is unknown. Source: Pfizer 2010.

[4] In a published study, progesterone was given intravenously to patients with advanced malignancies (ECOG PS< 2 ) at high doses (up to 10 g over 24 hours) 12 concomitantly with a fixed doxorubicin dose (60 mg/m$^2$ ) via bolus injection. Enhanced doxorubicin-induced neutropenia and thrombocytopenia were observed. Source: Pfizer 2010.

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| Anti-arrhythmic agents | | | | | |
|---|---|---|---|---|---|
| | **Increased risk of toxicity** | **CYP3A4** | **CYP2D6** | **P-gp** | **Wash-out** |
| amiodarone | | inhibitor | inhibitor | inhibitor** | 6 months |
| dronedarone | | | | inhibitor | |
| propafenon | | | inhibitor | inhibitor | |
| quinidine (kinidine) | | inhibitor | powerful* inhibitor | inhibitor** | |
| | | | | | |
| **Statins** | | | | | |
| atorvastatin | | inhibitor | | | |
| fluvastatin | | inhibitor | | | |
| lovastatin | | inhibitor | | | |
| pravastatin | | inhibitor | | | |
| | | | | | |
| **Anticonvulsants** *Hospital pharmacist's advice: the concentration of phenytoin and other antiepileptics can be affected by oncolytics, decreasing the antiepileptic effect. Dose adjustment is needed.* | | | | | |
| barbiturate agents | | inducer | | | |
| | *Hospital pharmacist's advice: interaction is only theoretical, no action is needed* | | | | |
| carbamazepine[5] | | inducer** | | inducer** | |
| fosphenytoin[5] | | inducer | | | |
| pentobarbital | | inducer | | | |
| phenobarbital *** | | inducer** | | inducer** | |
| phenytoin[5]*** | | inducer** | | inducer** | |
| primidone | | inducer** | | inducer** | |
| oxcarbazepine | | inducer | | | |
| valproic acid (depakine)[5] | | inhibitor | | | |
| | | | | | |
| **Antidepressants** | | | | | |
| bupropion | | | powerful* inhibitor | | |
| desipramine | | inhibitor | | | |
| duloxetine | | | inhibitor | | |
| fluoxetine | | powerful* inhibitor | | | |
| fluvoxamine | | inhibitor | | | 7 days |
| mirtazapine | | inhibitor | | | |
| nefazodone | | inhibitor | | | 7 days |
| norfluoxetine | | ? | inhibitor | | |
| paroxetine | | inhibitor | powerful* inhibitor | | |
| selegiline | | inhibitor | | | |
| sertraline | | inhibitor | inhibitor | | |
| tranylcypromine | | inhibitor | | | |
| trazodone | | inhibitor | | | |
| venlafaxine | | inhibitor | | | |

[5] The levels of carbamazepine, phenytoin and valproic acid can temporarily be affected by doxorubicin, with the risk of sub effective anti-epileptic dosage.

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| **Antipsychotics** | | | | | |
| clozapine | Hematologic | inhibitor | | | |
| haloperidol | | inhibitor | | | |
| olanzapine | | inhibitor | | | |
| pimozide | | inhibitor | | | |
| | **Increased risk of toxicity** | **CYP3A4** | **CYP2D6** | **P-gp** | **Wash-out** |
| risperidone | | inhibitor | | | |
| ziprasidone | | inhibitor | | | |
| | | | | | |
| **Thyreostatics ***** | | | | | |
| thionamids: e.g. | Hematologic | | | | |
| carbimazole | Hematologic | | | | |
| propylthiouracil | Hematologic | | | | |
| thiamazol / methimazole | Hematologic | inhibitor | | | |
| | | | | | |
| **Immune suppressive agents** | | | | | |
| azathioprine*** | Hematologic / Immune suppressive | | | | |
| cyclosporine/cyclosporine *** | Immune suppressive | inhibitor | | inhibitor** | |
| | *Hospital pharmacist's advice: The combination of anthracyclines and ciclosporin should be avoided* | | | | |
| interferon | Hematologic | | | | |
| sirolimus | | inhibitor | | | |
| tacrolimus | | inhibitor | | | |
| | | | | | |
| **Antibiotics** | | | | | |
| azithromycin | | inhibitor | | | |
| chloramphenicol*** | Hematologic | inhibitor | | | |
| clarithromycin | | powerful* inhibitor | | inhibitor | 7 days |
| ciprofloxacin | | inhibitor | | | |
| doxycycline | | inhibitor | | | |
| erythromycin | | powerful* inhibitor | | inhibitor ** | 7 days |
| norfloxacin | | inhibitor | | | |
| quinupristin | | inhibitor | | | |
| telithromycin | | inhibitor | | | |
| tetracycline | | inhibitor | | | |
| troleandomycin | | inhibitor | | | 7 days |
| | | | | | |
| **Antimycotics** | | | | | |
| amphotericin B*** | Nephrotoxicity | | | | |
| clotrimoxazole | | inhibitor | | | |
| fluconazole | | inhibitor | | | 7 days |
| flucytosine*** | Hematologic | | | | |
| itraconazole | | powerful* inhibitor | | inhibitor | 7 days |
| ketoconazole | | powerful* inhibitor | | inhibitor** | 7 days |

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| metronidazole | | inhibitor | | | |
| miconazole | | inhibitor | | | |
| posaconazol | | inhibitor | | | |
| sulconazole | | inhibitor | | | |
| terbinafine | | | inhibitor | | |
| voriconazole | | powerful* inhibitor | | | 7 days |
| | | | | | |
| **Anti (retro-) viral agents** *Hospital pharmacist's advice: In case of HIV-protease inhibitors, the interaction will always be discussed with the local pharmacist, doxorubicin is discouraged in this group.* | | | | | |
| atazanavir | | Inhibitor | | | |
| amprenavir | | Inhibitor | | | 7 days |
| boceprevir | | Inhibitor | | | |
| cobicistat | | powerful* inhibitor | | inhibitor** | |
| delavirdine | | Inhibitor | | | 7 days |
| efavirenz | | inducer | | | |
| fosamprenavir | | Inhibitor | | | |
| ganciclovir*** | Hematologic | | | | |
| indinavir | | Inhibitor | | | 7 days |
| interferon*** | Hematologic | | | | |
| lopinavir | | Inhibitor | | inhibitor** | 7 days |
| nelfinavir | | Inhibitor | | | 7 days |
| nevirapine | | Inducer | | | |
| ritonavir | | powerful* Inhibitor | powerful* inhibitor | inhibitor** | 7 days |
| saquinavir | | Inhibitor | | inhibitor | 7 days |
| simeprevir | | | | inhibitor** | |
| telaprevir | | | | inhibitor | |
| tipranavir | | | | inhibitor | |
| zidovudine*** | Hematologic | | | | |
| | | | | | |
| **Miscellaneous anti-infectious agents** | | | | | |
| clofazimine | | Inhibitor | | | |
| isoniazid | | Inhibitor | | | |
| mefloquine | | Inhibitor | | | |
| pentamidine | | Inhibitor | | | |
| primaquine | | Inhibitor | | | |
| quinine (kinine) | | Inhibitor | inhibitor | | |
| | | | | | |
| **Glucocorticoids[6]** | | | | | |
| betamethasone | | inducer | | | |
| cortisone (> 50 mg) | | inducer | | | 14 days |
| dexamethasone (> 1.5 mg) | | inducer | | inducer | 14 days |

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| hydrocortisone (> 40 mg | | inducer | | | 14 days |
| methylprednisolone (>8mg[8]), | | inducer | | | 14 days |
| prednisolone | | inducer | | | |
| prednisone (> 10 mg) | | inducer | | | 14 days |
| | | | | | |
| **Sedatives** | | | | | |
| dexmedetomidine | | inhibitor | | | |
| diazepam | | inhibitor | | | |
| midazolam | | inhibitor | | | |
| **propofol[9]** | | inhibitor | | | |
| | | | | | |
| **Pain medication** | | | | | |
| colchicine*** | Hematologic | | | | |
| diclofenac | | inhibitor | | | |
| dihydroergotamine | | inhibitor | | | |
| ergotamine | | inhibitor | | | |
| Fentanyl | | inhibitor | | | |
| lidocaine | | inhibitor | | | |
| paracetamol | | inhibitor | | | |
| | *Hospital pharmacists advice: This interaction is not clinically relevant, no action is needed* | | | | |
| | | | | | |
| **Antacids** | | | | | |
| *Hospital pharmacists advice: This interaction is not clinically relevant, no action is needed* | | | | | |
| rennies | Modify gastric acidity | | | | 1 hour before and after |
| mylanta / maalox (aluminum hydroxide, magnesium hydroxide simethicone) | Modify gastric acidity | | | | 1 hour before and after |
| tums | Modify gastric acidity | | | | 1 hour before and after |
| **Other GI agents** | | | | | |
| **aprepitant [10]** | | inhibitor | | | 7 days |

[8] Methylprednisolone at a single high dose (32mg) did not affect CYP3A4 activity and treatment with 8mg methylprednisolone daily for 9 days did not result in clinically significant induction of CYP3A3. (Villikka et al 2001)

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| cimetidine | | inhibitor | inhibitor | | 7 days |
| lansoprazole | | inhibitor | | inhibitor | |
| nizatidine | | inhibitor | | | |
| omeprazole | | inhibitor | | inhibitor | |
| | *Hospital pharmacists advice: This interaction is not clinically relevant, no action is needed* | | | | |
| pantoprazole | | | | inhibitor | |
| rabeprazole | | inhibitor | | | |
| | | | | | |
| **Histamine antagonists** | | | | | |
| azelastine | | inhibitor | | | |
| cimetidine | | inhibitor | | | |
| **clemastine**[11] | | inhibitor | | | |
| diphenhydramine | | | inhibitor | | |
| | | | | | |
| **Herbal or dietary ingredients or supplements** | | | | | |
| caffeine | | inhibitor | | | |
| | *Hospital pharmacists advice: This interaction is not clinically relevant, no action is needed* | | | | |
| cannabis oil | | | | inhibitor (10) | |
| citrus fruits (other than grapefruit: sour orange/bitter orange, pomelo, sweetie/oroblanco) | | inhibitor | | | 7 days |
| echinacea | | inducer (11) | | | 14 days |
| evening primrose oil | | inducer (12) | inhibitor (12) | | 14 days |
| ginkgo biloba | | inducer (12) | | | 14 days |
| ginseng | not conclusive(11, 13) | | | | 14 days |
| golden seal (yellow root, Hydrastis Canadensis) | | inhibitor (14) | inhibitor (14) | | |
| grape fruit (or juice) | | inhibitor | | | 7days |
| grape seed | | inhibitor (13) | | | 14 days |
| kava (piper methysticum) | not conclusive (13) | | | | 14 days |
| St. John's Wort (hypericum) | | inducer** | | inducer** | 14 days |
| turmeric (curcuma longa) | | inhibitor (15) | | inhibitor (16) | |
| valerian | not conclusive (14) | | | | 14 days |
| | | | | | |
| **Other** | | | | | |
| acetazolamide (Diamox) | | inhibitor | | | |
| aminoglutethimide | | inducer | | | |

[11] Pre-specified exceptions are described below

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html
*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## Table S3.1 Concomitant medications and products with possible interactions (continued)

| | Increased risk of toxicity | CYP3A4 | CYP2D6 | P-gp | Wash-out |
|---|---|---|---|---|---|
| bromocriptine | | inhibitor | | | |
| bosentan | | inducer** | | | |
| chlorzoxazone | | inhibitor | | | |
| cinacalcet | | | inhibitor | | |
| conivaptan | | inhibitor | | | |
| coumarins (vitamin K antagonists) | Possible fluctuation of coagulation times. Increased susceptibility to bleeding when thrombocytopenia occurs. | | | | |
| | *Hospital pharmacist's advice: Change to another anticoagulant is advised (e.g. LMWH)* | | | | |
| disulfiram | | inhibitor | | | |
| entacapone | | inhibitor | | | |
| glibenclamide/glyburide | | inhibitor | | | |
| hydralazine | | inhibitor | | | |
| live viruse vaccines | miscellaneous[12] | | | | |
| methadone | | inhibitor | | | |
| mirabegron | | | inhibitor** | | |
| modafinil | | inducer | | | |
| orphenadrine | | inhibitor | | | |
| oxybutynin | | inhibitor | | | |
| pergolide | | inhibitor | | | |
| pilocarpine | | inhibitor | | | |
| ranolazine | | inhibitor | | inhibitor | |
| sildenafil | | inhibitor | | | |
| ticlopidine | | inhibitor | | | |
| zalfirlukast | | inhibitor | | | |
| | | | | | |
| **Caution with (not strictly prohibited, consider monitoring)** | | | | | |
| digoxin | Doxorubicin can lower it's serum concentration | | | | |
| uric acid lowering agents | Doxorubicin can increase serum uric acid concentration (such as sulfinpyrazone*** and probenecid ***) | | | | |
| sorafenib | It might increase the doxorubicin dose | | | | |
| dexrazoxane[13] | It might result in lower response rates to doxorubicin | | | | |

[12] Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic agents including doxorubicin, may result in serious or fatal infections. Vaccination with a live vaccine should be avoided in patients receiving doxorubicin. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished. Source: Pfizer 2010

[13] In a clinical study of women with metastatic breast cancer, the concurrent use of the cardioprotectant, dexrazoxane, with the initiation of a regimen of fluorouracil, doxorubicin, and cyclophosphamide (FAC) was associated with a lower tumor response rate. Source: Pfizer 2010.

* Mentioned as powerful inhibitor/inducer in the KNMP Kennisbank.
Note: Relevance of an interaction also depends on properties of the substrate, such as therapeutical width, biological availability or another route of metabolism.
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html

** Mentioned as inhibitor/inducer in the KNMP Kennisbank
https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/G1126.html

*** Prohibited medication in the Phase I trial at Duke (IND#74,188)

## S3.2 Pre-specified exceptions (possible interactions accepted)

**1. Cyclophosphamide**

"The addition of cyclophosphamide to doxorubicin treatment does not affect exposure to doxorubicin, but may result in an increase in exposure to doxorubicinol, a metabolite. Doxorubicinol only has 5% of the cytotoxic activity of doxorubicin. Concurrent treatment with doxorubicin has been reported to exacerbate cyclophosphamide-induced hemorrhagic cystitis."(2) Concurrent cyclophosphamide treatment sensitizes the heart to the cardiotoxic effects of doxorubicin(17). However, since the AC regimen is frequently studied and used in daily practice, we anticipate a similar incidence of adverse events due to this interaction.

**6. Glucocorticoids (dexamethasone)**

Dexamethasone in the doses administered as premedication in this study induces CYP3A4 (7, 18), which could lower the doxorubicin concentration. However in the previous phase I and II dose finding studies (19), similar dosages of dexamethasone were administered (24 hours prior to treatment "dexamethasone 8 to 10 mg or an equivalent dose of a similar steroid consistent with local practice, every 12 hours x 3 doses" and 30 minutes prior to administration "IV Dexamethasone 20 mg"), therefore this interaction is accounted for in the maximum tolerable dose.

We will to administer dexamethasone in the premedication regimen as specified in the protocol, according to our local practice for the prevention of allergic reactions.

Additional dosages of glucocorticoids (above the specified dosages) are prohibited.

**9. Propofol**

A dosage-dependent inhibitory effect of propofol on cytochrome P450 3A4 has been described(20), indicating that a minimum clinical dosage could induce a significant inhibition of CYP 3A4 activity.

There is only one in vivo study were propofol decreased the clearance of midazolam, possibly via competitive inhibition of hepatic CYP3A4(21). Since no adverse events due to administration of propofol in combination with CYP3A4 substrates have been reported, we anticipate no severe interaction and administrate propofol as specified in the protocol.

**10. Aprepitant**

As described by Dushenkov et al. "coadministration of aprepitant with antineoplastics may result in SS pharmacokinetic alterations in serum levels of cytotoxics, with the best documentation for cyclophosphamide, ifosfamide and erlotinib. (…) To date, there are no data convincingly linking adverse outcomes due to coadministration of aprepitant and antineoplastics"(22).

Since the use of aprepitant as antiemetic in the dosages specified in the protocol is part of our hospital's standard practice for the AC chemotherapy regimen, and there is no convincing evidence against it, we will administer aprepitant as specified in the protocol. Additional dosages of aprepitant will be prohibited.

**11. Clemastine**

Clemastine may inhibit CYP3A4 activity and therefore alter doxorubicin metabolism. However clemastine is an essential part of the premedication regimen in our hospital for the prevention of allergic reactions. Since there are no reports in literature of clinically significant interactions with clemastine, and H1- antihistamine agents were also used in the dose finding study(19), we anticipate no severe interactions in our study and will administer clemastine as specified in the protocol.

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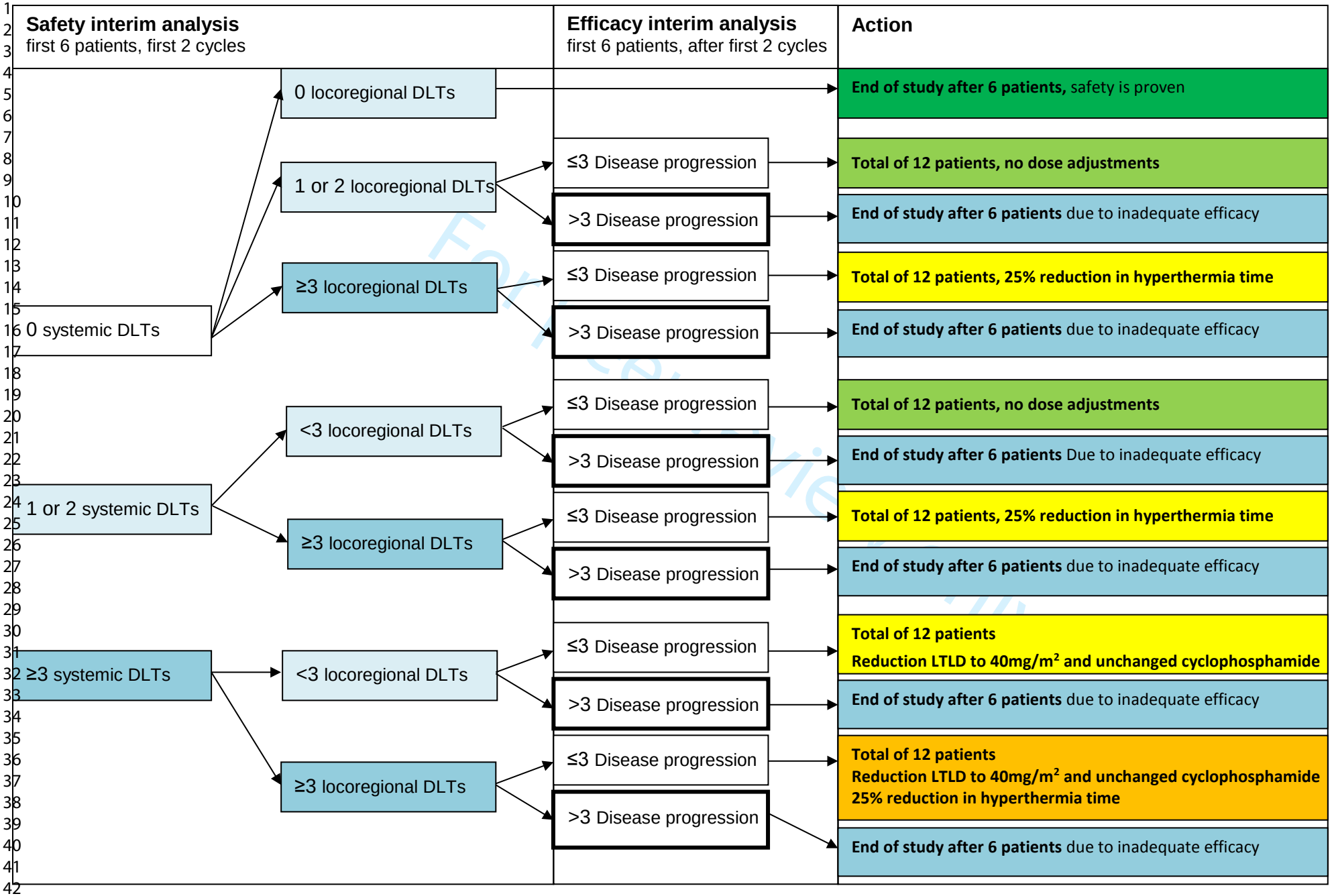
## Supplement 4 Flow-chart interim analysis



# Supplement 5 Data Safety Monitoring Board Charter

## DMC (DSMB) charter for the i-GO study

Version 3, 14-01-2019

| CONTENT | |
|---|---|
| **1. INTRODUCTION** | |
| Name (and sponsor's ID) of trial plus ISRCTN and/or EUDRACT number | • Trial name:<br>Image-guided targeted doxorubicin delivery with hyperthermia to optimize loco-regional control in breast cancer; the i-GO feasibility study.<br>Phase I Feasibility Study of High-Intensity Focused Ultrasound-Induced Hyperthermia (HIFU), Lyso-Thermosensitive Liposomal Doxorubicin (LTLD), and Cyclophosphamide for Metastatic Breast Cancer<br>• Trial sponsor: Imaging division and Cancer Center, University Medical Center (UMC) UTRECHT<br>• Type of trial: Investigational drug trial and investigational medical device trial<br>• Number of patients to be included: 6-12<br>• Number of sites: single center (UMC Utrecht)<br>• Estimated trial duration: 3 years<br>• EUDRACT number: 2015-005582-23<br>• METC protocol number: NL67422.041.18<br>• ClinicalTrials.gov Identifier: to be determined<br>• Principal investigator: B.B.M. Suelmann<br>• Coordinating investigator: J. S. de Maar |
| Objectives of trial, including interventions being investigated | Primary objective: To determine safety, tolerability and feasibility of the administration of LTLD + HIFU inducing local hyperthermia, combined with cyclophosphamide in metastatic breast cancer patients.<br>Secondary objective: Efficacy; to assess pathologic and clinically objective response from study treatment<br>A flow chart of the trial design is included (Figure 1). |
| Outline of scope of charter | *The purpose of this document is to describe the roles and responsibilities of the independent DMC for the i-GO trial, including the timing of meetings, methods of providing information to and from the DMC, frequency and format of meetings, statistical issues and relationships with other committees.* |
| **2. ROLES AND RESPONSIBILITIES** | |
| A broad statement of the aims of the committee | *"To safeguard the interests of trial participants, assess the safety and efficacy of the interventions during the trial, and monitor the overall conduct of the clinical trial."* |
| Terms of reference | *The DMC should receive and review the progress and accruing data of this trial and provide advice on the conduct of the trial to the Principal Investigator.*<br>*The DMC should inform the Principal Investigator and Head of Department of Medical Oncology if, in their view:*<br>*the results are likely to convince a broad range of clinicians, including those supporting the trial and the general clinical community, that the study treatment is clearly contraindicated or unsafe, and there was a reasonable expectation that this new evidence would materially influence patient management.* |

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| Specific roles of DMC | Interim review of the trial's progress including updated figures on recruitment, data quality, and main outcomes and safety data.<br>A selection of specific aspects could be compiled from the following list:<br>• assess data quality, including completeness (and by so doing encourage collection of high quality data)<br>• monitor recruitment figures and losses to follow-up<br>• monitor evidence for treatment harm (eg toxicity data, SAEs, deaths)<br>• Monitor the interim safety and efficacy analysis as specified in the study protocol in section 10.4<br>• decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated either for everyone or for some participant subgroups<br>• suggest additional data analyses<br>• advise on protocol modifications suggested by investigators or sponsors (eg to inclusion criteria, trial endpoints, or sample size)<br>• monitor compliance with previous DMC recommendations<br>• considering the ethical implications of any recommendations made by the DMC<br>• assess the impact and relevance of external evidence |
| **3. BEFORE OR EARLY IN THE TRIAL** | |
| Whether the DMC will have input into the protocol | All potential DMC members should have sight of the protocol/outline before agreeing to join the committee. Before recruitment begins the trial will have undergone review a research ethics committee. Therefore, if a potential DMC member has major reservations about the trial (eg the protocol or the logistics) they should report these to the PI and may decide not to accept the invitation to join. DMC members should be independent and constructively critical of the ongoing trial, but also supportive of aims and methods of the trial. |
| Whether the DMC will meet before the start of the trial | It is recommended that, if possible, the DMC meets before the trial starts or early in the course of the trial, to discuss the protocol, the trial, any analysis plan, future meetings, and to have the opportunity to clarify any aspects with the principal investigators. The DMC should meet within one year of recruitment commencing. |
| Any issues specific to the disease under study | Issues specific to the disease under study:<br>• The population consists of patients with metastatic breast cancer (stage IV disease), who have not received previous chemotherapy or surgery (previous antihormonal therapy is permitted)<br>• It concerns a non-curable disease. Median survival is approximately 2 years, however there is a great heterogeneity in survival, ranging from a few months to many years, with 10-15 % of patients surviving ten years and more.<br>• Most patients included in this trial will have the possibility to receive more treatment lines after this trial.<br>• Goal of the treatment in this stage of disease is to maintain or improve the quality of life and to improve survival.<br>• The natural course of the disease and the comorbidity are not always easy to distinguish from toxicity caused by the experimental treatment |

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| Any specific regulatory issues | The DMC should be aware of any regulatory implications of their recommendations. |
| Any other issues specific to the treatment under study | • The investigational drug (ThermoDox) and the co-intervention cyclophosphamide are chemotherapeutics<br>• As with the majority of chemotherapy regimens, toxicities can be expected including bone-marrow toxicity, nausea, fatigue, stomatitis, alopecia, constipation, and musculoskeletal chest pain. These adverse events are expected in standard treatment as well.<br>• All chemotherapy agents are potentially teratogenic and mutagenic.<br>• Specific regulations apply for the administration and handling of chemotherapy (UMC Utrecht protocols will be followed)<br>• The study treatment consists of an investigational drug as well as an investigational device. |
| Whether members of the DMC will have a contract | • Membership of the DMC (in agreement with the contents of this charter) will be accepted by the individual members and confirmed in writing and after submission of a signed and dated curriculum vitae (CV). The signed CV will be kept in the study file at the clinical trial bureau Medical Oncology.<br>• DMC members will sign a non-conflict of interest statement (Annex 1) in regard to this study which will be in the study file at the clinical trial bureau Medical Oncology. |
| **4. COMPOSITION** | |
| Membership and size of the DMC | The members will be independent of the trial (eg will not be involved with the trial in any other way or have some competing interest that could impact on the trial). Any competing interests, both real and potential, will be declared.<br>The members of the DMC for this trial are:<br>(1) *Hanneke van Laarhoven*<br>*Medical oncologist at the Academic Medical Center (AMC) Amsterdam*<br>*Clinician, experienced in the field of (medical) oncology and experienced in performing clinical trials*<br>(2) *Harm van Tinteren*<br>*Head of scientific administration/biometrics department at the Antoni van Leeuwenhoek (AVL) hospital. Biostatistical reviewer.*<br>(3) *Geertjan van Tienhoven*<br>*Radiation oncologist at the Academic Medical Center (AMC) Amsterdam*<br>*Clinician, experienced in the field of (radiation) oncology and experienced in performing clinical trials* |
| The Chair, how they are chosen and the Chair's role. (Likewise, if relevant, the vice-Chairman) | Hanneke van Laarhoven will be the chair of the DMC.<br>The Chair has previous experience of serving on DMCs and experience of chairing meetings, and is able to facilitate and summarize discussions. The Chair was chosen by the investigators. |
| The responsibilities of the DMC statistician | The DMC membership will include a statistician to provide independent statistical expertise. |
| The responsibilities of the trial statistician | The project team will not have a trial statistician, this is not considered necessary based on the trial design.<br>*The coordinating investigator* will produce the report to the DMC. |
| The responsibilities of the PI | The PI, may be asked, and should be available, to attend open sessions of the DMC meeting. |

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| **5. RELATIONSHIPS** | |
| Clarification of whether the DMC are advisory (make recommendations) or executive (make decisions) | The DMC makes recommendations to the investigators. |
| Payments to DMC members | Members will be reimbursed for travel expenses and for the costs of teleconferencing (if applicable). |
| The need for DMC members to disclose information about any competing interests | Competing interests should be disclosed. These are not restricted to financial matters – involvement in other trials or intellectual investment could be relevant. Although members may well be able to act objectively despite such connections, complete disclosure enhances credibility. (See Annex 1)<br>DMC members should not use interim results to inform trading in pharmaceutical shares, and careful consideration should be given to trading in stock of companies with competing products. |
| **6. ORGANISATION OF DMC MEETINGS** | |
| Expected frequency of DMC meetings | The DMC will meet approximately five times, during the trial. The exact frequency of meetings will depend upon trial events. The DMC will meet at least yearly. |
| Whether meetings will be face-to-face or by teleconference | The first meeting should ideally be face-to-face to facilitate full discussion and allow members to get to know each other. It is recommended that all subsequent meetings should be face-to-face if possible, with teleconference as a second option. |
| How DMC meetings will be organised, especially regarding open and closed sessions, including who will be present in each session | The format of the meetings will be:<br>1. Open session: **Introductory meeting**<br>Before start of the trial.<br>2. Closed session: **first data evaluation**<br>Once the first three patients completed two treatment cycles<br>(if necessary extra open session for clarification of specific concerns)<br>3. Closed session: **safety and efficacy interim analysis**<br>Once the first six patients completed two treatment cycles.<br>(if necessary extra open session for clarification of specific concerns)<br>4. (If applicable) Closed session: **further safety and efficacy analysis**<br>Once the first twelve patients completed two treatment cycles.<br>(if necessary extra open session for clarification of specific concerns)<br>5. Closed session: **follow up data evaluation**, **final evaluation**<br><br>The closed session will be restricted to the DMC members. The minutes of the closed session will be recorded by one of the members of the DMC. Minutes from the closed session will be recorded separately from the minutes of the open session and stored securely by the Chair. Closed session minutes, finalized by signature of the Chair, will be maintained in confidence and retained until discarded in accordance with applicable statutory regulation.<br>The open session will be attended by representatives of the study investigators (in general the coordinating investigator). Data presented in the open session may include enrolment data, individual AE data, baseline characteristics, overall data accuracy and compliance data or issues, and other administrative data. Minutes of the open session will be recorded by one of the members of the DMC. Minutes will be finalized upon signature of the Chair and maintained in the study file at the clinical trial bureau Medical Oncology in accordance with applicable statutory regulation. |

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| **7. TRIAL DOCUMENTATION AND PROCEDURES TO ENSURE CONFIDENTIALITY AND PROPER COMMUNICATION** | |
| Intended content of material to be available in open sessions | *Open sessions: Accumulating information relating to recruitment and data quality (eg data return rates, treatment compliance) will be presented. Toxicity details based on pooled data will be presented and total numbers of events for the primary outcome measure (safety, tolerability and feasibility) and other outcome measures may be presented, at the discretion of the DMC.* |
| Intended content of material to be available in closed sessions | *The same material will be available in the closed and open sessions.* |
| Will the DMC be blinded to the treatment allocation | Not applicable. |
| Who will see the accumulating data and interim analysis | The DMC members perform the interim analysis (safety and efficacy), based on the data provided by the coordinating investigator, and report their recommendations to the principal investigator.<br><br>DMC members do **not** have the right to share confidential information with anyone outside the DMC, including the PI. |
| Who will be responsible for identifying and circulating external evidence (eg from other trials/ systematic reviews) | Identification and circulation of external evidence (eg from other trials/ systematic reviews) is not the responsibility of the DMC members. The PI or the trials office team will collate any such information. |
| To whom the DMC will communicate the decisions/ recommendations that are reached | The DMC will report its recommendations in writing to the PI. |
| Whether reports to the DMC be available before the meeting or only at/during the meeting | The DMC will receive the report from the coordinating investigator at least 2 weeks before any meetings. |
| What will happen to the confidential papers after the meeting | *The DMC members should destroy their reports after each meetings. Fresh copies of previous reports will be circulated with the newest report before each meeting.* |
| **8. DECISION MAKING** | |
| What decisions/recommendations will be open to the DMC | Possible recommendations could include:<br>• No action needed, trial continues as planned<br>• Early stopping due, for example, to clear harm of a treatment, futility, or external evidence<br>• Stopping recruitment within a subgroup<br>• Extending recruitment or extending follow-up<br>• Sanctioning and/or proposing protocol changes |
| The role of formal statistical methods, specifically which methods will be used and whether they will be used as guidelines or rules | The planned statistical analyses are described in chapter 10 of the study protocol.<br>Specifically, an interim safety evaluation and an interim efficacy evaluation will be performed (section 10.4 of the study protocol) :<br>In summary, at the interim evaluations: |

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| | The trial will continue to accrue until a total of 12 subjects have been treated if both the following occur:<br>• a maximum of three of the first six subjects show disease progression after cycle 2; **AND:**<br>• either one or two systemic DLTs (dose limiting toxicities) were seen among the first two cycles of the first six subjects **OR**<br>•(if no systemic DLTs were seen) any locoregional DLT was seen among the first two cycles of the first six subjects<br><br>Specific criteria for dose adjustments for the entire study population are also specified in section 10.4 of the study protocol. |
| How decisions or recommendations will be reached within the DMC | Every effort should be made for the DMC to reach an unanimous decision. However, if this is not possible, the majority vote will decide.<br>It is important that the implications (eg ethical, statisticial, practical, financial) for the trial be considered before any recommendation is made.<br>The Chair will summarise discussions and encourage consensus; it may be best for the Chair to give their own opinion last. |
| When the DMC is quorate for decision-making | *Effort should be made for all members to attend. The coordinating investigator will try to ensure that a date is chosen to enable this. Members who cannot attend in person should be encouraged to attend by teleconference. If, at short notice, any DMC members cannot attend at all then the DMC may still meet if at least one statistician and one clinician, including the Chair will be present. If the DMC is considering recommending major action after such a meeting the DMC Chair should talk with the absent members as soon after the meeting as possible to check they agree. If they do not, a further teleconference should be arranged with the full DMC.* |
| Can DMC members who cannot attend the meeting input | If the report is circulated before the meeting, DMC members who will not be able to attend the meeting may pass comments to the DMC Chair for consideration during the discussions. |
| What happens to members who do not attend meetings | *If a member does not attend a meeting, it should be ensured that the member is available for the next meeting. If a member does not attend a second meeting, they should be asked if they wish to remain part of the DMC. If a member does not attend a third meeting, they should be replaced.* |
| Whether different weight will be given to different endpoints (eg safety/efficacy) | Safety and efficacy interim analyses are equally important and both determine whether the trial will be continued, as specified in section 10.4 of the study protocol. |
| Any specific issues relating to the trial design that might influence the proceedings, eg cluster trials, equivalence trials, multi-arm trials | The safety interim analysis and efficacy interim analysis will both be performed once the first six patients completed two treatment cycles.<br><br>It is possible (and expected) that when the sixth patient completes her second treatment cycle, the first patient will already have completed all treatment cycles. |

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| **9. REPORTING** | |
| To whom will the DMC report their recommendations/decisions, and in what form | The DMC will report their recommendations/decisions in the form of a letter to the PI and coordinating investigator, within 2 weeks. A copy of the letter will be kept in the study file at the Clinical trial bureau Medical Oncology. |
| Whether minutes of the meeting be made and, if so, by whom and where they will be kept | As described in chapter 6 of this charter minutes of the meetings will be taken by one of the DMC members and will be kept at the clinical trial bureau. |
| What will be done if there is disagreement between the DMC and the body to which it reports | *"If the DMC has serious problems or concerns with the PI's decision a meeting should be held. The information to be shown would depend upon the action proposed and the DMC's concerns. Depending on the reason for the disagreement confidential data will often have to be revealed to all those attending such a meeting. The meeting should be chaired by a senior member of the trials office staff or an external expert who is not directly involved with the trial."* |
| **10. AFTER THE TRIAL** | |
| Publication of results | At the end of the trial there may be a meeting to allow the DMC to discuss the final data with principal trial investigators and give advice about data interpretation.<br>The trial results will be published in a correct and timely manner. |
| The information about the DMC that will be included in published trial reports | DMC members should be named and their affiliations listed in the main report. A brief summary of the timings and conclusions of DMC meetings should be included in the body of this paper. |
| Any constraints on DMC members divulging information about their deliberations after the trial has been published | The DMC may discuss issues from their involvement in the trial when permission is agreed with the PI. |

I hereby declare that I have read the charter and that I agree with its contents.

Name: _____

**Signed:** _____ **Date:** _____

UMC Utrecht

## Supplement 6.1 Patient informed consent form in English

**The i-GO study: treatment of breast cancer using chemotherapy encapsulated in temperature sensitive nanoparticles, in combination with local warming of the tumour.**

- I have read the information letter. I was able to ask questions. My questions have been sufficiently answered. I had sufficient time to decide whether or not I will participate.
- I know that participation is voluntary. I also know that at any moment, I can decide not to participate after all or to quit the study. I don't have to provide a reason for that.
- I give consent to inform my general practitioner, treating medical specialist(s) and pharmacy that I participate in this study.
- I give consent to request information (medical data, laboratory results and previously made scans) from my general practitioner and treating medical specialist(s) from other hospitals.
- I give consent to notify my general practitioner and/or treating medical specialist(s) about unexpected findings that are or could be of importance to my health.
- I know that I cannot become pregnant during the study.
- I consent to collect and use my data and blood samples in the way and for the causes that are described in the information letter.
- I know that, in order to monitor the study, certain persons will have access to all my data. These persons are stated in the information letter. I give consent for access by these persons.
- I give consent to keep my data at the UMC Utrecht for 15 years after this study.
- I □ **do**
  □ **do not**
  give consent to use my personal data for future research on the topic of breast cancer, during the 15 year that the data have to be kept.
- I □ **do**
  □ **do not**
  give consent to approach me after this study for a follow-up study or other research on the topic of breast cancer.
- I know that, in case I have side effects of the treatment, coded data on the side effects will be provided to Profound Medical and Celsion. These data cannot be traced back to me personally.
- I □ **do**
  □ **do not**
  give consent to provide coded data (other than side effects) to Profound Medical and Celsion. These data cannot be traced back to me personally.

- I want to participate in this study.

Name study subject:

Signature: Date : __ / __ / __

UMC Utrecht

I declare that I have fully informed this subject on the mentioned study.
If, during the duration of the study, information will become available that could affect the subject's consent, then I will timely inform her about that.

Name investigator (or representative):

Signature: Date: __ / __ / __

UMC Utrecht

## Supplement 6.2 Patient informed consent form for Biobank research in English

### Biobank research of the i-GO study

(This is a separate part of the i-GO study, for which you can give consent separately)

- I □ **do**
  □ **do not** give consent to draw **extra blood** which will be coded and kept indefinitely in the Central Biobank of the UMC Utrecht, for future research on the topic of breast cancer.
- I □ **do**
  □ **do not**
  give consent to use my body material that has been obtained during breast biopsies, breast surgery or biopsies of metastases (to confirm my diagnosis or after the end of this study), to use this body material for further research and to keep it, as is explained in the patient information letter.
- I □ **do**
  □ **do not**
  give consent to keep my data at the UMC Utrecht for longer than 15 years and to use it for future research on the topic of breast cancer.
- I know that I can withdraw my consent to the Biobank research at any moment. I don't have to provide a reason for that.

Name study subject:

Signature: Date : __ / __ / __

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I declare that I have fully informed this subject on the mentioned study.
If, during the duration of the study, information will become available that could affect the subject's consent, then I will timely inform her about that.

Name investigator (or representative):

Signature: Date: __ / __ / __

**Reference to SPIRIT 2013 Checklist for:**

*Study protocol of the i-GO study, a phase I feasibility study of Magnetic Resonance guided High-Intensity Focused Ultrasound-induced hyperthermia, Lyso-Thermosensitive Liposomal Doxorubicin and cyclophosphamide in de novo stage IV breast cancer patients.*

| The SPIRIT 2013 Checklist contains recommended items to address in a clinical trial protocol and related documents.<br>The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" | | |
|---|---|---|
| **Section/item** | **Item no.** | **Mentioned in study protocol on page** |
| **Administrative information** | | |
| Title | 1 | Title page |
| Trial registration | 2a | Page 4, line12,<br>Page 26, line 16 |
| | 2b | Complete study protocol |
| Protocol version | 3 | Page 24, line 6 |
| Funding | 4 | Page 27, lines 6-9 |
| Roles and responsibilities | 5a | Page 24, line 14 through page 25 line 7<br>Page 26, lines 1-4 |
| | 5b | Page 24, lines 14-15 |
| | 5c | Page 24, line 14 through page 25 line 7 |
| | 5d | Page 24, line 14 through page 25 line 7 |
| **Introduction** | | |
| Background and rationale | 6a | Page 6-10 |
| | 6b | Not applicable |
| Objectives | 7 | Page 7, lines 2-8<br>Page 10, lines 19-22<br>Page 14, line 16<br>Page 23, lines 11-13 |
| Trial design | 8 | Page 10, lines 19-22 |
| **Methods: Participants, interventions, and outcomes** | | |
| Study setting | 9 | Page 24, lines 14-15 |
| Eligibility criteria | 10 | Page 11, line 15 trough page 14, line 14. |
| Interventions | 11a | Page 15, line 14 through page 19, line 4 |
| | 11b | Page 17, lines 19-22 |

| | | |
|---|---|---|
| | | Page 18, lines 12-17 |
| | 11c | Not applicable |
| | 11d | Page 19, lines 5-16<br>Supplement 3 |
| Outcomes | 12 | Page 14 line 15 through page 15 line 13. |
| Participant timeline | 13 | Figure 2 |
| Sample size | 14 | Page 11, lines 8-10 |
| Recruitment | 15 | Page 11, lines 10-14 |
| **Methods: Assignment of interventions (for controlled trials)** | | |
| Allocation | 16 | Not applicable |
| Blinding (masking) | 17 | Not applicable |
| **Methods: Data collection, management, and analysis** | | |
| Data collection methods | 18a | Page 25, line 8 through page 26 line 2. |
| | 18b | Page 20, lines 1-5. |
| Data management | 19 | Page 25, line 8 through page 26 line 10. |
| Statistical methods | 20a | Page 20, line 19, through page 21, line 3 |
| | 20b | Not applicable |
| | 20c | Page 20, lines 1-5. |
| **Methods: monitoring** | | |
| Data monitoring | 21a | Page 20, lines 14-17, supplement 5 |
| | 21b | Page 19 line 17 through page 20 line 17, supplement 4 |
| Harms | 22 | Page 16, line 20 through page 17 line 3,<br>page 24, lines 17-20,<br>page 18, lines 18-19 |
| Auditing | 23 | Page 20, line 14,<br>page 25, lines 6-7 |
| **Ethics and dissemination** | | |
| Research ethics approval | 24 | Page 4, line 8,<br>page 24, lines 4-6 |
| Protocol amendments | 25 | Page 24, lines 7-8 |
| Consent or assent | 26a | Page 24, lines 8-11, supplement 6 |
| | 26b | Page 18, lines 21-22 supplement 6 |

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|---|---|---|
| Confidentiality | 27 | Page 25, line 8 trrough page 26 line 10 |
| Declaration of interests | 28 | Page 28, line 2 |
| Access to data | 29 | Page 25, line 8 trrough page 26 line 10 |
| Ancillary and post-trial care | 30 | Page 24, line 20-21 |
| Dissemination policy | 31a | Page 24, line 11-12 |
| | 31b | Not applicable |
| | 31c | Not applicable |
| **Appendices** | | |
| Informed consent materials | 32 | Supplement 6 |
| Biological specimens | 33 | Page 18, line 21, through page 19 line 4 |