

Utility of gonadotropin-releasing hormone agonists for fertility preservation in young breast cancer patients: the benefit remains uncertain

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Background: Breast cancer in young women is typically characterised by aggressive disease, and treatment with adjuvant chemotherapy is generally recommended. Chemotherapy has conferred significant improvements in disease-free and overall survival for young women with breast cancer; however, with improved cure rates, long-term adverse effects of cytotoxic treatment, such as premature ovarian failure (POF) and infertility, have become increasingly important. A potential fertility preservation strategy is administration of gonadotropin-releasing hormone agonists (GnRHAs) during adjuvant chemotherapy.

Design: This review analyses and summarises the current evidence for use of GnRHa in preserving ovarian function in young breast cancer patients.

Results: Twelve trials, both non-randomised and randomised, have now been conducted assessing GnRHAs in fertility preservation in young breast cancer patients, with conflicting results. Limitations of the current data include the use of poorly sensitive end points for fertility preservation, variable age of enrolled patients and limited pregnancy data.

Conclusion: The utility of GnRHa as a fertility preservation strategy remains uncertain, and use outside of a clinical trial generally not recommended. Further research into this under-recognised issue is vital.

Key words: breast cancer, chemotherapy, fertility, GNRH analogue, young age

introduction

Around 12% of all newly diagnosed breast cancers are detected in women younger than 44 years [1]. Breast cancer in young patients is characterised by generally aggressive disease including higher incidence of hormone-insensitive, undifferentiated, and HER2-overexpressing tumours, and may be associated with unique biological features compared with older women [2–5]. Adjuvant chemotherapy has conferred significant improvements in disease-free and overall survival for young women [6, 7]. However, with improved cure rates, long-term adverse effects of cytotoxic treatment have become increasingly important [8]. Of particular concern for young breast cancer patients is the risk of premature ovarian failure (POF) and subsequent infertility following adjuvant chemotherapy [8, 9]. POF also causes other health-related consequences, including osteoporosis, hot flashes, sleep disturbance, and sexual dysfunction, which can negatively impact on short- and long-term quality of life [10–12].

Chemotherapy-induced infertility is an under-recognised problem. Infertility is often defined by amenorrhoea; however, this is an imperfect surrogate. Infertility can precede menopause by a number of years [13], and decreased ovarian reserve secondary to chemotherapy can occur even in women with regular menstruation [14]. Furthermore, given the increasing proportion of women who are delaying pregnancy until later in reproductive life, young breast cancer patients facing childlessness as a consequence of adjuvant chemotherapy is an issue of growing importance [15–17].

The ovaries are endowed with a fixed number of resting primordial follicles at birth, which constitutes the ovarian reserve. These follicles are slowly depleted throughout a woman's reproductive life. Gonadotoxic chemotherapy increases the rate of follicle loss through, presumably, apoptotic cell death [18–20]. As germ cells cannot be regenerated, cytotoxic damage in the ovary is progressive and irreversible [21].

Age and type of chemotherapy regimen are the major determinants of risk of POF [22–25]. Women >40 years have higher rates of chemotherapy-induced amenorrhoea (CIA) (49%–100%) compared with women younger than 40 (21%–71%) [26, 27], due to the relatively low number of oocytes

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remaining after this timepoint. Chemotherapy agents commonly used in breast cancer treatment carry variable risk of ovarian damage. Alkylating agents and topoisomerase II inhibitors damage both proliferating and resting primordial follicles and, as such, confer high risk of POF, while anti-metabolites have no or little effect on ovarian reserve [26, 28, 29]. The gonadotoxicity of taxanes is uncertain [30], although recent studies demonstrate increased amenorrhoea rates with sequential taxane-based regimens, compared with non-taxane-based regimens [31–34], suggesting that these agents also contribute to POF risk.

fertility preservation methods

Ovarian stimulation and embryo cryopreservation is the best-established technique for fertility preservation in young breast cancer patients, although, as 2 to 3 weeks are required for ovarian stimulation, it is unsuitable for patients requiring prompt commencement of adjuvant treatment. Pregnancy rates of 20%–40% for transfers of two to three embryos from *in vitro* fertilisation (IVF)-generated embryos have been reported [12]. Alternatively, oocytes can be frozen unfertilized. Lower live birth rates per injected oocyte are generally seen, around 2% for commonly used slow-freezing techniques [35]. However, with improvements in the technology, including the use of vitrification instead of slow-freezing, success rates are increasing [36, 37] and the American Society of Reproductive Medicine has recently removed the experimental label from this technique [38]. Both embryo and oocyte cryopreservations are carried out following ovarian stimulation, as without stimulation, the yield is typically very low [39]. Conventional ovarian stimulation causes supra-physiological estradiol (E2) levels, and therefore, may be unsuitable for women with estrogen receptor-positive (ER+) tumours [40, 41]. Instead, ovulation induction regimens incorporating tamoxifen or aromatase inhibitors (AIs) can be used [16, 42–45], resulting in attenuated E2 levels without compromising embryo or oocyte viability. Of note, BRCA1 mutations may increase the risk of low ovarian response from ovarian stimulation [46], potentially due to underlying decreased ovarian reserve [46, 47], thus increasing susceptibility to gonadotoxic effects of chemotherapy. Therefore, young women with BRCA1-associated breast cancer represent a particularly high-risk group for infertility following chemotherapy, even with utilisation of current fertility preservation strategies.

Where ovarian stimulation is not possible, ovarian tissue may be resected and cryopreserved. This remains an experimental technique, with limited data on the rate of successful pregnancies [12].

A fourth potential fertility preservation strategy is administration of gonadotropin-releasing hormone agonists (GnRHAs) during adjuvant chemotherapy. This approach has several advantages over other techniques: it is widely available and relatively inexpensive, does not require ovarian stimulation, or an invasive surgical procedure.

The rationale for use of GnRHa is based on an observation that prepubescent children treated with chemotherapy had differing rates of infertility later in life, where the prepubescent state appeared to confer some protection to female, but not

male, gonads [48]. Thus, induction of a prepubescent state with GnRHa may limit ovarian damage during chemotherapy. How this may occur is unknown, though various mechanisms have been suggested, including GnRHa-induced decrease in the number of primordial follicles entering the differentiation stage, reduction of ovarian perfusion due to a GnRHa-induced hypoestrogenic state, and decreased ovarian cell apoptosis, through either activation of GnRH receptors or upregulation of intragonadal antiapoptotic molecules [48]. However, due to a lack of substantiating data, the validity of these theories has been questioned [49]. In preclinical animal studies, GnRHa administration was shown to reduce the loss of primordial follicles following cyclophosphamide treatment [50–52], although data in humans are limited.

current evidence for GnRHa

The efficacy of GnRHAs in preserving ovarian function in breast cancer patients has been reported in 12 studies to date. While single-arm studies demonstrated encouraging results (Table 1), randomised trial data have been conflicting (Table 2). Furthermore, heterogeneity of study populations and procedures, and lack of a proven mechanism of action for ovarian protection with GnRHa [49] make interpretation of results more challenging.

non-randomised trials

Urruticoechea et al. reported outcomes from an institutional audit of 60 premenopausal women who received goserelin concurrent with chemotherapy [53]. Forty-two had resumption of menstruation at 12 months follow-up, and a further two patients after 17 months, giving an overall rate of menstrual resumption of 90%. At median follow-up of 43 months, 10 patients had attempted pregnancies, 7 of whom were successful.

An update of results from this group was recently reported [54]. From 125 patients treated with goserelin during chemotherapy, 84% recovered menstrual function, after a median duration of 6 months (range 1–43). Forty-two pregnancies and 30 healthy births were recorded from 42 patients attempting pregnancy (71%).

Fox et al. reported, in abstract form, an institutional audit of 24 premenopausal women treated with chemotherapy plus leuprolide [55]. After a mean follow-up of 6 months post-treatment (range 2–12) menstruation had returned in 23 (96%). From eight patients attempting pregnancy, there were six pregnancies in five patients, resulting in one live birth, one ongoing pregnancy, three miscarriages and one termination. Three patients who became pregnant did so only after additional fertility treatment.

A single-arm phase II study from Maisano et al. [56] assessed the ovarian protective effect of leuprolide in 19 premenopausal women with ER-negative (ER-) early breast cancer (EBC) treated with adjuvant FEC (5-fluorouracil–epirubicin–cyclophosphamide) chemotherapy. The primary end point of menstruation resumption within 12 months of completion of chemotherapy was achieved in all patients, after median of 5 months (range 3–8). Premenopausal follicle-stimulating hormone (FSH) and E2 levels were recorded in all patients after

Table 1. Non-randomised trials of GnRHa in fertility preservation

Author Year	Urruticoechea [53] 2008	Wong et al. [54] 2012	Fox et al. [55] 2003	Maisano et al. [56] 2008	Recchia et al. [57] 2006
Number of patients	50	125	24	19	100
Study type	Retrospect cohort/audit	Retrospect cohort/audit ^a	Audit/retro cohort (abstract only)	Phase II single arm	Phase II single arm
Treatment	CT + goserelin	CT + goserelin	CT + leuprolide	CT + leuprolide	CT + goserelin
Timing of GnRHa	Start: 0–14 days pre-CT End: end of CT ^b	Start: 0–14 days pre-CT End: end of CT	Start: 0–14 days pre-CT End: end of CT	Start: 10–14 days pre-CT End: end of CT	Start: 7 days pre-CT End: after 1 (62%) or 2 years (38%) ^c
CT type	Any (all but one patient received A + C)	Any	A + C ± T	A + C	C or A + C, including HDCT + ASCT
Maximum age (years)	<45	<45	NR	≤40	≤50
Median age (years) [range]	Mean 34 (20–43)	35 (20–45)	35 (23–42)	36.5 (26–40)	43 (27–50)
Premenopausal definition	Regular menstruation	Regular menstruation	NR	Actively menstruating within 6 weeks pre-CT FSH <40 IU/L and E2 >20 pg/mL	Actively menstruating and FSH <10, LH <0.8, E2 20–693
%ER+	72%	69%	NR	0%	52%
Marker of ‘fertility preservation’	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation and premenopausal FSH, E2, LH, progesterone levels
Primary end point/ major objectives	Recovery of menstruation and Subsequent pregnancies	Recovery of menstruation Rate of successful conception median time to recovery of menses	Recovery of menstruation within 12 months	Recovery of menstruation or return to premenopausal FSH and E2 levels at 12 months post-CT	Recovery of menstruation
Median f/u (range)	43 months (21–64)	58 months (4–119)	NR	3 years (1–5 years)	75 months (minimum 32 months)
Rate of recovery of menstruation	90% within 17 months	84% at 6 months post-CT	96% within 12 months post-CT At mean f/u 34 months, 15/21 (71%) with regular menstruation	100% within 12 months	67% after completion of all Rx, including 5 of 9 pts treated with HDCT Return of menstruation: 100% versus 56% for patients <40yo versus. >40yo
Median time to recovery of menstruation (range)	NR	6 months (1–43)	5.7 months (2–12)	5 months (3–8)	NR
Pregnancies	7 of 10 women attempting pregnancy successful with 6 births, 1 termination and 1 woman with ongoing pregnancy	42 pregnancies and 30 healthy deliveries from 42 patients attempting pregnancy	5 of 8 women attempting pregnancy successful (3 requiring fertility treatment) resulting in 3 miscarriages, 1 termination, 1 pregnancy ongoing, 1 live birth	4 pregnancies in 4 patients, 4 normal deliveries	3 pregnancies, resulting in 2 births and 1 termination

FSH	Correlation seen between resumption of menstruation and premenopausal FSH and E2 levels	MR	NR	FSH and E2 returned to premenopausal levels within 6 months	Baseline and 6 monthly for FSH, LH, P, E2
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^aFollow-up of Urruticoechea et al. study.
^bOption for 2 years of goserelin after CT: unknown how many patients treated with a longer course of ovarian suppression.
^cER+patients treated with an AI during 1 or 2 years of goserelin then switched to tamoxifen after 1 or 2 years, except if ongoing amenorrhea and contraindication to tamoxifen and/or high risk. GnRH_a, gonadotropin-releasing hormone agonist; CT, chemotherapy; A, antracycline; T, taxane; C, cyclophosphamide; HDCT, high-dose chemotherapy; ASCT, autologous stem cell transplant; NR, not reported; FSH, follicle-stimulating hormone; LH, luteinising hormone; E2, estradiol; P, progesterone; ER+, estrogen receptor positive; f/u, follow up.

a median follow-up of 6 months. At 3 years median follow-up, there had been four successful pregnancies in four patients.

In a second single-arm phase II trial [57] 125 premenopausal women, of whom 100 had adequate follow-up, were treated with goserelin plus variable adjuvant breast cancer chemotherapy regimens. Half (52%) of the patients had ER+ disease, and received AI and GnRH_a after chemotherapy for up to 2 years, followed by tamoxifen, or continuation of AIs in patients with contraindication to tamoxifen. After a median follow-up of 75 months, 67% of patients had recovery of menstruation and premenopausal hormone levels. The menstruation recovery rate was higher in patients <40 years, compared with patients ≥40 (100% versus 56% for younger versus older patients, respectively). There were two successful pregnancies in two patients, and one additional patient had a voluntary termination.

randomised trials

In a trial from Badawy et al. [58], 80 premenopausal EBC patients were randomly assigned to receive adjuvant FAC (5-fluorouracil–doxorubicin (Adriamycin)–cyclophosphamide), with or without goserelin. The primary end point of POF at 3 months post completion of chemotherapy demonstrated a significant benefit for patients receiving goserelin. Menstruation resumption was reported in 35 (90%) versus 13 (33%) patients treated with, respectively, goserelin/chemotherapy versus chemotherapy alone, *P* < 0.001. The pregnancy rate was not reported. Importantly, no information was given on either breast cancer hormonal status or tamoxifen use, limiting interpretability of results.

The ZIPP (Zoladex In Premenopausal Patients) randomised study was designed to compare the efficacy of different endocrine therapy regimens in EBC patients treated with or without adjuvant chemotherapy ± radiotherapy. A subset of patients were also enrolled in a prospectively planned substudy assessing ovarian function [59]. The premenopausal status was defined as the last menstrual period within the preceding 6 months, including regular or irregular menstruation, without a specific age cut-off. As such, perimenopausal patients were not excluded.

Eligible patients were randomised to one of four treatment arms: (i) control (no endocrine therapy), (ii) goserelin, (iii) tamoxifen, and (iv) goserelin plus tamoxifen. Due to uncertainty about the role of adjuvant tamoxifen at the time of trial design, all patients received endocrine therapy for 2 years, commenced concurrently with chemotherapy, regardless of ER status. In addition to endocrine therapy randomisation, patients with node-positive disease received CMF (cyclophosphamide–methotrexate–5-fluorouracil), plus radiotherapy if four or more nodes were involved.

From 408 patients recruited in the main ZIPP study, 285 were enrolled in the ovarian function substudy, 25 of whom were excluded due to recurrent disease. From 260 assessable patients, 123 received six cycles of CMF. At completion of endocrine therapy, amenorrhoea rates of 85% (control), 95% (tamoxifen), 97% (goserelin), and 92% (goserelin + tamoxifen) were reported. Twelve months after completion of all therapy (36 months follow-up), there was a significant decrease in the

Table 2. Randomised trials of GnRHa in fertility preservation

Author	Bawady et al. [58]	Sverrisdottir et al. [59]	Del Mastro et al. [60]	Leonard et al. [61]	Gerber et al. [63]	Munster et al. [64]	Elgindy et al. [65]	SWOG 0230
Year	2009	2009	2011	2010	2011	2012	2013	Pending
Patients (n)	80	285	281	227	60	49	100	416 planned (not reached)
Study type	Phase II RCT	Substudy from combined analysis of four RCTs using a core protocol	Phase III RCT	Phase III RCT (abstract only)	Phase II RCT	Phase III RCT	Phase II RCT	Phase III RCT
Treatment arms	CT + goserelin versus CT	TAM ^a ± CT Goserelin ± CT Goserelin/ TAM ± CT TAM ± CT	CT + triptorelin versus CT	CT + goserelin versus CT	CT + goserelin versus CT	CT + triptorelin versus CT	'Delayed CT' + triptorelin versus CT 'Early CT' + triptorelin + cetrorelix ^b versus CT	CT + goserelin versus CT
Timing of GnRHa	Start: 14 days pre-CT End: end of CT	Start: CID1 of CT End: after 2 years	Start: ≥7 days pre-CT End: end of CT ^c	Start: before or at CID1 End: end of CT	Start: >14 days pre-CT End: end of CT	Start: 28 to ≥7 days pre-CT End: end of CT	<i>Triptorelin alone</i> Start: 10–14 days pre-CT End: end of CT <i>Triptorelin + cetrorelix</i> Start: <7 days pre-CT End: end of CT	Start: 7 days pre-CT End: end of CT
CT type	FAC	CMF	A, A + T, or CMF	C ± A ± T	AC ± T	FAC, FEC, AC, AC-T	FAC	3–8 months of C ± A
Maximum age (years)	≤40	No age cut off	≤45	NR	≤45	<45	≤40	≤49
Median age (range)	30 [26–33]	45 [29–55] ^c	39 [24–45]	NR	37 [26–47]	39 [21–43]	33 [18–40]	Pending
Premenopausal definition	Regular menstruation FSH <10 IU/L	LMP <6 months before study entry, including irregular cycles	Actively menstruating during 6 weeks pre-CT	Regular menses in 12 months preceding surgery	Regular menstruation FSH <15 in follicular phase	Regular menstruation (three or more periods in 6 months, lasting ≥2 days, 21–35 days apart FSH <40 IU/L	Regular menstruation (three or more consecutive periods within 21–35 days)	LMP < 6 weeks pre-randomisation or FSH E2 in the premenopausal range
%ER+	NR	45%	81%	NR ^c	0%	73%	0%	NR (~0%)
Marker of 'fertility preservation'	Resumption of menstruation or spontaneous ovulation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation	Resumption of menstruation

Primary end point	Rate of POF (no menstruation/spontaneous ovulation) 3 months post-CT	Recovery of menstruation	Rate of CIA (no menstruation and post-menopausal FSH/E2 levels) for 12 months post-CT	Rate of amenorrhea 12 months after start of CT	Rate of normal ovarian function (defined as 2 periods 21–35 days apart within 5–8 months post-CT) at 6 months post-CT	Uninterrupted or restored menstruation during f/u of at least 2 years post-CT	Rate of regular menstruation at 12 months after completion of CT	Rate of ovarian failure (amenorrhea) at 2 years
Median f/u (range)	NR (3–8 months)	NR Menstruation questionnaires obtained up to 36 months	12 months post-CT	NR	6 months (primary end point) Also f/u at 12, 24, 48 months post-CT	18 months (5–43 months) after CT	NR All patients followed for at least 12 months	Planned f/u at 1, 2, and 5 years
Rate of recovery of menstruation	90% (goserelin) versus 33% (control), $P < 0.001$	At 6 months post ET cessation : 36% (goserelin) versus 10% (control), 13% (TAM), 7% (goserelin + TAM), $P = 0.006$	91.1% (triptorelin) versus 74.1% (control) $P < 0.001$	NR No statistically significant difference between treatment arms (further details not published)	70% (goserelin) versus 56.7% (control), $P = 0.284$	88.5% (triptorelin) versus 90.5% (control), $P = \text{ns}$ Trial stopped early for fertility	At 12 months post-CT Delayed CT: 72% (triptorelin) versus 52% (control), $P = 0.15$ Early CT: 80% (triptorelin + cetorelix) versus 48% (control), $P = 0.39$	Pending
Median time to recovery of menstruation	NR	NR	6.7 months (triptorelin) versus not reached (control)	NR	6.1 (goserelin) versus 6.8 (control) months, $P = \text{ns}$	5.0 (triptorelin) versus 5.8 months (control), $P = 0.58$	NR	Pending
Pregnancies	No data on pregnancies	No data on pregnancies	Three pregnancies in triptorelin arm, one in control arm	No data on pregnancies	One pregnancy in each group	Two pregnancies in the control arm	Three pregnancies, one in early CT + triptorelin + cetorelix arm, 1 in early CT control arm and 1 in delayed CT + triptorelin arm	Pending

Continued

Table 2. Continued

Author	Bawady et al. [58]	Sverrisdottir et al. [59]	Del Mastro et al. [60]	Leonard et al. [61]	Gerber et al. [63]	Munster et al. [64]	Elgindy et al. [65]	SWOG 0230
Hormone assays	Higher post-CT FSH (15.2 versus 8.3 IU/ml) and lower E2 (75 versus 279 pg/ml) in the control group versus The goserelin group	NR	No statistically significant differences between either FSH or E2 levels in triptorelin versus control groups during 12 months f/u	NR (reported separately as pooled analysis, i.e. no comparison between treatment arms)	LH lower in goserelin gp versus control gp, <i>P</i> = 0.015. FSH also lower in control gp, though <i>P</i> = ns	Higher FSH levels in both groups at 18 months versus baseline (4 and 5 versus 19 and 15 for control and triptorelin groups, respectively), <i>P</i> = NR	No significant difference between FSH, LH or E2 levels for treatment and control arms in either early CT or delayed CT groups	Will assess day 2–4 FSH, E2, inhibin B during month 12/13 and 24/25, or anytime if amenorrhoeic

^aET given for 2 years irrespective of ER status, with ET concurrent with CT.

^bCetrorelix (GnRH antagonist) was given only to prevent a 'flare' response from GnRHa therapy, and was discontinued once E2 levels were confirmed to be <50pg/ml, with GnRHa continuing until the end of CT.

^c65% of CMF-treated patients ≥45 years.

^dPatients with ER+ tumours and return of menstruation offered an option of restarting triptorelin for up to 2 years (number treated with extended ET not given).

^ePredominantly ER-negative.

GnRHa, gonadotropin-releasing hormone agonist; CT, chemotherapy; TAM, tamoxifen; A, anthracycline; T, taxane; C, cyclophosphamide; CMF, cyclophosphamide/methotrexate/fluorouracil; FAC, fluorouracil/doxorubicin/cyclophosphamide; FEC, fluorouracil/epidubicin/cyclophosphamide; AC (±T), doxorubicin/cyclophosphamide (±taxane); NR, not reported; LMP, last menstrual period; FSH, follicle-stimulating hormone; LH, luteinising hormone; E2, estradiol; P, progesterone; ER+, estrogen receptor positive; CIA, chemotherapy-induced amenorrhea; f/u, follow up; ns, not statistically significant.

amenorrhoea rate in patients treated with goserelin alone (64%) compared with persisting high rates for the other groups: 90% (control), 87% (tamoxifen), and 93% (goserelin + tamoxifen), *P* = 0.006. Interestingly, no decrease in amenorrhoea was seen in the combined goserelin + tamoxifen group, while the lower amenorrhoea rate associated with single-agent goserelin was restricted to patients who received chemotherapy, not those treated with endocrine therapy alone. The definition of premenopausal status, which allowed inclusion of perimenopausal patients, likely contributed to the very high amenorrhoea rates reported across all groups. Pregnancy rates after treatment were not reported.

A phase III trial from Del Mastro et al. [60] assessed the efficacy of triptorelin in preventing CIA when added to CMF or anthracycline-based adjuvant or neoadjuvant chemotherapy for EBC. For patients with ER+ disease (80%) randomly assigned to triptorelin, in the event of return of menstruation after chemotherapy, triptorelin was restarted and continued concurrently with tamoxifen for a total of 2 years.

With 281 enrolled patients, at a median follow-up of 12 months post-chemotherapy, the CIA rate was significantly higher with chemotherapy alone compared with triptorelin/chemotherapy: 26% versus 9% respectively, *P* < 0.001. In a multivariate analysis, only triptorelin, but not age or chemotherapy type, was associated with the rate of CIA, inconsistent with previously reported risk factors for POF [22–25]. Twenty-four months after all patients had completed chemotherapy, one successful pregnancy in the control arm, and three pregnancies in the triptorelin group, resulting in two live births and one voluntary termination, were reported.

In contrast with the preceding trials, four randomised trials have failed to confirm any preservation of menses from GnRHa through adjuvant chemotherapy.

The OPTION (Ovarian Protection Trial In premenopausal breast cancer patients) randomised phase III trial has only been reported in abstract form [61], limiting its interpretability. OPTION was designed to assess the ovarian protective effect of goserelin compared with no goserelin in patients with ER– EBC treated with anthracycline- and/or cyclophosphamide-based adjuvant chemotherapy, with the primary end point being amenorrhoea rate at 12 months after completion of chemotherapy.

Despite initially estimated accrual of 400 patients (www.clinicaltrials.gov), revised sample size calculations indicated that 210 patients were required to achieve primary end point, and thus, after enrolment of 227 patients, the trial was closed. At the time of reporting, 173 patients had adequate follow-up data, and 140 had adequate information about menstrual bleeding [61]. No difference between treatment arms was reportedly found [62]; however, data on actual amenorrhoea rates between arms have not been published.

The phase II ZORO (ZOledex Rescue of Ovarian function) trial included 60 premenopausal patients with ER– breast cancer randomly assigned to receive neoadjuvant anthracycline/cyclophosphamide-based chemotherapy with or without goserelin [63]. From 60 randomly assigned patients, 56 (28 in each arm) completed planned chemotherapy ± goserelin. For the primary end point of normal ovarian function, regular menstruation was reported in 70% versus 57% of patients

treated with goserelin/chemotherapy versus chemotherapy alone, respectively, though this difference did not reach statistical significance, $P = 0.284$. Similarly there was no significant difference in the median time to resumption of menstruation, 6.1 versus 6.8 months for goserelin versus control group respectively, $P = 0.304$. Of note, this trial was powered to detect a difference of 30% between treatment arms. As such, the possibility of a smaller benefit for goserelin cannot be excluded. Interestingly, FSH and LH levels were higher in the control arm than the GnRHa arm, reaching statistical significance for LH, $P = 0.015$, suggesting a possible impact of GnRHa on ovarian function, although interpretation is difficult with results of these assays reported in graphical form only. There was limited pregnancy data available, with one pregnancy being reported in each treatment arm.

A randomised trial from Munster et al. [64] compared ovarian function in EBC patients treated with adjuvant anthracycline-based \pm taxane chemotherapy with or without triptorelin. Ovarian function was defined as resumption of normal menstruation during follow-up of at least 2 years post-chemotherapy. FSH, inhibin A, and B levels were also measured. Follow-up was planned to extend to 5 years; however, the trial was ceased for futility at interim analysis, at which time less than half of anticipated accrual had been reached.

Of the 49 patients enrolled, 27 patients received triptorelin/chemotherapy and 22 received chemotherapy alone, with one patient from each arm not assessable. Most patients had ER+ disease (73% and 74% for triptorelin and control groups, respectively). After a median follow-up of 18 months (range 5–43 months), resumption of menstruation was seen in 88% versus 90% for triptorelin versus control groups respectively, $P = 0.97$. Similarly, there was no significant difference in time to resumption of menstruation, 5.8 versus 5.0 months for triptorelin versus control groups, respectively, $P = 0.58$. Furthermore, there was no difference in FSH levels between treatment arms either pre- or post-chemotherapy. However, mean FSH levels were higher in both the treatment and control groups at 18 months after completion of chemotherapy compared with baseline measurements: 4.3 versus 19.0 IU/l for baseline and 18-month levels, respectively, for the control group, and 5.2 versus 15.7 IU/l for the triptorelin group, indicating potential ovarian damage in both the groups that was not adequately detected by amenorrhoea rates. Two pregnancies were reported, both in the control group. The number of patients attempting pregnancy was not given.

A phase II randomised trial from Elgindy et al. [65] was designed to assess the potential benefit of triptorelin in two different groups of premenopausal ER– EBC patients. The ‘delayed chemotherapy’ (DC) group, i.e. patients not requiring immediate cytotoxic treatment, were randomly assigned to chemotherapy plus triptorelin or chemotherapy alone. Patients requiring immediate commencement of chemotherapy were allocated to the ‘early chemotherapy’ (EC) group, and randomly assigned to receive chemotherapy with or without triptorelin plus cetrorelix, a GnRH antagonist. The primary end point of the rate of regular menstruation at 12 months post-chemotherapy was 72% (triptorelin) versus 52% (control), $P = 0.15$, for the DC group, and 60% (triptorelin + cetrorelix) versus 48% (control), $P = 0.39$, for the EC group. The difference

was not statistically significant in either group. Of note, power calculations were based on a large predicted decrease in amenorrhoea rate from 65% to 20% with the addition of GnRHa (\pm GnRHa), thus the negative results do not preclude a smaller benefit from triptorelin.

pending studies

The POEMS (Prevention Of Early Menopause Study)/SWOG-0230 phase III RCT (NCT01530607) was recently closed without meeting its accrual target of 416 patients, due to difficulties with drug supply and funding [66]. This trial was designed to compare the ovarian protective effects of goserelin when added to adjuvant or neoadjuvant chemotherapy containing 3–8 months of an alkylating agent with or without an anthracycline in women with ER– EBC, thereby removing the potential confounding effect of endocrine therapy on relevant outcomes.

The primary end point is the ovarian failure rate at 2 years after completion of chemotherapy, as determined by amenorrhoea persisting for 6 months and a post-menopausal FSH level. Secondary outcomes include assessment of ovarian reserve using FSH, E2, and inhibin B levels, and fertility, assessed by 2- and 5-year pregnancy rates. Lower than expected accrual may affect study power significantly, while inclusion of patients up to 49 years will likely impact on the ovarian reserve and fertility end points.

discussion

Based on current evidence, the role of GnRHa as ovarian protection agents remains controversial and unproven. The results are conflicting, and heterogeneity across trials further impacts on interpretability of the data.

In particular, current evidence is limited by the fact that regular menstruation has generally been equated with fertility. However, CIA is an insensitive marker of infertility, with infertility typically preceding menopause by around 10 years [13]. Moreover, the absence of menstruation does not imply infertility *per se*, as non-menstruating women may still be fertile. Transient amenorrhoea occurs frequently in premenopausal women during chemotherapy, while menstruation will resume in a proportion of these patients within 6 months of completion of chemotherapy [26]. Two studies [58, 63] report the primary end point of menstruation resumption measured after a follow-up of <12 months, thus temporary amenorrhoea rates may have confounded the findings. Also of concern is that, in most studies, there is lack of control for the confounding effects of tamoxifen, with tamoxifen being a known independent risk factor for amenorrhoea [10, 62].

Patient age for eligibility varies considerably between trials. Only three studies limited age for eligibility to 40 years [56, 58, 65]. While prevention of premature menopause has important health consequences for women over 40 years, the proportional benefits in terms of fertility preservation are likely to be minimal, with very low ovarian reserve expected in most women over 40 [13].

In order for GnRHa to prevent ovarian damage, treatment should commence at least a week before chemotherapy.

Table 3. AMH and inhibin data from trials of GnRHa in breast cancer patients

	Number of patients with AMH/inhibin levels (%)	Assay	Comparison by treatment arm	Results from secondary analyses of alternate markers of ovarian function (AMH or inhibin)
Gerber et al. [63]	17 (28%)	AMH Inhibin B AFC	No	Low AMH correlated with increased age Correlation with amenorrhea not specifically reported Formal comparison by treatment arms not possible due to limited patient numbers, though AMH level >0.2 ng/ml seen in 33% (3 of 9 patients) versus 50% (4 of 8 patients) for control versus GnRHa arms, respectively ($P =$ not reported) In the 10 patients with available AFC, 2 patients with a higher (>3) follicle count also had higher AMH levels All patients with available samples ($n = 17$) had inhibin B levels <10 ng/l ^a
Munster et al. [64]	NR	Inhibin B	No	Post-treatment inhibin B levels correlated inversely with FSH levels in patients who resumed menstruation FSH levels correlated with menstrual status and did not differ between treatment arms In patients with resumed menses, inhibin B levels in both treatment arms were < 45 pg/ml ^a , Statistical analysis by treatment arm not reported
Elgindy et al. [65]	NR	AMH AFC	Yes	AMH decreased significantly from baseline after CT, regardless of treatment arm (2.0–2.3 versus. 0.2–0.4 ng/ml for pre- and post-CT respectively) No difference in AMH levels between control and GnRHa arms at 12 months post-CT: AMH for both arms ~0.2ng/ml for the early CT group; AMH for both arms ~0.4 ng/ml for the delayed CT group ^b AFC decreased from baseline after CT in both control and experimental arms
Leonard et al. [73]	67 (30%)	AMH	No	AMH significantly higher before CT compared with post-CT, regardless of treatment arm, $P < 0.01$ Lower pre-treatment AMH levels in either treatment group predicted amenorrhea at 12 months follow-up. Limited data has been reported on differences in AMH between treatment arms
Giraudi et al. [74] ^c	26 (NR)	AMH	No	Mean post-CT AMH levels were significantly lower than pre-CT levels (2.04 versus 0.59 pre- versus post-CT respectively $P = 0.0003$), while FSH increased significantly (8.64 versus 23.13, $P = 0.017$) No association between AMH levels and probability of resumption of menstruation seen

^aAn inhibin B level of <45 ng/l indicates decreased ovarian reserve and poor fertility.

^bValues approximate, derived from graphical representation of results presented in article, no P values given for comparison of treatment arms

^cSubstudy from Del Mastro et al., reported separately. Not reported how many of the 280 patients enrolled in the main study were included in this analysis.

GnRHa, gonadotropin-releasing hormone agonist; AMH; anti-Mullerian hormone; CT, chemotherapy FHS; follicle-stimulating hormone; AFC, antral follicle count.

However, in several studies, some patients received GnRHa at the commencement of chemotherapy [53–55, 59, 61], where potential activity would be diminished.

High rates of menstruation resumption have been reported in several studies [63–65], potentially resulting in limited power to detect any benefit from GnRHa. Importantly, low amenorrhoea rates *per se* should not be interpreted as evidence that chemotherapy does not impact on fertility, as amenorrhoea, as discussed, is an insensitive marker of fertility, and chemotherapy may still cause subclinical but irreversible depletion of ovarian reserve. The ‘gold standard’ for assessing preserved fertility is successful pregnancy. In the trials of GnRHa included in this review, limited, if any, data on pregnancy rate is presented, primarily due to the prolonged

follow-up required for this end point. Wong et al. [54] have reported the most extensive pregnancy data, where 30 of 42 (71%) women attempting pregnancy were successful. However, with all women in this study receiving GnRHa, no definitive conclusions on the impact of GnRHa on pregnancy are possible.

Rather than the pregnancy rate, a more useful intermediate marker of ‘preserved fertility’ in a woman is the assessment of ovarian reserve. Primordial follicles present in the ovaries decrease in both quantity and quality throughout a woman’s reproductive life. Around 6 to 7 years before menopause, periods become irregular, and once the follicle number falls below ~1000, menopause ensues [13]. Cytotoxic agents, in particular alkylating agents, can increase the rate of loss of primordial follicles, leading to POF. The primordial follicle

number cannot be measured directly with current techniques; however, several alternate approaches, including anti-Müllerian hormone (AMH), inhibin B and antral follicle count (AFC), may be useful surrogate measures, with AMH appearing to be the most favourable.

AMH is secreted by granulosa cells in small antral follicles, with levels considered to be directly proportional to the primordial follicle number. AMH, when adjusted for age, has been shown to be a more accurate predictor of time of natural menopause than either FSH or AFC [67], and is a sensitive marker of likelihood of ovarian response in an IVF treatment programme [68].

Several studies in breast cancer patients have demonstrated potential utility of AMH, inhibin, or FSH, as biomarkers predicting infertility risk. Decreased AMH and inhibin levels, and higher FSH levels, compared with age-matched healthy controls, have been shown to correlate with decreased ovarian reserve following chemotherapy [14, 69, 70]. Additionally, increased rates of CIA have been associated with lower pre-treatment AMH, inhibin and higher FSH levels [70–72].

From the trials of GnRHa in EBC, data on hormone levels, where available, are conflicting. GnRHa treatment was associated with lower post-treatment FSH levels in the Bawady et al. trial, and lower FSH and LH levels in the ZORO trial [63], while, conversely, Munster et al. [64] and Elgindy et al. [65] reported no difference in FSH between treatment arms. Data specifically relating to the impact of GnRHa treatment on AMH levels are very limited. While some studies included AMH and/or inhibin B assessment (Table 3), these assays were carried out only in a minority of patients, with analyses reported only as exploratory [63, 64], or in abstract form [73, 74].

Importantly, while comparisons of AMH or inhibin between treatment arms has generally not been possible due to limited patient numbers, assessment of AMH and/or inhibin B indicate reduced ovarian reserve following chemotherapy [63–65, 73, 74], even in the setting of high rates of menstruation resumption [64].

Data regarding AMH as a predictor of infertility risk following chemotherapy in breast cancer patients are limited and requires further validation. Nonetheless, AMH appears to be a more sensitive biomarker than amenorrhoea, is easier to implement than AFC, and correlates more strongly with AFC than inhibin B, E2, FSH, or LH [75]. Additionally, as AMH does not form a part of the pituitary–ovarian axis [68], levels do not vary during the menstrual cycle [76, 77] unlike inhibin and FSH, nor are AMH levels affected by GnRHa or tamoxifen [70]. Thus, AMH would be of interest to incorporate into future trials assessing chemotherapy-induced infertility.

conclusion

Fertility preservation is an increasingly recognised issue in young breast cancer patients and of growing importance. Chemotherapy-induced infertility can cause long-term psychological distress and decreased quality of life, and should be discussed with all young breast cancer patients, ideally at the time of initial diagnosis. Early referral to a reproductive specialist will decrease time delays and increase the number of

women who can feasibly undergo fertility preservation strategies including embryo/oocyte preservation [78].

Where ovarian stimulation is not possible, GnRHa treatment remains an unproven entity. The data from POEMS/SWOG-0230 are awaited with interest, although as this trial utilised menstruation rates as the primary measure of ovarian function, and did not reach its predetermined accrual target, the utility of GnRHa may remain uncertain, regardless of the findings. In order to define the role of GnRHa in the prevention of chemotherapy-induced infertility, studies which incorporate both an appropriate age cut-off (~40 years) and a more sensitive marker of ovarian reserve, such as AMH, are needed.

With current data indicating uncertain efficacy of GnRHa in fertility preservation, these agents should ideally only be administered in the context of a clinical trial, and their use in standard practice is discouraged. However, it is acknowledged that clinical trials are not always available and difficult situations can arise, where a young breast cancer patient may have no other viable fertility preservation options. In these exceptional circumstances, given the absence of clear evidence of harm, coupled with the potential negative long-term effects of infertility on the quality of life, consideration of GnRHAs for fertility preservation outside of a clinical trial setting may be warranted, provided there is careful discussion with the patient of the lack of proven benefit.

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