GnRH-analogues and oral contraceptives for fertility preservation in women during chemotherapy

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BACKGROUND: For preserving fertility in women during chemotherapy, the character of invasive techniques, such as ovarian cryopreservation and other techniques, await further experience. Meanwhile, non-invasive techniques have attempted to minimize the gonadotoxic effect of chemotherapy, by using gonadotrophin-releasing hormone-analogues (GnRH-a) or oral contraceptives (OC). METHODS: We performed a computerized MEDLINE search to identify articles published on fertility preservation using GnRH-a or OCs. RESULTS: Nine human-controlled studies reported the use of GnRH-a and four reported the use of OCs in parallel to chemotherapy. All nine studies analysing the effect of GnRH-a found lower rates of premature ovarian failure (POF) in patients receiving GnRH-a compared with the controls. Summarizing the studies resulted in 11.1% incidence of POF in patients who received GnRH-a compared with 55.5% incidence in the controls. Evidence using the fertility preserving effect of OC is limited. Two studies showed lower POF rates in OC-treated patients. The summarized data revealed a POF rate of 13.2% in patients who received OCs compared with that of 29.8% in the controls. CONCLUSIONS: The published clinical studies provide evidence, but do not prove statistically, that GnRH-a co-treatment reduces gonadotoxicity. Owing to the retrospective and non-randomized nature of most of the studies, definite conclusions concerning the reduction of POF by GnRH-a can still not be unequivocally drawn. As GnRH-a and OC have no serious side effects and as GnRH-a can even reduce chemotherapy-induced complications, such as severe menometrorrhagia, GnRH-a are considered by many clinicians as a clinically useful co-treatment in chemotherapy. The published clinical studies on OC also suggest a possible effect on the reduction of POF under certain conditions.

Keywords: gonadotoxicity; premature ovarian failure (POF); chemotherapy; GnRH-agonist; oral contraceptives

Introduction

In the last two decades, the survival rates for many of the malignancies that affect young adults have markedly improved. For many of these malignancies, survival rates exceed 80–90%. Therefore, the remote effects of cancer treatment have recently gained a ubiquitous worldwide interest and protection against iatrogenic infertility caused by chemotherapy assumes high priority. Chemo- and/or radiotherapy can permanently impair reproductive functions (Madsen *et al.*, 1995), and preserving fertility in female patients is crucial since a high percentage of these young patients will develop premature ovarian failure (POF) due to follicular damage (Familiari *et al.*, 1993). About 50% of women, over 25 years of age, and 20% of women, <25 years of age, who are treated with MOPP (Mechloroethamine, Vincristine, Procarbazine and Prednisone) will develop POF (Schilsky *et al.*, 1981). The most common significant long-term toxicity in

premenopausal women receiving chemotherapy is POF. The impact of POF after chemotherapy and its associated infertility is of great importance to the individual patient and their families.

The only unequivocal and clinically available option is the cryopreservation of embryos or fertilized oocytes after IVF (*in vitro* fertilization) before chemotherapy (Blumenfeld, 2007a, 2007b). Whereas this alternative is relevant to those who have a long-term partner, it may be unacceptable to some single women. Furthermore, IVF might not be relevant in a substantial number of patients who need urgent chemotherapy, or in diseases which can be possibly aggravated by the increased pharmacological levels of sex steroids, such as systemic lupus erythematosus (SLE) or breast cancer.

Cryopreservation of unfertilized metaphase II oocytes has been successful in rodents, and recently its efficiency and clinical applicability in humans has increased, both by conventional slow programmed cryopreservation and more recently due to the

vitrification technology (Borini *et al.*, 2007; Lornage and Salle, 2007; Yavin and Arav, 2007).

Transplantation of ovarian tissue has been of great interest since the first baby was born after autologous transplantation of ovarian tissue (Donnez *et al.*, 2004). But, to date, the success in humans is limited and currently only five successful deliveries in humans have been reported (Blumenfeld, 2007a).

Furthermore, some of the described techniques are also invasive, causing additional stress to the patients. The ideal treatment to preserve fertility in cancer patients would be a medication that can easily be applied orally or by subcutaneous injection.

Several investigators have therefore focused their interest on gonadotrophin-releasing hormone-analogues (GnRH-a) or oral contraceptives (OC) in order to analyse their fertility preserving effects as a co-treatment in parallel to chemotherapies.

Rationale for using GnRH-a for fertility preservation

The possibility of administering an adjuvant treatment that may minimize the gonadal damage caused by an otherwise successful treatment is obviously attractive. Glode *et al.* (1981) have tested this hypothesis almost three decades ago, using a murine model and concluded that an agonistic analogue of GnRH appeared to protect male mice from the gonadal damage normally produced by cyclophosphamide. Decreased secretion of the pituitary gonadotrophins followed by decreasing gonadal function may possibly protect against the sterilizing effects of chemotherapy. Although previous suggestions have been made (Glode *et al.*, 1981), claiming that primordial germ cells fare better than germ cells that are part of an active cell cycle, this hypothesis has not been seriously tested clinically, until the last decade. Whereas several investigators have demonstrated that GnRH-a can inhibit chemotherapy-induced ovarian follicular depletion in the rat, uncertainty and even skepticism remained regarding human application (Bohlmann *et al.*, 2003; Oktay *et al.*, 2007). It was argued that the human ovary has lower concentrations of ovarian GnRH-receptors and may not necessarily exhibit the same response as rats (Lobo, 2005; Lutchman Singh *et al.*, 2005; Blumenfeld, 2007a).

The chances of preserving gonadal function following combined modality treatment are significantly better for girls than for boys (Johnson *et al.*, 1985; Ortin *et al.*, 1990). In contradiction to the results reported in adults, MOPP chemotherapy in prepubertal girls with Hodgkin's lymphoma (HL) did not result in POF (Ortin *et al.*, 1990). Since ovarian function was preserved in most long-term female survivors who were treated prepubertally for lymphoma, but only in a minority of similarly treated adult patients (Ortin *et al.*, 1990; Blumenfeld, 2007b), it was clinically logical and therefore tempting to create a temporary prepubertal milieu in women of reproductive age before and during the chemotherapeutic insult (Blumenfeld, 2007a). As GnRH-a and OCs reduce FSH levels, it can be speculated that these medications can positively influence the vicious cycle of chemotherapy-induced follicle depletion, FSH increase and accelerated recruitment of further follicles (Fig. 1).

Ataya *et al.* (1995) have shown in their prospective randomized study in female Rhesus monkeys, that GnRH-a may protect the ovary from cyclophosphamide-induced gonadal damage. Administration of GnRH-a in parallel with cyclophosphamide has
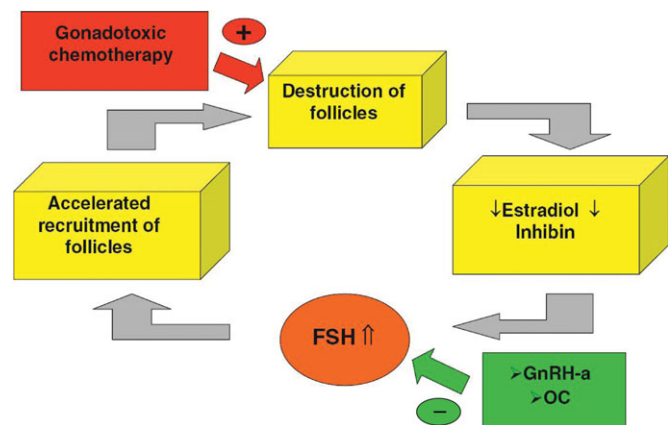

Figure 1: Vicious pathophysiologic cycle and the protective effect of GnRH-a or OCs against chemotherapy-associated gonadotoxicity.
The gonadotoxic chemotherapy destroys follicles causing a decrease in estrogen and inhibin secretion, bringing about an increase in FSH concentration due to the negative feedback. The increased FSH causes an enhanced recruitment of follicles which are further destroyed by chemotherapy. The administration of GnRH-a or OC may prevent the increased FSH concentration, thus rescuing the follicles from accelerated atresia. FSH, follicle-stimulating hormone; GnRH-a, gonadotrophin-releasing hormone agonist; E_2, estradiol; OC, oral contraceptives.

significantly decreased the daily rate of follicular decline and the total number of follicles lost during the chemotherapeutic insult, as compared with cyclophosphamide alone (without GnRH-a). More recently, Imai *et al.* (2007) have demonstrated that GnRH-a may decrease the *in vitro* gonadotoxic effect of chemotherapy, independently of the hypogonadotropic milieu. These investigators have shown direct *in vitro* protection from doxorubicin-induced granulosa cell (GC) damage by a GnRH-a, indicating some direct, but not yet fully understood, effect of GnRH-a on the ovary.

As opposed to young girls, most prepubertal boys who receive chemotherapy or radiotherapy suffer from azoospermia; therefore there is little rationale to expect a significant benefit from a similar GnRH-a co-treatment to preserve fertility in men (Johnson *et al.*, 1985; Ortin *et al.*, 1990; Blumenfeld, 2007b). Additionally, the encouraging protective effect of GnRH-a as a co-treatment appears to be limited to chemotherapy, as a study in rats has shown that GnRH-a could provide no protection from ovarian damage caused by irradiation (Blumenfeld, 2007b).

Methods

We have conducted a computerized MEDLINE search for all the human studies using either GnRH-a (agonists and/or antagonists) or OC in parallel to chemotherapy for preservation of ovarian function and minimizing gonadotoxicity. The 'Pubmed' database was searched for the terms: fertility preservation, GnRH-a, OCs, chemotherapy and gonadotoxicity, from 1980 to 2008. All the relevant publications were included, but studies without controls were not included in the tables. The studies with a control group are summarized in Tables I and II. Relevant abstracts were retrieved from proceedings of international meetings on the subject or from those cited in the retrieved publications.

Table I. Rate of POF following GnRH-a as co-treatment during chemotherapy (peer-reviewed papers with control groups only, abstracts not included).

	Age (year) (GnRH-a)	GnRH-a	Control	Age(year) (Control)
Waxman et al. (1987) (Lymphoma)	NA	50.0% (4/8)	66.7% (6/9)	
Blumenfeld et al. (2000) (Systemic lupus erythematosus)	18–35	0% (0/8)	55.6% (5/9)	20–35
Pereyra Pacheco et al. (2001) (Lymhoma, leukaemia, thymoma)	15–20	0% (0/12)	100% (4/4)	16–20
Dann et al. (2005) (Non-Hodgkin Lymphoma)	18–40	0% (0/7)	17% (1/6)	21–40
Somers et al. (2005) (Systemic lupus erythematosus)	24–28	5.0% (1/20)	30.0% (6/20)	25–28
Elis et al. (2006) (Non-Hodgkin Lymphoma)	17–40	0% (0/3)	8.7% (2/24)	17–40
Castelo-Branco et al. (2007) (Lymphoma)	14–45	10.0% (3/30)	76.9% (20/26)	14–45
Blumenfeld et al. (2008) (Hodgkin Lymphoma	14–40	3.1% (2/65)	63.0% (29/46)	14–40
Huser et al. (2008) (Hodgkin Lymphoma)	18–35 median=32.5	20.8% (15/72)	71.1% (32/45)	18–35 median = 29
Total		11.1% (25/225)	55.5% (105/189)	

Table II. Rate of POF following OC as co-treatment during chemotherapy (peer-reviewed papers with control groups only, abstracts not included).

	Age (year)	OC	Control
Whitehead et al. (1983) (Lymphoma)	23 (median)	44.4% (4/9)	37.1% (13/35)
Longhi et al. (2003) (Osteosarcoma)	NA	15.8% (3/19)	4.2% (3/71)
Behringer et al. (2005) (Hodgkin Lymphoma)	15–40	10.1% (7/69)	44.1% (64/145)
Elis et al. (2006) (Non-Hodgkin Lymphoma)	17–40	0% (0/9)	8.7% (2/24)
Total		13.2% (14/106)	29.8% (82/275)

Clinical studies using GnRH-a for ovarian protection

Haemato-oncology

The largest study including >111 patients was published by ourselves (Blumenfeld et al., 2008) (Table I). In this study, a monthly depot injection of GnRH-a (D-TRP6-GnRH-a, Decapeptyl C.R., 3.75 mg, Ferring, Switzerland) was administered, for various indications, starting before chemotherapy for up to 6 months, in parallel to, and until the end of chemotherapeutic treatment. In an attempt to reduce a possible selection bias, they included every possible patient in their fertile years into either the study or control group. GnRH-a was offered to every referred female patient before chemotherapy. Those who either did not accept the offer or were referred after the commencement of chemotherapy were included in the control group in addition to historical controls (20 patients) treated during the decade before starting the GnRH-a co-treatment protocol (Blumenfeld et al., 2008).

Patients receiving GnRH-a were compared with a control group of patients of comparable age, who were similarly treated with chemotherapy but without the GnRH-a adjuvant. The cumulative doses of each chemotherapeutic agent and the mean or median radiotherapy exposure did not differ between the groups. The length of follow-up did not differ significantly between the control and the study groups (1–22 versus 1–18 years, respectively), despite the fact that some of the controls were historical controls, since the proportion of the historical controls was a minority (20/46; Blumenfeld et al., 2008).

The main significant difference was the rate of POF, which was <10% in the GnRH-a co-treatment group versus >40% in the control-group (Blumenfeld et al., 2008). The relatively advanced ages (35–40 years) of the patients in the study group, who developed POF, suggest that minimizing follicular loss may be efficient only in the younger patients whose follicular reserve is above a certain limit. In patients older than 37, this reserve may not be sufficient. In keeping with Ataya et al.'s (1995) observation that

GnRH-a significantly decreases the cyclophosphamide-associated follicular loss, but does not eliminate it completely, it is understandable that the beneficial effect of GnRH-a is age-limited. Therefore, for aggressive chemotherapy protocols, such as BEACOPP or escalated BEACOPP (Behringer et al., 2005), limiting GnRH-a co-treatment to women up to age 35–37 years may be considered (Blumenfeld et al., 2008).

In the treated group, 48 pregnancies occurred in 34 patients, who were 18–33 years old at chemotherapy administration, compared with 22 pregnancies in 16 patients who were ages 16–26 years at chemotherapy administration in the control group (NS) (Blumenfeld et al., 2008). Although the difference is not statistically significant (α error, due to insufficient power and relatively small number of patients), the older age (at the chemotherapy administration) of some of the patients who spontaneously conceived, in the treatment group, as compared with younger age (at chemotherapy insult) of the controls, suggests a possible prolongation of fertility potential by several years, possibly to beyond 30, enabled by the GnRH-a co-treatment. One of the patients who has spontaneously conceived and successfully delivered a healthy neonate 3 years after being treated with escalated BEACOPP and GnRH-a at 32 years, became menopausal a year after delivery, at the age of 35. Similarly, Edgar and Wallace (2007) and Sklar et al. (2006) have recently found that the survivors of childhood cancer have an 8% risk of becoming prematurely menopausal by the age of 40, compared with <1% POF in the general population. This is in agreement with our and others' findings suggesting <7–10% of POF in young women treated with GnRH-a in parallel to chemotherapy (simulating a prepubertal exposure) compared with 40–50% of POF in controls (Blumenfeld, 2007a; Blumenfeld et al., 2008). This supports the hypothesis that the induction of a prepubertal milieu by the GnRH-a co-treatment may be one of the mechanisms responsible for the minimized gonadotoxic effect of chemotherapy in women in the reproductive age. As reported by Ataya et al.

(1995), GnRH-a significantly decreases the cyclophosphamide-associated follicular loss, but does not eliminate it completely. It is therefore understandable that the beneficial effect of the GnRH-a is age-limited, and the treated survivors should be encouraged to conceive, if interested, and in agreement with their haemato-oncologist, as soon as their situation permits, but not before 1 year after chemotherapy.

Most recently, Huser et al. (2008) have reported similar results in an experimental case–control study with historical controls. Both, the experimental and control groups were of similar age and received the same protocols. They (Huser et al., 2008) treated 72 young HL female patients with GnRH-a in parallel to chemotherapy in 2004–2005, and compared these 72 patients to a historical control group of 45 patients treated in 2002–2003, without GnRH-a. They (Huser et al., 2008) also found a significantly lower rate of POF in the study group (20.8%) than in control group (71.1%) ($P < 0.001$). In keeping with our findings in HL (Blumenfeld et al., 2008), the study of Huser et al. (2008) also suggests that the efficiency of GnRH-a for the prevention of POF is decreased in patients using aggressive chemotherapy, such as eight courses of BEACOPP or escalated BEACOPP regimen.

Similar experience and results regarding the protective effect of GnRH-a was reported in adolescent females by Pereyra Pacheco et al. (2001). Whereas all 12 GnRH-a treated patients (15–20 years) resumed cyclic ovarian function, similarly to four positive pre-adolescent controls (treated at 3–7.5 years), the patients in the chemotherapy alone (without GnRH-a) group experienced hypergonadotropic amenorrhea in spite of their similar young, adolescent age (16–20 years). However, it should be noted that the small number of patients in each group raises the possibility of an α-type error, therefore, these results await validation by larger studies. Similarly, Castelo-Branco et al. (2007) have prospectively treated 30 haemato-oncologic patients with GnRH-a in addition to chemotherapy and compared them with 26 controls of similar age (14–45 years) and treatment, and who did not receive the agonist. The control group was composed of patients who were treated for Hodgkin disease during the same period and who had identical disease stages and chemotherapy schedules, but did not wish to wait for the effect of GnRH-a on ovarian function before starting chemotherapy. Whereas 20 of the 26 (76.9%) controls suffered POF, only three out of the 30 (10.0%) patients in the study group developed POF, two of them after BMT.

Waxman et al. (1987) conducted a prospective study of using GnRH-a in parallel to chemotherapy in both male and female patients. In spite of the low number of eight women treated with GnRH-a, they concluded that there was no significant difference between the resulting POF in the treatment group and in the controls (Table I). It needs to be noted that the power of the study was too low to show a difference between the GnRH-a group of women (4 of 8 patients with POF) and the controls (6 of 9 with POF) (Waxman et al., 1987). Furthermore, the pituitary desensitization and hypogonadotropic milieu might have been insufficient, since Waxman (1987) himself claimed that the used analogue (buserelin) might have not been appropriate.

We have recently described a case report where spontaneous pregnancy and normal delivery occurred after repeated autologous bone marrow transplantation (BMT) and GnRH-a treatment

(Blumenfeld et al., 2007). BMT almost invariably induces ovarian failure, irrespective of patient age or treatment protocol (Lobo, 2005; Blumenfeld et al., 2007). A large survey (Salooja et al., 2001) of fertility after stem cell transplantation (SCT) involving 37 362 patients found that only 0.6% of patients conceived after one autologous or allogenic SCT. The estimated odds for spontaneous conception after two BMTs are negligible (Salooja et al., 2001). The administration of GnRH-a before and in parallel to chemotherapy suggests that it may have minimized the gonadotoxic effect of chemotherapy and increased the chance of spontaneous ovulation and successful conception and delivery (Blumenfeld et al., 2007).

In non-HL, in some retrospective, small and underpowered studies, a significant difference between the GnRH-a versus control groups regarding long-term POF (Dann et al., 2005; Elis et al., 2006) could not be found. The GnRH-a group in these two studies consisted of only seven and three patients, respectively, therefore valid conclusions cannot be drawn.

Breast cancer

In premenopausal patients with breast cancer, GnRH-a also reduces chemotherapy-associated POF as revealed by several studies. In breast cancer, several phase II studies evaluated the effect of ovarian suppression with GnRH-a in preserving fertility and ovarian function (Recchia et al., 2002, 2006; Recchia, 2007; Fox et al., 2003; Urriticoechea et al., 2004; Del Mastro et al., 2006).

Recchia et al. (2006) reported that all their breast cancer patients who were younger than 40 years at the time of chemotherapy administration and who received GnRH-a co-treatment in addition to chemotherapy resumed cyclic ovarian function, with excellent 5- and 10-year survival rates. More recently, Recchia (2007) updated the data of GnRH-a treated patients. In this recent report (abstract), the group treated with GnRH-a in addition to chemotherapy included 130 women. After a median follow-up of 79 months, amenorrhea was observed in none of the patients <40 years and in 49% of patients over 40 years. Four pregnancies were observed: three ended at term and one was voluntarily terminated. The projected overall survival rates at 5 and 10 years were 94 and 85%, respectively. The main drawback of this study is the absence of a parallel control group. On the other hand, the importance of this study is the excellent survival rates, which does not support the expressed theoretical risk that hormonal manipulation of ER positive cancer may negatively affect the response of the malignant cells to chemotherapy. Recchia (2007) concluded that in premenopausal patients with high risk early breast cancer, the addition of GnRH-a to adjuvant therapy and total estrogen blockade in estrogen receptor positive patients is well tolerated, protects long-term ovarian function and seems to improve the expected clinical outcome.

Similarly, Del Mastro et al. (2006) have treated 30 patients with breast cancer of a median age of 38 years (range 29–47) with CEF (cyclophosphamide, epirubicin, 5-fluorouracil) chemotherapy regimen. Out of 17 patients, 16 (94%) younger than 40 years resumed cyclic ovarian function, concluding that GnRH-a given before and during chemotherapy may prevent premature menopause in the majority of patients (Del Mastro et al., 2006). However, similar to Recchia's studies (2006, 2007), this study also did not include a control group, but revealed good survival

rates and a low rate of premature menopause despite chemotherapy.

A recent meta-analysis published in *the Lancet* (Cuzick *et al*., 2007), based on data from 11 906 premenopausal women with early breast cancer randomized in 16 trials, has concluded that the addition of GnRH-a to tamoxifen, chemotherapy or both, reduced recurrence by 12.7% (95% confidence interval, 2.4–21.9%; $P < 0.02$) and death after recurrence by 15.1% (95% confidence interval, 1.8–26.7%; $P < 0.03$). Although GnRH-a were not administered in parallel to chemotherapy in all included studies, this meta-analysis weakens the previously raised hypothetical speculation that GnRH-a may decrease the efficacy of chemotherapy in estrogen receptor positive breast cancer. Furthermore, the publications of Recchia's group (2002, 2006, 2007) and Del Mastro *et al*. (2006) who administered GnRH-a in parallel to chemotherapy reported on excellent 5 and 10 years survival, which are as good as, if not better than, most of the studies which did not use GnRH-a in similarly treated breast cancer patients (96 and 91%, respectively) (Sutton *et al*., 1990; Mattle *et al*., 2005).

The results of a few ongoing, phase III, prospective randomized controlled trials such as the ZORO study [prevention of chemotherapy-induced ovarian failure with goserelin in breast cancer patients (ZORO, Zoladex Rescue of Ovarian function)] in Germany and the Southwest Oncology Group led USA. Intergroup Trial S0230 are still awaited.

GnRH-a for fertility preservation in non-malignant diseases

The GnRH-a co-treatment may also be applied to young women receiving cytotoxic chemotherapy for non-cancerous, benign diseases. Cyclophosphamide therapy for SLE, a disease predominantly affecting women of childbearing age, causes an unacceptably high incidence of irreversible POF (Somers *et al*., 2005).

In lupus patients treated with cyclophosphamide, 60% suffered from POF and hypergonadotropic amenorrhea (Blumenfeld *et al*., 2000; Manger *et al*., 2006). Whereas the POF rate was <50% in women <30 years, it was 60% between 30 and 40 years.

Manger *et al*. (2006) tested the concentrations of FSH and LH, before, during, and after cyclophosphamide treatment in 63 premenopausal women with SLE without ovarian protection concluding that most of them suffered POF, and they therefore initiated the ongoing PREGO-Study (Prospective Randomized study on protEction against GOnadal toxicity).

In keeping with the results by Blumenfeld *et al*., (2000), whereby co-treatment with GnRH-a may significantly decrease the cyclophosphamide-associated ovarian failure, Somers *et al*. (2005) have also demonstrated that the treatment with GnRH-a in parallel to cyclophosphamide therapy was associated with a significant reduction of POF in young women with severe SLE. In their study, POF developed in one of 20 women treated with GnRH-a (5%) compared with that in six of 20 controls (30%) matched by age and cumulative cyclophosphamide dose (matched odds ratio 0.09, $P < 0.05$). Kaplan–Meier estimates demonstrated improved cumulative ovarian protection over time in the GnRH-a-treated group ($P = 0.04$).

GnRH-a for prevention of menometrorrhagia during treatment

Heavy uterine bleeding is a frequent and serious phenomenon in premenopausal women with haematological malignancies (Meirow *et al*., 2006; Quaas and Ginsburg, 2007). The menometrorrhagia may be associated with thrombocytopenia due to the neoplasia itself, or may be a side effect of chemotherapy, especially after aggressive conditioning for BMT (Meirow *et al*., 2006; Quaas and Ginsburg, 2007). Indeed, haemorrhagic complications represent the second leading cause of mortality in adults with leukaemia, with only infection being a more common cause of death (Chang *et al*., 2001; Quaas and Ginsburg, 2007).

Quaas and Ginsburg (2007) have reviewed the published literature on the prevention and treatment of uterine bleeding in haematologic malignancy. They concluded that most publications use menstrual suppression with GnRH-a in haematological malignancy, although no prospective randomized trials were published. Review of the identified literature suggested that the medical prevention of menometrorrhagia with GnRH-a therapy is highly effective for the prevention of uterine bleeding in haematologic malignancy (Quaas and Ginsburg, 2007).

Meirow *et al*. (2006) have retrospectively evaluated young female oncology patients with regular menstrual cycles undergoing myelosupressive treatments receiving either depo-medroxyprogesterone acetate, or GnRH-a, or no treatment before the administration of myelosupressive chemotherapy. Only patients who later developed severe thrombocytopenia (<25 000 platelets/ml) were included in their study. Severe or moderate menorrhagia was documented in none of the 39 women who received GnRH-a, in nine patients (21.4%) who received depo-medroxyprogesterone acetate, and in nine controls, untreated patients (40%; $P = 0.02$). Furthermore, fewer calls for urgent gynaecological consultations were documented in the GnRH-a group compared with the untreated group ($P < 0.0001$). They also concluded that female patients undergoing myelosupressive therapy are at high risk of developing significant menorrhagia secondary to the chemotherapy-associated thrombocytopenia. GnRH-a treatment was more effective and therefore clinically superior to gestagens for the prevention of bleeding disorders in these patients.

GnRH antagonists instead of, or in combination with, GnRH-a

As GnRH antagonists suppress gonadotrophin levels immediately after administration, it has been suggested that future studies should examine GnRH antagonists instead of agonists for the achievement of a faster pituitary–ovarian desensitization, eliminating the waiting period of 7–14 days needed by the GnRH-a to achieve down-regulation (Meirow *et al*., 2004; Blumenfeld *et al*., 2007).

Meirow *et al*. (2004) attempted to determine whether administration of the GnRH antagonists, cetrorelix, before exposure to increasing doses of cyclophosphamide affected the number of surviving primordial follicles (PMF) in the mice ovary. Ovaries exposed to cyclophosphamide at doses of 50 and 75 mg/kg had significantly fewer PMF than those in the control group ($P < 0.01$). In each of the cyclophosphamide groups used,

pretreatment with cetrorelix resulted in significantly higher numbers of PMF: in the 50 mg/kg cyclophosphamide group, only 14% were destroyed (Cetrorelix group) compared with 53% (without Cetrorelix) ($P < 0.001$), whereas in the 75 mg/kg cyclophosphamide group, only 35% of PMF were destroyed (with Cetrorelix) versus 54% in animals treated without Cetrorelix ($P < 0.004$). The interaction between the effect of cetrorelix and the different doses of cyclophosphamide did not reach statistical significance. Meirow et al. concluded that administration of the GnRH antagonists to mice significantly decreases the extent of ovarian damage induced by the chemotherapeutic agent cyclophosphamide. The use of different sub-sterilizing doses of cyclophosphamide suggested that the extent of protection achieved by the antagonist is dose-dependent and decreases with increasing cyclophosphamide doses. The results of this study (Meirow et al., 2004) may suggest a possible similar beneficial effect in women undergoing chemotherapy.

However, a more recent study (Danforth et al., 2005) has concluded that in contrast to the 'well-known effects of GnRH-a to reduce chemotherapeutic destruction of PMF, GnRH antagonists do not protect the ovary from the damaging effects of cyclophosphamide'. In this study (Danforth et al., 2005), administration of cyclophosphamide to adult mice caused a nearly 50% reduction in the number of the PMF. GnRH antagonists did not prevent the depletion of PMF caused by cyclophosphamide. Surprisingly, both tested antagonists, Antide and Cetrorelix, caused a significant reduction in the number of the PMF, even without cyclophosphamide.

This observation, although preliminary, casts doubt on the assumption that GnRH antagonists may substitute for the agonists in the future, for minimizing the chemotherapy-associated gonadotoxicity (Danforth et al., 2005). In a controversial response, Gupta and Flaws (2005) raised the provocative question: 'GnRH-a and the ovary: do GnRH antagonists destroy PMF?' They concluded that the findings of Danforth et al. (2005) are interesting and novel because they provide further support that GnRH-a protect against chemotherapy. In addition, the study of Danforth et al. (2005) is the first to demonstrate that a GnRH antagonist decreases the number of PMF and that this likely stems from a direct effect of the antagonist on the ovary (Gupta and Flaws, 2005).

A similar conclusion has been reached by Peng et al. (2007) while examining the effects of GnRH-a on chemotherapy-induced ovarian function damage in rats. In an attempt to investigate the effects of GnRH-a or an antagonist on cyclophosphamide-induced ovarian damage in rats, they concluded that in the rat model, GnRH-a prevents the ovarian function damage induced by cyclophosphamide, but the GnRH antagonist does not show a similar protective effect.

On the other hand, GnRH antagonists may be useful in combination with GnRH-a to achieve a faster down-regulation, as compared with the agonist alone. Mardesic et al. (2004) have tested this combination, in six young women (aged 15.4 ± 0.7) years with haematological malignancies before the onset of cytotoxic chemotherapy. They concluded that this combination of agonist and antagonist induced a reliable and long-lasting suppression of gonadotrophin secretion within 96 h in all patients, allowing cytotoxic therapy to be started without any delay.

Recently, Mardesic et al. (2008, abstract, 9th Symposium on GnRH-a in Human reproduction and cancer, Berlin, February 10–12) have presented similar data and conclusions from a larger group of patients similarly treated with a GnRH antagonist and agonist combination. All their young patients similarly treated resumed cyclic ovarian function, but all those who received aggressive chemotherapy conditioning before BMT turned prematurely menopausal and suffered POF (Mardesic et al., 2008, abstract, 9th Symposium on GnRH-a in Human reproduction and cancer, Berlin, February 10–12).

OC for fertility preservation

Chapman and Sutcliffe (1981) have attempted to determine if suppression of ovarian function by OC would provide protection against ovarian cell death secondary to chemotherapy in young women, 18–31 years old. By means of menstrual history, serum gonadotrophin levels and ovarian biopsy, ovarian function was evaluated in six young women with untreated HL. Each woman was given a standard six cycles of MVPP therapy (nitrogen mustard, vinblastine, procarbazine and prednisone). At the time of initiation of MVPP therapy, they were placed on combination of OCs. Six to twelve weeks after the last cycle, three women were biopsied and the menstrual history was reported in all cases. This follow-up was repeated at intervals of 4–12 months up to 29 months. Ovarian biopsies obtained prior and after therapy revealed primordial and primary follicles. Normal menses were established in the five women who discontinued OCs at the end of MVPP therapy and one conceived (Chapman and Sutcliffe, 1981). The pregnancy and the regular menses in the three women not on hormonal agents up to 2 years after stopping MVPP therapy encouraged the authors to believe that these women will not experience POF in the next few years. They concluded that suppression of ovarian function by combination OC may protect the ova against an otherwise certain injury by the chemotherapeutic drugs.

However, Longhi et al. (2003) reached different conclusions. They treated 31 women, suffering from localized osteosarcoma of the extremities, with high-dose ifosfamide, methotrexate, adryamycin, cis-platinum and an OC in an attempt to prevent POF. Their control group included 71 patients treated with similar protocols without OC or other treatment to protect ovarian function. There were no significant differences between the two groups. POF occurred in 3/19 in the OC group and in 3/71 controls (Longhi et al., 2003). Furthermore, in the OC group, two patients suffered a complication of thrombophlebitis, which is a possible drawback of using OC during chemotherapy, since both may increase the thrombophilic tendency. These investigators concluded that the OC during chemotherapy do not protect ovarian function in patients receiving high-dose chemotherapy (Longhi et al., 2003).

Similarly, Whitehead et al. (1983) also found that the combination of OC pill throughout chemotherapy did not protect their treated female patients from chemotherapy-induced ovarian damage. They studied 44 female lymphoma patients who had been treated with MVPP, at a median age of 23 years. The 17 women who subsequently suffered POF were significantly older (median, 30 years) than those who maintained cyclic ovarian function (median, 22 years). All the patients >36 years developed chemotherapy-induced POF. There were nine patients, who developed POF or oligomenorrhea despite OC co-treatment throughout

chemotherapy, suggesting that OCs did not protect from chemotherapy-induced ovarian damage (Whitehead *et al.*, 1983).

In a retrospective study, Elis *et al.* (2006) examined the fertility status of women treated for aggressive non-HL. A cohort of 36 women with aggressive non-HL in first remission, who were treated in five university-affiliated hospitals in Israel, was evaluated. All women were younger than 40 years at diagnosis and received frontline protocols, including cyclophosphamide and adryamicin, mostly CHOP. Menstrual cycle characteristics, as well as pregnancies before the diagnosis, during treatment and in first complete remission, were evaluated. Three patients received GnRH-a, whereas nine received OC together with cytotoxic treatment. There was no significant difference between those patients who received fertility-preserving measures compared with the remainder concerning regular menstrual cycle recovery or pregnancies (Elis *et al.*, 2006). However, the small numbers underpowers this study from reaching any conclusion.

On the other hand, Behringer *et al.* (2005) found, in a larger study, a statistical association between the use of OC and return of menstrual cycle. After a follow-up of 3.2 years, over half of the women receiving eight cycles of BEACOPP had amenorrhea. Amenorrhea correlated with advanced-stage HL ($P < 0.0001$), age >30 years ($P = 0.0065$), and non-usage of OCs ($P = 0.0002$) during chemotherapy (Behringer *et al.*, 2005). Unlike the other four studies, they have found that OC usage may reduce POF (10.1% in the treated group versus 44.1% in controls).

Suggested mechanisms of gonadotoxic protection by GnRH-a

Creating a prepubertal, hypogonadotropic milieu

The hypogonadotropic state, generated by GnRH-a, simulates a prepubertal hormonal milieu. It has been speculated and hypothesized that the gonadotoxic chemotherapy induces an accelerated rate of follicular demise with a subsequent decrease in the production of inhibins and estrogens (Fig. 1). The generated diminution in estrogen and inhibin production and plasma concentration will result in an increase in follicle-stimulating hormone (FSH) secretion due to the negative feedback effect on the hypophysis and hypothalamus. The generated supraphysiologic FSH levels will accelerate the rate of preantral follicle maturation and recruitment to enter the unidirectional process of maturation, which being further subjected to the gonadotoxic effects of chemotherapy, ends in an accelerated rate of follicular demise. The administration of GnRH-a may interrupt this destructive vicious cycle by inducing pituitary desensitization, which prevents the increase in FSH concentrations despite low inhibin and sexsteroids levels (Fig. 1) (Lobo, 2005; Blumenfeld, 2007a).

Flaws *et al.* (1997) have suggested a possible detrimental effect of high gonadotrophin concentrations on primordial and primary follicles. Transgenic mice for β-LH demonstrating high levels of LH have a number of follicles comparable to that of wild-type controls at birth. However, they show a significant premature loss of their primordial and primary follicles, several weeks after being exposed to high LH concentrations, in agreement with the suggested pathophysiologic hypothesis (Fig. 1). It has also been found by several other investigations (Zheng *et al.*, 1996; Oktay *et al.*, 1997; Patsoula *et al.*, 2003) that primary and PMF express

mRNA for FSH and LH receptors. These findings support the concept that even immature follicles such as the primordial and primary follicles may be gonadotrophin dependent (Babu *et al.*, 2001; Adriaens *et al.*, 2004; Knight and Glister, 2006).

Even if one assumes that PMF are gonadotrophin independent, because gonadotrophin receptors have not been unequivocally proven on their primordial GCs, they are dependent on many growth factors (GFs) such as activins and bone morphogenic protein (BMP)-4, -7 and -9 (Knight and Glister, 2006). These, and possibly other similar GFs that are secreted by the more mature follicles, may induce the maturation of the PMF (Knight and Glister, 2006). The secretion of these GFs by the more advanced and mature, preantral follicles are induced by FSH stimulation (Knight and Glister, 2006). The administration of GnRH-a brings about pituitary desensitization, thus preventing the secretion of GFs by the FSH-dependent follicles; and secondarily preserving more PMF in the uncommitted, 'dormant' stage, and minimizing their ultimate destruction by alkylating agents (Knight and Glister, 2006; Blumenfeld, 2007a).

Although the initiation of PMF differentiation and growth and the early stages of folliculogenesis can occur without gonadotrophins, FSH may affect the rate of preantral follicle growth (Webb *et al.*, 2004). Thus, the older hypothesis that primordial and primary follicles are gonadotrophin independent may need re-evaluation and reassessment.

Decreased utero-ovarian perfusion

Another possible explanation for the beneficial effect of GnRH-a on decreasing chemotherapy-associated gonadotoxicity is the decrease *in utero*-ovarian perfusion resulting from the hypoestrogenic state generated by pituitary—gonadal desensitization (Saitta *et al.*, 2001; Kitajima *et al.*, 2006; Blumenfeld, 2007a).

High estrogen levels increase ovarian perfusion in a rat model of ovarian stimulation, and this effect was significantly and dose-dependently inhibited by administration of a GnRH-a (Kitajima *et al.*, 2006). The decreased utero-ovarian perfusion in the hypoestrogenic milieu generated by GnRH-a induced pituitary desensitization may result in a lower total cumulative exposure of the ovaries to the chemotherapeutic agents, secondarily resulting in decreased gonadotoxic effect.

A direct effect on GnRH receptors

Primate and human gonads also contain GnRH-receptors, as do the ovaries of rodents (Grundker and Emons, 2003; Leung *et al.*, 2003; Harrison *et al.*, 2004).

Grundker and Emons (2003) have demonstrated that GnRH-receptor activation by their ligands decrease cellular apoptosis. More recently, Imai *et al.* (2007) have demonstrated that GnRH-a may decrease the *in vitro* gonadotoxic effect of chemotherapy, independently of the hypogonadotropic milieu. These investigators have shown direct *in vitro* protection from doxorubicin-induced GC damage by GnRH-a.

The possible role of sphingosine-1-phosphate

An interesting speculation is associated with the emerging recognized role of sphingosine-1-phosphate (S-1-P) and related molecules involved in chemotherapy-induced oocyte apoptosis

(Perez *et al*., 1997; Morita *et al*., 1999, 2000; Paris *et al*., 2001, 2002; Tilly, 2001).

S-1-P is a pleiotropic lipid mediator of cell growth, survival, invasion, vascular maturation and angiogenesis, which are all processes that are involved in cell viability and cancer progression (Spiegel *et al*., 1998; Spiegel and Milstien, 2003). Intracellular S-1-P levels are regulated by the balance between its synthesis by sphingosine kinases and degradation by S-1-P lyases and phosphatases (Alvarez *et al*., 2007). It may be hypothesized that GnRH-a may up-regulate the ovarian S-1-P (Blumenfeld, 2007a). Targeted disruption of the Bax gene in mice or, more recently, targeted expression of the Bax antagonist, Bcl-2, to the female mouse germ line can protect the oocytes from the gonadotoxic effect of doxorubicin (Morita *et al*., 1999; Reynolds, 1999). The S-1-P molecule can also prevent doxorubicin-induced oocyte death *in vitro* (Perez *et al*., 1997; Morita *et al*., 2000). Moreover, oocytes which are deficient of acid sphingomyelinase which generates ceramide, (Kolesnick and Kronke, 1998) are resistant to the apoptosis induced *in vitro* by chemotherapy (doxorubicin) (Morita *et al*., 2000). Intrabursal administration of S-1-P prevented the destruction of the ovarian follicles, induced by massive irradiation (Morita *et al*., 2000; Paris *et al*., 2001). Furthermore, Paris *et al*., (2002) have demonstrated that S-1-P can protect the female germ line from radiation without a discernible propagation of genomic damage at all the biologic and cytogenetic tested levels. It may be speculated that the GnRH-a adjuvant co-treatment positive effect may be possibly associated with an intragonadal increase in S-1-P. However, this concept of GnRH-a acting on S-1-P metabolism is still speculative and needs further evaluation.

Possible protection of ovarian stem cells

A few years ago, Johnson *et al*. (2004) presented revolutionary data whereby rodent ovaries may possess mitotically active germ cells that continuously replicate themselves. According to these investigators (Johnson *et al*., 2004), these germ line stem cells (GSC) may exist in the mouse ovary and replenish the PMF pool. Their hypothesis challenges the basic doctrine of reproductive biology, whereby mammalian females are born with a fixed reserve of germ cells (potential oocytes) and lose the capacity for germ-cell renewal during fetal life (Johnson *et al*., 2004; Gargett, 2007). The dogma that mammalian oogenesis does not occur after birth was established and upheld for more than half a century. Therefore, these revolutionary publications (Johnson *et al*., 2004, 2005) have raised serious criticism and antagonism (Byskov *et al*., 2005; Gargett, 2007).

Following this revolutionary and yet unaccepted concept, it may theoretically be speculated that the GnRH-a protective effect may possibly be through protection of the undifferentiated GSCs, which ultimately generate *de novo* PMF (Blumenfeld, 2007a). This hypothetical speculation is supported by our observation of temporary high, reversible FSH concentrations in about one-third of our patients several months after the chemotherapy. However, it remains to be proven if this effect can be attributed to GnRH-a.

Summary

The review of all published studies using GnRH-a or OC clearly demonstrate that the provided data are too limited to provide conclusive statistical evidence concerning the reduction of POF. On the other hand, most studies, analysing the effect of GnRH-a, and one large statistically significant study, studying the effect of OC, have shown a reduction of POF in patients receiving GnRH-a or OC during chemotherapy. Therefore, many oncologists treat young women with GnRH-a as major positive effects, such as the potential to reduce POF and the definite reduction of menometrorrhagia, seem to outweigh the minor negative side effects such as hot flushes. Nevertheless, to unequivocally recommend GnRH-a or OC as a proved fertility preserving treatment, large prospective randomized studies are still needed. The results of additional studies are awaited to address the safety and efficiency of oocytes, follicles or ovary cryopreservation and the most efficient way of using the cryopreserved–thawed tissue. Similarly, the results of multicenter, prospective, randomized studies are urgently awaited to substantiate the *in vivo* effect of GnRH-a as an unequivocal means for minimizing follicular apoptosis. Success in the prevention of chemotherapy-induced ovarian failure will not only improve prospects for future fertility, but should prevent other adverse effects of premature menopause, such as bone density loss, sexual dysfunction and vasomotor symptoms.

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