What lies behind chemotherapy-induced ovarian toxicity?

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Abstract

Seminal advances in anticancer therapy as well as supportive care strategies have led to improved survival rates, posing an emphasis on preserving an optimum quality of life after cancer treatment. This recognition has paved the way to an increasing research of long-term side effects, both clinical and preclinical and to an ongoing design of a supportive care system to evaluate and treat long-term adverse effects of anticancer treatments, including the impact on fertility. As with many adverse effects induced by anticancer treatments, the literature comprised mostly clinical data with regard to chemotherapy-induced gonadotoxicity, while understanding of the biological mechanism is lagging. The impact of anticancer treatments on female fertility depends on the women's age at the time of treatment, the chemotherapy protocol, the duration, and total cumulative dose administered. Several suggested mechanisms that underlie chemotherapy-induced gonadotoxicity have been described. This review illustrates the clinical evidence, as well as its supportive preclinical studies, while proceeding from the 'bedside to the bench work' and provides an insight to what lies behind chemotherapy-induced gonadotoxicity.

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Introduction

Novel approaches in early detection and effective management strategies have led to increased rates of cancer survivors throughout the past three decades. In 2008, ~1 435 000 people were diagnosed in the US with cancer, 4% (~57 500 cases) of them were under the age of 35. The most frequent malignancies in people under 40 are breast cancer, cervical cancer, sarcoma, non-Hodgkin's lymphoma, leukemia, and melanoma (Jemal *et al.* 2008). Out of an estimated 2.4 million breast cancer survivors in the US, 10% are of childbearing age. Seminal advances in anticancer therapy as well as supportive care strategies result in improved survival rates, thus yielding an ancillary focus on preservation of an optimum quality of life after cancer treatment. This recognition has paved the way to an increasing research of long-term side effects, both clinical and preclinical and to an ongoing design of a supportive care system to evaluate and treat long-term adverse effects of cancer treatments, including the impact on fertility.

As a long-term adverse outcome of anticancer treatments, the literature comprised mostly clinical data, while understanding of the biological mechanism is lagging. This review illustrates the clinical evidence as well as its supportive preclinical studies, while going from the 'bedside to the bench work' and providing an insight into what lies behind gonadotoxicity.

The impact of anticancer treatments on fertility: clinical evidence

Several studies have assessed reproductive success after cancer itself and following anticancer treatments. The Childhood Cancer Survivor Study (CCSS) found that female survivors and partners of male survivors were substantially less likely to have live births compared with their siblings. Recently published, the updated CCSS demonstrated that ~30% of male childhood cancer survivors had permanent infertility (Sklar *et al.* 2006). Men aged 15–44 years, who received either testicular radiation at a dose of more than 7.5 Gy, or who were treated with procarbazine or cyclophosphamide were less likely to achieve a pregnancy. Men diagnosed in early childhood were more likely to father a pregnancy than those diagnosed in adolescence (Green *et al.* 2010). A study published in 2008 found an increase in the use of assisted reproductive technologies (ART) with both male and female cancer patients, and a significant decrease in first-time parental probability in female patients compared with the general population (Magelssen *et al.* 2008). Two Scandinavian cohort studies compared ~25 000 childhood, adolescent, and young adult survivors with their siblings. These studies found that the relative probability of a cancer survivor having a child was reduced by about 50% for women and about 30–57% for men (Madanat *et al.* 2008, Schover 2008).

There are several obstacles to drawing clear conclusions regarding the gonadotoxic potential of various anticancer treatments. The effects of chemotherapy and radiation therapy on fertility depend on the patient's age, chemotherapeutic regimens, dose and duration, the size and location of the radiation field, type of cancer (mainly in male patients), and pretreatment fertility status of the patient. The major drawback in assessing the rate of infertility is the use of inaccurate parameters like amenorrhea as gonadal outcomes in most of the studies, as this is only a surrogate marker for infertility, while many women with regular menstrual cycles are not fertile.

Hormonal measurements to appraise the ovarian reserve have been evaluated in recent studies. FSH and estradiol (E_2) measured on day 3 of the menstrual cycle reflect the population of maturing follicles and are indirectly associated with ovarian reserve. Inhibin- β , secreted by the granulosa cells lining the follicles, is directly associated with the loss of oocytes; however, the assay is not very reliable. Anti-Müllerian hormone (AMH), secreted by the granulosa cells of follicles, varies relatively little through the menstrual cycle and can thus be measured at any time. AMH levels appear to be more sensitive predictors of the ovarian reserve loss; they become abnormal earlier than follicular FSH (Lobo 2005). Ultrasound imaging of the ovaries early in the menstrual cycle (days 2–4) with a count of antral follicles (measuring 2–10 mm in both ovaries combined) is also a reliable measurement of ovarian reserve. Nevertheless, large, prospective studies are warranted to confirm that these are indeed reliable markers for evaluating chemotherapy-induced ovarian failure.

The impact of anticancer treatments on female fertility

The impact of anticancer treatments on female fertility depends on the women's age at the time of treatment, the chemotherapy protocol, the duration, and total cumulative dose administered.

Age

The high susceptibility of the ovaries to chemotherapy-induced toxicity derives from the physiological decline in the number of oocytes from birth to menopause. Approximately, 90% of oocytes within the female ovary will undergo physiological apoptosis during the fetal or postnatal life (Littley *et al.* 1989, Johnson *et al.* 2004). The cellular machinery of the follicles has an inherent high susceptibility to the apoptosis exerted by many chemotherapeutic agents. Chemotherapy-induced ovarian failure is age-dependent, with older age being associated with a greater loss of ovarian reserve. This is due to strong negative correlation between age and the

nongrowing follicle pool, representing ovarian reserve (Anderson & Cameron 2007, Kelsey & Wallace 2010). Assessing ovarian reserve in young patients may be challenging due to the rise in AMH level before the peak at around age 25 years. Nevertheless, a recent study performed on prepubertal and postpubertal girls treated with chemotherapy indicated that AMH may be used as a clinically useful marker of damage to the ovarian reserve (Brougham *et al.* 2012).

Type of treatment

Anticancer treatments can result in subfertility or infertility due to its impacts on the hypothalamic–pituitary–gonadal axis, causing a hypogonadic state in which the ovary is not adequately stimulated, or due to direct gonadotoxic damage to the germ cells. Cranial irradiation >35–40 Gy can impair hypothalamic pituitary function, resulting in hypogonadism through GnRH or FSH/LH deficiency (Gonfloni *et al.* 2009). Radiation to the pelvis may induce uterine fibrosis, which may complicate future pregnancies. There is no evidence for chemotherapy-induced uterine toxicity (Critchley & Wallace 2005).

Chemotherapy

Alkylating agents are overall more toxic to the ovary than other chemotherapy classes, although in preclinical studies, anthracyclines and platinum compounds have also been shown to be toxic to germ cells (Bines *et al.* 1996, Ben-Aharon *et al.* 2010). Several clinical studies have demonstrated that chemotherapy-related amenorrhea (CRA) rates varied from 30 to 76%, depending on the average age of the cohort and the chemotherapeutic protocol used (Goodwin *et al.* 1999, Burstein & Winer 2000, Stone *et al.* 2000, Parulekar *et al.* 2005, Hart 2008, Abusief *et al.* 2010). CRA occurs in over 90% of patients treated with high-dose chemotherapies, induction therapies before bone marrow transplantation, or total body irradiation (TBI). Virtually all women undergoing induction chemotherapy and TBI before transplantation are irreversibly sterilized (Lobo 2005). There is very limited data regarding infertility risk for patients undergoing newer chemotherapy regimens or targeted biological agents.

Mechanisms of chemotherapy-induced ovarian toxicity

It has been formerly implied that the pathogenesis of chemotherapy-induced ovarian toxicity involves loss of ovarian reserve, and hence several studies have associated it with the mechanism of primary ovarian failure where the loss of ovarian reserve is accelerated (Dnistrian *et al.* 1983, Dowsett & Richner 1991).

A recent study implied that the ovarian response of cancer patients who undergo controlled ovarian hyperstimulation before chemotherapy is diminished even before oncological treatment (Domingo *et al.* 2012). Several suggested mechanisms that underlie chemotherapy-induced gonadotoxicity have been described and are depicted in Fig. 1.

Direct ovarian toxicity

Several preclinical studies described the pattern of ovarian impairment, mostly following exposure to alkylating agents. Histological observations revealed apoptosis of primordial follicles (PMF) and primarily apoptosis of pregranulosa cells (Nicosia *et al.* 1985, Perez *et al.* 1997, Meiorow 1999, 2000, Meiorow *et al.* 1999). The observed reduction in the population of PMF was dose-dependent (Meiorow 1999, Meiorow *et al.* 1999). Dividing granulosa cells that populate antral follicles are mostly affected by chemotherapies, while the effect on dormant PMF is variable. The rapid and temporary loss of menses in many patients implies this toxic effect on the mature follicles. Direct effect on oocytes has been observed as some chemotherapies cross the blood–follicle barrier and reach the oocytes enclosed within the follicles (Perez *et al.* 1999, Bar-Joseph *et al.* 2010). Lately, an alternative mechanism has been suggested to explain the variable loss of follicular reserves, the ‘burn-out’ theory on which the destruction of the growing follicles following chemotherapy results in a decrease in cell-derived paracrine growth factors that inhibit PMF

recruitment, such as AMH. This may cause an enhanced recruitment of dormant follicles into the pool of actively growing follicles, resulting in a decrease of PMF pool (Meiorow *et al.* 2010).

Ovarian toxicity mediated by an acute vascular insult

Several studies have documented signs of fibrosis in the cortical stroma, and changes in the capillaries were observed in ovaries exposed to chemotherapy (Marcello *et al.* 1999, Meiorow *et al.* 2007, Oktem & Oktay 2007). Using innovative real-time molecular imaging, we observed an acute reduction in ovarian blood flow and disintegration of the vessel wall following *in vivo* administration of doxorubicin. This phenomenon was doxorubicin-unique and was not evident following exposure to other classes of chemotherapies (Ben-Aharon *et al.* 2010, Bar-Joseph *et al.* 2011).

Oxidative stress

There is limited data from mouse models regarding the initiation of oxidative stress following chemotherapy and irradiation. Thus, it has been shown that antioxidant enzymes may play important roles in follicular survival. Oxidative stress has been associated with cyclophosphamide toxicity in granulosa cells of mature follicles (Chang *et al.* 1993, Dirven *et al.* 1994). The active metabolite of cyclophosphamide, 4-hydroxycyclophosphamide (4HC), is formed following oxidation by cytochrome P450 enzymes.

Figure 1 Suggested mechanisms for ovarian toxicity. Different classes of chemotherapies exert ovarian toxicity via distinct organ or cellular cascades of events: 1) direct ovarian toxicity or ‘ovotoxicity’ has been described primarily for alkylating agents that may directly deplete follicular pool. 2) Vascular toxicity may mediate end-organ (i.e. ovarian) damage and has been illustrated in anthracycline-treated mice by live high-resolution imaging. 3) Direct cellular effects on various components of the ovary have been shown for several classes of chemotherapies that differ on their specific cellular targets. Bold titles represent the well-established evidence for chemotherapy-induced toxicity.

4HC spontaneously converts into the reactive metabolite phosphoramidate mustard. Treatment with cyclophosphamide results in a depletion of glutathione, a crucial cellular antioxidant, and a rise in reactive oxygen species that mediates apoptosis in granulosa cells (Tsai-Turton *et al.* 2007, Devine *et al.* 2012).

Gonadotoxicity by class of chemotherapy

Alkylating agents

Cyclophosphamide is an alkylating agent that serves as a backbone in many chemotherapeutic regimens and is considered a prototype for gonadotoxic chemotherapy. Cyclophosphamide is not cell cycle-specific, and hence may affect cells that are not actively dividing, such as oocytes or PMF. It has been shown to induce temporary or permanent amenorrhea, and may cause reduced fertility or infertility. Age confers a major determinant for the risk of amenorrhea due to cyclophosphamide-containing protocols, while rates rise above the age of 35 years and reach >80% for women over 40 years old (Parulekar *et al.* 2005). A recent study provided a long follow up of Hodgkin's lymphoma patients and indicated an increase in risk of premature ovarian failure of 23% per year of age at treatment (hazard ratio, 1.23), corrected with dose of alkylating chemotherapy administered (van der Kaaij *et al.* 2012).

Destruction of follicles at all stages of development in a dose-dependent manner has been reported in pre-clinical studies and in human ovarian tissue as well. In mice, a minimal exposure to cyclophosphamide results in a significant annihilation of PMF (Meirow 1999, Meirow *et al.* 1999). Using a novel xenografting model to characterize the impact of chemotherapy on human PMF reserve, a single injection of cyclophosphamide resulted in a drastic reduction in PMF density (Reh *et al.* 2008). This cyclophosphamide-induced toxicity was documented in other studies as well (Jarrell *et al.* 1987, Plowchalk & Mattison 1991, Ataya *et al.* 1995, Meirow 1999, Meirow *et al.* 1999). In a study that evaluated the reproductive performance and teratogenicity of cyclophosphamide in mice, it has been suggested that the effect of cyclophosphamide on the gametes is influenced by the stage of oocyte maturation at the time of exposure to the agent. Early post-chemotherapy fertilization results in higher rates of pregnancy failure and malformations.

Anthracyclines

Anthracyclines are widely integrated in a variety of anticancer regimens. Doxorubicin represents a key player of the anthracyclines; it accumulates in both nucleus and mitochondria, initiates oxidative stress, and induces chromosomal obliteration by inhibiting topoisomerase-II. In clinical and cohort studies, there is a

wide range of amenorrhea incidence following doxorubicin-based protocols that reflects a tight correlation between the age of the patient and a worsening effect of doxorubicin. While in women between 40 and 49 years of age, doxorubicin-containing regimens were associated with amenorrhea rate of 96% (Bines *et al.* 1996), in younger women treated without alkylating chemotherapy, cumulative amenorrhea rates ranged between <10% (Brusamolino *et al.* 2000, Zekri *et al.* 2008, van der Kaaij *et al.* 2012) and ~34% (Cobleigh *et al.* 1995, Elis *et al.* 2006). If menstruation returned after treatment, cumulative premature ovarian failure risk was independent of age at treatment.

However, preclinical studies illustrate a pronounced gonadotoxic effect to doxorubicin. It has been shown that doxorubicin induces apoptosis in mammalian oocytes and decreases their survival *in vitro* significantly (Perez *et al.* 1997, 1999, Takai *et al.* 2007, Ting & Petroff 2010). Most of the studies were performed on ovulated metaphase II (MII) oocytes. We demonstrated that doxorubicin crosses the blood-follicle barrier and directly affects the germinal vesicle (GV) oocytes that represent a more accurate physiological model of oocytes encountering chemotherapy. We observed different sensitivities of GV and MII oocytes to doxorubicin, manifested by different intensity of apoptotic responses, where GV oocytes are more vulnerable. Doxorubicin exerts its toxic effect in oocytes via the mitochondria, concomitant with its known effect on the chromosomes, induction of endoplasmic reticulum (ER)-stress, and a possible increase in intracellular Ca^{2+} , leading to apoptosis, as illustrated in Fig. 2 (Bar-Joseph *et al.* 2010). This cascade had been formerly introduced in cardiomyocytes (Jang *et al.* 2004). Our former results indicated an acute doxorubicin-induced ovarian toxicity, manifested by a dramatic reduction in ovulation rate that was partially recovered at later phases and a significant reduction in the population of secondary and PMF 1 month following treatment, as seen in histological sections.

We have established an innovative *in vivo* real-time molecular imaging platform to track the effect of chemotherapies on the gonads. *In vivo* magnetic resonance imaging (MRI) depicted a decrease in ovarian size and a marked periovarian edema (Ben-Aharon *et al.* 2010). We used microscopic ultrasound using micro-bubbles as a contrast agent to visualize the acute effect of doxorubicin on the ovarian vasculature and revealed a 33% decrease in ovarian blood volume already 3 min after doxorubicin injection. Visualization of ovarian microvasculature was obtained by fluorescence optical imaging system, equipped with a confocal fiber microscope. It depicted a pattern of an acute vessels injury, where the wall of the blood vessels became irregular and the fluorescence signal displayed in the small vessels was gradually diminished (Bar-Joseph *et al.* 2011). Based on other *in vitro* and *in vivo* studies that imply the toxicity of doxorubicin to the endothelium, the

Figure 2 Proposed cellular signal transduction for doxorubicin-induced toxicity within mammalian oocytes. Suggested pathway of doxorubicin-induced apoptosis within oocytes involves chromosomal disintegration, activation of the mitochondria, and elevation of superoxides, followed by ER-stress induction. This cascade exerts activation of PERK and caspase-12, a possible increase in intracellular calcium reflected by calpain involvement, and inactivation of PARP. The proposed doxorubicin-induced apoptotic signal transduction observed in oocytes resembles the one studied in cardiomyocytes.

acute reduction in gonadal and femoral blood flow and the impairment of the blood vessels wall may mediate the toxic effect doxorubicin exerts on the ovary.

Platinum compounds

Members of the platinum-based compounds family are a cornerstone of the current antineoplastic treatments in various cancers and are specific inducers of various types of chromosomal damage and DNA cross-links (Blommaert & Saris 1995). Cisplatin is the prototype and mostly investigated family member. Evidence for platinum-induced ovarian toxicity has been published only with regard to cisplatin. Clinical data is very limited, showing mild to moderate rates of amenorrhea following cisplatin-based treatments (Maneschi *et al.* 1994, Meiorow 2000, Nozaki *et al.* 2009). The pattern of ovarian injury was studied in an *in vitro* human tissue model that depicted histological and immunohistochemical changes that accompany PMF destruction: pregranulosa cell swelling with marked accumulation of cytokeratin, pregranulosa cell nuclear swelling, PMF architecture disruption with disappearance of the lumen, and its oocyte (Meiorow 2000, Gonfloni *et al.* 2009). Few studies that appraised cisplatin toxicity in various tissues

indicated that the cell cycle phase is a crucial parameter for cisplatin-induced damage, as DNA replication is necessary for the expression of cisplatin effect (Higdon *et al.* 1992). It has been shown that cisplatin induces a reduction in ovulation rate in rats and a decline in the levels of AMH and inhibin- α (Higdon *et al.* 1992, Yeh *et al.* 2006, 2008). It has been implied that cisplatin-induced ovarian toxicity may derive from a decline in the level of hyperpolarization-activated cation channels in the ovary. These channels are widely distributed in the ovary and may mediate the effect on oocytes, granulosa cells, and theca cells and contribute to decreased ovarian function (Yeh *et al.* 2009). The well-documented cisplatin-induced nephrotoxicity also involves differential changes in membrane channels in the kidney (Kishore *et al.* 2000). Another proposed mechanism for cisplatin-induced ovarian toxicity is that cisplatin leads to the accumulation or activation of p63, a member of the p53 family, and eventually to oocyte death. It was postulated that the DNA damage caused by cisplatin has been shown to activate the c-Abl tyrosine kinase as cisplatin treatment induced a 1.5-fold increase in c-Abl mRNA level, as well as c-Abl nuclear accumulation and cleavage. The putative role of c-Abl in the pathogenesis of cisplatin-induced damage rationalized the targeting of

c-Abl activity with imatinib (see also below), which led to a significant rescue of primary and PMF in ovaries treated simultaneously with cisplatin and imatinib (Gonfloni *et al.* 2009).

Taxanes

Taxanes are widely used in various malignancies and have become a pivotal cornerstone in the adjuvant treatment of breast cancer, replacing in specific instances the alkylating-based regimens. Paclitaxel and docetaxel act on the cytoskeleton: they stabilize microtubules and disrupt normal polymerization/depolymerization, leading to an arrest of the cells at the G2–M phase of the cell cycle. The evidence for the potential gonadotoxicity taxanes may confer limited and inconsistent. Few clinical studies have found no additional increase in amenorrhea rates in women treated by taxane-containing regimens, or a mild increase in a reversible amenorrhea (Davis *et al.* 2005, Berliere *et al.* 2008, Reh *et al.* 2008, Abusief *et al.* 2010, Pérez-Fidalgo *et al.* 2010). Nevertheless, several prospective studies have shown that the incidence of amenorrhea in taxane-based chemotherapy regimens was higher than in anthracycline-based chemotherapy regimens (Fornier *et al.* 2005, Oktay *et al.* 2005, Han *et al.* 2009). Women older than 40 years had a greater risk of amenorrhea, with a greater likelihood of irreversibility, than younger women (Petrek *et al.* 2006, Tham *et al.* 2007).

In rats, paclitaxel treatment resulted in a decreased number of PMF compared with control (Yucebilgin *et al.* 2004), indicating that paclitaxel is gonadotoxic. It was also documented that rats exposed to high-dose paclitaxel exhibited loss of fertility, manifested by blocked ovulation, although in a later follow up there was not a significant difference in the number of fetuses, implantation sites, and resorption, suggesting that paclitaxel effect on the ovary may be reversible (Kai *et al.* 1994). Other studies demonstrated that paclitaxel induces apoptosis mainly in mature follicles and to a lesser extent in a less mature population; accordingly, E₂ production was affected and decreased serum levels were observed (Tarumi *et al.* 2009). A clue for the nontoxic effect of paclitaxel on oocytes was provided while treatment with paclitaxel on *in vitro* matured porcine oocytes had significantly positive effects on morphology, distribution, and ultrastructure of mitochondria and lipid droplets in the oocytes (Fu *et al.* 2009). In contrary to doxorubicin, the mechanism of potential paclitaxel-induced ovarian toxicity is not mediated by acute vascular injury (Bar-Joseph *et al.* 2011).

Vinca alkaloids

No increased risk of ovarian failure was detected in patients treated with plant alkaloids (Meirow 2000,

Zhou *et al.* 2010). Nevertheless, vincristine has been reported as an aneuploidy inducer (Meirow 1999, Meirow *et al.* 1999).

Antimetabolites

Fluoropyrimidines are considered as the backbone of adjuvant treatment for colorectal cancer. They act primarily on cells that are actively synthesizing DNA (S-phase of the cell cycle). The widely used agents are 5-fluorouracil (5-FU) and capecitabine, an oral fluoropyrimidine, which has been shown to confer survival rates similar to 5-FU. The clinical data regarding the impact of fluoropyrimidines on fertility are limited mainly due to the older age of patients and paucity of premenopausal population in the trials. Standard 5-FU-based chemotherapy is considered to have minimal effects on female fertility. There are few reports of premature ovarian failure following 5-FU adjuvant therapy (Azem *et al.* 2004, Twelves *et al.* 2005). The antimetabolites methotrexate and 5-FU in the cyclophosphamide, methotrexate, 5-FU (CMF) regimen have not been associated with an increased rate of amenorrhea. While the amenorrhea rate of methotrexate and 5-FU in the adjuvant setting reached 9%, the rate in the standard CMF regimens, using oral cyclophosphamide, was 69% (Bines *et al.* 1996).

A study performed on a mouse model shed light on a possible correlation between a potential toxic effect of 5-FU to ovarian function and a circadian timing of administration of the agent. When 5-FU was administered during the estrous phase (immediate postovulatory) of the fertility cycle, female mice suffered a greater subsequent loss of fertility, determined by a decreased successful pregnancy rate, than mice administered with 5-FU during the metestrous, diestrous, or proestrous stages (Hrushesky *et al.* 1999).

Biological agents

Imatinib

Imatinib mesylate is a highly effective, targeted agent used widely to treat Philadelphia-positive leukemia and acts through selective inhibition of tyrosine kinases in other selected cancers. The treatment may last for a long time, offering a prolonged remission. Although mammalian ovaries express several kinases as c-kit, c-abl, and platelet-derived growth factor receptor that are inhibited by imatinib (Hutt *et al.* 2006), its impact on fertility capacity is not clear. The clinical evidence for a potential gonadotoxic effect is conflicting and is presented as case reports and not as a prospective trial (Hensley & Ford 2003, Christopoulos *et al.* 2008, Malozowski *et al.* 2008, Zamah *et al.* 2011). Recent study on leukemic mouse model depicted that imatinib mesylate at therapeutic doses showed no effects on either folliculogenesis or

spermatogenesis, suggesting that the agent does not reduce fertility (Schultheis *et al.* 2012). Furthermore, it has been suggested that imatinib may exhibit a protective effect on chemotherapy-induced gonadotoxicity. As described earlier, cisplatin rapidly promotes the accumulation of TAp63, a p53 homolog, and ultimately causes cell death. TAp63 expression correlates with oocytes radio sensitivity and is essential for their ensuing DNA damage-induced death within the PMF (Suh *et al.* 2006). Treatment with imatinib offsets these cisplatin-induced effects and further impairs cisplatin-induced depletion of the follicle reserve, suggesting further research of imatinib as a potential protectant of chemotherapy-induced gonadotoxicity (Gonfloni *et al.* 2009). Nevertheless, imatinib should be avoided before or during pregnancy, due to a higher risk of congenital malformations. The potential impact of other tyrosine kinase inhibitors (TKIs) has not been studied yet; few case reports suggest TKIs do not affect fertility (Conchon *et al.* 2010, Oweini *et al.* 2011).

Trastuzumab

Trastuzumab, a MAB targeting the extracellular domain of the HER2 protein, administered in combination with chemotherapy followed by an extended period of trastuzumab only in the adjuvant setting, improves survival in women with either early or metastatic breast cancer. There is no valid data regarding the impact of trastuzumab on fertility. Unpublished data of a toxicological study conducted in cynomolgus monkeys revealed no evidence of impaired fertility following exposure to high-dose trastuzumab (Genentech 2010: U.S. BL 4851301 Supplement: Trastuzumab Genentech, Inc.). In a clinical study on breast cancer patients, the addition of trastuzumab to chemotherapy did not affect amenorrhea rates (Abusief *et al.* 2010). Exposure to trastuzumab during pregnancy can result in oligohydramnios and oligohydramnios sequence manifesting as pulmonary hypoplasia, skeletal abnormalities, and neonatal death (Witzel *et al.* 2008).

AntiVEGF therapy

Bevacizumab. No published studies have addressed the impact of bevacizumab on fertility. A clinical report of two patients who were treated with bevacizumab due to choroidal melanoma described a transient amenorrhea following bevacizumab treatment (Newman *et al.* 2011). In 2011, the FDA added a warning label to bevacizumab with regard to ovarian function. Results published by Genentech, the developing company, demonstrate that the incidence of ovarian failure was higher in premenopausal women receiving bevacizumab in combination with adjuvant chemotherapy, compared with those receiving chemotherapy alone for adjuvant treatment for colorectal cancer (34 vs 2% respectively).

The use of bevacizumab in the adjuvant setting has not been approved (Genentech 2011: U.S. BL 125085 Supplement: Bevacizumab Genentech, Inc.).

Tamoxifen

Tamoxifen, a selective estrogen receptor modulator, has been widely used in hormone responsive breast cancer. It shares a multifaceted mechanism of tissue-specific activation or inhibition of estrogen signaling. The clinical data with regard to the effect of tamoxifen on fertility are biased with its subsequent use following chemotherapy in most of the studies. Nevertheless, newer technologies of genetic profiling enable the selection of patients with hormone responsive breast cancer that are being treated with endocrine treatment only as a concept of 'tailored' therapy; future studies will therefore reveal the sole effect of tamoxifen on fertility. The pre-clinical data are not consistent: few studies have implied that the addition of tamoxifen to cyclophosphamide resulted in decreased follicle loss and improved reproductive function in rats (Kim *et al.* 2010, Ting & Petroff 2010). Another toxicological study on rats that employed a higher dose of tamoxifen showed a disturbance of estrus cycle and a decrease in the number of pregnant rats that were considered to be related to ovarian histopathological changes (Tsujioka *et al.* 2009). Two clinical studies indicate that tamoxifen may affect the follicular pool, resulting in a delayed fall in AMH in a group of patients receiving tamoxifen and goserelin. AMH level continued to further decline, more than the expected physiological decline caused by age alone (Zhou *et al.* 2010, Anderson & Cameron 2011). In another follow-up study of menstrual functioning in premenopausal women with invasive breast cancer who received adjuvant chemotherapy with or without tamoxifen, patients receiving tamoxifen therapy were twice as likely to remain amenorrheic compared with those not receiving tamoxifen. However, this association was not statistically significant among women younger than 40 years of age at diagnosis (Abusief *et al.* 2010). There was no difference in AMH level between tamoxifen users and no-users, suggesting that only growing follicles were influenced by tamoxifen (Rosendahl *et al.* 2008). Tamoxifen is contraindicated during pregnancy due to the reports of craniofacial abnormalities associated with tamoxifen use in the first trimester.

Implications for research and practice

In order to provide young patients with reliable information about the gonadotoxic potential of their anticancer treatment, a better and accurate knowledge regarding the effect of classes of chemotherapy and biological agents is required, as the current data are limited. The available evidence of chemotherapy-induced ovarian toxicity displays several limitations:

the use of nonuniform definitions of assessing the ovarian reserve, different combinations of drugs in the various studies, small sample size, the heterogeneity of the cohort (mainly wide age range and lack of exclusion of patients with other possible causes of subfertility), and the short follow up of the majority of the studies. Most of the included studies employed resumption of menstrual cycle as an indicator for preserved ovarian function. Numerous studies have demonstrated that menstruating is not an accurate marker and does not reflect reproductive capacity, particularly in women with breast cancer who continue with hormonal therapy (such as tamoxifen) after the cessation of chemotherapy. Other hormonal measurements include: FSH, inhibin- β , AMH, and antral follicle count. In recent studies, AMH shows promise as a reliable hormonal biomarker of reduced ovarian reserve in women who have been previously treated with anticancer treatments. The CCSS represents a well-characterized cohort study that appraises detailed information on reproductive parameters in a large cohort and hence enables to determine the specific long-term effect on fertility, manifested in pregnancies and the occurrence of premature ovarian failure. The fact that childhood cancer survivors who continued to have spontaneous menses more than 5 years after their cancer diagnosis had a risk of developing premature menopause that was 13-fold higher than that in siblings, with a cumulative incidence of 8% by age 40 years, along with the significant increased use of ART by childhood cancer survivors (Sklar *et al.* 2006), indicates that the resumption of menses may in some cases be insufficient to predict the future fertility state and that the actual premature ovarian failure rates may be higher than the reported incidence of amenorrhea.

Notably, there is no evidence that the prepubertal ovary is protected from the effects of chemotherapy (Kelsey & Wallace 2010). However, young patients, due to their larger PMF pool, may have a window of opportunity for fertility before the onset of a premature menopause.

A prospective registry of reproductive outcomes for a long-term follow-up while documenting menstruation, pregnancy rates, and the use of ART along with biomarkers evaluation would enable a better characterization of the actual effect of anticancer treatments on ovarian function. Future preclinical research on specific mechanisms of gonadotoxicity of older and newer drugs would unravel what lies behind gonadotoxicity and may enable the development of novel strategies to preserve fertility.

As the rate of young cancer survivors has grown, fertility issues have become more relevant. Providing cancer patients with timely information related to the potential gonadotoxicity and options for fertility preservation are imperative. Referring the patients to a reproductive specialist may partially alleviate the emotional burden that accompanies the commencement

of anticancer treatment and later on facilitates the transition from a cancer patient to a cancer survivor.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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