Fruitful progress to fertility

Chemotherapy can save the lives of many individuals with cancer. Unfortunately, it usually causes infertility after treatment, posing a concern for these people who will face a lifetime condition that considerably limits the quality of their lives. Advances in the field of oncofertility have brought hope to cancer survivors who long to plan a family; however, standard approaches only rely on cryopreservation of sperms and eggs before treatment and do not prevent infertility. In 'Bedside to Bench', Min Xu, Mary Ellen Pavone and Teresa Woodruff examine a study where individuals treated with gonadotropin-releasing hormone (GnRH) agonists before cancer therapy showed a decreased risk of infertility. How these agonists work to suppress and protect ovarian function and increase fertility in women after treatment is still unclear and begs further investigation at the bench. In 'Bench to Bedside', Amander Clark, Bart Phillips and Kyle Orwig discuss potential experimental options to preserve and restore male fertility after chemotherapy. These approaches will shed light into mechanisms of male fertility and spermatogenesis and may be the alternative to sperm freezing, which is not suitable for prepubertal boys and men unable to make sperm.

■ BEDSIDE TO BENCH

Preserving oocytes from chemodestruction

Min Xu, Mary Ellen Pavone & Teresa Woodruff

More than 12% of women diagnosed with breast cancer in the US are under the age of 45 (ref. 1). Adjuvant therapies commonly used to improve disease-free and overall survival, including chemotherapy, hormonal therapy or both also worsen patients' quality of life. In women of childbearing age, one of the most disconcerting effects of chemotherapy is the potential for early menopause and subsequent loss of fertility. Concerns about fertility among young female individuals with cancer seem to influence the choice of, and adherence to, particular treatment regimens[2].

No standard approaches for preventing risk of infertility during cancer therapy currently exist; however, assisted reproductive technologies, such as embryo or oocyte cryopreservation, or ovarian tissue cryopreservation can be used to preserve fertility before the start of fertility-damaging treatment[3]. Protecting the endocrine function of the ovary is also important for patients with cancer but presents some complexity for hormone-receptor–positive breast disease.

An unresolved and controversial question in the field of oncofertility is whether suppressing ovarian function with the use of gonadotropin-releasing hormone (GnRH) agonists may protect against chemotherapy-induced premature menopause. A recently published human study shows that patients with breast cancer have a reduced risk of

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early menopause when treated with GnRH analogs[4]. From the clinical perspective, this is an important finding—any way in which we can protect or extend ovarian function for young patients with cancer without the use of time-consuming or invasive procedures, such as hormone injections or removal of an ovary, is considered a medical advance. From the basic-science perspective, however, the study results are somewhat perplexing and are driving many reproductive scientists back to the bench to investigate how GnRH analogs might be having a protective effect.

The mechanism of action probably does not involve central regulation of the gonadotropins, as simple inhibition of gonadotropin production would not alter the steady pace of primordial follicle activation. So, one is left with an equally intriguing role for GnRH and its analogs in the primate ovary. Data in rodents support this notion, whereas the absence of detectable GnRH receptors in primate primordial follicles is a conundrum. This basic-science tangle and the equally knotted clinical data need to be resolved so physicians and patients can know whether this medical intervention will have the intended effect.

The PROMISE-GIM6 study (Prevention of Menopause Induced by Chemotherapy: A Study in Early Breast Cancer Patients-Gruppo Italiano Mammella 6) is the largest randomized clinical trial to evaluate the ability of a GnRH agonist to preserve ovarian function after adjuvant or neoadjuvant chemotherapy[4–7]. The reports from prior studies did not reach the bar of significance due to low numbers of patients, different measures

of ovarian reserve and menstrual function and disease and treatment status[8]. Notably, the authors showed a significantly lower incidence of early menopause—absence of menstruation and postmenopausal estradiol levels a year after the last cycle of chemotherapy—in premenopausal women receiving chemotherapy and a GnRH agonist compared to those receiving chemotherapy alone. Multivariate analyses indicated that only treatment with GnRH agonist, not patient age or type of chemotherapy, was associated with this reduction in infertility risk[4].

Although promising, the results of this study should be interpreted with caution. Recovery of the menstrual cycle and premenopausal hormone values should not be regarded as surrogate markers of oocyte quality and follicular reserve, only somatic cell function—fertility cannot be presumed to be normal by this simple hormone assessment. Although the levels of hormone receptor in breast tissue should not influence the effect of GnRH analogs on reproductive endpoints, accounting for these differences within the cohort may also be valuable. Furthermore, studies assessing the long-term safety of GnRH agonists, or testing the degree and longevity of preserved ovarian function, are the next needed steps.

Thus, the study by Del Mastro et al.[4] provides evidence supporting a potentially valuable alternative method for preserving ovarian function in young women facing probable premature menopause. Nevertheless, whereas suppressing ovarian function with GnRH agonists may protect against chemotherapy-induced
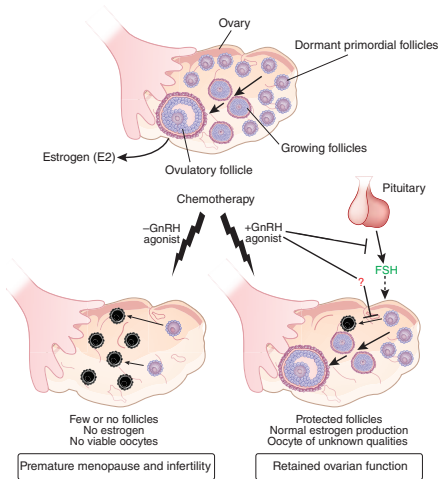
Figure 1 Potential role of GnRH agonist in preserving ovarian function and fertility during cancer treatment. Normal ovarian function relies on the steady release of follicles from the dormant primordial follicle pool, which is not thought to depend on peripheral hormones such as FSH. FSH 'recruits' follicles from the activated pool, causing growth of the somatic cells that ends with maturation of an oocyte in a large ovulatory follicle. The follicle produces increasing amounts of the steroid hormone estradiol. GnRH analogs (both agonists and antagonists) have been tested as agents that can mitigate the effects of chemotherapeutics on ovarian function by blocking new follicle growth (where rapidly dividing somatic cells can succumb to drug effect similar to growing cancer cells) or by directly acting on primordial follicles and somehow blocking their entry into the growing pool. The direct target of GnRH agonist effect is still not known.

premature menopause, how this happens remains unclear. Answering this question may provide even better targets in the ovary that would medically protect the irreplaceable primordial follicle pool against off-target chemotherapeutic destruction.

One commonly suggested ovary-specific mechanism is alteration of the gonadotropic hormone milieu upon GnRH agonist administration, such that primordial follicles are prevented from entering the growing phase, when follicles are particularly vulnerable to chemotherapeutic damage (**Fig. 1**). But primordial follicle activation is thought to be a gonadotropin-independent process[9,10], and the rate of primordial follicle atresia is fairly constant before menopause[11] despite, for example, remarkable changes in gonadotropin levels during pregnancy and lactation. Therefore, it is not clear how GnRH agonist-induced changes in gonadotropin levels could slow primordial follicle activation. GnRH agonists may also act directly on the ovary, independent of gonadotropins. If this is the case, then the GnRH receptor should

be expressed in primordial follicles, and, although GnRH receptors have been found in the rodent ovary[12], it is all but absent in human primordial follicles[13]. Moreover, the rodent literature is mixed, with some studies reporting that GnRH analogs are fertoprotective[14] and some showing little effect[15].

Whether GnRH can signal through other receptors on the follicle or surrounding support cells remains to be illustrated (**Fig. 1**). Crucial preclinical models are urgently needed, together with longer and larger clinical studies, to determine whether GnRH analogs work through suppression of the gonadotropins (in which case rodent and primate studies should have similar outcomes) or at the level of the ovary (although receptor levels, if present, cannot yet be detected in primates).

Regardless of how GnRH agonists prevent primordial follicle activation, two other essential questions need to be investigated through large studies. First, can the immature oocyte survive chemotherapeutic damage and potentially even repair itself[16]? The depletion of the oocyte pool by chemotherapy is the fundamental cause of permanent amenorrhea and premature ovarian failure in patients with cancer. Most of the agents causing infertility, including cyclophosphamide and cisplatin, are independent of the cell cycle and function by directly attacking DNA, which means they can damage both dividing somatic cells and the dormant germ cell. The oocyte may be damaged and yet survive—permitting follicle development and hormone production—but may not be capable of producing live offspring. Pregnancy data, menstrual characteristics, fetal anomalies and reproductive lifespan will all be important to follow over the next decade in the Del Mastro cohort.

The second fundamental question is what noninvasive methods best assess the number of primordial follicles and the oocyte quality within primordial follicles, the two markers that determine the capacity for ovarian functional restoration and future fertility. Although the resumption of regular menstrual cycles and plasma follicle-stimulating hormone (FSH) level have served as the surrogate markers of post-chemotherapy fertility for many years; these markers are just indices of ovarian steroidogenic function rather than predictors of future fertility. Recently, Anti-Müllerian hormone (AMH) has been proposed as a better marker of ovarian reserve[17]. However, AMH is produced by the follicles that have been newly activated and have left the dormant pool, so it does not accurately reflect the 'dormant' follicle number. Clearly,

better markers or physical measures of the primordial pool are an imperative for basic science.

The clinical observation that GnRH agonists have a protective effect on the ovarian function of young patients with breast cancer undergoing chemotherapy have re-ignited interest in understanding how GnRH analogs might be affecting the primordial follicle pool. However, the clinical utility of GnRH agonists in fertility preservation during chemotherapy will remain unresolved until the mechanism of action on ovary is fully investigated, the oocyte repair process is understood and direct markers for primordial follicle number and quality are found.

Reproductive research and clinical investigation on the cause of infertility requires patients, and patience, to follow the cohort over the next several years. If GnRH analogs work independently of cancer agent and target either the pituitary or the ovary, this easily administered drug could be a much-needed answer for all young patients with cancer. A large, multicenter initiative in rodents with clear, agreed-upon endpoints in partnership with global clinical leadership could allow the field to definitively answer a question and find the medical solution to fertility management for young patients with cancer that is urgently needed.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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