# Fertility Preservation in Women With Malignancy: Future Endeavors

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**ABSTRACT:** The area of fertility preservation is constantly developing. To date, the only noninvestigational and unequivocally accepted methods for fertility preservation are cryopreservation of embryos and unfertilized oocytes. This article is one of several in a monogram on fertility preservation. The debate, pros and cons, and equivocal data on the use of GnRH analogues for fertility preservation are elaborated by 3 other manuscripts, in this monogram. A repeat of the arguments, pros and cons of this debatable issue, would be a repetition and redundancy of what is already included in this monogram. The subject of ovarian cryopreservation for fertility preservation is also elaborated by several other authors in this monogram. It is possible that, in the not too far future, the technologies of in vitro maturation of primordial follicles to metaphase 2 oocytes, and the "artificial ovary," will turn clinically available. These technologies may bypass the risk of resuming malignancy by autotransplantation of cryopreserved-thawed ovarian tissue in leukemia and diseases where malignant cells may persist in the cryopreserved ovarian tissue. We summarize here the suggested options for future endeavors in fertility preservation.

**KEYWORDS:** Fertility preservation, GnRH analogues, chemotherapy, premature ovarian failure (POF), premature ovarian insufficiency (POI)

## GnRH Antagonists

There is no unequivocal evidence that antagonist pretreatment is beneficial in terms of ovarian protection. The GnRH antagonists suppress gonadotropin levels immediately, whereas the GnRH agonists (GnRHa) administration needs a waiting period of 7 to 14 days to achieve pituitary desensitization and hypogonadotropic milieu.[1] Therefore, the antagonists may theoretically offer clinical benefits over GnRHa, whereas the antagonists do not cause the "flare-up effect," as they directly compete with GnRH for binding to its receptors. Whereas growing follicles are believed to be more susceptible to the cytotoxic effects of chemotherapy than resting, nonactive, primordial follicles (PMFs), it has been hypothesized that chemotherapy should be started after the "flare-up effect,"[1] although no robust data exist on comparison of exact dating of chemotherapy in relation to GnRHa administration. Thus, the antagonists can be administered immediately before chemotherapy to induce the targeted hypogonadotropic milieu and cause quick reversal of the gonadotropin suppression on their withdrawal.

On the other hand, as chemotherapy often is administered for several months, the long-term administration of daily antagonist may be cost-prohibitive and may not offer clinically significant advantages.

In mice, administration of a GnRH antagonist before 2 doses of cyclophosphamide resulted in significantly higher numbers of PMF.[2] In the lower dose (50 mg/kg) cyclophosphamide group, only 14% of the PMF were lost (antagonist group) compared with 53% (without antagonist) ($P < .001$), whereas in the higher cyclophosphamide insult (75 mg/kg), only 35% of the PMFs were destroyed (with antagonist) versus 54% in the control mice (without antagonist) ($P < .004$).[2] This study suggested that the extent of protection achieved by the antagonist is dose-dependent decreasing with escalating cyclophosphamide doses.[2] In contrast, another study[3] concluded that contrary to the "well-known effects of GnRHa to reduce chemotherapeutic destruction of PMFs, GnRH antagonists did not protect the ovary from the damaging effects of cyclophosphamide." In this study,[3] 2 different GnRH antagonists did not prevent the depletion of PMF caused by cyclophosphamide. More alarming, both tested antagonists, cetrorelix and antide, brought about a significant loss in the number of the PMFs, even without cyclophosphamide.[3] Therefore, it may be possibly unsafe to use GnRH antagonists as a substitute for the agonists for fertility preservation and for minimizing chemotherapy-associated gonadotoxicity.[3] Peng et al[4] published similar results and reached comparable conclusions on examining the effects of GnRH analogues on chemotherapy-induced gonadotoxicity in rats. In an attempt to investigate the effects of GnRHa or an antagonist on cyclophosphamide-induced gonadotoxicity in rats, they found that GnRHa significantly prevented the ovarian function damage induced by cyclophosphamide, but the GnRH antagonist did not exhibit a similar protective effect.[4] Gupta and Flaws[5] emphasized the necessity to determine whether GnRH antagonists had a similar effect in humans before definitively stating that the antagonists reduce PMFs and do not protect the ovaries from chemotherapy-induced gonadotoxicity.

In the clinical setting, Mardesic et al[6] combined GnRH antagonists with GnRHa to achieve a faster hypogonadotropic milieu in 6 young women with hematological malignancies.

They concluded that this combination successfully suppressed gonadotropin levels within 96 hours in all patients, allowing for almost immediate chemotherapy.[6] Later on, the same group[7] presented data from a larger group of patients similarly treated with a GnRH antagonist and agonist combination. All of these young patients resumed cyclic ovarian function, but those treated with aggressive conditioning chemotherapy before bone marrow transplantation (BMT) had premature ovarian failure (POF).[7]

More recently, Li et al[8] tested the antagonist-agonist combination in a rat model; concluding it could enhance the protective effect of the ovary from cisplatin-induced gonadotoxicity. Whereas cisplatin completely abolished normal cyclicity, it was preserved in 25.0%, 33.3%, 66.7%, and 41.7% of the rats in the cisplatin + GnRHa, antagonist in long-term and short-term combination groups, respectively.[8] It cannot be categorically stated yet whether the antagonists by themselves can reduce chemotherapy-induced gonadotoxicity.

Nevertheless, in July 2018, an orally active GnRH antagonist has been approved by the US Food and Drug Administration, for treating endometriosis-associated pain, for the first time.[9,10] The therapeutic potential of orally active antagonists is enormous, bypassing the inconvenience of the injectable agonists.[9,10] Besides possibly replacing the agonists for suppression of estrogen-dependent diseases, they may well provide an attractive alternative for fertility preservation, if, indeed, the antagonists will prove to be efficient like the agonists in decreasing the gonadotoxic insult of chemotherapy.

### Sphingosine-1-Phosphate/FTY720 (S1P Analogue, Fingolimod)

Obviously, prevention of chemotherapy-induced POF/POI (premature ovarian insufficiency) is preferable to current fertility preservation techniques of treating it after its occurrence. Sphingosine-1-phosphate (S1P) inhibits ceramide-associated apoptosis.[11,12] Its administration to mice in which human ovarian slices were xenografted resulted in improved angiogenesis and vascular density and decreased follicle apoptosis.[13] In vivo administration of S1P to mice in high doses completely preserved primordial and growing follicles despite irradiation.[11] Indeed, S1P pretreatment could significantly reduce irradiation-associated PMF loss not only in rats[14] but also in primates and in xenografted human ovarian slices.[15] Although this pretreatment seemed to protect against the gonadotoxic effect of radiotherapy and chemotherapy in most[11-17] but not all studies,[14] its clinical extrapolation is not straightforward. It is impossible to administer it systemically due to the risk of possibly interfering with the chemotherapy effect on malignant cells. Theoretically, local administration might reduce the possibility that S1P would interfere with the therapeutic effects of chemotherapy.[18,19] Furthermore, its plasma half-life is very short.[18,19] It is not clinically practical to infuse S1P into the ovaries continuously through indwelling catheters, as has been successfully done in adult female nonhuman primates.[15]

However, it is reassuring that continuous intraovarian S1P administration through indwelling catheters before radiation did not compromise the health and well-being of the generated neonates.[15] The clinical challenge is to develop a technology whereby S1P or its agonists may be directly delivered to the gonads, minimizing the gonadotoxic effect of chemotherapy and/or radiotherapy, without interfering with the therapeutic effect on destroying the malignant cells.

### AS101—Ammonium Trichloro (dioxoethylene-O,O′) Tellurate

The groups of Meirow and Sredni have investigated the tellurium-based AS101 compound which affects the phosphoinositide 3-kinase (PI3K)/phosphatase and tensin homolog/Akt/PKB signaling pathway.[18,20-28] This compound, AS101, is a nontoxic immunomodulator that may be administered orally as a chemoprotective and radioprotective agent and has been shown to reduce the gonadotoxic effect of alkylating agents such as cyclophosphamide in rodents.[18,20-28] The ability of oral administration and noninterference with the antineoplastic activity of alkylating agents in vivo or in vitro and its additive and synergistic anticancer activity with cyclophosphamide makes AS101 an ideal target for possible clinical studies.[18,20-28] The possibility for systemic administration makes it a more suitable target for clinical investigation as a fertility-preserving agent compared with S1P, as it does not prevent apoptosis of the malignant cells. Furthermore, it did not increase fetal malformations in mice pups, suggesting that the protected follicles from the gonadotoxic effects of chemotherapy and radiotherapy were genetically normal.[18,20-28] Unfortunately, although more than 2 decades have passed since the publication of these studies demonstrating the additive and synergistic anticancer activity of AS101 in rodents, the groups that demonstrated its unique qualities as a possible noninvasive inexpensive fertility preservation agent have not roused interest in clinical extrapolation experiments.[18,20-28] It is anticipated that others may rise the glove and examine its possible beneficial clinical effects.

### Anti-Müllerian Hormone

A possible attractive agent for fertility preservation is anti-Müllerian hormone (AMH), with its main advantage being that it is an endogenous hormone with ovarian specific and exclusive activity; therefore, theoretically, no or minimal side effects are anticipated.[18,20] In addition to its inhibitory effect on follicle-stimulating hormone (FSH) receptor activation, AMH inhibits follicle activation.[29-31] Its depletion leads to excessive activation and exhaustion of the PMF pool.[20,31] Experiments in AMH knockout mice and in vitro culture of ovarian tissue suggest that AMH has an inhibitory effect on PMFs' exit from their dormant pool and their recruitment for folliculogenesis.[20,30,31] Meirow's group has demonstrated that cyclophosphamide has significantly reduced AMH messenger RNA (mRNA) expression in mice, in vivo, and PMF loss by cyclophosphamide active metabolite, in vitro.[20,31]

Most recently, Sonigo et al[32] have demonstrated that AMH prevents primordial ovarian follicle loss and fertility alteration in cyclophosphamide-treated mice. The administration of recombinant AMH prevented cyclophosphamide-induced PMF loss.[33] Injection of AMH significantly decreased FOXO3A phosphorylation, a main actor of PMF activation, implying a protective role of AMH against chemotherapy-induced follicular loss.[32] Thus, concomitant AMH administration during chemotherapy might offer a new option for preserving young patients' fertility. Whether its administration for fertility preservation may be clinically extrapolated awaits further experimentation.

## Granulocyte Colony-Stimulating Factor

Granulocyte colony-stimulating factor (G-CSF) is a cytokine and hormone produced by several different tissues, which may significantly reduce the gonadotoxic effect of several chemotherapeutic agents such as busulfan, cyclophosphamide, and cisplatin and protect the PMF pool.[20,33,34-37] It may also reduce microvascular damage and decrease the markers of DNA damage in oocytes of small follicles.[20,34-37] Mice treated with G-CSF in parallel to gonadotoxic chemotherapy delivered increased litter numbers versus controls.[20] Whereas G-CSF may reduce microvascular damage, decreasing ischemia has been postulated as a possible protective mechanism, in addition to possible direct antiapoptotic effects.[20,36,37] Although G-CSF is a clinically accepted treatment for minimizing chemotherapy-induced neutropenia, its additional clinical testing for fertility preservation awaits clinical validation.[20]

## Imatinib

A tyrosine kinase inhibitor, imatinib (Gleevec), is a chemotherapeutic agent that inhibits the c-Abl apoptotic pathway.[20,33] The latter, c-Abl, is a kinase inhibitor that activates tumor protein p63 (TP63), also known as transformation-related protein 63.[20,38-40] The TP63 is encoded in humans by the TP63 gene, a transcription factor that promotes the apoptosis of PMFs induced by DNA damage.[20,33,38] C-Abl can switch TP63 transcription and the apoptosis induced by cisplatin[38] and doxorubicin.[38-45] It may also be important in maintaining genomic integrity by preventing DNA damage in mitotic and meiotic cells.[20,38-40,42] Whereas some investigators have found that imatinib may protect against the cisplatin-induced gonadotoxicity in mice,[20,38,39,43] others have challenged this protective effect.[20,44,45] Obviously, this controversy is not yet settled, and additional studies are awaited to determine the safety and efficacy of imatinib.

## Dexrazoxane (ICRF-187)

Dexrazoxane is a catalytic topoisomerase 2 (TOPII) inhibitor, an iron-chelating ethylenediaminetetraacetic acid derivative clinically used to diminish the toxic effects of doxorubicin on the heart and skin without diminishing the antineoplastic effect of chemotherapy.[46-48] Dexrazoxane disrupts doxorubicin-iron binding, mitigating the oxidative stress and catalytically inhibiting TOPII, thus preventing doxorubicin-induced double-stranded DNA breaks.[46] Dexrazoxane is clinically administered before doxorubicin for minimizing cardiotoxicity in high-risk patients.[46] It mitigated the acute gonadotoxicity induced by doxorubicin in mice and increased fecundity, pup weight, litter size, and number of deliveries after doxorubicin treatment.[46] Several recent studies have demonstrated that dexrazoxane may protect the ovaries from the gonadotoxic effect of doxorubicin in rodents [46-48] and in nonhuman primates.[49]

These preliminary data suggest that the clinically available dexrazoxane may be extrapolated to provide ovarian protection and fertility preservation for young female patients with cancer combined with gonadotoxic chemotherapy.[46-49] Whereas dexrazoxane can be easily administered, it may provide a timely, cost-effective, and safe noninvasive method for fertility preservation in prepubertal girls, for whom ovarian cryopreservation is the only available option.[46]

## Luteinizing Hormone

Recently, Rossi et al[50] have reported a protective effect of luteinizing hormone (LH) on the PMF pool of rodent prepubertal ovaries against cisplatin-induced gonadotoxicity. In vitro exposure of prepubertal ovarian fragments to LH-generated antiapoptotic signals is by a subset of ovarian somatic cells expressing LH receptor through cAMP/PKA and Akt pathways.[50] Such signals, reducing the oocyte level of proapoptotic TAp63 protein and favoring the repair of the cisplatin-damaged DNA in the oocytes, prevented their apoptosis.[50] Furthermore, in vivo administration of LH to prepubertal female mice inhibited the depletion of the PMF reserve caused by cisplatin and preserved fertility.[50]

These findings are surprising and contradict the beneficial effect of GnRHa in postpubertal women along with chemotherapy by simulating a prepubertal hypogonadotropic milieu.[1,41,51-55] Even more intriguing, Flaws et al[56] found that chronically elevated LH depleted PMFs in the mouse ovary. According to their findings, transgenic mice for β-LH, with high levels of LH, have at birth a similar number of follicles as wild-type controls.[56] However, after several weeks of exposure to increased LH concentrations, they suffer significant premature loss of their primordial and primary follicle pool, in keeping with the suggested pathophysiologic "vicious cycle"[1,41,51-55] (Figure 1). These contradicting publications underscore the need for more data to elaborate the role of increased LH levels in prepubertal animals to determine whether that role is protective from the gonadotoxic effect of some chemotherapeutic agents, such as cisplatinum, or detrimental to the PMF pool[50,56] (Figure 2).

## Curcumin and Capsaicin

Curcumin and capsaicin are naturally occurring phytochemicals commonly used as food additives in the east, known to possess significant health benefits as anti-inflammatory medications,
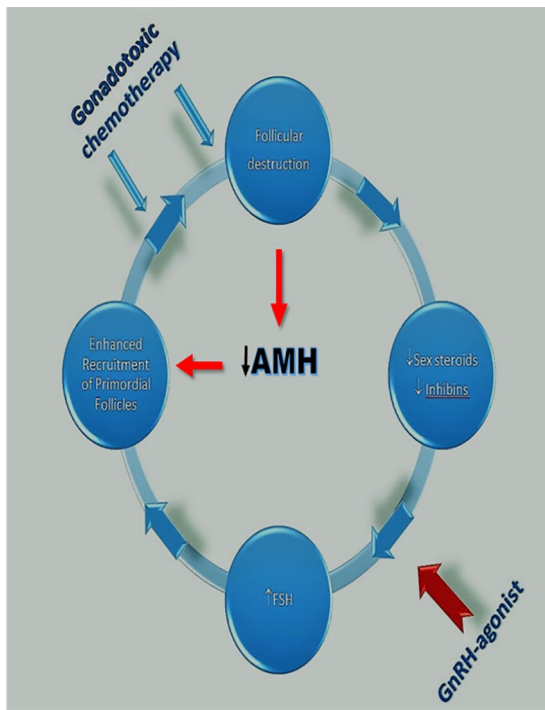
**Figure 1.** Suggested pathophysiologic vicious cycle for explanation of the possible beneficial role of GnRHa in minimizing the gonadotoxic effect of chemotherapy. Alkylating agents such as cyclophosphamide destroy many follicles; therefore, the levels of estrogens and inhibins are reduced, bringing about an increase in FSH secretion, due to decreased negative feedback. The increased FSH recruits many follicles to enter the unidirectional path of folliculogenesis, to be destroyed by the alkylating agents. The GnRHa, by its effect to prevent increased gonadotropin concentration, may minimize this vicious cycle. The AMH may also play an important role in this pathophysiologic process. AMH indicates anti-Müllerian hormone; FSH, follicle-stimulating hormone.



**Figure 2.** Schematic cartoon summarizing the possible future beneficial effects of various agents in minimizing the gonadotoxic effect of chemotherapy. In addition to the present modalities of GnRHa co-administration, cryopreservation of embryos, ova, or ovarian tissue, future endeavors may consist of the generation of gametes from induced pluripotent stem cells (iPC), in vitro maturation (IVM) of PMFs stored in the cryopreserved ovarian tissue, generation of an in vitro "artificial ovary," and use of many possible agents. Such possible protective agents include AMH, tamoxifen, melatonin, Shilajit, resveratrol, imatinib (Gleevec), mangafodipir, sphingosine-1-phosphate (S1P), AS101, ceramide-1-phosphate (C1P), granulocyte colony-stimulating factor (G-CSF), dexrazoxane, and possibly others (???). AMH indicates anti-Müllerian hormone.

analgesics, and anticancer agents.[57] Recently, treatment of alkylating agent induced POI in rats with curcumin and capsaicin decreased the gonadotoxic effect of cyclophosphamide.[57] The beneficial effect of curcumin and capsaicin on reducing ovarian cytotoxicity was attributed to improving tissue oxidative stress, ovarian reserve markers, and histopathologic parameters[57] (Figure 2).

These encouraging results await clinical extrapolation before offering curcumin and capsaicin cotreatment in parallel to chemotherapy as an additional conservative treatment approach for minimizing POI in patients with cancer.

## Shilajit

Shilajit is a traditional Indian medicine containing antioxidant agents believed to possess gametogenic protective effects. To evaluate the ability of Shilajit to prevent radiation-induced ovarian toxicity, Kececi et al[58] have treated rats with this agent in parallel to radiation. They have found that whereas most of the follicles have undergone atresia in the radiation group, normal-looking PMFs were detected in the radiation + Shilajit group.[58] Furthermore, the follicular expression of apoptotic markers such as p53, Bax, and caspase 3 was decreased in the

radiation + Shilajit–cotreated rats compared with the radiation-only group.[58] Despite this preliminary, encouraging publication, many more preclinical data need to be generated before possible clinical extrapolation of Shilajit as a gonadal protective agent against the gonadotoxic effects of radiotherapy and/or chemotherapy (Figure 2).

## Mangafodipir

Mangafodipir is a manganese chelate and superoxide dismutase mimetic agent.[59] It has been recently tested as a gonadoprotective agent in human nonluteinized granulosa cell line cultures treated with cisplatin, paclitaxel, and other toxic agents, and in rodents, in vivo.[59] Mangafodipir inhibited the chemotherapeutic induced loss of PMFs and granulosa cell apoptosis in vivo and attenuated the apoptosis induced by anticancer drugs in vitro[59] did not affect anticancer drug antitumor effects. Furthermore, mangafodipir minimized chemotherapy-induced ovarian toxicity without interfering with the antitumor activities of these medications.[59] Again, despite this preliminary, encouraging publication, additional preclinical data should be generated before possible clinical extrapolation of mangafodipir as a gonadal protective agent against the gonadotoxic effects of chemotherapy (Figure 2).

## Resveratrol

Recently, the possible beneficial effect of resveratrol in chemically induced POF has been investigated in rats.[60] The authors have performed Western blot analysis of PI3K, p-PI3K, Akt, p-Akt, mTOR, p-mTOR, Bax, Bcl-2, and caspase 3 in ovarian
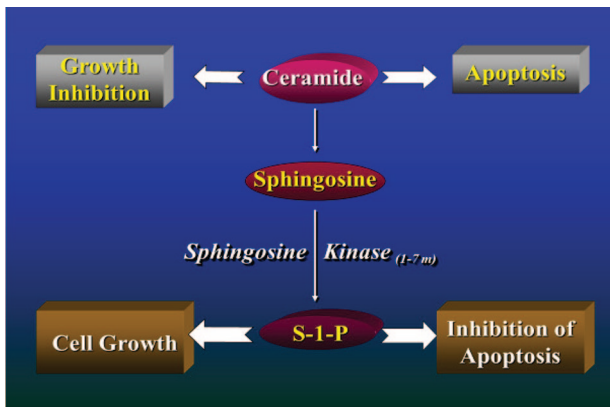
**Figure 3.** The balance between ceramide and sphingosine-1-phosphate (S1P), the first inducing apoptosis and cell death, whereas the second induces cell growth. Therefore, the cells' fate to die or survive and thrive depends on the balance between ceramide and S1P.

tissues and evaluated the expression of p-Akt, p-mTOR, Bax, Bcl-2, and caspase 3 using immunohistochemistry.[60] They also determined the serum malondialdehyde and malondialdehyde by enzyme-linked immunosorbent assay.[60] Compared with controls, these investigators have found that serum malondialdehyde decreased and malondialdehyde increased in resveratrol-treated groups.[60] They have also measured increased expression of p-PI3K, p-Akt, p-mTOR, and Bcl-2 and decreased expression of Bax and caspase 3 in the ovaries of rats with POF.[60] Whether resveratrol will have any future clinical role in treating women with POF after gonadotoxic chemotherapy or radiotherapy is premature to determine at present, and many more experimentations are needed to possibly answer this question (Figure 2).

### Ceramide-1-Phosphate
Whereas the ovarian protective effects of S1P are well known for over 2 decades,[11-17] recent findings suggest that also ceramide-1-phosphate (C1P) may have similar protective effects.[61] Paradoxically, Pascuali et al[61] have recently found that local administration of C1P reduced the ovarian toxicity induced by cyclophosphamide through protection of follicular reserve, inhibition of apoptosis, improvement of stromal vasculature, and restoration of hormone levels while protecting fertility, oocyte quality, and uterine morphology. These results are surprising, and even paradoxical, as S1P and ceramide have antagonistic intracellular effects.[62] Ceramide-1-phosphate may regulate various intracellular processes such as cell proliferation, cell migration, angiogenesis, and apoptosis.[61] Ceramide-1-phosphate can modulate vascular development and apoptosis in ovaries affected by chemotherapy.[61] Indeed, there is ample evidence to suggest that the balance between S1P and ceramide and/or sphingosine levels in cells may be an important determinant of cell fate to longevity or apoptosis[62] (Figure 3).

Nevertheless, the addition of C1P to cyclophosphamide prevented the destruction of ovarian follicles.[61] Furthermore, the addition of C1P to the alkylating agent minimized the cyclophosphamide decrease in AMH and estradiol levels and

the increase in FSH concentrations ($P < .01$).[61] Whereas cyclophosphamide has significantly increased the apoptotic index (TUNEL-positive follicles/total follicles) in preantral and early antral stages, compared with control ovaries, C1P protected follicles from this increase.[61] Ceramide-1-phosphate also protected the blood vessels from the chemotherapy-induced negative effects.[61] Most importantly, C1P preserved normal fertility, compared with the cyclophosphamide-treated rodents, and prevented the increase in abnormal oocytes.[61] The authors declared reservation and limitations of their study, claiming that the in vivo animal experimental model was already used by several authors, and further studies on the safety of this sphingolipid are required[61] (Figure 2).

### Targeted Cancer Therapy-Immunotherapy Instead of Chemotherapy
Recently, remarkable advances have been made in cancer immunotherapy and in targeted drug delivery technologies that can significantly prevent gonadotoxic side effects of systemic chemotherapy. The concept of immunotherapy instead of gonadotoxic chemotherapy and radiotherapy for combating cancer is a relatively recent endeavor with enormous potential. Although it is still in the experimental stages, remarkable advances in clinical applications have occurred in the past several years.[63] More specifically, adoptive cellular therapy using chimeric antigen receptor (CAR)-modified T cells targeted to CD19 has demonstrated substantial clinical efficacy in patients with B-cell acute lymphoblastic and chronic lymphocytic leukemia and B-cell non-Hodgkin lymphoma.[63] Early-phase clinical trials are ongoing to assess CAR T-cell efficacy and safety in several other malignant diseases.[63]

### Generating Oocytes From Induced Pluripotent Stem Cells
Recent advances in regenerative medicine and reproductive engineering have enabled the generation of germ cells in vitro from rodent-induced pluripotent and embryonic stem cells.[64-66] Hayashi and Saitou[65] have transformed rodent embryonic and induced pluripotent embryonic stem cells into primordial germ cell–like cells (PGCLCs) that were aggregated with somatic cells of female embryonic gonads, the precursors for adult ovaries. This process lasted about 8 days.[65] The aggregations were then transplanted under the ovarian bursa, in which PGCLCs developed into germinal vesicle oocytes in approximately 1 month. The PGCLC-derived germinal vesicle oocytes were further matured into MII ova within an additional day by IVM, and these MII ova were fertilized with spermatozoa by in vitro fertilization (IVF) to generate healthy and fertile offspring.[63] Although this breakthrough is a remarkable achievement, extrapolation to humans awaits the resolution of several cardinal technical and ethical problems.[33,63-65]

Another similar and related issue is the possible existence of progenitor stem cells in adult mammalian ovaries, including

the human ovary.[66] Characterization and definition of such controversial cells are undergoing in several leading laboratories.[66] There are no answers, yet, as to why these cells do not generate postnatal de novo folliculogenesis and whether future technology may possibly overcome POI by ovarian rejuvenation.[61] In the current issue of this journal, Tilly's group elegantly elaborate on this new endeavor.

### "Artificial Ovary"—IVM of PMF to MII Fertilizable Oocytes

Although the first newborn mice, generated from follicles grown in vitro in 3 dimensions alginate hydrogels, have been reported,[67-76] there are still many technical requirements for advance in IVM for primates, from the stage of PMF to mature, fertilizable MII ova. Woodruff's group[67,71-73,75] has developed and applied new biomaterials for the in vitro and in vivo growth of ovarian follicles with the ultimate goal of providing new options for preserving both fertility and endocrine function in patients. Another leading group in this endeavor is the group of Telfer.[68,69,74] It is anticipated and hoped that in not too long time, one of these leading groups, or others, will come up with the clinical breakthrough of IVM of mature, fertilizable MII oocytes from the stage of resting PMF.[67-77] In the current issue of this journal, Tilly's group elegantly elaborate on this new endeavor.

The research concentrated on isolation of ovarian follicles at specific stages of development, removing any contaminating cells and recombining the follicles within a supportive matrix to form an "artificial ovary" for subsequent reimplantation or ex vivo development. Another direction aimed at the IVM of oocytes from cryopreserved ovarian fragments.[67-77] Despite promising preliminary results, significant amount of additional investigation is needed before an "artificial" ovary can be clinically extrapolated.

### Very Small Embryonic-Like Stem Cells

Recently, very small embryonic-like stem cells (VSELs) have been identified in adult human ovaries and testes, in menopausal women or patients with POF/POI and azoospermic testicular biopsies from survivors of childhood cancer.[78-83] Due to their quiescent nature, VSELs may survive gonadotoxic chemotherapy and were detected in chemoablated gonads of rodents, at the mRNA and protein levels and by flow cytometry.[78-83] Thus, the surviving VSELs may spontaneously differentiate into oocyte-like structures and sperm when inhibitory factors are overcome in vitro.[78-83] Moreover, transplantation of mesenchymal cells enabled the rejuvenation of chemoablated mouse ovaries and even generated live births.[78-83] Thus, endogenous VSELs that have survived the gonadotoxic chemotherapy may possibly regenerate nonfunctional gonads in cancer survivors.[78-83] However, to enable the nonfunctional gonads to become functional and generate fertilizable gametes, exposure to a healthy niche either in vitro or in vivo is necessary.[78-83] Mesenchymal cells can create such an environment by secreting trophic and growth factors, required for the differentiation of VSELs into functional gametes.[78-83] Presence of VSELs may

explain the occurrence of spontaneous conceptions after BMT and after ovarian slices autotransplantation.[78-83] However, this explanation is not unequivocally accepted. Once validated and unanimously accepted by the scientific community, it could obviate the need to remove a whole gonad for cryopreservation before gonadotoxic chemotherapy.[78-83] Indeed, in keeping with the VSEL concept of Bhartiya's group,[83] Fazeli et al[84] have recently described that transplanting mesenchymal cells may restore fertility in chemoablated gonads.

### Ethical Issues—Combination of Methods

Many unknown and equivocal matters remain to be addressed in fertility preservation. Therefore, the data appear to suggest that clinical medicine is still far from having a ubiquitous solution for all survivors interested in future fertility and raising children. None of the suggested methods for fertility preservation guarantees unequivocal success in achieving pregnancy and delivering healthy infants. Therefore, several modalities need to be offered and practiced for maximizing patients' odds for future fertility. American Society of Clinical Oncology (ASCO) has updated the practice guideline for fertility preservation,[85] recommending that health care providers discuss all fertility preservation options with patients as early as possible before beginning gonadotoxic therapy, so as to allow for the widest array of options, including the GnRHa cotreatment for fertility preservation.

Whereas not all methods are 100% successful, young patients deserve to be informed of all the possible options to reduce gonadotoxicity and preserve ovarian function. In our opinion, GnRHa cotreatment should be offered in addition to IVF and cryopreservation of embryos, ova, and ovarian tissue for fertility preservation. There is no contraindication to ovarian biopsy for cryopreservation combined with GnRHa adjuvant cotreatment and follicular aspiration, as done in the *FertiPROTEKT* consortium.[86] In cases where chemotherapy has induced POF/POI, as is frequently the case in total body irradiation and BMT, the patient has cryopreserved ova, embryos, or PMFs to fall back upon. However, in conventional chemotherapy regimens such as those commonly practiced for young patients with breast cancer and lymphoma, GnRHa cotreatment may preserve ovarian function and prevent POF without necessitating the use of cryopreserved ova, embryos, or ovarian tissue.

### Author Contributions

The author has conceived and written the manuscript.

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