

# Ovarian suppression using luteinizing hormone-releasing hormone agonists during chemotherapy to preserve ovarian function and fertility of breast cancer patients: a meta-analysis of randomized studies

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**Background:** The role of temporary ovarian suppression with luteinizing hormone-releasing hormone agonists (LHRHa) in the prevention of chemotherapy-induced premature ovarian failure (POF) is still controversial. Our meta-analysis of randomized, controlled trials (RCTs) investigates whether the use of LHRHa during chemotherapy in premenopausal breast cancer patients reduces treatment-related POF rate, increases pregnancy rate, and impacts disease-free survival (DFS).

**Methods:** A literature search using PubMed, Embase, and the Cochrane Library, and the proceedings of major conferences, was conducted up to 30 April 2015. Odds ratios (ORs) and 95% confidence intervals (CIs) for POF (i.e. POF by study definition, and POF defined as amenorrhea 1 year after chemotherapy completion) and for patients with pregnancy, as well hazard ratios (HRs) and 95% CI for DFS, were calculated for each trial. Pooled analysis was carried out using the fixed- and random-effects models.

**Results:** A total of 12 RCTs were eligible including 1231 breast cancer patients. The use of LHRHa was associated with a significant reduced risk of POF (OR 0.36, 95% CI 0.23–0.57;  $P < 0.001$ ), yet with significant heterogeneity ( $I^2 = 47.1\%$ ,  $P_{\text{heterogeneity}} = 0.026$ ). In eight studies reporting amenorrhea rates 1 year after chemotherapy completion, the addition of LHRHa reduced the risk of POF (OR 0.55, 95% CI 0.41–0.73,  $P < 0.001$ ) without heterogeneity ( $I^2 = 0.0\%$ ,  $P_{\text{heterogeneity}} = 0.936$ ). In five studies reporting pregnancies, more patients treated with LHRHa achieved pregnancy (33 versus 19 women; OR 1.83, 95% CI 1.02–3.28,  $P = 0.041$ ;  $I^2 = 0.0\%$ ,  $P_{\text{heterogeneity}} = 0.629$ ). In three studies reporting DFS, no difference was observed (HR 1.00, 95% CI 0.49–2.04,  $P = 0.939$ ;  $I^2 = 68.0\%$ ,  $P_{\text{heterogeneity}} = 0.044$ ).

**Conclusion:** Temporary ovarian suppression with LHRHa in young breast cancer patients is associated with a reduced risk of chemotherapy-induced POF and seems to increase the pregnancy rate, without an apparent negative consequence on prognosis.

**Key words:** luteinizing hormone-releasing hormone agonists, fertility preservation, ovarian function preservation, breast cancer, premenopausal patients

## introduction

The majority of young premenopausal women diagnosed with early-stage breast cancer are candidates to receive a systemic treatment that includes chemotherapy [1]. Chemotherapy may

cause acute and chronic side-effects, including premature ovarian failure (POF) [2]. Despite chemotherapy-induced POF is reported to be favorably associated with survival outcomes [3], its occurrence is associated with several health-related consequences such as infertility, hot flashes, sleep disturbance, sexual dysfunction, and osteoporosis, as well as potentially influences the development of cardiovascular disease and dementia as women age [4, 5]. As a consequence, POF can cause psychological distress and negatively impact on short- and long-term global

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health of young breast cancer survivors [6]. Moreover, even patients who continue to menstruate after treatment remain at risk of infertility due to the detrimental effect of chemotherapy on ovarian reserve [7]. The concerns related to the possible loss of ovarian function and fertility can affect treatment decisions of a significant percentage of young patients at the time of breast cancer diagnosis [8].

Pregnancy after breast cancer is safe even in patients with endocrine-sensitive disease [9]. With a rising trend of delaying childbearing, more breast cancer patients are diagnosed without having completed their families and thus, it is vital to provide reliable fertility preservation methods for these young women. Recently, the 2015 St Gallen International Expert Consensus panel and the National Comprehensive Cancer Network (NCCN) guidelines have been updated to acknowledge the role of luteinizing hormone-releasing hormone agonists (LHRHa) in preventing chemotherapy-induced POF of hormone receptor-negative breast cancer [10, 11]. On the contrary, other guidelines are still hesitating to recommend this technique [12–14]. To date, the role of this approach in fertility preservation remains controversial for both the conflicting results shown in several randomized clinical studies and the lack of data on subsequent pregnancies [12, 13]. Moreover, due to both preclinical and clinical evidence suggesting potential antagonism with concurrent administration of anti-estrogen therapy and chemotherapy [15–17], and the possible detrimental effect of the lack of chemotherapy-induced amenorrhea on prognosis [3, 18, 19], some concerns exist on the safety of the concurrent use of LHRHa and chemotherapy in patients with endocrine-sensitive breast cancer [20].

Previously, we published a meta-analysis evaluating the role of temporary ovarian suppression with LHRHa during chemotherapy to reduce POF across different tumor types. We found that LHRHa was associated with a significant reduction in the occurrence of treatment-related POF, yet with significant heterogeneity observed [21]. In addition, few studies reported POF as the number of menstruating patients after the end of chemotherapy with limited information on subsequent pregnancies and impact on disease-free survival (DFS). Recent emerging data suggest that different tumor types like lymphoma can compromise ovarian function even before chemotherapy initiation [22], which would possibly contribute to differential effect of LHRHa on reducing POF according to the underlying disease. Thus, in this meta-analysis, we aim to reliably study the effect of temporary ovarian suppression with LHRHa during chemotherapy as a fertility preservation method, in breast cancer patients, not only addressing the impact on POF but also on subsequent pregnancy rates and DFS.

## methods

The study design was a quantitative synthesis of randomized trials aiming to evaluate the efficacy of temporary ovarian suppression with LHRHa during chemotherapy as a strategy to reduce POF and preserve fertility in young breast cancer patients, and the impact of this technique on patients' prognosis.

## study objectives

The primary objective of the study was to compare the incidence of treatment-related POF between patients treated with concurrent

temporary ovarian suppression with LHRHa during chemotherapy and those who received chemotherapy-alone. For this analysis, the definition of POF and the time-point of evaluation used in each eligible study were considered.

Secondary objectives were: (i) to compare the incidence of treatment-related POF, evaluated as amenorrhea (i.e. absence of menses) 1 year after the end of chemotherapy; (ii) to compare pregnancy rates; and (iii) to evaluate the impact of concurrent administration of LHRHa and chemotherapy on DFS. For the secondary analyses, only the studies with the availability of the required information were included.

## data sources and search strategy

A literature search using PubMed, Embase, and the Cochrane Library was carried out with no date restriction up to 30 April 2015. The search strategy entailed inputting the keywords related to 'chemotherapy', 'breast cancer', 'luteinizing hormone-releasing hormone', 'gonadotropin-releasing hormone', and 'ovarian function' in 'adult'. Specific keywords for each database and free text terms were combined with Boolean operators. According to different terms and rules of searching for each database, the effective combination of search terms was designed and set up by one reviewer (DU) and discussed with other two reviewers (ML and LDM). Two reviewers (ML and DU) independently evaluated the titles and abstracts of the identified studies; a third author (LDM) reviewed the search results to apply the eligibility criteria to both sets of search outcomes. A computerized search of the abstracts, reported at the American Society of Clinical Oncology (ASCO) Annual Meetings, at the ASCO Breast Cancer Symposium, at the European Society for Medical Oncology (ESMO) Annual Meetings and at the San Antonio Breast Cancer Symposium (SABCS) from 2004 onwards until April 2015, was run to identify relevant unpublished studies. Finally, cross-referencing from relevant studies and review articles on the topic was carried out to confirm retrieval of all possible pertinent trials.

The work was done and reported according to the PRISMA guidelines for reporting of systematic reviews [23].

## selection of the articles

Eligible studies had to fulfill the following inclusion criteria: (i) randomized trials designed to evaluate the efficacy of the addition of LHRHa to chemotherapy in terms of POF and/or fertility after chemotherapy; (ii) studies conducted in early-stage premenopausal breast cancer patients who were candidates for neo-adjuvant and/or adjuvant chemotherapy; and (iii) the odds ratio (OR) for POF and/or pregnancies had to be reported or could be computed from the data presented in the selected studies.

Studies excluded from the analysis were those with the following characteristics: (i) randomized trials designed to evaluate the efficacy of the addition of LHRHa to chemotherapy in terms of POF and/or fertility after chemotherapy in patients with autoimmune disease or tumors other than breast cancer; (ii) non-randomized studies conducted to evaluate the role of LHRHa during chemotherapy as a strategy to preserve ovarian function and/or fertility; and (iii) ongoing studies which had not yet been presented or published at the time of the literature search. No language restriction was applied.

For each eligible study, we collected study design, number of patients enrolled overall and into the two treatment arms, main eligibility criteria of patients enrolled in each study, type of chemotherapy administered, type and duration of LHRHa used in the experimental arm, use of adjuvant endocrine therapy following chemotherapy, main adverse events during chemotherapy, number of patients who developed POF, number of patients with menstrual resumption at 1 year after the end of chemotherapy, number of patients with pregnancy following breast cancer treatment and pregnancy outcomes, and number of DFS events.

## statistical analysis

ORs were calculated for the effect of temporary ovarian suppression with LHRHa during chemotherapy compared with chemotherapy-alone for POF and 12-month amenorrhea. An OR <1 indicates the use of LHRHa during chemotherapy yielded lower probability of developing POF and/or 12-month amenorrhea. The OR for pregnancies was calculated as the odds of patients with pregnancy in the LHRHa group divided by the odds of patients with pregnancy in the control group. An OR >1 indicates the use of LHRHa increased the probability of subsequent pregnancies. Hazard ratio (HR) was calculated for the effect of temporary ovarian suppression with LHRHa during chemotherapy compared with chemotherapy-alone for DFS. A HR <1 indicates the use of LHRHa reduced the probability of developing DFS events. For each point estimate, 95% confidence intervals (CIs) were computed.

The Mantel-Haenszel method was used to obtain fixed-effects model estimates of the pooled OR [24]; the fixed-effects model for

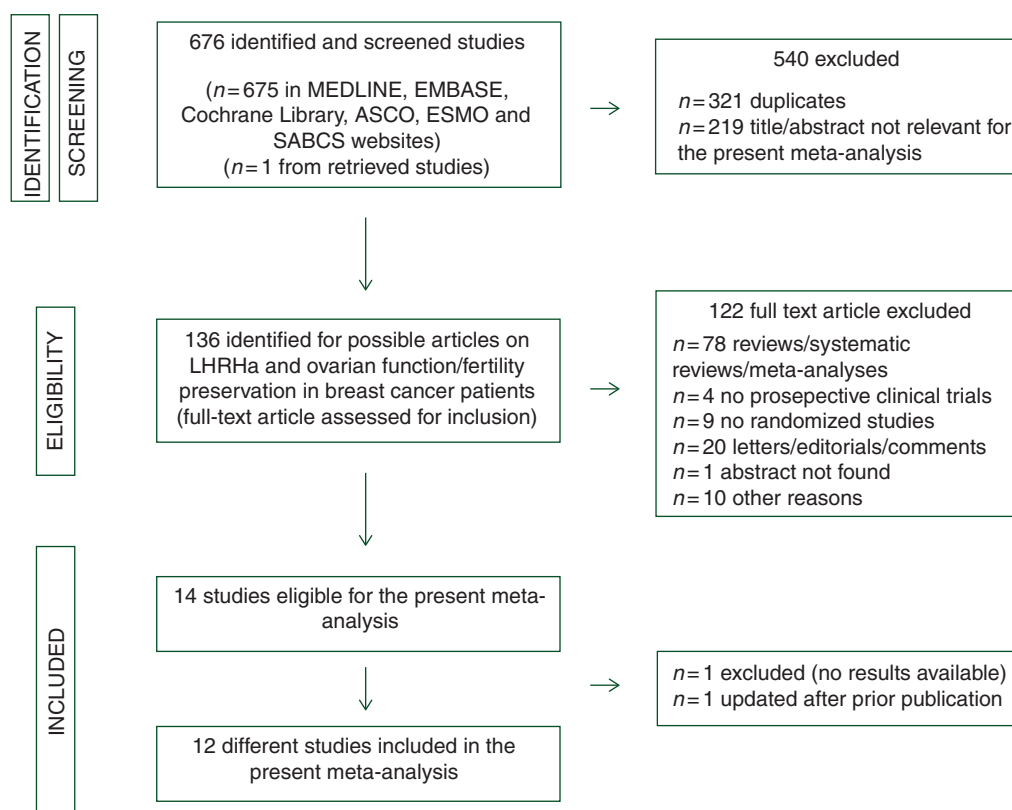
HR was computed by the inverse variance method [25]. Standard checks of the homogeneity assumption were carried out [26]. In the presence of significant heterogeneity among studies, the random-effects model is generally considered more appropriate than the fixed-effects model [27]. For this reason, pooled OR or HR estimates using the random-effects model were computed with the method of DerSimonian and Laird [28]. The Higgins'  $I^2$  index was computed to obtain a quantitative measure of the degree of inconsistency in the results of the studies included [27]. A visual inspection of the funnel plot for study size against treatment effect [29] and the Harbord's asymmetry test were used to assess the likelihood of publication bias [30]. All reported  $P$ -values were two-sided.

To assess whether the pooled OR/HR estimates were stable or strongly dependent on one or few of the studies included in the meta-analysis, sensitivity analyses were conducted by iteratively recalculating the pooled OR/HR estimates after exclusion of each single study.

All statistical analyses and the generation of forest plot were carried out using Stata Software Version 12.3 (StataCorp LP).

## results

The search strategy returned 676 entries: after the exclusion of 662 irrelevant publications, 14 potentially eligible trials were identified (Figure 1) [31–44]. Three studies were published only in abstract form: one study was presented in the poster session at the 2014 SABCS [43], another reported long-term outcomes



**Figure 1.** The PRISMA flow chart summarizing the process for the identification of the eligible studies. ASCO, American Society of Clinical Oncology; ESMO, European Society for Medical Oncology; SABCS, San Antonio Breast Cancer Symposium; LHRHa, luteinizing hormone-releasing hormone agonists.

of one previously published trial [40], and the third was presented at the 2010 ASCO Annual Meeting in abstract form, but no results have yet been reported [41]. The authors of the last study were contacted but no additional information could be obtained, and thus, this study was excluded leaving 12 studies (1231 patients) to be included in the present meta-analysis (Figure 1 and Table 1).

Different chemotherapy regimens were used in the different studies (Table 2). None of the studies used a placebo-controlled arm to allow blinding.

In two studies, patients could have received other forms of endocrine agents in addition to chemotherapy with or without concurrent LHRHa [32, 36]. In the study by Sverrisdottir et al. [32], all patients undergoing adjuvant chemotherapy were randomized in four different treatment arms: LHRHa, LHRHa + tamoxifen, tamoxifen, or no additional therapy. For the purpose of the present analysis, two different comparisons were considered: chemotherapy + LHRHa versus chemotherapy-alone (i.e. 'Sverrisdottir 1'), and chemotherapy + LHRHa + tamoxifen versus chemotherapy + tamoxifen (i.e. 'Sverrisdottir 2'). Elgindy et al. [36] randomly allocated patients into four different treatment arms: early chemotherapy-alone, early chemotherapy + LHRHa + LHRH antagonist (the LHRH antagonist was administered until obtaining a suppression of estradiol levels), delayed chemotherapy, and delayed chemotherapy + LHRHa. In the two delayed arms, chemotherapy was started at least 10 days after study inclusion. This study was analyzed considering the following comparisons: early chemotherapy-alone versus early chemotherapy + LHRHa + LHRH antagonist (i.e. 'Elgindy 1'), and delayed chemotherapy versus delayed chemotherapy + LHRHa (i.e. 'Elgindy 2').

### treatment-related POF according to study definition

For the purpose of this analysis, the definition of POF and the time-point of evaluation used in each eligible study were considered. The following different definitions of POF were used in the identified studies: no resumption or absence of menses [32, 33, 35, 36, 38, 42, 44], no resumption of spontaneous menstruation and ovulation [31], no resumption or absence of menses and postmenopausal levels of follicle-stimulating hormone (FSH) [39, 43], and no resumption or absence of menses and postmenopausal levels of both FSH and estradiol ( $E_2$ ) [34, 37]. The occurrence of POF was evaluated at different time-points after the end of chemotherapy: 6 months [33, 38], 8 months [31], 12 months [34, 36, 37, 42–44], 24 months [35, 39], and 36 months [32].

Overall, 320 POF events were recorded in 1231 patients, 114 of 616 (18.5%) patients treated with LHRHa during chemotherapy, and 206 of 615 (33.5%) undergoing chemotherapy-alone. A highly significant reduction in the risk of POF (OR 0.36, 95% CI 0.23–0.57,  $P < 0.001$ ) was observed in patients receiving LHRHa during chemotherapy, although with significant heterogeneity ( $I^2 = 47.1\%$ ,  $P_{\text{heterogeneity}} = 0.026$ ; Figure 2).

The funnel plot (Figure 3) and the Harbords asymmetry test ( $P = 0.276$ ) showed no evidence of publication bias. A satisfactory stability of the estimated OR was suggested by the sensitivity analysis, showing only marginal fluctuations (supplementary Table S1, available at *Annals of Oncology* online). The estimated OR computed excluding each study at a time, ranged from 0.33

and 0.41, with all results showing statistical significance (supplementary Table S1, available at *Annals of Oncology* online).

### amenorrhea 1 year after the end of chemotherapy

The efficacy of temporary ovarian suppression with LHRHa during chemotherapy was also evaluated in terms of treatment-related POF, defined as amenorrhea 1 year after the end of chemotherapy. A total of eight studies reported the number of patients with the absence of menses 12 months after the end of chemotherapy independently from the definition of POF used in the trials [33–37, 42–44].

Overall, 326 events of amenorrhea were recorded in 882 patients, 136 of 439 (31.0%) patients treated with LHRHa during chemotherapy, and 190 of 443 (42.9%) undergoing chemotherapy-alone. In patients receiving LHRHa during chemotherapy, a significant reduction in the risk of amenorrhea 1 year after the end of chemotherapy was observed (OR 0.55, 95% CI 0.41–0.73,  $P < 0.001$ ; Figure 4). No significant heterogeneity was observed ( $I^2 = 0.0\%$ ,  $P_{\text{heterogeneity}} = 0.936$ ). Supplementary Table S2, available at *Annals of Oncology* online summarizes the sensitivity analysis.

### patients with pregnancy

A total of five studies reported the number of patients with pregnancies after breast cancer treatment [33, 35, 36, 39, 40]. Of 359 patients treated with LHRHa during chemotherapy, 33 (9.2%) became pregnant, compared with 19 (5.5%) among 347 women undergoing chemotherapy-alone. A significant higher chance of becoming pregnant was observed for patients treated with LHRHa during chemotherapy (OR 1.83, 95% CI 1.02–3.28,  $P = 0.041$ ; Figure 5). No significant heterogeneity was observed ( $I^2 = 0.0\%$ ,  $P_{\text{heterogeneity}} = 0.629$ ). Supplementary Tables S3 and S4, available at *Annals of Oncology* online present the pregnancy outcomes reported in the studies and the sensitivity analysis, respectively.

### disease-free survival

Three studies reported DFS events [39, 40, 43]. In the study by Li et al. [43], median follow-up was 35.6 months for patients treated with LHRHa during chemotherapy, and 32.8 months in those treated with chemotherapy-alone; the overall median follow-up was 4.1 years in the POEMS-SWOG S0230 trial [39] and 7.3 years in the PROMISE study [40]. In the study by Li et al. [43], all patients had hormone receptor-positive disease whereas in the POEMS-SWOG S0230 trial all patients had hormone receptor-negative breast cancer [39]. In the PROMISE study, 226 of 281 (80.4%) patients had hormone receptor-positive disease [34].

A total of 60 DFS events occurred in the 307 (19.5%) patients treated with LHRHa during chemotherapy, and 60 in the 319 (18.8%) women undergoing chemotherapy-alone (supplementary Table S5, available at *Annals of Oncology* online). No difference in terms of DFS events was observed (HR 1.00, 95% CI 0.49–2.04,  $P = 0.939$ ; Figure 6), although with significant heterogeneity ( $I^2 = 68.0\%$ ,  $P_{\text{heterogeneity}} = 0.044$ ). Supplementary Table S6, available at *Annals of Oncology* online provides the sensitivity analysis.

**Table 1.** Main characteristics of the randomized studies included in the present meta-analysis

Authors	Year	Patients randomized, <i>n</i> (control/experimental)	Median age (control/ experimental)	Hormone receptor status, <i>n</i> (pos/neg)	Use of endocrine therapy	Type of LHRHa used	Definition of POF	Timing of POF evaluation
Li et al. [44]	2008	32/31	NR	NR	NR	Goserelin	No resumption of menses	NR
Badawy et al. [31]	2009	39/39	29.2/30	NR	NR	Goserelin	No resumption of menses and ovulation	8 months
Sverrisdottir et al. [32]	2009	66/57	45–45/45–46	NR	Yes (tamoxifen)	Goserelin	No resumption of menses	36 months
Del Mastro et al. [34]	2011	133/148	39/39	226/51	Yes (tamoxifen) <sup>a</sup>	Triptorelin	No resumption of menses and postmenopausal levels of FSH and E <sub>2</sub>	12 months
Lambertini et al. [40]	2014							
Gerber et al. [33]	2011	30/30	38.5/35.0	0/60	No	Goserelin	No resumption of two consecutive menstrual periods	6 months
Sun et al. [42]	2011	50/50	33/32	NR	NR	Goserelin	No resumption of menses	NR
Munster et al. [35]	2012	22/27	38/39	16/20	Yes (tamoxifen)	Triptorelin	No resumption of menses	12 months
Elgindy et al. [36]	2013	50/50	32.3–32.8/33.2–33.0	0/100	No	Triptorelin	No resumption of menses	12 months
Song et al. [37]	2013	94/89	40.3/42.1	150/33	Yes (tamoxifen) <sup>b</sup>	Leuprolide	Postmenopausal levels of FSH and E <sub>2</sub> in the absence of menstrual activity	12 months
Karimi-Zarchi et al. [38]	2014	21/21	37	0/42	No	Dipherelin	No resumption of menses	6 months
Li et al. [43]	2014	108/108	39/37.5	216/0	Yes (tamoxifen)	Goserelin	Amenorrhea for the prior 12 months and postmenopausal levels of FSH	12 months
Moore et al. [39]	2015	113/105	38.7/37.6	0/218	No	Goserelin	Amenorrhea for the prior 6 months and postmenopausal levels of FSH	24 months

<sup>a</sup>If the patients resumed menstrual activity during the 12-month period of observation after the end of chemotherapy, triptorelin could be restarted until ovarian function had been suppressed for at least 2 years.

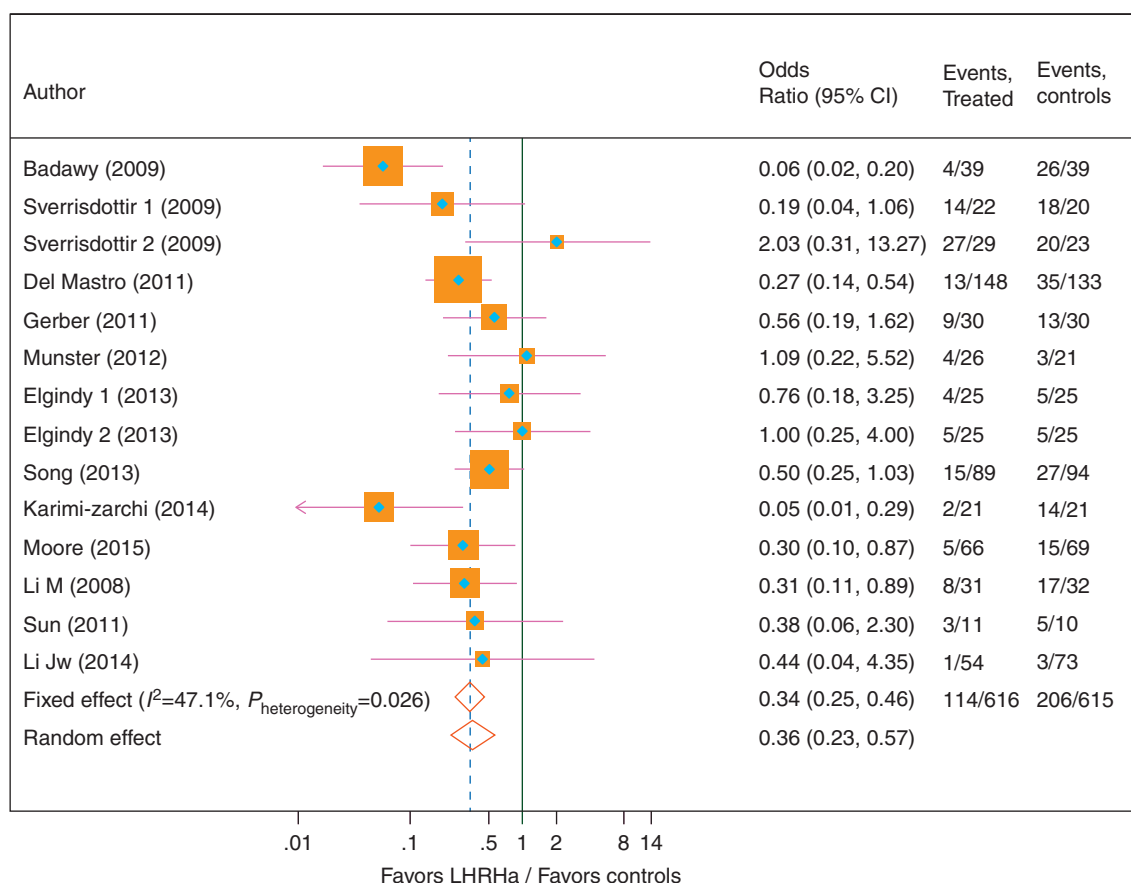
<sup>b</sup>If the patient resumed menstrual activity during follow-up, leuprolide was restarted and continued for 24 months to induce therapeutic ovarian suppression.

LHRHa, luteinizing hormone-releasing hormone agonist; pos, positive; neg, negative; POF, premature ovarian failure; NR, not reported; FSH, follicle-stimulating hormone; E<sub>2</sub>, estradiol.

**Table 2.** Number of cycles, type, and dose of chemotherapy administered in the randomized studies included in the present meta-analysis

Authors	Year	Type of chemotherapy	Cycles, <i>n</i>	Patients treated with anthracycline, <i>n</i> (control/experimental)	Patients treated with taxane, <i>n</i> (control/experimental)	Patients treated with cyclophosphamide, <i>n</i> (control/experimental)	Median dose of cyclophosphamide (control/experimental)
Li et al. [44]	2008	AC or AC → D	4	32/31	NR	63	NR
Badawy et al. [31]	2009	FAC	6	39/39	0/0	39/39	NR
Sverrisdottir et al. [32]	2009	CMF	6	0/0	0/0		NR
Del Mastro et al. [34]	2011	CMF or E → CMF or EP → CMF or	4–8	122/143	64/87	125/142	4008/4080
Lambertini et al. [40]	2014	ED → CMF or AC or EC or FEC or AC → D or EC → D or EC → P or FEC → P or FEC → D or ED					
Gerber et al. [33]	2011	FEC → T or EC → T or FEC or FAC or TAC or FEC → GEM	6–8	30/30	16/16	30/30	NR
Sun et al. [42]	2011	NR	NR	NR	NR	NR	NR
Munster et al. [35]	2012	AC or AC → P or FEC or FAC	4–8	22/27	5/8	22/27	NR
Elgindy et al. [36]	2013	FAC	6	50/50	0	50/50	5.680–5.528/5.564–5.536
Song et al. [37]	2013	AC or AC → D	4–6	94/89	25/32	94/89	3217.0/3094.5
Karimi-Zarchi et al. [38]	2014	TAC	NR	42	42	42	NR
Li et al. [43]	2014	NR	NR	NR	NR	NR	NR
Moore et al. [39]	2015	AC or CAF or TAC or CEF or AC → T or CMF	NR	102/96	NR	113/105	NR

AC, doxorubicin and cyclophosphamide; AC → D, AC followed by docetaxel; FAC, 5-fluorouracil, doxorubicin and cyclophosphamide; CMF, cyclophosphamide, methotrexate and 5-fluorouracil; E → CMF, epirubicin followed by CMF; EP → CMF, epirubicin, paclitaxel followed by CMF; ED → CMF, epirubicin, docetaxel followed by CMF; EC, epirubicin and cyclophosphamide; FEC, 5-fluorouracil, epirubicin and cyclophosphamide; AC/EC → D, AC or EC followed by docetaxel; EC → P, EC followed by paclitaxel; FEC → P, FEC followed by paclitaxel; FEC → D, FEC followed by docetaxel; ED, epirubicin and docetaxel; AC → P, AC followed by paclitaxel; TAC, docetaxel, doxorubicin and cyclophosphamide; CEF, cyclophosphamide, epirubicin and 5-fluorouracil; NR, not reported.



**Figure 2.** Odds ratios for premature ovarian failure by study definition, of patients treated with concurrent LHRHa versus those treated with chemotherapy-alone (controls). The squares on the odds ratio plot are proportional to the weight of each study. LHRHa, luteinizing hormone-releasing hormone agonists; CIs, confidence intervals.

## discussion

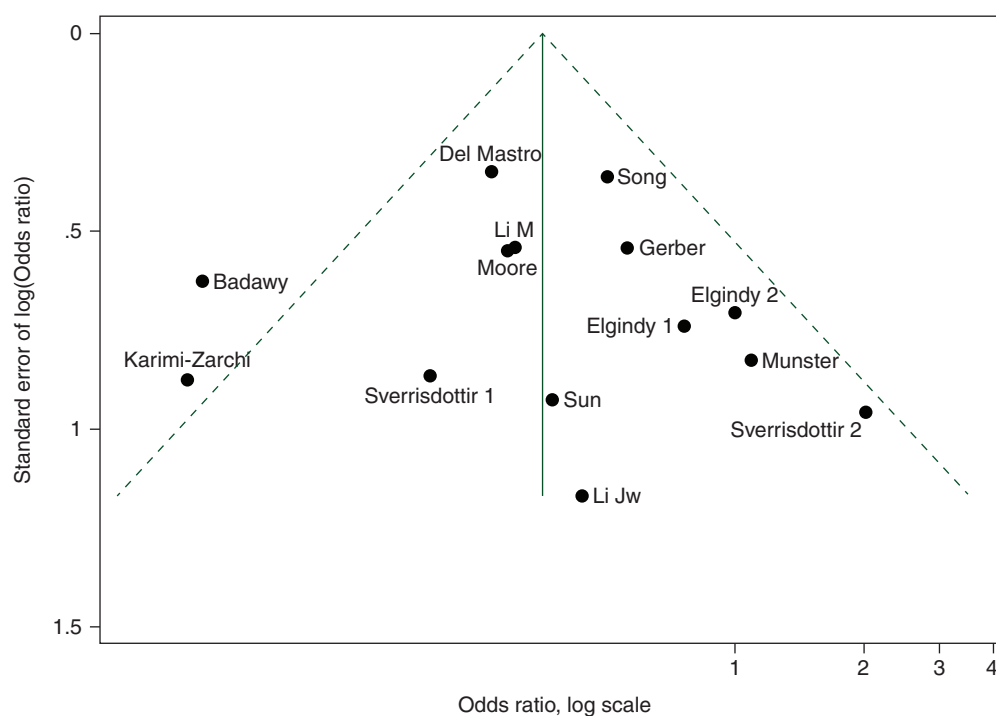
This is the largest, updated meta-analysis to assess the role of temporary ovarian suppression with LHRHa as a strategy to reduce POF and preserve fertility in breast cancer patients. The use of LHRHa showed to be an effective strategy reducing the risk of treatment-related POF and resulting in a significantly larger number of menstruating women 1 year after the end of chemotherapy. Albeit the numbers remain relatively low, its use almost doubled the chance of achieving subsequent pregnancies, suggesting a possible role of this technique as a promising new way to preserve fertility. When the results of the three studies reporting DFS were pooled together, no negative effect on patients' prognosis was observed.

To date, a total of 13 randomized trials with different aims, results, and methods have been conducted to assess the efficacy of this strategy [31–44]. The results from some of these studies have been previously analyzed in other meta-analyses [21, 45, 46]. However, these meta-analyses suffer of several limitations (i.e. to include also non-randomized studies, to include only part of the randomized studies, not to be restricted to trials conducted in breast cancer patients, and to analyze the effect of LHRHa only in terms of POF), and thus, no conclusive results are available so far. Indeed, despite this extensive research effort, there is still active debate about the efficacy of this strategy [47, 48]; the 2013 ASCO and ESMO practice guidelines consider the

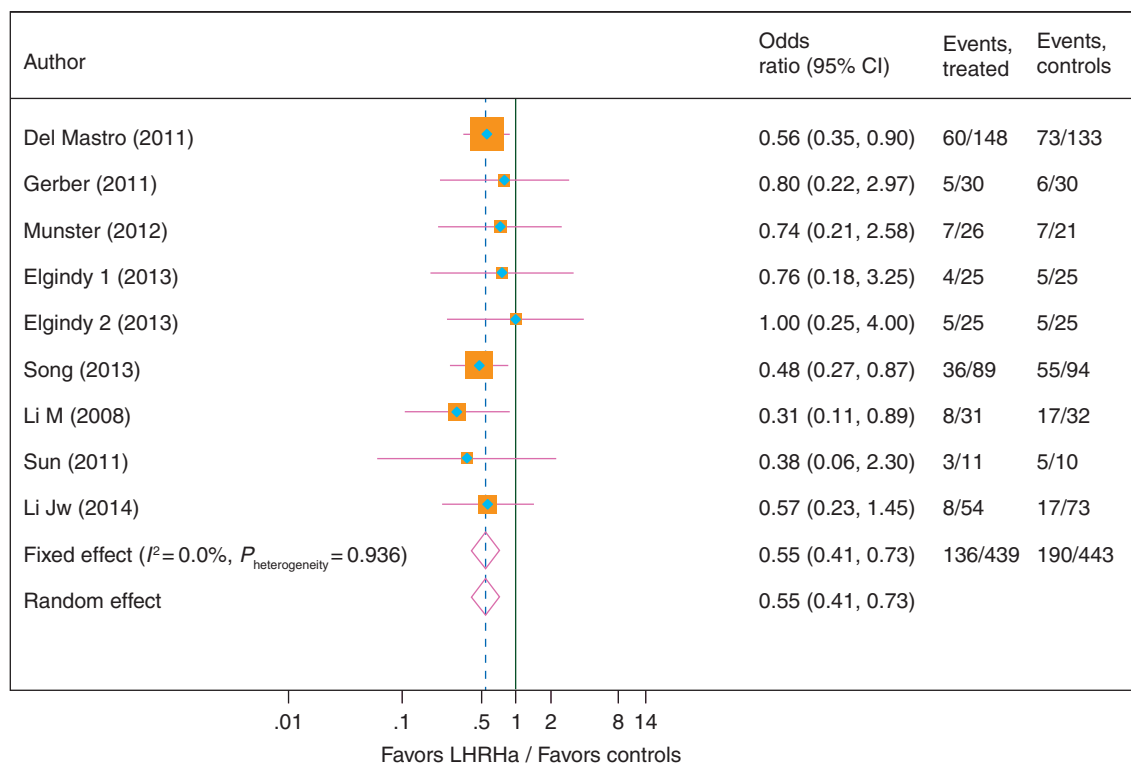
use of temporary ovarian suppression with LHRHa during chemotherapy, an experimental strategy to preserve fertility [12, 13]. However, these guidelines were completed before two of the largest studies were available [39, 40].

In the present analysis, temporary ovarian suppression with LHRHa during chemotherapy showed to significantly reduce the occurrence of treatment-related POF in breast cancer patients (OR 0.36,  $P < 0.001$ ). However, we found a high heterogeneity of the effect of LHRHa on reducing POF: one possible reason could be the different characteristics of the studies included (i.e. different definition of chemotherapy-induced POF used). In attempt to address the issues of the single trials, we then evaluated the protective effect of ovarian suppression with LHRHa restricting the analysis to the eight studies with available information on the rate of amenorrhea 1 year after the end of chemotherapy, which is a widely adopted definition [49]. The addition of LHRHa confirmed to have beneficial effect (OR 0.55,  $P < 0.001$ ), with no heterogeneity.

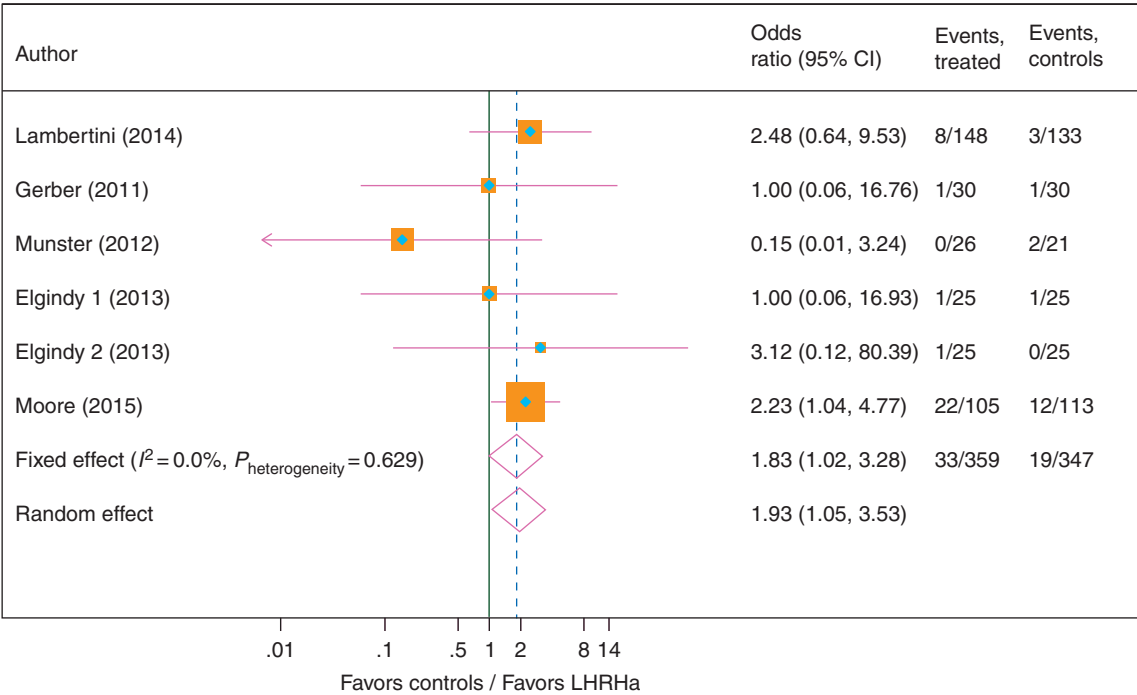
To date, there is paucity of data available on recovery of ovarian function at longer time-points. In the ZORO study and the trial by Munster et al., most of the patients in both treatment arms reported regular menses 24 months after the end of chemotherapy [33, 35]. In the POEMS-SWOG S0230 trial, among patients with available primary end point data (defined as the absence of menses and postmenopausal levels of FSH, 2 years after the end of chemotherapy), the use of LHRHa during



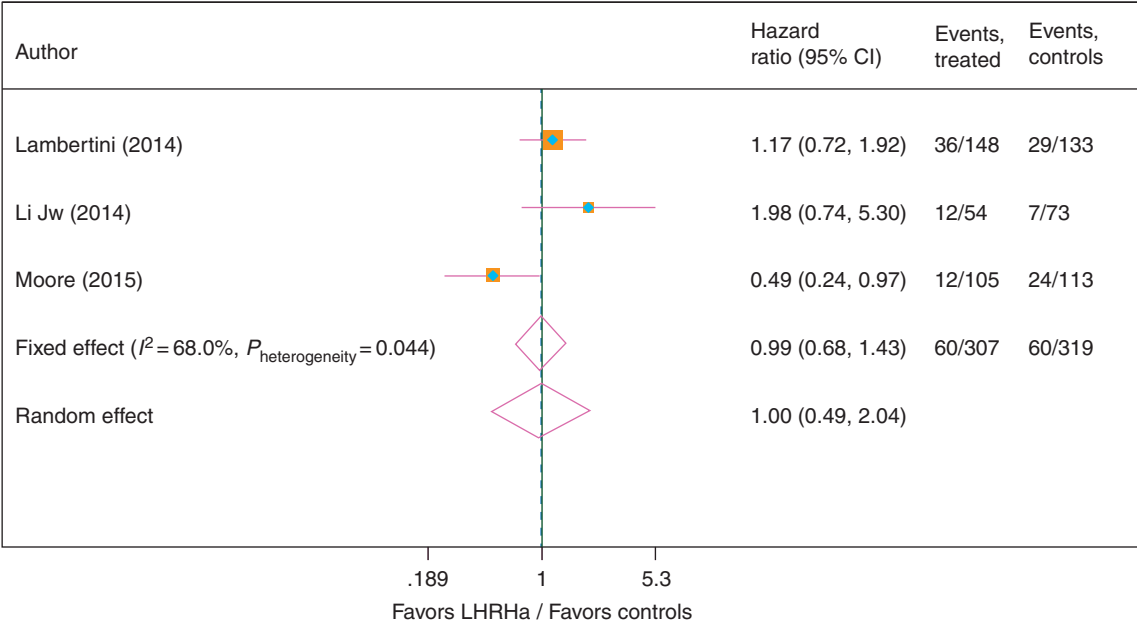
**Figure 3.** Funnel plot with pseudo 95% confidence limits of the LHRHa effect, estimated from individual studies (horizontal axis) against the study size (vertical axis). Publication bias is unlikely as suggested by the symmetric inverted funnel shape. LHRHa, luteinizing hormone-releasing hormone agonists.



**Figure 4.** Odds ratios for premature ovarian failure defined as amenorrhea 12 months after the end of chemotherapy, of patients treated with concurrent LHRHa versus those treated with chemotherapy-alone (control). The squares on the odds ratio plot are proportional to the weight of each study. LHRHa, luteinizing hormone-releasing hormone agonists; CIs, confidence intervals.



**Figure 5.** Odds ratios for women with pregnancy comparing patients treated with concurrent LHRHa versus those treated with chemotherapy-alone (control). The squares on the odds ratio plot are proportional to the weight of each study. LHRHa, luteinizing hormone-releasing hormone agonists; CIs, confidence intervals.



**Figure 6.** Hazard ratio for disease-free survival in patients treated with concurrent LHRHa versus those treated with chemotherapy-alone (control). The squares on the hazard ratio plot are proportional to the weight of each study. LHRHa, luteinizing hormone-releasing hormone agonists; CIs, confidence intervals.

chemotherapy significantly reduced the occurrence of treatment-induced POF (8% versus 22%; OR 0.30,  $P=0.04$ ) [39]. Sverrisdottir et al. [32] observed a significant increase in the proportion of menstruating women 36 months after chemotherapy in patients enrolled in the LHRHa arm (36% versus 10%;  $P=0.006$ ).

In the updated analysis of the PROMISE-GIM6 study, the 5-year cumulative incidence estimate of menstrual resumption was statistically significantly higher in the chemotherapy plus LHRHa arm than in the chemotherapy-alone arm (72.6% versus 64.0%; age-adjusted HR 1.48,  $P=0.006$ ) [40].

Although resumption of menses is a clinically relevant and reproducible outcome, it is not a perfect surrogate for adequate ovarian function/reserve and does not necessarily translate into fertility restoration. However, although ovarian suppression with LHRHa during chemotherapy has been studied as a strategy to preserve ovarian function and not fertility, there is evidence suggesting a possible utility of this technique to preserve fertility [50]. So far, in all the randomized studies included, a total of 33 patients treated with LHRHa and chemotherapy became pregnant when compared with 19 of those undergoing chemotherapy-alone. A significantly greater chance of becoming pregnant was observed for patients treated with LHRHa during chemotherapy (OR 1.83,  $P = 0.041$ ). However, this result might be influenced by the underlined methods of the studies: specifically, none of the trials aimed primarily to study fertility outcomes, and limited information on the number of patients interested in future pregnancies at the time of randomization and on those who attempted to become pregnant are available. Nevertheless, the most valid denominator for comparing pregnancy outcomes seems to be the entire randomized population as carried out in the present analysis: in fact, multiple biases are possible also when using the number of patients attempting pregnancy (e.g. attempted pregnancy could be influenced by the intervention assignment, and some pregnancies might occur in women who did not report an attempt to become pregnant as observed in the POEMS-SWOG S0230 trial) [51]. Moreover, the short median follow-up of the studies might be a possible explanation of the limited number of pregnancies observed, especially in those trials including patients with hormone receptor-positive disease who received adjuvant endocrine therapy for at least 5 years [35, 40], thus delaying attempts to pregnancy.

Importantly, safety concerns have been raised with the use of LHRHa as a strategy to preserve ovarian function and fertility, for both a possible increase in the side-effects during cytotoxic therapy, a possible detrimental effect of the lack of treatment-induced amenorrhea on prognosis and a potential negative interaction with chemotherapy.

The administration of LHRHa is associated with some adverse effects (e.g. headache, hot flashes, vaginal dryness, sweating, mood changes, insomnia, urogenital symptoms, and thromboembolic events). However, no significant difference in the incidence of grade 3 or 4 side-effects has been reported in the studies when LHRHa was added during chemotherapy [34, 39].

Chemotherapy-induced amenorrhea has demonstrated to have an independent prognostic value being associated with improved survival outcomes in young breast cancer patients, with a larger benefit in women with hormone receptor-positive disease [3, 18, 19]. However, the concern on the lack of the occurrence of chemotherapy-induced amenorrhea can be overcome by re-administering LHRHa at the time of ovarian function recovery as a part of adjuvant endocrine treatment [52].

The safety concerns on the potential negative interaction between endocrine therapy and chemotherapy are based on preclinical and clinical evidence, suggesting antagonism with the use of tamoxifen concurrently with chemotherapy [15–17]. However, LHRHa has different pharmacodynamic properties when compared with tamoxifen and, more than a decade ago, the three available randomized studies that investigated the impact of adding concurrent ovarian function suppression (obtained pharmacologically or with

surgery or radiotherapy) to chemotherapy did not demonstrate any difference in patients' prognosis [53–55]. Moreover, in the recently published TEXT trial, excellent survival outcomes were reported with the use of LHRHa concomitantly to chemotherapy [56]. In our meta-analysis, survival data could be retrieved only from three studies and the result showed high heterogeneity (probably due to the inclusion of different population according to hormone receptor status and with different length of follow-up). Owing to these limitations, the lack of association between DFS and LHRHa administered concurrently with chemotherapy observed in our meta-analysis (HR 1.00,  $P = 0.939$ ) should be considered with caution. Furthermore, follow-up data from the other studies are warranted to corroborate these findings.

Several limitations of the present analysis should be acknowledged including the different definitions of POF used, and the few studies reporting the number of patients attempting and achieving pregnancy, and limited data on survival outcomes. Also, the studies differed in the duration of LHRHa co-treatment and length of follow-up. Moreover, all data extracted are not based on individual patient data, but were retrieved from published articles or proceedings of major conferences. For this reason, it was not possible to investigate the impact of other important factors (i.e. patients' age, type and dose of chemotherapy, and use of adjuvant tamoxifen) on the results of the present meta-analysis. However, these limitations should not significantly influence the overall interpretation of our findings thanks to the strict methodology used and the attempts to overcome the heterogeneity of the results obtained from the analysis of the primary objective. Our data add new potential insights on the role of LHRHa during chemotherapy for ovarian function and fertility preservation in premenopausal breast cancer patients, in terms of both efficacy and safety of the strategy.

In conclusion, temporary ovarian suppression with LHRHa during chemotherapy is associated with a reduced risk of chemotherapy-induced POF and seems to increase the pregnancy rate in young breast cancer patients, with no apparent negative impact on patients' prognosis. The use of LHRHa during chemotherapy might be considered as an option for women interested in preserving their ovarian function, thus reducing the chance of developing the negative consequences of early menopause, and might also play a role in increasing the likelihood of becoming pregnant after cessation of chemotherapy.

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## disclosure

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## Designs of preoperative biomarkers trials in oncology: a systematic review of the literature

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**Background:** The identification of predictive and pharmacodynamics (PD) biomarkers of efficacy of anticancer-targeted therapies is not always straightforward. To address this problem, preoperative trials have been set up. The present study aimed at evaluating how these trials are designed.

**Design:** We retrieved all preoperative oncology trials, defined as preoperative trials having a PD end point.

**Results:** Only 56 trials met our selection criteria. Of these, 27 trials (48%) were randomized. Forty-nine trials (88%) evaluated at least a noncytotoxic agent. In 37 trials (66%), a single agent was administered. The most prevalent tumor type was breast cancer (59%). Median duration of accrual was 28 months (range: 9–98). In these trials, there was a mean of two patients included per month (range: 0–7). The date of surgery was fixed before study entry in 35 trials (62%), while surgery was set up after preoperative therapy in the remaining 21 trials (38%). In the former trials, median duration of preoperative therapy was 17 days (range: 1–112), whereas in the latter trials it ranged from 4 to 29 weeks. The primary end point was a PD end point in 26 of the 45 trials (58%) in which it was mentioned. One percent of patients could not undergo surgery as per protocol due to an adverse event. Statistically significant predictive and PD biomarkers were identified in 17 (30%) and 27 trials (48%), respectively.

**Conclusion:** Preoperative biomarkers trials are infrequent but safe and feasible. These trials often permit the identification of predictive and PD biomarkers.

**Key words:** preoperative, biomarker, pharmacodynamic, predictive, oncology, trials

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