PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	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.
AUTHORS	de Maar, Josanne Sophia; Suelmann, Britt B.M.; Braat, Manon N.G.J.A.; van Diest, Paul; Vaessen, Paul; Witkamp, Arjen J.; Linn, S. C.; Moonen, Chrit T.W.; van der Wall, Elsken; Deckers, Roel

VERSION 1 – REVIEW

REVIEWER	Kui Luo Sichuan University, China
REVIEW RETURNED	13-Jun-2020

GENERAL COMMENTS	The author aimed to provide a new study protocol of phase I study on the combination of LTLD and hyperthermia in breast cancer, which has certain innovation. However, there are still some problems occur in this manuscript. From my point of view, the protocol is well-done and provides interesting research value. Based on the considerations above, I suggest a minor modification before publication. 1. Page6 line13: 'We aimed to increase doxorubicin levels in the primary tumor'. Is there any clue proving that the existing problem of neo-adjuvant and/or adjuvant chemotherapy is the insufficient level of chemotherapy drug? 2. Exit criteria should be included in the study protocol. 3. To estimate the appropriate dose of LTLD, dose escalation protocol or dose reduction protocol is needed, rather than simply setting a single start dose. 4. Some nanomedcines for breas cancer studies may be added to Introduction Section in the revised manuscript, such as Advanced Materials, 2020, 32(14), 1907490; Advanced Science, 2020, 7(6), 1903243; Chemical Engineering Journal, 2020, 391, 123543; Advanced Materials, 2019, 31(35), 1901586.

REVIEWER	Tadahiko Shien Okayama University Hospital, Japan
REVIEW RETURNED	26-Jun-2020

GENERAL COMMENTS	This is a design paper of new phase I trial to confirm the efficacy and safety of new treatment for breast cancer (LTLD with MR-HIFU). This new treatment is interesting and unique. I think that this manuscript indicated the details of this trial, completely. If the editors can allow that the design paper can be accepted this journal, I think

	that this paper has enough value. However, I cannot agree this treatment strategy using LTLD with MR-HIFU for de novo-stage IV breast cancer patients. They should receive effective systemic treatment including AC regimen to prolong their survival. We already know that standard systemic therapy is effective both local and metastatic disease. Moreover, limited local therapy for primary tumor does not have prognostic efficacy for them. I do not think that this treatment strategy is useful for de-novo stage IV breast cancer patients.

REVIEWER	Jun-Beom Park The Catholic University of Korea
REVIEW RETURNED	12-Aug-2020

GENERAL COMMENTS	Thank you very much for submitting your research to BMJ Open. The reviewer would like to make several comments on your article. 1.What is the novelty of report? 2.How did the authors calculate the sample size? How did the authors arrive at the number of participants number? 3.Do the authors have preliminary data regarding lyso-thermosenstive liposomal doxorubicin.? What is the benefits for the participants? 4.It seems that the initial goal regarding the endpoint improvements was not met. Do you assume that conducting the further study seems suitable? 5.Please provide a more detailed information regarding the number of participants. 6.Please go over the exclusion criteria. 7.Pleae provide detailed information regarding the statistical analysis. Thank you very much.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Kui Luo
Institution and Country
Sichuan University, China
The author aimed to provide a new study protocol of phase I study on the combination of LTLD and hyperthermia in breast cancer, which has certain innovation. However, there are still some problems occur in this manuscript. From my point of view, the protocol is well-done and provides interesting research value. Based on the considerations above, I suggest a minor modification before publication.
Response of the authors:
Thank you very much for your compliments and your valuable suggestions.

1. Page 6 line13: ‘We aimed to increase doxorubicin levels in the primary tumor’. Is there any clue

proving that the existing problem of neo-adjuvant and/or adjuvant chemotherapy is the insufficient level of chemotherapy drug?
Response of the authors:
Preclinical data has proven that an increased doxorubicin concentration in the primary tumor leads to increased tumor growth delay (Ponce et al. 2007, Besse et al. 2019).
Furthermore, in clinical practice, it is thought that a higher chemotherapy dose leads to increased effect. This has been 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 with longer survival in patients with liver metastasis of colorectal carcinoma (Moehler et al. 1995) a and with better tumour response in lung cancer patients (van der Veldt et al. 2013), respectively.
In a study comparing different dose schedules of the adjuvant AC regimen, the highest dosages (60mg/m2 doxorubicin and 600mg/m2
cyclophosphamide) were most effective, and this is currently
the standard of care (Budman et al. 1998). However increasing the systemic dosage further is limited by the occurrence of unacceptable side effects. A randomized study evaluating even higher doxorubicin dosages (60mg/m2 versus 75mg/m2 and 90 mg/m2
) did not find a difference in diseasefree 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 (Henderson et al. 2003). By combining LTLD with local hyperthermia we aim to increasing local tumor drug concentration and thereby local response without interfering with systemic efficacy or toxicity.
Changes in manuscript:
- Reference Besse et al., 2019 was added
- The rationale described above was added to the manuscript on page 6.
2. Exit criteria should be included in the study protocol.
Response of the authors:
We have established the following exit criteria:
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.
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 (Supplement 2).”
Changes in manuscript:
- We have added a paragraph on reasons for study withdrawal on page 18 and 19.
- Detailed information on dose adjustments can be found in Supplementary materials 2
3. To estimate the appropriate dose of LTLD, dose escalation protocol or dose reduction protocol is needed, rather than simply setting a single start dose.
Response of the authors:
The optimal dose for LTLD has been previously determined at 50 mg/m2
(Zagar et al. 2014), which is
therefore the starting dose for this study. For this study a dose reduction protocol has been established, including LTLD dose reductions for systemic toxicity (e.g. myelosuppression, abnormal liver tests and mucositis) and hyperthermia time reductions for locoregional toxicity (e.g. postprocedural pain and skin effects). In general, in case of systemic dose limiting toxicity, LTLD dose will
be decreased from 50mg/m2
to 40mg/m2 and cyclophosphamide dose will remain unchanged. In case
of locoregional dose limiting toxicity, hyperthermia duration will be decreased from 60 minutes to 45 minutes.

Changes in manuscript:
We have included the dose reduction protocol in Supplement 2 and we refer to this supplement on page 17. In addition, we updated Supplement 4 to a recent amendment of the protocol.
4. Some nanomedicines for breast cancer studies may be added to Introduction Section in the revised manuscript, such as Advanced Materials, 2020, 32(14), 1907490; Advanced Science, 2020, 7(6), 1903243; Chemical Engineering Journal, 2020, 391, 123543; Advanced Materials, 2019, 31(35), 1901586.
Response of the authors:
Thank you for the suggestion. Although we highly appreciate the content of these papers, we do not feel that they are appropriate in the context of our present study.

Reviewer: 2
Reviewer Name
Tadahiko Shien
Institution and Country
Okayama University Hospital, Japan
1. This is a design paper of new phase I trial to confirm the efficacy and safety of new treatment for breast cancer (LTLD with MR-HIFU). This new treatment is interesting and unique. I think that this manuscript indicated the details of this trial, completely. If the editors can allow that the design paper can be accepted this journal, I think that this paper has enough value. However, I cannot agree this treatment strategy using LTLD with MR-HIFU for de novo-stage IV breast cancer patients. They should receive effective systemic treatment including AC regimen to prolong their survival. We already
know that standard systemic therapy is effective both local and metastatic disease. Moreover, limited local therapy for primary tumor does not have prognostic efficacy for them. I do not think that this treatment strategy is useful for de-novo stage IV breast cancer patients.
Response of the authors:
We thank the reviewer for his thoughtful comments. With this study we aim to provide a proof of principle for the combination treatment MR-HIFU and LTLD in de novo stage IV patients, while determining its safety and feasibility. We hypothesize that this treatment will in the future provide a benefit in the neoadjuvant setting. We agree that 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.
As you rightfully state, these patients should receive an effective systemic treatment including the AC regimen. In this study, their treatment will greatly resemble the AC regimen, which is the standard of care for these patients in our hospital, and our goal is to maintain equivalent systemic efficacy. Conventional doxorubicin will be replaced by LTLD and MR-HIFU hyperthermia to the primary tumour, and cyclophosphamide will be administered according to the standard-of-care. In the absence of hyperthermia, LTLD releases ThermoDox slowly (~1%/min), therefore the systemic effect of doxorubicin is still guaranteed. We have taken several precautions to mitigate the risk of insufficient efficacy compared to the standard of care AC regimen. First, the dosage of LTLD was chosen based on the highest tolerable dose, which was also used in the phase 3 clinical LTLD studies. We expect a similar or perhaps even stronger systemic effect of LTLD at 50mg/m2
compared to doxorubicin at
60mg/m2
, based on the pharmacokinetic data shown in Supplement 1. Second, if this dose will lead to systemic dose limiting toxicity (and thus a higher systemic effect than the standard-of-care AC regimen), only the LTLD dose will be decreased and the cyclophosphamide dose will remain unchanged. Third, as we outlined in the strength and limitations, conducting this study in de novo stage IV patients has the benefit that both local and systemic response to the treatment can be monitored. If a patient has disease progression after two cycles, study participation will end and she will continue with standard of care treatment, i.e. the LTLD will be replaced by conventional

doxorubicin. The cyclophosphamide will continue in the schedule as proposed, as this is the standard of care schedule. In case the systemic efficacy is less than expected (four or more of the first six participants show distant disease progression after two cycles) the trial will be stopped.
In light of the recently presented results of the E2108 trial at ASCO (Khan et al. 2020), indicating that local treatment does not affect outcomes of stage IV breast cancer patients, we have concluded that the (previously conflicting) data on this topic are now convincing enough. 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 we do expect at least an equally effective treatment, based on the pharmacokinetic studies mentioned in the discussion and highlighted in Supplement 1. Study participants will participate altruistically in the interest of future patients in the neoadjuvant setting.
Changes in manuscript:
- The rationale described above was added to the manuscript on page 7 and 8.

Reviewer: 3
Reviewer Name
Jun-Beom Park
Institution and Country
The Catholic University of Korea
1.What is the novelty of report?
Response of the authors:
This will be the first-in-human study to evaluate LTLD with MR-HIFU hyperthermia in breast cancer patients.
2.How did the authors calculate the sample size?
How did the authors arrive at the number of participants number?
Response of the authors:
We have not conducted a formal sample size calculation because this is a phase I trial. The small samples size was chosen because this is the first study evaluating the combination of MR-HIFU hyperthermia, LTLD and cyclophosphamide. Since the LTLD and hyperthermia doses could be chosen
based on previous studies, this study is comparable to a phase Ib study with two cohorts of 3 patients to begin with. The rationale to proceed from 6 to 12 patients is presented in Supplement 4.
Changes in manuscript:
- We have added a statement about the sample size on page 11.
3.Do the authors have preliminary data regarding lyso-thermosenstive liposomal doxorubicin.?
What is the benefits for the participants?
Response of the authors:
The previous studies using LTLD have been described in the introduction.
In light of the recently presented results of the E2108 trial at ASCO (Khan et al. 2020), indicating that local treatment does not affect outcomes of stage IV breast cancer patients, we have concluded that the (previously conflicting) data on this topic are now convincing enough. 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 we do expect at least an equally effective treatment, based on the pharmacokinetic studies mentioned in the discussion and highlighted in Supplement 1. Study participants will participate altruistically in the interest of future patients in the neoadjuvant setting.
Changes in manuscript:
- We have highlighted this topic on page 7 and 8.
4.It seems that the initial goal regarding the endpoint improvements was not met.
Do you assume that conducting the further study seems suitable?
Response of the authors:
We have not previously performed a study, this is the first phase 1 trial evaluating the safety of this

combination treatment in breast cancer patients. The study has not started recruitment yet.
5.Please provide a more detailed information regarding the number of participants.
Response of the authors:
We will start with 6 participants, and during the interim analysis the decision whether or not to proceed to 12 participants will be made. The rationale to proceed from 6 to 12 patients is presented in Supplement 4. Patients who stopped study participation because MR-HIFU induced hyperthermia was
insufficient in two separate treatment cycles will be included in the analysis for safety and feasibility. However if they did not experience a DLT, they will be replaced with an additional patient in the interim safety evaluation. If four patients have to end study participation because MR-HIFU induced hyperthermia was insufficient in two separate treatment cycles, the study will be terminated, because of insufficient feasibility.
Changes in manuscript:
- We added a section on this topic on page 20.
6.Please go over the exclusion criteria.
Response of the authors:
We reviewed the exclusion criteria and elaborated on the exclusion criterion about gadolinium-based contrast agents. We did not find any reasons to modify the other exclusion criteria.
Changes in manuscript:
- We have adapted this exclusion criterion on page 14.
7.Please provide detailed information regarding the statistical analysis.
Response of the authors:
The plan for statistical analysis is outlined in the section on data analysis on page 21. We will conduct descriptive statistics, as this is a single arm phase 1 trial.
Thank you very much.

VERSION 2 – REVIEW

REVIEWER	Tadahiko Shien Okayama University Hospital, Japan
REVIEW RETURNED	27-Sep-2020

GENERAL COMMENTS	This is a study protocol of new treatment procedure for Breast cancer patients. There was no additional comments. I think that this article has enough value to be accepted to this journal.

REVIEWER	Jun-Beom Park The Catholic University of Korea, Republic of Korea
REVIEW RETURNED	28-Sep-2020

GENERAL COMMENTS	Dear Authors I extent my sincere thanks for submitting your manuscript to BMJ Open for the review. Authors have answered all the queries very nicely. This paper is of excellent merit and it seems fully acceptable for publication in BMJ Open. Thank you very much.