Symposium article

Fertility after treatment for Hodgkin’s disease

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**Background:** The investigational endeavors of ovarian cryopreservation await the clinical experience of auto- or xenotransplantation, in vitro maturation of thawed primordial follicles, their in vitro fertilization and embryo transfer. Although promising, this experience is not yet available. Moreover, the risk of possible reimplantation of malignant stem cells with the thawed cryopreserved ovary has been raised following experimental animal observations. Therefore, until these innovative endeavors prove successful, we have attempted to minimize the gonadotoxic effect of chemotherapy by the co-treatment with a gonadotropin-releasing hormone agonistic analog (GnRH-a) to induce a temporary prepubertal milieu. The immunoreactive inhibin-A and -B in these patients was measured before, during and following the gonadotoxic chemotherapy.

**Methods:** A prospective clinical protocol was undertaken in 60 women aged 15–40 years with lymphoma, 10 with leukemia and 10 undergoing chemotherapeutic treatments for non-malignant diseases such as systemic lupus erythematosus or other autoimmune diseases. A monthly injection of depot D-TRP6-GnRH-a was administered from before starting the chemotherapy until its conclusion, up to a maximum of 6 months. Hormonal profile [follicle-stimulating hormone (FSH), luteinizing hormone (LH), E$_2$, T, P4, insulin-like growth factor (IGF)-1, IGF-BP3 and prolactin] was taken before starting the GnRH-a/chemotherapy co-treatment, and monthly thereafter until resumption of spontaneous ovulation. This group was compared with a control group of 60 women who have been treated with similar chemotherapy.

**Results:** Whereas all but three (40, 36 and 34 year old) of the surviving patients within the GnRH-a/chemotherapy co-treatment group resumed spontaneous ovulation and menses within 12 months, less than half of the patients in the ‘control’ group (chemotherapy without GnRH-a co-treatment) resumed ovarian function and regular cyclic activity ($P <0.05$). The remaining 55% experienced premature ovarian failure (POF). Temporarily increased FSH concentrations were experienced by about one-third of the patients resuming cyclic ovarian function, suggesting reversible ovarian damage in a larger proportion of women than those experiencing POF. Inhibin-A and -B decreased during the GnRH-a/chemotherapy co-treatment but increased to normal levels in patients who resumed regular ovarian cyclicity, and/or spontaneously conceived, as compared with low levels in those who developed POF.

**Conclusions:** If these preliminary data are consistent in a larger group of patients, GnRH-a co-treatment should be considered in every woman of reproductive age receiving chemotherapy, in addition to assisted reproductive technologies and the investigation into ovarian cryopreservation for future in vitro maturation, autotransplantation or xenotransplantation.

**Key words:** chemotherapy, GnRH analogs, gonadotoxicity, premature ovarian failure

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Introduction

As survival rates for young treated Hodgkin’s disease (HD) patients continue to improve, protection against iatrogenic infertility caused by chemotherapy assumes higher priority [1–4].

HD is the most common malignancy in the population aged 15–24 years [5]. Prolonged survival of >90% is now expected for a high proportion of young patients treated with cytotoxic chemotherapy for HD [1, 5–7]. This is due to the introduction of effective chemotherapy protocols [1, 5, 8–10]. Recent advances in the understanding of the molecular biology of lymphomas permit an additional dimension: the identification of molecular characteristics associated with a poor prognosis, such as patterns of gene expression that are associated with protection against apoptosis, chemotherapy resistance, or the stimulation of angiogenesis. If such attempts prove successful,
the goal of 100% cure without serious late secondary effects, despite its apparent elusiveness a few decades ago, will have been positioned clearly on the horizon [7]. Premature ovarian failure (POF) is a common long-term consequence of chemotherapy [1, 6, 8–12] and radiotherapy [6, 13]. Whereas the cytotoxic-induced damage is reversible in other tissues of rapidly dividing cells, such as bone marrow, gastrointestinal tract and thymus [6, 14], it appears to be progressive and irreversible in the ovary, where the number of germ cells is limited, fixed since the fetal life, and cannot be regenerated [1, 6].

The preponderance of the data support the concept of a direct correlation between dose intensity and POF [1].

Infertility represents one of the main long term consequences of combination chemotherapy given for Hodgkin’s lymphoma (HL), leukemia and other malignancies in young women [1, 2, 5, 15–23]. The impairment of gonadal function after chemotherapy is much more frequent in men than in women, occurring in up to 90% of post pubertal males [1, 2, 24]. Because dividing cells are known to be more sensitive to the cytotoxic effects of alkylating agents than are cells at rest, it has been suggested that inhibition of the pituitary–gonadal axis would reduce the rate of spermatogenesis and oogenesis and thereby render the germinal epithelium less susceptible to the effects of chemotherapy [1, 2, 6, 24, 25]. Several alternatives have been attempted to try to preserve fertility in young women undergoing chemotherapy treatment.

Alternatives for fertility preservation

Cryopreservation of ‘mature’ metaphase II oocytes
Cryopreservation of ‘mature’ metaphase II oocytes usually after hMG/hCG (human menopausal gonadotropin/human chorionic gonadotropin) ovarian stimulation. Although successful in rodents [20, 21, 26–28], it is still far from being a clinical alternative in humans [29, 30]. Moreover, a concern of possible chromosomal aberration associated with the freezing of MII oocytes has been raised [26, 31]. A future possible alternative may be the retrieval of human immature oocytes for cryopreservation and in vitro maturation after thawing [20, 21, 26, 32, 33].

Cryopreservation of fertilized ova or embryos
A much more clinically available option is the cryopreservation of fertilized ova or embryos after in vitro fertilization (IVF) before chemotherapy [1, 34]. However, this alternative is relevant to married women or those who have a partner, and almost inapplicable to the very young, single woman. Moreover, the ovarian stimulation with hMG/hCG before IVF-egg retrieval requires the initiation of chemotherapy to be postponed, which is often contraindicated by the hematologists and oncologists [1, 26]. Furthermore, the increase in estradiol concentrations, caused by hMG/hCG ovarian stimulation, may aggravate the clinical situation of patients with breast carcinoma or other estrogen-sensitive tumors, or that of systemic lupus erythematosus patients, by inducing a flare-up of the autoimmune disease [1]. Preliminary clinical results in our and other centers suggest that it may be possible to retrieve immature ova from developing antral follicles, mature them in vitro for a few days, fertilize them by intra-cytoplasmic sperm injection and subsequently cryopreserve the few embryos generated. This option may enable an incomplete resolution, although limited, for preservation of future fertility, without postponing the initiation of chemotherapy and without exposure of these patients to ovarian stimulation and hyperestrogenism.

Transplantation of ovarian tissue
An old suggestion [35] that has recently been the focus of intense investigation is the transplantation of ovarian tissue [1–3, 18–23, 26, 33–44].

However, a concern over possible associated malignancy in the transplanted ovary has been raised [1, 3, 26, 40]. Therefore, future endeavors may concentrate on cryopreservation of ovarian fragments, before chemotherapy, and thawing these fragments, dispersion of the primordial/primary follicles, in vitro maturation and fertilization. Although not clinically available yet, the enormous progress of the assisted reproductive technologies (ART) in the last two decades lends hope that in the next few years this option may indeed turn into a practical option [39]. Until then, every effort should be made to offer all the possible options available at present for minimizing the gonadotoxic effect of chemotherapy.

A recent editorial in Fertility and Sterility by Oehninger [45] has challenged the pertinent question of whether ovarian autotransplantation will have a role in reproductive and gynecological medicine for preservation of ovarian function. This has been successfully accomplished in rats, sheep and other animals [35–46].

Such therapy could provide a source of ovarian tissue that, when autotransplanted, would maintain an adequate estrogenic milieu that protects against heart disease and osteoporosis. It has been suggested that it may be best to restrict ovarian transplantation to women whose ovaries are disease free, because cancer can be transmitted with ovarian tissue grafts in animals [40].

A second role for this therapy could be in the form of ‘oocyte banking’ as a strategy to preserve the reproductive potential of younger women or girls before cancer therapy [26, 37–48]. Cryopreservation of ovarian tissue before initiation of oncological treatment, followed by autologous transplantation after remission, could provide a means of protecting fertility [39, 45, 49]. Obviously, an option for these cases involves oocyte retrieval (today probably better accomplished with gonadotropin stimulation, although the natural cycle will be preferable when improved oocyte in vitro maturation protocols become available), followed by IVF and embryo cryopreservation [39, 45].
The potential therapeutic use of ovarian autotransplantation mandates continued efforts to achieve better results with ovarian tissue cryopreservation (i.e. freezing of the isolated germ cell at different stages of maturity, cumulus-encased oocytes, follicles and sliced tissue) and with in vitro oocyte maturation procedures. Ovarian tissue can be recovered at the time of laparotomy, or ovarian biopsy specimens can be taken by laparoscopy. When autografting to an orthotopic site succeeds in restoring ovulatory menstrual cycles, hormonal replacement therapy and medical intervention in the process of conception may not be needed [1, 37, 39, 45]. Restoration of fertility to oophorectomized sheep by autografts stored at −196°C has been reported [36]. Alternatively, oocytes could be recovered from an ectopically located graft and matured in vitro or, after gonadotropin stimulation, mature oocytes could be retrieved and used in ART [39, 43, 45].

Cryopreservation of ovarian tissue has several potential advantages over both oocyte and embryo freezing. Hundreds of immature oocytes may thus be cryopreserved without the necessity of ovarian stimulation and delay in initiating cancer treatment [50]. Cryopreservation of ovarian tissue is of great benefit, since immature oocytes are relatively quiescent, smaller, and lack zona pellucida and cortical granules [50]. These properties make them far more tolerant to freezing and thawing injuries than mature oocytes. Furthermore, it has been hypothesized that primordial follicles have a greater potential to repair sublethal damage to organelles and other intracellular structures during their prolonged growth phase [50]. However, a significant follicular loss occurs with freezing, thawing and grafting. It is unknown as yet how well and how long a given frozen–thawed ovarian segment will function after autotransplantation in human [50].

There are three optional methods for utilization of cryopreserved ovarian tissues: Autotransplantation, xenotransplantation and in vitro maturation.

**Autotransplantation**

Cryopreserved ovarian tissue can be autografted either orthotopically or heterotopically, but as yet it is not known which will turn to be more practical and effective. The expected relatively short life span of frozen–thawed ovarian grafts is a concern [50]. Where few follicles remain and early graft exhaustion is expected, it may be more reasonable to use the heterotopic approach [50]. Bearing in mind the potential risk of transmission of microscopic metastatic disease, attempts to confirm the safety of ovarian tissue transplantation, based on the absence of malignant cells by light microscopy, may not be reassuring enough [50, 51]. A recent study [51] suggested that it is impossible to exclude the risk of cancer transmission in hematogenous diseases (such as leukemia) or systemic neoplasms.

Experimental studies have shown that the ischemia that occurs in the graft before revascularization results in the loss of virtually the entire growing follicle population in addition to the ~50% of primordial follicles [17, 42, 44, 52].

In another recent study [17], the ovarian autograft resulted in a two- to seven-fold increase in peripheral follicle-stimulating hormone (FSH) concentration in the face of a re-established E2 secretion, which is in agreement with studies using ovarian cortical autografts in sheep [41]. Elevated basal FSH in regularly menstruating women has been reported to be associated with slower follicular growth [53]. In turn, it has been reported in sheep [41], the most likely cause of the increase in FSH associated with restoration of ovarian function after autografting is a decline in inhibin secretion. Lower inhibin-A and -B levels in older cycling women are considered to be early markers of female reproductive aging [54], and thus, overall, the endocrine changes associated with human ovarian autografting are similar to those observed in aged women. This study concluded that hormonal "protection" and potentially even fertility can be restored after fresh or cryopreserved heterotopic ovarian autografting in women, albeit for only a short reproductive span [18].

**Xenotransplantation**

Using this option, the possibility of cancer cells transmission and relapse is eliminated since cancer cells do not penetrate the zona pellucida. Another advantage of this technique is the possibility of its application to patients for whom hormonal treatment is contraindicated (such as in breast cancer). It has recently been demonstrated [51, 55] that after subcutaneous transplantation of human ovarian cortex into mice, exogenous gonadotropin stimulation generated follicular growth in 51% of grafts [55]. Revel et al. [56] have demonstrated follicle maturation and subsequent formation of corpus luteum in human ovarian tissue xenografted subcutaneously into mice. The advantages of subcutaneous xenografting are simplicity, convenient monitoring of follicular development and direct access to follicle aspiration [50]. However, there is a serious concern of possible transmission of xenooes and animal pathogens to human.

**In vitro maturation**

Developing an in vitro culture system contains multiple variables that may affect oocyte integrity, since the development of primordial follicles to the preovulatory stage in humans may take more than 6 month [57]. The in vitro growth and maturation of human primordial follicles, followed by IVF, is a very attractive and desirable option, but it is technically challenging due to the prolonged growing phase and lack of knowledge of the optimal conditions for growth and maturation of human oocytes. Although preliminary encouraging experiments have been recently generated in animal models.
the ability to completely grow and mature human primordial follicles in vitro, however, will not be available until the development of an optimal culture system, which depends on the acquisition of a full understanding of the signal and control mechanism of follicular growth [45, 50, 58].

In summary, several urgent issues need to be resolved for the clinical application of ovarian transplantation and/or in vitro maturation to be successful and clinically applicable. Furthermore, unequivocal information is needed on the optimal dehydratation times, cooling and thawing rates, and identification of the most effective cryoprotectant [50]. The most crucial factor for tissue survival is the degree of ischemia-reperfusion injury after transplantation [50]. Indeed it has been reported that more primordial follicles suffer demise because of ischemia than because of freezing injury [50]. Indeed, using a sheep autograft model [59], only 5% of the primordial follicles survived autografting. The optimal site for transplantation is also not yet known precisely [50]. The main hurdle with ovarian transplantation is ischemia-reperfusion injury during revascularization [50]. It is therefore crucial to find a way to minimize the hypoxic injury, which may significantly jeopardize the future success of ovarian transplantation. Ovarian tissue cryopreservation has yet to prove itself in terms of its main goal: a human pregnancy [45, 58]. Indeed, many centers around the world have begun extracting ovarian tissue in clinical settings, wagering that, by the time their patients need this tissue, the optimal manner for its use and the proper technology will have been developed [58]. Therefore, it appears that at the present time we should be prudent and reserve ovarian tissue cryopreservation for patients who have nothing to lose, when the intensity of anticancer treatments (such as in bone marrow transplantation) will render them menopausal [58].

The study by van Eye Corleta et al. [46] confirmed that ovarian grafts with sliced tissue resulted in a lower degree of ischemic or degenerative changes than intact transplanted ovaries. In various animal models (rat, rabbit, monkey) it has been demonstrated that autotransplantation of ovaries can result in a prompt revascularization of the gland. The transplanted ovary is (or becomes after grafting) able to produce substances that promote and direct angiogenesis [60]. Recent studies have shown that the increase in gonadotropin secretion after ovarian transplantation in the rat contributes to revascularization of the graft by upregulating the gene expression of two major angiogenic factors: vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β [60]. More information is needed to determine whether the oocytes from autotransplanted ovarian tissue are competent to achieve full maturation, fertilization and embryo development.

Interesting, perplexing and challenging questions arise. (i) Could the addition of angiogenic and other growth factors (such as VEGF, TGF-β or others) to the transplanted ovary enhance the anchorage and number of functionally competent cells? (ii) What is the survival rate of the grafts? (iii) Could the functional longevity be prolonged by addition of survival factors? (iv) Is the developmental potential of the female gamete preserved after transplantation (with or without an intermediate cryopreservation step)? (v) Could gene expression (regulatory genes of growth, steroidogenesis, oocyte maturation) be manipulated before transplantation (or after) to achieve better results or to treat specific abnormalities of the germ cell line [45]?

All these questions and concerns about ovarian autotransplantation in the human need to be addressed in preclinical animal studies. Better cryopreservation methods of human ovarian tissue and oocytes must be sought. Only then should clinical studies be considered. Appropriate discussions not only at the scientific but also at the ethical level are mandatory to establish whether these techniques will benefit reproductive and gynecological medicine patients [1, 39, 45, 58].

Chemotherapy and gonadotropin-releasing hormone—a co-treatment

The possibility of administering an adjuvant treatment that might limit the gonadal damage caused by an otherwise successful treatment program is attractive [1, 4, 5, 61]. Glode et al. [62] tested this hypothesis using a murine model and concluded that a gonadotropin-releasing hormone agonistic analog (GnRH-a) appeared to protect male mice from the gonadal damage normally produced by cyclophosphamide. It may be that decreased secretion of the pituitary gonadotropin, by decreasing gonadal function, could protect against the sterilizing effects of chemotherapy. Although previous suggestions have been made [63, 64] 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 recently [1, 65–67]. Whereas several investigators have demonstrated that GnRH-a inhibits chemotherapy-induced ovarian follicular depletion in the rat [62–65], uncertainty remains about human application [1, 65–67]. The human ovary has lower concentrations of ovarian GnRH receptors and may not necessarily exhibit the same response as rats [1, 65, 66]. Ataya et al. [65] have found that GnRH-a protected the ovary against cyclophosphamide-induced damage in Rhesus monkeys by significantly decreasing the total amount of follicle loss during the chemotherapeutic insult, and by decreasing the daily rate of follicular decline. Chapman et al. [10] have found that of their female patients treated for HD, 69% developed POF if they were <29 years old, while 96% developed POF if their age was >30 years.

Advances in the treatment of all stages of HL and non-Hodgkin’s lymphoma (NHL) with chemotherapy and irradiation have led to a long-term survival of 90%, or even more in several groups of patients [1, 7, 67–70]. The improved long-term survival of relatively young patients treated for lymphoma focused attention to the gonadal toxicity of the combined chemotherapy and radiotherapy. Whereas 86% of men had azoospermia after the COPP/ABVD regimen [69], only...
48–77% of women receiving chemotherapy for lymphoma exhibited hypergonadotropic amenorrhea and ovarian failure [69–71]. Moreover, a long-term follow-up of 240 children, aged ≤15 years, treated with MOPP (mechlorethamine, vincristine, procarbazine and prednisone) for HD showed azoo-
spermia in 83% of the boys, whereas only 13% of the girls suffered ovarian failure [72]. The chances of maintaining
gonadal function following combined modality treatment are
significantly greater among girls than boys [1, 66, 72]. In con-
trast to the results reported in adults, the MOPP chemotherapy
in girls with HD did not induce ovarian failure [73]. Since
ovarian function was preserved in most long-term survivors
who were treated prepubertally for lymphoma or leukemia
[72–74], but only in a minority of similarly treated adult
patients [69], it is clinically logical and therefore tempting to
temporarily create a prepubertal milieu in women in
the reproductive age before and during the chemotherapeutic
insult [1, 66, 67, 70].

It has been reported that 64% of adult female patients
undergoing cancer therapy experienced one or more of the
symptoms of ovarian failure [15, 75]. Whereas previous stu-
dies [15, 24, 76] suggested profound gonadal toxicity in men
after adjuvant chemotherapy in patients with or without
GnRH-a protection, for either malignant lymphoma [24] or
germ cell tumors [76], the situation in females may be
completely different. Ataya et al. [65] have shown, 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 signifi-
cantly 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) [65].

We have therefore administered a monthly depot i.m.
injection of GnRH-a [D-TRP6-GnRH-a (Decapeptyl C.R.),
3.75 mg (Ferring, Cologne, Germany)] to 60 young patients
with lymphoma, after they gave informed consent, starting
7–10 days before chemotherapy for up to 6 months, parallel
to chemotherapy [1, 66]. The study was approved by the institu-
tional Committee for Clinical Human Experimentation. Of the
58 evaluable patients (Table 1), only three (5%) developed
irreversible hypergonadotropic amenorrhea. All the others
(95%) resumed cyclic ovarian function, and 13 patients spon-
taneously conceived 18 times (Table 1). These patients were
compared with a control group of 60 patients of similar age
(14–40 years), who were similarly treated with chemotherapy
(with or without following radiotherapy) but without GnRH-a
adjuvant. These control group patients were either not referred
to early enough before starting chemotherapy (in most cases)
or were historical controls, treated in the few years before
starting our GnRH-a clinical protocol [66]. Neither the age nor
the ratio of HD to NHL differed between the two groups
(Table 1). Similar doses of radiotherapy exposure (Table 1) and
ratios of patients treated by radiotherapy in addition to
chemotherapy were experienced by the two groups. More-
ever, the cumulative doses of each chemotherapeutic agent
did not differ between the groups. The only significant dif-
ference was the rate of POF: three of 58 (5%) in the GnRH-a
co-treatment group versus 32 of 50 (55%) in the ‘control’
(chemotherapy without GnRH-a) group (P <0.01).

This preliminary experience is encouraging. Whereas most
of the survivors of the chemotherapy (with or without radio-
therapy) who received the GnRH-a co-treatment resumed
ovulatory menses (55 of 58, 95%), only 26 of the 58 women
(45%) who were treated with chemotherapy with or without
mante irradiation (control group) had normal ovarian func-
tion and more than half of these women (32 of 58) experienced

<table>
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<tr>
<th>Patients (total)</th>
<th>60</th>
<th>60</th>
<th>NS</th>
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<tr>
<td>Evaluable patients</td>
<td>58</td>
<td>58</td>
<td>NS</td>
</tr>
<tr>
<td>HD</td>
<td>36/60 (60%)</td>
<td>36/60 (60%)</td>
<td>NS</td>
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<td>NHL</td>
<td>24/60 (40%)</td>
<td>24/60 (40%)</td>
<td>NS</td>
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<td>Age (years)</td>
<td>14–40</td>
<td>14–40</td>
<td>NS</td>
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<tr>
<td>Radiotherapy</td>
<td>36/60 (60%)</td>
<td>35/60 (58%)</td>
<td>NS</td>
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<tr>
<td>Radiation dose (cGy) (mean ± standard deviation)</td>
<td>2320 ± 1521</td>
<td>1882 ± 1993</td>
<td>NS</td>
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<tr>
<td>Pregnancies</td>
<td>18 in 13 women</td>
<td>13 in 8 women</td>
<td>NS</td>
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<tr>
<td>Age of pregnant women at chemotherapy (years)</td>
<td>18–33</td>
<td>16–24</td>
<td>NS</td>
</tr>
<tr>
<td>Cyclic ovarian function</td>
<td>55/58 (95%)</td>
<td>26/58 (45%)</td>
<td>&lt;0.01</td>
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<tr>
<td>POF</td>
<td>3/58 (5%)</td>
<td>32/58 (55%)</td>
<td>&lt;0.01</td>
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HD, Hodgkin’s disease; NHL, non-Hodgkin’s lymphoma; POF, premature ovarian failure; NS, not significant.

Table 1. Comparison of clinical data and the rate of POF in the two groups of young women undergoing chemotherapy with or without GnRH-a co-treatment
premature ovarian failure (POF) and hypergonadotropic amenorrhea [1,66]. Buserelin, another GnRH-agonistic analog, administered by others [61] in a small group of young male patients in parallel to chemotherapy, with or without irradiation, failed to protect from azoospermia associated with chemotherapy. As opposed to young girls, most prepubertal boys receiving chemotherapy and radiotherapy suffered azoospermia; therefore, there is little rationale to expect a significant benefit from the GnRH-a co-treatment in men [66, 72–74, 77]. In keeping with this hypothesis, Johnson et al. [24] concluded that no improvement in post-treatment fertility could be demonstrated by GnRH-a co-treatment in six men receiving chemotherapy for advanced lymphomas. Contrary to the apparent protective effect of GnRH-a co-treatment with chemotherapy, no protection from ovarian damage caused by irradiation to rats could be provided by the GnRH agonist [78].

Neither the age, nor the dosages of the various cytotoxic drugs were significantly different between the study and control groups [1, 66, 70]. The only significant difference between the two groups was the incidence of POF and hypergonadotropic amenorrhea (55% versus 5%, P < 0.01; Table 1).

Similar experience and results regarding the protective effect of GnRH-a agonist chemotherapy associated gonadotoxicity was recently reported by Pereyra-Pacheco et al. [79] in adolescent females. Whereas all their GnRH-a-treated patients resumed cyclic ovarian function, all the patients in the chemotherapy alone (without GnRH-a) group experienced hypergonadotropic amenorrhea in spite of their young age [79]. Eighteen pregnancies occurred spontaneously in 13 patients in the GnRH-a group, in our series, compared with 13 pregnancies in eight patients in the chemotherapy alone (control) group. Whereas these numbers are not significantly different, it should be emphasized that whereas all the pregnancies in the control group occurred in patients who were at an age of 16–24 years when exposed to chemotherapy, the ages of the 13 patients who conceived in the GnRH-a/chemotherapy co-treatment were 18–33 years at exposure to chemotherapy (Table 1). This difference may suggest a possible prolongation of the ‘fertility window’ by almost 10 years by the GnRH-a adjuvant treatment in parallel to chemotherapy to young HD women.

Notwithstanding all the above, two recent studies in the New England Journal of Medicine [80, 81] reported that observational studies give results similar to randomized controlled trials. They found ‘little evidence that estimates of treatment effects in observational studies reported after 1984 are either consistently larger than or quantitatively different from those obtained in randomized, controlled trials’ [80]. Moreover, ‘the results of well-designed observational studies, do not systematically overestimate the magnitude of the effects of treatment as compared with those in randomized, controlled trials on the same topics’ [81].

Few of our young patients, two in the study group, and several in the control group, who later underwent high-dose chemotherapy and autologous bone marrow transplantation due to recurrence of the disease, have turned prematurely menopausal. This is in line with recent experience that such intensification regimens carry a high risk of permanent infertility and POF [16, 26, 66, 82–87]. It has been well established that chemotherapy with total body irradiation followed by allogeneic or autologous bone marrow transplantation causes permanent elevation of gonadotropin levels, hypoestrogenism and amenorrhea in 92–100% of female patients [1, 16, 66, 82, 84]. Future endeavors will obviously need to challenge the long-term infertility problem of young women treated with chemotherapy.

Future endeavors may also use GnRH antagonists instead of agonists for achievement of a faster pituitary-ovarian desensitization, eliminating the waiting period of 7–14 days needed by the GnRH-a to achieve downregulation [1, 66, 67, 88].

**Future endeavors**

If the protective effect observed in our preliminary study of GnRH-a and chemotherapy on future ovarian function is confirmed in larger and prospective randomized studies, it may become mandatory to use this co-treatment protocol in every woman undergoing chemotherapy. Thus, ovarian protection may enable the preservation of future fertility in survivors and prevent the bone demineralization and osteoporosis associated with hypoestrogenism and ovarian failure [1, 2, 6, 66, 69, 88].

This GnRH-a co-treatment may be also applied to young women receiving cytotoxic chemotherapy for non-malignant, benign diseases. Since almost one-quarter of young women with systemic lupus erythematosus at reproductive age may develop POF after cyclophosphamide pulse therapy [1, 6, 89, 90], GnRH-a co-treatment may be offered to these young women in parallel to the cytotoxic treatment, as well as to any woman with an autoimmune disorder treated by gonadotoxic chemotherapy, mainly alkylating agents [1, 2, 90].

Until now, women undergoing aggressive cytotoxic therapy such as that experienced in bone marrow transplantation, could preserve their future fertility potential only by undergoing egg retrieval and IVF with embryo cryopreservation for future thawing and embryo transfer after several years of no evidence of the malignant disease [1, 39, 47]. However, this ART is only pertinent to female patients who have a partner (husband, or a partner who is the selected father of their future children). Owing to the high prevalence of lymphoma and leukemia at young ages, many of these young women may be single. Unfortunately, although the technology of sperm cryopreservation is widely utilized, the technology for cryopreservation of unfertilized ova is not yet successful. It may be possible to freeze unfertilized eggs, but upon thawing the fertilizability is very low, and it is therefore unpractical for clinical use at present. Although intense investigational efforts are being conducted in many medical centers [1, 38, 39, 47,
by follicular aspiration, controlled ovarian hyperstimulation by hMG/hCG, with or without GnRH-a co-treatment, needs to be experienced, as usually practiced in IVF programs [91]. This may further postpone the initiation of cytotoxic chemotherapy for another 2 weeks or more, and may be relatively contraindicated in breast cancer or other sex hormone-sensitive tumors. Owing to these shortcomings, the possibility of ART by IVF combined with embryo cryopreservation for future embryo transfer may not be applicable to all the young women with malignant diseases who were single, unmarried and not yet interested in conception [1, 79]. Therefore, for all these young patients, the GnRH-a co-treatment, and possibly in the future GnRH antagonists as well, parallel to gonadotoxic chemotherapy may offer an increased chance of preserving their unconsumed fertility potential. This practical option may be widely experienced clinically, until the technology of cryopreservation of immature prophase I or unfertilized, metaphase II oocytes is available for young women undergoing gonadotoxic chemotherapy for various clinical indications [38, 39, 47]. The use of GnRH antagonists instead of or in combination with GnRH agonists awaits future clinical testing.

Recently, several molecules have been identified that are required for chemotherapy-induced oocyte apoptosis [92–94]. While much of this work has relied on gene knockout mice, it has identified a small lipid antagonist of the pro-apoptotic second messenger ceramide, termed sphingosine-1-phosphate (S-1-P), as a potent protective molecule in vitro. Mouse oocytes that were exposed to doxorubicin did not degenerate in a pathological fashion, but initiated programmed cell death [95]; i.e. the unwanted destruction of oocytes by anticancer therapy occurs by the same mechanism that is clinically desirable in tumor cells. Therefore, controlled manipulation of the oocyte apoptosis programme emerged as a tantalizing prospect for therapeutic development to protect the germ line from anticancer treatments [96]. Indeed, 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, protects oocytes from doxorubicin [95, 97]. Unfortunately, comparable genetic technologies are not yet feasible in humans.

To circumvent this limitation, experiments in mice tested the possibility that a small-molecule inhibitor of a key step in the oocyte-death programme could be administered in vivo to protect the ovaries from radiotherapy-induced damage [94]. However, a crucial question was which step to target for optimal therapeutic outcome. Previous work had shown that suppression of caspase activity in models of Bax-driven apoptosis eventually activates a default pathway of cell death, which is more akin to primary necrosis, probably because the mitochondria are still ‘damaged’ by Bax in a caspase-independent manner [98]. As oocyte death that is induced by anticancer therapy is Bax dependent [95], caspase inhibition was ruled out as a possibility. From this, a ‘pre-Bax’ step in the oocyte-death programme was chosen: the pro-apoptopic molecule ceramide.

Many somatic cell types generate ceramide, by either synthetic or hydrolytic mechanisms, in response to stresses such as radiation and chemotherapy [99]. Ceramide can act at many points in the programmed cell death pathway, such as ‘capping’ of death receptors [100] and helping Bax to insert into mitochondrial membranes [101]. However, the actions of ceramide do not go unchecked. In hematopoietic cells, the ceramide metabolite S-1-P counteracts its pro-apoptotic effects (or those of the stresses that generate it [102, 103]). S-1-P also inhibits doxorubicin-induced oocyte death in vitro [94, 95]. Furthermore, oocytes that lack acid sphingomyelinase—a hydrolytic enzyme that generates ceramide [99]—are resistant to doxorubicin-induced apoptosis in vitro [94]. Therefore, S-1-P was tested as a small-molecule inhibitor of ceramide-promoted apoptosis in oocytes that are exposed to anticancer therapy in vivo.

In this study [92, 94], young adult female mice were given a single injection of S-1-P into the bursal cavity that surrounds each ovary. Then, 2 h later, they were given enough ionizing radiation to kill over three-quarters of the primordial oocyte reserve. Two weeks after irradiation, the ovaries were analyzed. No differences were observed between mice that had not been irradiated versus those that had been protected by S-1-P in vivo before irradiation. In contrast, irradiated females that had not been given S-1-P showed a pronounced loss of oocytes and a reduced embryonic developmental potential of the remaining oocytes [94]. With preliminary data from in vivo mounting trials supporting the conclusions that S-1-P preserves a normal level of fertility in female mice that are exposed to anticancer therapy [104], minimizing the gonadal toxicity of such treatments in female cancer patients might one day prove feasible.

Whether the GnRH-a adjuvant co-treatment positive effect is direct or possibly associated with an intraovarian increase in S-1-P or another similar protective molecule, is a question of tremendous scientific interest and clinical impact. It awaits further investigation.

Since most of the methods involving ovarian or egg cryopreservation are not yet clinically established and successful, one should be very careful in providing these young patients with all the information concerning the various modalities to minimize gonadal damage and to preserve ovarian activity and future fertility. Furthermore, combining the various modalities for a specific patient may increase the odds of preservation of future fertility. There is no contraindication to ovarian biopsy for cryopreservation combined with GnRH-a administration and follicular aspiration for IVF and embryo freezing where the patient has a spouse/partner. In cases where the chemotherapy has caused POF, as is frequently the case in bone marrow transplantation, the patient has cryopreserved primordial follicles and/or frozen embryos to fall back upon.
However, in cases where conventional chemotherapy regimens such as those commonly used for young lymphoma patients are applied, the GnRH-a co-treatment may preserve ovarian function without necessitating the usage of cryopreserved ova or embryos.

Of course, we hope that in the future many of the unanswered questions may receive appropriate scientific answers. Until then, let us be very cautious in supplying our patients with all of the possibly relevant information. Holding back part of the whole information may violate the ancient dictum ‘primum non nocere’.

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References


71. Ratcliffe MA, Lanham SA, Reld DM, Dawson AA. Bone mineral density (BMD) in patients with lymphoma: the effects of chemo-


