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Review

Fertility Preservation for Young Female Cancer Patients

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Abstract. *Young female cancer patients are still being poorly counseled with regard to the negative impact of treatment on their fertility and on their options for fertility preservation. Today, many possibilities exist for fertility preservation, such as ovarian suppression with GnRH analogues, ovarian tissue cryopreservation, in vitro maturation or IVF after ovulation induction with aromatase inhibitors. A pregnancy after cancer treatment does not seem to limit the prognosis. This review focuses on both the effect of cancer treatments on fertility and on the various assisted-reproduction innovations that are available to provide the cancer patient with the option of future pregnancies. It is currently a time of uncertainty and revolution concerning the role of ovarian suppression and other fertility preservation measures in the management of early breast cancer, but developments in the near future promise to be very exciting.*

It is estimated that in 2010, every 250th adult will be a survivor of childhood cancer (1). In developed or Westernized countries, women are using better methods of contraception and are delaying childbearing for social or financial reasons so that an increasing number of women are anxious to preserve their fertility when early-stage cancer is discovered (2-5).

In addition, increasing numbers of patients with nonmalignant autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus, as well as hematological diseases (6), are being successfully treated with chemotherapy or radiation therapy. Such cytotoxic therapy often results in premature ovarian failure (POF) (7). Premature ovarian failure, defined as menopause before the age of 40 years or hypergonadotropic amenorrhea, occurs in up to 0.9% of women in the general population. Such patients with POF have to face years of menopause and psychological problems, or else years of hormone replacement therapy. However, this substitution therapy is not capable of replacing the reproductive function of the ovaries.

This article reviews the literature on this topic, discusses the effects of cancer treatment on female fertility and presents the options currently available – thanks to advances in assisted-reproduction technology – for the conservation of fertility in women undergoing such treatments.

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Ovarian Anatomy and Physiology

The peak number of oocytes present in the ovaries *ca.* 6.8×10^6 occurs at 5 months gestation. After this point, there is no further proliferation of germ cells and a progressive atresia occurs. At birth, this number decreases to $1\text{-}2\times10^6$ and at puberty there are only 300,000 oocytes remaining. From these follicles approximately 300-500 will develop to mature oocytes, while the rest will become atretic. At the age of 51 years, the average age of natural menopause in women in developed countries, there are about 1,000 left (3).

In healthy women, at approximately 37.5 years of age, an accelerated atresia of oocytes begins, associated with an increase in the level of follicle-stimulating hormone (FSH) (8). As atresia continues, both the number and quality of oocytes fall below a critical level, and the rate of aneuploidy increases. This process leads to a greater risk of spontaneous abortion once pregnancy occurs. This central doctrine of age-dependant follicle depletion has been challenged by recent data that suggest a presence of ovarian stem cells in mice, which could presumably lead to a replenishment of follicles (9), a theory that at least in humans cannot be supported.

The life cycle of the ovary has four major developments (10). Firstly, the phase of embryogenesis whereby populations of primordial germ cells and somatic cells become an integrated ovary mass containing oocytes and granulosa cells located within primordial follicles. The first phase of oocyte

maturation starts *in utero* and is gonadotropin independent.

Secondly, the phase of folliculogenesis in which oogenesis, granulogenesis and thecogenesis occur as a recruited primordial follicle grows and develops to the preovulatory follicle, or dies by atresia. This phase begins at puberty and is gonadotropin dependent.

Thirdly, the phase of ovulation whereby the oocyte, triggered by the luteinizing hormones, transforms into a mature egg which is secreted into the oviduct to await fertilization.

Finally the phase of luteogenesis whereby the follicle luteinises into the corpus luteum, which if implantation does not occur, dies by a process termed luteolysis.

The Effect of Cancer Treatment on Female Fertility

Radiotherapy-induced ovarian damage. Ionizing radiation has adverse effects on gonadal function at all ages. The degree and persistence of the damage depends on the dose, the irradiation field and the patient's age, with older women being at greater risk of damage (11). Cranial irradiation for brain tumors with doses to the hypothalamic–pituitary area in excess of 30 Gy can in time cause hypogonadotropic hypogonadism in children (12). The ovaries are exposed to significant doses of radiation when radiotherapy is used in the treatment of cervical and rectal cancer, and with craniospinal radiotherapy for central nervous system malignancies. This can also happen when the pelvic lymph nodes are irradiated for hematological malignancies, such as Hodgkin's disease, and with total body irradiation before bone-marrow transplantation (11). Gosden *et al*. demonstrated that there is dose-related depletion of primordial follicles in mouse ovaries after increasing radiation doses of 0.1, 0.2 and 0.3 Gy. This explains sterilization with total depletion of the primordial follicle reserve after exposure to high doses of radiotherapy and premature ovarian failure at lower doses that cause only partial depletion of the primordial follicle reserve (13).

Various reports have been published on the radiation dosage necessary to cause loss of ovarian function. Lushbaugh and Casarett have shown that women under 40 years of age are less sensitive to radiation-induced ovarian damage, with an estimated dose of 20 Gy being required to produce permanent ovarian failure, in comparison with 6 Gy in older women (14). Chiarelli *et al*. observed a dose-dependent and distribution-dependent relationship between the risk of premature ovarian failure and the total dose of abdominal pelvic irradiation: with doses <20 Gy, the relative risk was 1.02; at 20-35 Gy, the relative risk increased to 1.37; and with doses >35 Gy, the relative risk of premature ovarian failure was 3.27. The percentage of women who suffered infertility correlated with increasing dosages of abdominal pelvic irradiation. Treatment with 20-35 Gy caused a 22% rate of infertility and doses >35 Gy led to a 32% rate of infertility (15).

There is also a known radiation effect on the uterus and subsequent pregnancy outcomes. Uterine radiation is associated with infertility, spontaneous miscarriage and intrauterine growth retardation (16). Direct effects on the uterus after irradiation include irreversible changes in the uterine musculature and blood flow, as well as hormone-resistant endometrial insufficiency. A review by Critchley and Wallace (17) indicates that physiological sex steroid replacement therapy may improve uterine characteristics in some patients after irradiation at a young age.

It is also known that there is a higher rate of obstetric complications in patients who have received radiation treatment, in comparison with the general population. Complications include spontaneous abortions (38% *vs*. 12%), preterm labor (62% *vs*. 9%) and low-birthweight infants (62% *vs*. 6%). However, as long as radiation is not administered during pregnancy, there is no risk of subsequent teratogenicity (18). These findings confirm studies on women exposed to the atomic bomb and on offspring conceived and born to them following exposure, which have shown that the incidence of spontaneous abortion is greater, but that the children do not suffer from an increased rate of mutations or major congenital anomalies in comparison with the normal population (19). Fenig *et al*. (20) reported an increase in low-birthweight infants and spontaneous abortions, especially if conception occurred less than a year after radiation exposure. They advised delaying pregnancy for a year after the completion of radiation therapy.

Chemotherapy-induced ovarian damage. All chemotherapeutic drugs act by interrupting vital cell processes and arresting the normal cellular proliferation cycle. Frequently, chemotherapeutic agents are used in combination because of synergistic effects, but this also leads to an increase in their adverse effects. In animal experiments, Meirow *et al*. demonstrated that regular menses and a normal reproductive outcome after chemotherapy are not certain indicators of whether the ovarian follicular reserve has survived the treatment unaffected (21). The authors also postulated that patients who recover from ovarian failure after high-dose chemotherapy or radiotherapy treatments should not delay childbearing for too many years. These patients should try to conceive after a few years of a disease-free interval, but not <6-12 months after the treatment, due to the possible toxic effects of the treatment on growing oocytes (22).

The risk of chemotherapy-related amenorrhea depends on the patient's age, the specific chemotherapeutic agents used, and the total dose administered. Older women have a higher incidence of complete ovarian failure and permanent infertility in comparison with younger women (11, 23). This can be explained by the larger primordial follicle reserve of the latter, which declines with age. Alkylating agents have a severe effect on human fertility. They can cause ovarian fibrosis and follicular

and oocyte depletion (24). According to Meirow (22), alkylating agents are associated with the greatest risk among all chemotherapeutic agents for inducing ovarian failure (odds ration (OR) of 3.98 in comparison with unexposed patients). In a study that examined the development of ovarian failure after cyclophosphamide treatment for lupus erythematosus, the POF incidence was 26% , with the major determining factors being the patient's age at the start of therapy and the cumulative dose (25). Animal experiments have also shown an increase in abortions and fetal malformations (10 times higher than in the control group) in pregnancies resulting from oocytes exposed to cyclophosphamide at different stages of oocyte maturation (11). Meirow also indicated that the effect of cyclophosphamide in mice is not an "all-or-nothing" phenomenon and that it causes follicular destruction in exponential proportion to increasing doses. Cisplatin and its analogs have also been investigated. Meirow estimated that cisplatin causes ovarian failure with an OR of 1.77. Studies of cisplatin treatment in female mice have demonstrated that different types of chromosomal damage are induced with genetic effects in the oocytes, resulting in early embryonic mortality and marked aneuploidy (21). Vinca alkaloids are known as aneuploidy inducers. According to Meirow, the OR for ovarian failure was about 1 (21). Many animal experiments have shown high levels of aneuploidy in oocytes exposed to vinblastine (26), which means that these damaged oocytes could produce malformed fetuses.

Further types of chemotherapeutic agents include antimetabolites and anthracycline antibiotics Insufficient data are available on the effects of antimetabolites on female germ cells. Anthracycline antibiotics such as adriamycin and bleomycin are female-specific mutagens and have been shown to induce dominant lethal mutations in maturing/preovulatory oocytes in female mice. Etoposide induced preferential pericentric lesions and aneuploidy in oocytes (27).

In everyday practice, women are rarely subjected to just one chemotherapeutic agent, so that the results of single-agent administration cannot be determined (23). Studies that have monitored pregnancies in women exposed to chemotherapy before conception have not reported increased rates of miscarriage or congenital abnormalities in comparison with the general population (21). Since these pregnancies occurred long after treatment had ceased, it can be assumed that there are correction mechanisms within the oocyte or that there are undetected miscarriages at a very early stage due to dominant lethal mutations.

Fertility Preservation Strategies

To date, the most effective approach is embryo cryopreservation. The human embryo is very resistant to damage caused by cryopreservation. The post-thaw survival rate of embryos is in the range of 35-90% , while implantation rates are between 8% and 30% . If multiple embryos are available for cryopreservation, cumulative pregnancy rates can be more than 60% (28). Delivery rates per embryo transfer using cryopreserved embryos are reported to be in the range of 18-20% (28). However, this approach requires *in vitro* fertilization and a participating male partner. If many mature oocytes are retrieved, there is an opportunity to carry out several attempts at embryo transfer from a single cycle. This option may not be acceptable to prepubertal or adolescent girls (29).

Cryopreservation of mature oocytes (after gonadotropin stimulation). Oocyte banking is more problematic than cryopreservation of sperms or embryos. The first obstacle is the sensitivity of oocytes to chilling, probably because of the sensitivity of the spindle apparatus and the higher lipid content of the cells. Cooling and exposure to cryoprotecting agents (CPAs) affect the cytoskeleton and may aggravate the already high incidence of aneuploidy in human oocytes (30). Exposure to CPAs also causes hardening of the zona pellucida, so that all oocyte cryopreservation protocols involve intracytoplasmic sperm injection (ICSI) as a precaution. Fertilization has to be carried out about 3-5 hours after thawing while the oocyte remains fertile. Further disadvantages of this method are that cancer patients may not have more than one opportunity for oocyte harvesting before undergoing potentially sterilizing treatment, since a cycle of controlled stimulation requires several weeks, and there is normally a delay of a few months before treatment cycles. The success of the method is also dependent on the total number of eggs harvested (<10 oocytes means very low chances of pregnancy). However, with the introduction of ICSI and the publication of reassuring data (31), efforts to cryopreserve oocytes have resumed in recent years, with conventional slow cooling–rapid thawing protocols and later with vitrification. To date, more than 4,300 oocytes have been cryopreserved and more than 80 children have been born, mostly with the conventional slow cooling method. The overall live birth rate per cryopreserved oocyte is about 2% , which is much lower than that with IVF using fresh oocytes (32). These data were confirmed by a recent metaanalysis from Oktay *et al*., who found that the live birth rate per injected oocyte was approximately 2% for the most commonly used slow-freezing technique. Pregnancy rates were one third to one fourth of the success rates seen with unfrozen oocytes (33).

Cryopreservation of immature oocytes after in vitro maturation (IVM) (without gonadotropin stimulation). Oocytes are recovered for IVM from fresh tissue or follicular aspirates before the dominant follicle emerges during the mid-follicular phase of the menstrual cycle (normally 8-10 mm in diameter). Cryopreservation difficulties include the different optimal times of equilibration for the oocyte and its smaller cumulus cells. At present, the reported success of IVM in young

Table I. *Ovarian suppression with GnRH agonists in breast cancer patients.*

First author (ref.)	Year	Patients (n)	Chemotherapy Regimen	Pregnancies (%)	Births (%)	Menses 1 year after therapy (n)	Menses at the end of follow-up (n)	Outcome
Recchia	2006	100	26% CMF, 11% FEC, 54% CMF+E, 9% HCST	3%	2%	100%		Ovarian function preservation
Fox	2003	24	AC, AC-T, FAC, AT-CMF	21%	8%	96%	75%	Ovarian function preservation
Del Mastro	2006	29	100% FEC	-	-	94%	92%	Ovarian function preservation
Urruticoechea	2007	50	78% FEC, 14% AC, 8% AC-T	16%	16%	86%	90%	Ovarian function preservation
Total		203						

women with polycystic ovaries is a pregnancy rate of approximately 25-30% per cycle, with a high miscarriage rate (34). Oocytes can be recovered from unstimulated ovaries as well as from children, and egg harvesting is less expensive and risky and can be repeated frequently. However, this procedure requires further advances in cryotechnology.

GnRH analogue treatment (gonadotropin-releasing hormone analog, GnRH-a). Multiple small studies have evaluated the utility of this treatment in order to preserve ovarian function during cytotoxic therapy. Rendering the ovarian follicular development quiescent by suppression of gonadotropins has been proposed to protect women from damage by cytotoxic therapy. This research has suggested that receipt of GnRH-a throughout treatment may increase a woman's likelihood of remaining premenopausal after chemotherapy, although there has been an intensive debate concerning the existence of FSH (Follicle stimulating hormone) receptors in primordial follicles and GnRH-a receptors in the human ovary (35, 36). The protection cannot involve induction of quiescence in the already dormant primordial follicle, but may involve direct effects of GnRH analogues or indirect effects of gonadotropin suppression on the whole ovary (35, 37).

Meirow was unable to demonstrate a protective effect of GnRH after ablative chemotherapy and radiotherapy in patients undergoing bone-marrow transplantation (38). Waxman *et al.* found that buserelin was not effective for fertility preservation in humans. However, it is possible that complete pituitary ovarian suppression was not achieved, which might be a necessary prerequisite for these drugs to work (39). It has been shown that GnRH-a did not prevent ovarian follicle loss in a human ovarian xenograft model (40).

Blumenfeld and other research groups were able to demonstrate that the GnRH agonists are well tolerated and may protect long-term ovarian function (Table I, 41-46). However these studies included a small number of patients. Blumenfeld has reported on what is probably the largest group of women (55 lymphoma patients) who were started on GnRH-a 7-10 days before chemotherapy treatment. The rate of POF was 5% in the GnRH-a/chemotherapy group *versus* 55% in the group receiving chemotherapy alone (41).

The treatment with GnRH-a should begin at least 10 days before the beginning of chemotherapy because of the initial flare-up effect which causes undesirable ovarian stimulation. The application should continue throughout the chemotherapy period in the form of depot-injections, so that the down-regulating effect remains for at least two weeks after the end of chemotherapy. In the case of estrogen-sensitive tumors, a tamoxifen therapy can be initiated after the GnRH-a treatment. However, available studies are limited by small sample size, lack of a randomized control group and lack of definitive information regarding actual fertility outcomes. Randomized controlled trials are currently underway internationally to evaluate this strategy in women with cancer.

The Southwestern Oncology Group is running an ongoing randomized evaluation among women with hormone receptor-negative Stage I-IIIA breast cancer to receive, or not, goserelin throughout treatment. In the UK, the OPTION trial is similar, but is also including women with hormone receptor-positive disease. The potential benefit of ovarian suppression in addition to tamoxifen for women with hormone receptor-positive breast cancer is currently under active investigation in the SOFT trial. Other prospective randomized trials such as the Zoladex Rescue of Ovarian Function study in Germany, the Italian multicenter study for breast cancer patients, the German Hodgkin Lymphoma group multicenter study, the UK lymphoma multicenter study the Spanish Lymphoma multicenter study, and the PREGO (Prevention of gonadal toxicity and preservation of gonadal function and fertility in young women with systemic lupus erythematosus treated by cyclophosphamide) will give definitive evidence of the role of GnRH-a in ovarian function preservation (36).

In a recent review by Octay *et al.* (36), the possible hazards of a GnRH-a treatment for fertility preservation purposes have been sufficiently described. Not only are GnRH-a expensive

Table II. *Pregnancies and outcome after ovarian tissue cryopreservation and transplantation.*

First author (ref)	Year	Diagnosis	Age (years)	Outcome
Donnez	2004	Hodgkin's lymphoma	25	Spontaneous pregnancy, live birth
Meirow	2005	Non Hodgkin's lymphoma	28	IVF, live birth
Demeestere	2007	Hodgkin's lymphoma	29	Spontaneous pregnancy, live birth
Rosendahl	2006	Hodgkin's lymphoma	28	ICSI, biochemical pregnancy
Demeestere	2007	Hodgkin's lymphoma	31	Spontaneous pregnancy, miscarriage, then live birth
Andersen	2008	Hodgkin's lymphoma	25	IVF, miscarriage
Andersen	2008	Hodgkin's lymphoma	26	IVF, live birth
Andersen	2008	Ewing sarcoma	27	IVF, live birth

and cause severe menopausal symptoms, but in addition, the direct effects of GnRH agonists on human cancer cells are not sufficiently understood. A variety of human cancers, including those of the breast, ovary and endometrium express GnRH receptors. These receptors mediate several effects, such as inhibition of proliferation, induction of cell-cycle arrest and inhibition of apoptosis, induced, for example, by cytotoxic drugs (47). Thus, it cannot be excluded that GnRH agonist therapy concomitant with cytotoxic chemotherapy might reduce the efficacy of chemotherapy for breast cancer. There are however data from randomized studies as well as the results of the Early Breast Cancer Trialists' Collaborative Group meta-analysis and the results from the GnRH-agonists in Early Breast Cancer Overview group that did not show a different outcome in patients who had received concurrent ovarian suppression with the chemotherapy compared with patients treated with chemotherapy alone (48-50). In a recent meta-analysis data from 11,906 premenopausal women with early breast cancer randomised in 16 trials were examined. When used as the only systemic adjuvant treatment, GnRH agonists did not significantly reduce recurrence (28.4% relative reduction, 95% confidence interval (CI) consistent with 50.5% reduction to 3.5% increase, p=0.08) or death after recurrence (17.8%, 52.8% reduction to 42.9% increase, p=0.49) in hormone-receptor-positive cancer. Addition of GnRH agonists to tamoxifen, chemotherapy or both, reduced recurrence by 12.7% (2.4-21.9, p=0.02) and death after recurrence by 15.1% (1.8-26.7, p=0.03). GnRH agonists showed similar efficacy to chemotherapy (recurrence: 3.9% increase, (95% CI: 7.7% reduction to 17.0% increase); death after recurrence: 6.7% reduction (20.7% reduction to 9.6% increase), both not significant. No trials had assessed a GnRH agonist *versus* chemotherapy with tamoxifen in both arms. GnRH agonists were ineffective in hormone receptor-negative tumours (48).

On a more practical level, up to 97% of patients suffer from hypoestrogenic symptoms when using a GnRH-a along with chemotherapy (45). Furthermore, when used for >4 months, patients may experience bone loss, which may be irreversible with longer durations of use (51).

The American Society of Clinical Oncology points out that there is insufficient evidence regarding the safety and effectiveness of GnRH-a and other means of ovarian suppression on female fertility preservation at this time and recommends that women interested in ovarian suppression for this purpose are encouraged to participate in clinical trials (52).

These studies show that these agents are promising but still at a very early experimental stage.

At present, cryopreservation of ovarian tissue appears to a very promising way of providing the cancer patient with a realistic chance of fertility preservation – a prospect that is also extremely important for psychological reasons (61). The cryopreservation of ovarian cortical strips has emerged in recent years as an easy, fast, and inexpensive technique and has already yielded the first five live births (Table II, 62-66).

However, is not possible to estimate accurately how many women have had ovarian tissue cryopreserved in the last 10 years.

The idea of cryopreserving ovarian tissue is based on the finding that the ovarian cortex harbors primordial follicles that are more resistant to cryoinjury than mature oocytes, because the oocytes they contain have a relatively inactive metabolism and lack a metaphase spindle, zona pellucida and cortical granules (67). The clinical indications are almost identical with those for the oocyte but there are fewer logistical restrictions and does hormonal stimulation is not required when time is short and there is a greater fertility potential because of the far larger number of oocytes preserved. Ovarian tissue cryopreservation may be the only acceptable method for any prepubertal or premenarchal female patients receiving chemotherapy or pelvic radiotherapy (68). Follicular viability after cryopreservation and thawing has been demonstrated in several studies (69-71).

The risks of ovarian tissue cryopreservation include reimplantation of the primary tumor, malignant transformation as well as risks related to the invasiveness of the procedure (72). Limiting factors of this method are its current experimental status, the availability of the procedure in some selected centers and the limited life of the ovarian grafts. Questions in the field of ovarian tissue cryopreservation that

are still unanswered include the optimal site for retransplantation, the size of the ovarian grafts and the effect of gonadotropin stimulation (61).

Pregnancy after Cytotoxic Therapy

In a recent review of a number of studies in a population of over 15,000 women with more than 1,100 breast cancer patients, it was demonstrated that there are no conclusive data at present that suggest any deleterious effects, such as an increased risk for relapse, due to subsequent pregnancy in women with a history of breast cancer. A limiting factor of this analysis is that none of these studies was a randomized, controlled trial. It is however impossible to perform a randomized trial regarding this specific issue, since no woman can be denied the right to become pregnant. Fertility preservation options should therefore be discussed with all cancer patients (4).

Regarding the miscarriage rate, studies that have monitored pregnancies in women exposed to chemotherapy before conception were unable to detect any increased rates of miscarriage or congenital abnormalities in comparison with the general population. Since these pregnancies occurred long after the cytotoxic treatment, it can be assumed that correction mechanisms exist within the oocytes or that there are undetected miscarriages as a result of dominant lethal mutations at a very early stage (73).

The optimal timing of a subsequent pregnancy after cancer is unclear and depends on the patient's prognosis, age and personal situation. Meirow and Schiff postulated that patients who recover from ovarian failure after high-dose chemotherapy or radiotherapy treatments should not delay childbearing for too many years. These patients should try to conceive after a disease-free interval of a few years, but not <6-12 months after the treatment, due to the possible toxic effects of the therapy on growing oocytes (73). A delay of 2-3 years after the cancer treatment is conventionally recommended, so that the period associated with the greatest risk of recurrence has passed before a pregnancy. In patients with hormone-positive breast cancer, tamoxifen and GnRH-a do not cause permanent amenorrhea, but this treatment can last up to 5 years, during which a pregnancy is contraindicated (74).

Conclusion

The field of fertility preservation for female cancer survivors has been growing rapidly and the public as well as the scientific interest has increased tremendously. This comes as a result of ever more successful outcomes of cancer treatment and fast growing reproductive technologies.

Although there are some studies that show a positive effect of GnRH-a on fertility preservation there are not sufficient evidence-based data to establish their use as a first-line therapy. There are at this point some prospective randomized studies under way, but the long-awaited results will probably not be published for a couple of years. In the meantime, the use of GnRH-a in cancer patients should be offered within a clinical trial after adequate counseling of the patients regarding the possible influence on chemotherapy effectiveness.

The cryopreservation of ovarian cortical strips has already yielded the first live births and is being offered increasingly to patients undergoing cancer treatment. There are important advances in this reproductive technology that will help to enhance its success and its availability for cancer victims.

In everyday routine work, better interdisciplinary cooperation between gynecological and pediatric oncologists, surgeons, immunologists and endocrinologists is necessary so that individualized options for fertility preservation can be offered in advance of surgical procedures or cancer treatments.

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