Expert Opinion

Leuprolide acetate: a drug of diverse clinical applications

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Leuprolide acetate is a synthetic nonapeptide that is a potent gonadotropin-releasing hormone receptor (GnRHR) agonist used for diverse clinical applications, including the treatment of prostate cancer, endometriosis, uterine fibroids, central precocious puberty and *in vitro* fertilization techniques. As its basic mechanism of action, leuprolide acetate suppresses gonadotrope secretion of luteinizing hormone and follicle-stimulating hormone that subsequently suppresses gonadal sex steroid production. In addition, leuprolide acetate is presently being tested for the treatment of Alzheimer's disease, polycystic ovary syndrome, functional bowel disease, short stature, premenstrual syndrome and even as an alternative for contraception. Mounting evidence suggests that GnRH agonist suppression of serum gonadotropins may also be important in many of the clinical applications described above. Moreover, the presence of GnRHR in a multitude of non-reproductive tissues including the recent discovery of GnRHR expression in the hippocampi and cortex of the human brain indicates that GnRH analogs such as leuprolide acetate may also act directly via tissue GnRHRs to modulate (brain) function. Thus, the molecular mechanisms underlying the therapeutic effect of GnRH analogs in the treatment of these diseases may be more complex than originally thought. These observations also suggest that the potential uses of GnRH analogs in the modulation of GnRH signaling and treatment of disease has yet to be fully realized.

Keywords: Alzheimer's disease, central precocious puberty, contraception, endometriosis, functional bowel disease, GnRH analog, gonadotropin-releasing hormone, gonadotropin-releasing hormone receptor, *in vitro* fertilization, leuprolide acetate, polycystic ovary syndrome, premenstrual syndrome, prostate cancer, short stature, uterine leiomyomata

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1. GnRH analogs: an overview

After the discovery of gonadotropin-releasing hormone (GnRH) in 1971, a number of GnRH agonists and antagonists were developed throughout the 1970s and 1980s for the treatment of a range of diseases and conditions [1,2]. These drugs are thought to mediate their effects by modulating the concentration of circulating hypothalamic–pituitary–gonadal (HPG) hormones. Leuprolide acetate was introduced in 1985 for the treatment of prostate cancer as an alternative to surgical castration and estrogen therapy [3-6]. Subsequently, over the last 20 years, leuprolide acetate has become a well-established drug for the treatment of endometriosis, uterine fibroids and central precocious puberty (CPP) [7-11], as well as for use in *in vitro* fertilization (IVF) techniques [17,18]. In addition, this drug is now being investigated as a potential therapeutic for Alzheimer's disease (AD [12-14]), polycystic ovary syndrome (PCOS [15,16]), functional bowel disease [19], short stature [10,11,20],

GnRH	pGlu 1	His 2	Trp 3	Ser 4	Tyr 5	Gly 6	Leu 7	Arg 8	Pro 9	Gly 10	NH_2
Leuprolide	pGlu	His	Trp	Ser	Tyr	DLeu	Leu	Arg	Pro	Gly	NEt
Goserelin	pGlu	His	Trp	Ser	Tyr	DSer (tBu)	Leu	Arg	Pro	Gly	NH_2
Nafarelin	pGlu	His	Trp	Ser	Tyr	DNal (2)	Leu	Arg	Pro	Gly	NH_2
Triptorelin	pGlu	His	Trp	Ser	Tyr	DTrp	Leu	Arg	Pro	Gly	NH_2
Histrelin	pGlu	His	Trp	Ser	Tyr	DHis (ImBzl)	Leu	Arg	Pro	Gly	NH_2
Buserelin	pGlu	His	Trp	Ser	Tyr	DSer (tBu)	Leu	Arg	Pro	Gly	NEt
Deslorelin	pGlu	His	Trp	Ser	Tyr	DSer	Leu	Arg	Pro	Gly	NEt

Figure 1. Structure of GnRH and GnRH analogs.
GnRH: Gonadotropin-releasing hormone.

premenstrual syndrome (PMS) [21] and even as an alternative for contraception [22-25].

Leuprolide acetate is the largest selling GnRH agonist [201,202], but it is not without competition from other agonists (**Figure 1**). For example, goserelin, the second largest selling GnRH agonist, was introduced in 1983 for the treatment of prostate cancer and in 1985 for the treatment of breast cancer [26,27]. Another GnRH agonist, histrelin, has been used mainly for CPP but was later reformulated as an annual subcutaneous implant for prostate cancer [28,29]. GnRH agonists have also been developed for nasal administration; nafarelin, used for endometriosis, and buserelin, used for prostate and breast cancer, are administered multiple times in a day but have a lower bioavailability. Buserelin is also offered as a daily subcutaneous injection or in a 2- or 3-month depot form, although it is not commercially available in the US [30,31]. Triptorelin, a 1- or 3-month subcutaneous or intramuscular injection has also been used for both prostate cancer and endometriosis since the 1980s [32,33]. Finally, deslorelin has been experimentally tested for the induction of ovulation in mares [31,34] but this GnRH agonist implant has also been used in trials for contraception in animals and in humans for CPP [35-38]. In this review, the authors discuss the mechanism of leuprolide acetate action and its present and future clinical applications.

2. Leuprolide acetate

2.1 GnRH and GnRH receptors

GnRH is a small decapeptide that serves as an important connection between the neural and endocrine systems. This oligopeptide, pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-amide, is synthesized and stored in the medial basal hypothalamus [39] and is released in a pulsatile fashion. It acts on anterior pituitary gonadotropes, which express GnRH receptors (GnRHRs), to signal both the synthesis and secretion of the gonadotropin hormones – luteinizing hormone (LH) and follicle-stimulating hormone (FSH) – from the pituitary into the circulation [39]. GnRH has a short half-life of 3 – 4 min [39,40] and the amplitude and frequency of GnRH pulses directly regulate the divergent production of LH and FSH [40,41]. The maintenance of LH and FSH release is directly regulated by GnRH pulse frequency and amplitude as continuous (non-pulsatile) GnRH administration or long-lasting GnRH agonists desensitize GnRHR, thereby decreasing/inhibiting the release of LH and FSH by the pituitary [40,42]. Due to its direct regulation of LH, FSH and, in turn, sex steroid production and gametogenesis, GnRH plays a central role in both reproductive function and general hormonal control via precise regulation of the HPG axis. It is for this reason that GnRH agonists were initially developed as a method to regulate reproductive function [43], prior to the discovery that they inhibited tumor growth [3,44].

The actions of GnRH and its analogs are mediated by high-affinity receptors for GnRH found on the membranes of the pituitary gonadotrophs [45]. Mammalian GnRHR is a seven transmembrane-spanning G-protein-coupled receptor of 328 amino acids in length [46]. In addition to its well-characterized expression on pituitary gonadotropes, this receptor is expressed in a multitude of other human tissues, including the placenta, uterus, ovary, prostate gland, breast, liver, heart, skeletal muscle and kidney [47-49]. The authors have also recently found the receptor to be highly expressed in the human hippocampi and cortex [50]. The mechanism of GnRH signaling via GnRHRs has been well reviewed elsewhere [41,51].

2.2 Mechanisms of action and pharmacology

Leuprolide acetate is a synthetic nonapeptide analog of GnRH, with the chemical name 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide acetate (salt; **Figure 2**). Leuprolide acetate is available in many different dose and administration forms [203]. This super agonist is more potent than the natural GnRH peptide due to its increased affinity for GnRH receptors [31] and longer half-life (~ 3 h versus 3 – 4 min compared with endogenous GnRH) [31,52,53]. Bioavailability is similar for both the intravenous and subcutaneous routes of administration. There is < 5% of the dose that is recoverable following administration of the 3.75-mg depot.

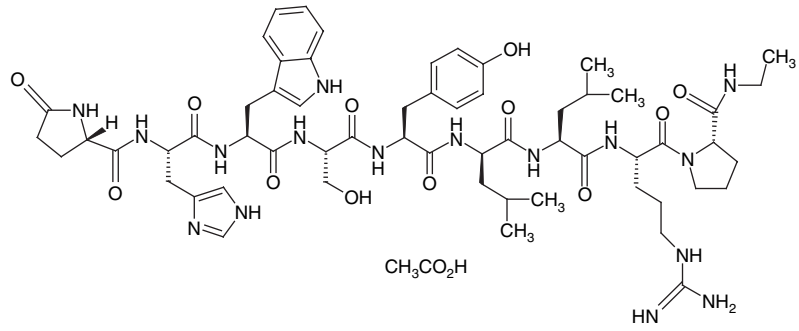

Figure 2. The chemical structure of leuprolide acetate [208].

Finally, although no official studies have been conducted, it is speculated that there are no drug–drug interactions [54,55].

Defined as an agonist, leuprolide acetate functions as an inhibitor of gonadotropin secretion when administered in therapeutic doses continuously. Both animal and human studies demonstrate that leuprolide acetate works by initially inducing a sharp increase in the pituitary secretion and serum levels of LH and FSH, which subsequently leads to an increase in serum sex steroids within 3 days of the initial treatment. However, continuous stimulation of the pituitary by chronic administration of GnRH agonists produces an inhibition of the hypophyseal–gonadal axis that is postulated to be due to the 'downregulation' of pituitary receptors for GnRH, desensitization of the pituitary gonadotrophs and a suppression of circulating levels of LH, FSH and sex steroids within 2 – 4 weeks [56]. This downregulation of GnRH signaling for the secretion of LH/FSH and sex steroids is thought to be the basis for clinical applications in gynecology and oncology. For males treated with leuprolide acetate, serum gonadotropins/testosterone decrease to castration levels whereas serum gonadotropins fall to postmenopausal levels in premenopausal women. These actions of leuprolide acetate (and all GnRH analogs) are completely reversible on discontinuation of treatment [54,55].

2.3 Safety, tolerability and adverse effects

Overall, leuprolide acetate is considered to be a very safe and tolerable drug [54,55]. The biggest concern with the use of GnRH analogs is the initial stimulatory effect it produces; this is an issue for all diseases treated using this drug. As a super agonist, when the drug is administered, a sharp, initial increase known as a 'flare up' occurs in the serum concentrations of LH, FSH and sex steroids. This can exacerbate the condition at first, before hormone levels drop to postmenopausal/castration levels. Flare up is associated with worsening symptoms of the particular condition being treated. Each disease, therefore, carries its own individual risks. For CPP, the sharp increase in hormones leads to the continuation of pubertal signs such as menses in girls and the development of secondary sex characteristics for both boys and girls. In endometriosis and uterine fibroids, the risk involves growth and proliferation of the tumor cells and bleeding. Finally, for patients with prostate cancer, the first few weeks of treatment with leuprolide acetate may incur bone pain and worsening of their existing symptoms such as hematuria and urinary tract obstruction. During this short period, all patients need to be monitored carefully. In addition, in this regard, the dosing needs to be examined so that it is high enough and consistent enough for complete, effective desensitization of the pituitary gonadotropes [54,55].

Due to the suppression of sex hormones by leuprolide acetate, it is contraindicated in women who are or may become pregnant [57]. General side effects are comparable to those experienced during menopause and andropause [54,55,58]. These symptoms may include nausea, amenorrhea, changes in bone mineral density (BMD), decreased libido, depression, hot flashes/sweats, insomnia, headache, weight gain and impotence [57]. In women, fertility can be affected while on therapy but studies indicate a full reversibility within 24 weeks when the treatment is discontinued. Of these general side effects mentioned, the largest risk incurred with the use of leuprolide acetate is the potential loss of BMD. As previously discussed, administration of leuprolide acetate or any GnRH agonist induces a sharp decrease in serum estradiol production and hypothalamic feedback. Estradiol has been shown to be important for bone health in both men and women, even though women are 4 times as likely to acquire osteoporosis during menopause. Therefore, it is important to monitor BMD during clinical use of leuprolide acetate. Recent studies and clinical trials, especially for endometriosis, are now focusing on 'add-back' hormone replacement therapy (estradiol/progesterone) without affecting or compromising the effects of leuprolide acetate, in order to minimize this negative effect on bone [57-59].

3. Present clinical applications

3.1 Prostate cancer

An estimated 218,890 new cases of prostate cancer (33% of all new cancer cases) will be diagnosed in 2007, with an estimated 27,050 deaths (9% of all cancer deaths), making

Table 1. GnRH agonists: comparative clinical applications.

GnRH agonists	Present clinical applications
Leuprorelin (leuprolide acetate)	Prostate cancer, breast cancer, endometriosis, uterine fibroids, CPP, *in vitro* fertilization
Goserelin	Prostate cancer, breast cancer, endometriosis, uterine fibroids, assisted reproduction, endometrial thinning, CPP
Buserelin	Prostate cancer, endometriosis, uterine fibroids, assisted reproduction
Histerelin	Prostate cancer, uterine fibroids, CPP
Nafarelin	Endometriosis, uterine fibroids, CPP, *in vitro* fertilization
Triptorelin	Prostate cancer, breast cancer, endometriosis, uterine fibroids, assisted reproduction, endometrial thinning, CPP

CPP: Central precocious puberty; GnRH: Gonadotropin-releasing hormone.

it the most commonly diagnosed and the second deadliest cancer in men [204]. The death rate for prostate cancer is decreasing partly due to earlier diagnosis and better treatment options. Treatment options for the early stages of prostate cancer include surgery and radiation. Treatment options for more advanced disease rely on suppression of testosterone production, either via orchiectomy or with hormonal ablation therapy [205]. Despite the fact that orchiectomy is a relatively simple and safe procedure, its psychologic effect can be significant and therefore it is often rejected by patients in favor of other medical therapies such as hormonal therapies [60,61]. Hormone ablation therapy has become the primary means of suppressing testosterone and hormonal agents used include both agonists and antagonists of GnRH, estrogens (such as diethylstilbestrol) and antiandrogens. The following sections describe the benefits and disadvantages of different hormone ablation therapies.

3.1.1 GnRH agonist therapy

Long-acting, synthetic GnRH agonists have been used in advanced prostate cancer for ~ 20 years for reducing serum testosterone levels (**Table 1**) [62]. Several GnRH agonists are available for use in early and advanced prostate cancer (**Table 1**) and some (abarelix and ganirelix) are in development. After the initiation of GnRH agonist therapy, up to 33% of patients will have transient elevations in serum testosterone and dihydrotestosterone that may result in tumor flare, including invading symptoms as well as skeletal pain, cord compression, uremia, paralysis or (in isolated cases) death in patients with advanced metastatic disease [63]. These complications may be prevented by short-term administration of an antiandrogen (e.g., flutamide, nilutamide and bicalutamide) [64]. Following treatment, the concentration of serum testosterone decreases to < 50 ng/dl by 14 – 28 days [63,65]. Abarelix, a GnRH antagonist, induces medical castration more rapidly than combined androgen blockade and without a testosterone surge that can result in tumor flare as described in Section 2.3 [66]. Earlier hormonal therapy may halt disease progression and increase survival for some patients [67] despite the fact that GnRH agonists eventually become ineffective at suppressing tumor growth [6]. Calcium supplementation in the diet of the patient, as recommended for menopausal women, may help to prevent bone loss and the development of osteoporosis in men on GnRH agonists.

3.1.2 Competing therapies for prostate cancer

3.1.2.1 Estrogen therapy

Estrogen therapy was the predominant medical form of hormone manipulation and an alternative to orchiectomy until the introduction of GnRH agonists in the 1980s (**Table 2**) [68]. However, the concentration of diethylstilbestrol (5 mg) required to produce a therapeutic effect comparable to that of orchiectomy was associated with excess mortality from deep vein thrombosis, myocardial infarction and transient ischemic attack in ≥ 20% of patients [69]. Some, but not all, studies have demonstrated that lower doses of diethylstilbestrol have a more favorable side effect profile [70-72]. Many of the side effects occurring with oral estrogen therapy may be modulated by parenteral administration and, because diethylstilbestrol offers the option of suppressing testosterone production without causing bone loss and potentially leading to osteoporosis, estrogen use is being revisited [73].

3.1.2.2 Antiandrogen therapy

Antiandrogen monotherapy may be an alternative for patients with locally advanced disease [27]. Although monotherapy with bicalutamide has an equivalent effect to castration on overall survival, it does not seem to have a benefit for those patients with metastatic disease [4]. The dose that shows equivalence to castration (150 mg) is not approved by the FDA. Although antiandrogen therapy has gynecomastia and breast pain as its common side effects, it does offer improvements in quality-of-life benefits related to sexual interest and physical capacity compared with other hormonal approaches [61].

3.1.2.3 Combined androgen blockade therapy

Combined androgen blockade uses either a steroidal antiandrogen (bicalutamide, flutamide or nilutamide) or a nonsteroidal antiandrogen (megestrol or cyproterone) in combination with a GnRH agonist. These block gonadal hormone synthesis, as well as blocking androgens synthesized outside the gonads from binding to their receptors, in order to prevent androgen signaling to the prostate tumor. However, combined androgen blockade does have additional side effects, including liver function abnormalities, diarrhea and

Table 2. Overview of present treatments for conditions and diseases where GnRH agonists are used clinically or are contemplated for clinical use.

Disease	Present treatments
Clinical use	
Prostate cancer	GnRH agonists, antiandrogens (flutamide, bicalutamide and so on), orchiectomy, prostatectomy, estrogens, radiation therapy, high-intensity focused ultrasound, chemotherapy
Endometriosis	GnRH agonists, synthetic androgen, oral contraceptives, progestins, suppressive steroids (danazol, gestrinone), surgery (laparoscopy, laparotomy, hysterectomy)
Uterine leiomyoma	GnRH agonists, surgery (myomectomy or hysterectomy)
Central precocious puberty	GnRH agonists
IVF techniques	GnRH agonists, acupuncture, adjunctive interventions (intracytoplasmic sperm injection, zygote intrafallopian transfer, gamete intrafallopian transfer, preimplantation genetic diagnosis)
Contemplated	
Alzheimer's disease	Anticholinesterase inhibitors (donepezil, ENA-713, galantamine, tacrine); NMDA receptor antagonist (memantine)
Functional bowel disease	Stress and diet management (fiber), antispasmodic drugs (anticholinergic agents), antidepressants (SSRIs)
Polycystic ovary syndrome	Anti-androgens (birth control pills, flutamide and so on) and insulin-lowering medications (metformin and so on)
Premenstrual syndrome	Hormonal therapy, antidepressants (SSRIs)
Short stature	Growth hormone, growth hormone + leuprolide acetate, anabolic steroid + leuprolide acetate

GnRH: Gonadotropin-releasing hormone; IVF: *In vitro* fertilization; SSRI: Selective serotonin re-uptake inhibitor.

other gastrointestinal symptoms (primarily with flutamide), pulmonary toxicities, decreased light accommodation, alcohol intolerance and the development of rashes (with nilutamide) that may limit its application [74,75].

3.1.2.4 Other therapies

Due to the fact that tumors eventually become androgen independent with GnRH agonist treatment, intermittent hormone therapy, antiandrogen therapy, nutritional supplementation and variations in the length of hormonal therapy for specific cancer stages are being investigated. Intermittent hormonal therapy has been postulated as a means of allowing tumors to recover their ability to undergo apoptosis. Following an initial period of androgen deprivation (≥ 8 months is required for maximal loss of tumor mass; [32]), removal of GnRH agonist therapy allows testosterone levels to return above castration levels with the androgen-independent cells no longer having a growth advantage. Early experiments in animals have demonstrated a 3-fold prolongation of progression to the hormone-refractory state using this strategy [33]. These hypotheses are now being evaluated in several clinical trials.

3.2 Endometriosis and uterine leiomyoma

Endometriosis, affecting ~ 10% of women who are of child-bearing age, is a gynecologic disorder defined by the presence of endometrial-like tissue located outside of the uterine or endometrial cavity. These lesions are typically found on the ovaries but have been known to grow in many other areas of the pelvic peritoneum. Endometriosis accounts for 15 – 25% of the pelvic pain experienced by women and correlates with the major symptom of dysmenorrhea [76,77]. The benign cells comprising these lesions respond to the normal cyclical changes in HPG axis hormones, directly leading to pain and bleeding. Therefore, treatments have been developed to modulate HPG hormone signaling to prevent endometrial cell growth and bleeding and have included progestogens, synthetic androgens, oral contraceptives and GnRH agonists (**Table 2**) [76,77].

A multitude of clinical studies have specifically focused on the efficacy of leuprolide acetate, among other GnRH agonists, for hormonal regulation of endometriosis. Despite the inability of leuprolide acetate to effectively eliminate these collections of endometrial tissue, which are only amenable to surgery, this drug has proven to be effective in the treatment of symptoms, as well as reducing the size of the lesions. Typically, leuprolide acetate was used in these studies in depot suspension (both the 3.75 mg monthly or 11.25 mg 3 monthly), not exceeding a total treatment period of 6 months [76,78]. One study, involving 52 women, analyzed the safety of leuprolide acetate 3.75 mg administered monthly for 6 months, compared with placebo. By month 3 of treatment, leuprolide acetate clearly had a safe and positive effect in relieving dysmenorrhea and pelvic pain [79]. A number of clinical trials comparing leuprolide acetate to danazol (800 mg/day; a synthetic androgen proven in its

treatment of endometriosis), concluded that leuprolide acetate was equally as effective as danazol in relieving the clinical symptoms as well as reducing the size of the endometrial lesions, albeit there being differences in the side effects experienced by the patients [80-83]. Leuprolide acetate seemed to cause a greater decrease in BMD than danazol; however, one particular trial indicated that danazol also has side effects as it lowered the levels of high-density lipoprotein [82]. Another study compared leuprolide acetate to a subcutaneous depot of medroxyprogesterone acetate. Medroxyprogesterone acetate is equally successful in reducing pain and other symptoms, as leuprolide acetate has been shown to do multiple times, but, again, it had less of an effect on BMD than leuprolide acetate [78]. Present research is focusing on the use of leuprolide acetate, with the addition of 'add-back' therapy consisting of estrogen and/or progesterone to limit side effects. Preliminary studies indicate that add-back of sex steroids with leuprolide acetate treatment provides extended pain relief and preservation of BMD [58,59].

Uterine leiomyomata, also referred to as myomas or fibroids, are benign tumors that arise from smooth muscle cells located within the uterus. Myomas are known to be the most common benign tumor and occur in 20 – 50% of women during their reproductive period. Much as with endometriosis, the symptoms observed include pelvic pressure/pain, dysmenorrhea, bleeding and dysfunction of both the reproductive and adjacent organs. Myomas are responsible for 40% of hysterectomies performed in the US [84]. As with endometriosis, these tumors are responsive to their hormonal environment and therefore hormone suppression using GnRH agonists, especially leuprolide acetate, is used in the management of this disease. Leuprolide acetate is typically used as a preoperative measure to shrink tumor size (~ 3 months). Leuprolide acetate is thought to suppress estradiol and progesterone signaling in reducing uterine leiomyoma size. This effect is completely reversible on termination of treatment, with the tumor being capable of returning to its original size [84,85].

Goserelin and triptorelin have also been used to treat menorrhagia and induce endometrial thinning prior to hysteroscopic endometrial ablation or resection [86].

3.3 Central precocious puberty

Puberty is defined as the process of biologic, psychologic, social and cognitive maturation into adulthood. This transition during development typically occurs in girls and boys at an average age of 11.5 and 13.5 years, respectively. These changes are thought to be regulated by GnRH secretion into the hypophysial portal circulation leading to downstream activation of the HPG axis [87]. CPP is a rare disease (1:5000 to 1:10000) characterized by the early onset of secondary sexual characteristics in females who are ≤ 8 years of age and in males ≤ 9 years of age (idiopathic or neurogenic). Treatment goals are aimed at both physical and psychologic levels including ensuring social/mental well-being, prevention of early menarche and maintenance of normal body proportions and height growth. The use of GnRH agonists in the treatment of CPP dates back to the 1980s when these drugs were first designed. A multitude of studies have investigated the effects of GnRH agonists, specifically the routes and frequency of administration in order to make it as child friendly as possible [87]. At present, the starting dose of leuprolide acetate in the US is 7.5 mg/month, while in Europe the starting dose is 3.75 mg/month.

Leuprolide acetate for CPP has progressed from daily subcutaneous injections to monthly intramuscular or subcutaneous depot injections in a variety of doses. The minimal necessary dose required for the monthly leuprolide acetate depot in order to achieve complete desensitization in CPP was assessed in 1992 following measurement of LH pulses, LH concentration and pulse frequency [88]. The conclusions indicated an incomplete desensitization with the use of 7.5-mg monthly injections for some children with CPP, which could exacerbate or promote the disease. Based on this and other findings, a later study emphasized the importance for a GnRH stimulation test used to biochemically monitor the hormonal suppression that is intended to occur with the use of leuprolide acetate [89]. Complete suppression of the gonadotropins, where LH levels are < 2 IU/l, is crucial to the effectiveness of this and all GnRH agonist drugs. Therefore, it is recommended to administer a GnRH stimulation test within the first 3 months of treatment and every 6 months thereafter as needed [89]. As dosing studies continued, a group in France assessed the efficacy of a 3-month leuprolide acetate depot at 11.25 mg/dose [90]. The results of this 6-month open trial indicated successful gonadotropin suppression in children weighing > 20 kg (this quarterly preparation has also been used to treat both endometriosis as well as prostate cancer). Stabilization of puberty progression and a decrease in growth rate were clinical signs of success, as well as the overall tolerance in children due to the reduction in the number of total yearly injections [90]. In contrast, a more recent study performed at Stanford University, California, US indicated that the 1-month dose of leuprolide acetate 7.5 mg was more effective at suppressing gonadotropin levels, pubertal stage and height than either the 1-month dose of 3.75 mg or 3-month dose of 11.25 mg [91].

3.4 *In vitro* fertilization techniques

Leuprolide acetate has proven to be beneficial in controlled ovarian hyperstimulation and IVF outcome when combined with oral contraceptives or with FSH stimulation for more complete androgen suppression [92-94]. The value of GnRH agonists in assisting reproductive treatment stems from their ability to reduce the incidence of a premature LH surge and, therefore, premature luteinization. Additionally, the decrease in bioavailable LH results in lower ovarian androgen concentrations leading to improved oocytes, an increase in

the number and synchronization of developing follicles and an increase in the number of oocytes obtained at retrieval [17,95]. It has been established that the most effective protocol combining GnRH agonists with gonadotropins is the 'long protocol' [95,96]. The purpose of this protocol is to suppress the pituitary before inducing ovulation and in this respect the GnRH agonist is administered in the early follicular phase or the midluteal phase of the previous cycle.

As the GnRH agonists have different chemical structures (**Figure 1**), formulations, potencies, serum half-lifes and routes of administrations, a multitude of studies have looked at the different effects of GnRH agonists in IVF treatment. The most recent study looked at two different modes of administration for leuprolide acetate [96]. One group of 52 women on oral contraceptives were instructed to take daily subcutaneous injections (0.5 mg) of leuprolide acetate, while the other 51 patients were administered a single-dose depot of leuprolide acetate (1.88 mg). Results indicated no significant differences for the number and quality of the oocytes retrieved between both groups. Additionally, the pregnancy and implantation rates were similar. Both methods of administration, the multi-low dose or the single depot were equally effective; however, based on convenience, comfort and cost, the single-depot leuprolide protocol is a much more attractive option for the patients.

Other clinical studies focused on the differences between GnRH agonists. In a comparison of a single depot-dose of goserelin (3.6 mg) and multiple daily doses (1 mg) of leuprolide acetate, the results showed comparable pituitary suppression as well as ovulation induction [95]. The only difference observed was that those patients taking goserelin required more ampoules of human menopausal gonadotropins for superovulation [95]. Leuprolide acetate has also been compared with triptorelin for IVF [97]. As depot preparations have been found to be as effective as daily doses and with patient preference for such a mode of administration, two long-acting depots were compared in this study: triptorelin depot of 3.75 mg and leuprolide acetate depot of 3.75 mg. The results showed statistically higher implantation and clinical pregnancy rates for those patients receiving the leuprolide acetate. It is speculated that these differences may be due to variations in metabolism and potency between GnRH agonists, as mentioned above [97]. Finally, a third study chose to compare three different GnRH agonists: buserelin intranasal spray, nafarelin intranasal spray and long-acting leuprolide acetate depot [98]. Both buserelin and nafarelin were nasal sprays that required multiple daily doses, whereas leuprolide acetate was a single intramuscular injection (3.75 mg). Aside from the occasional allergic nasal reaction in the naferelin group, the three GnRH agonists performed similarly and were equally effective in ovulation induction for IVF. However, as previously mentioned, despite the lack of statistical differences between these GnRH agonists among the IVF parameters measured, leuprolide acetate depot has become the preferred option based on patient convenience, cost and compliance [98].

4. Potential clinical applications

Leuprolide acetate has also been implemented for use in patients with AD, functional bowel disease, PCOS, PMS, short stature, paraphilia and autism, adding to its list of wide-ranging applications [12,19,21].

4.1 Alzheimer's disease

The potential of leuprolide acetate for use in the treatment of neurologic diseases was first indicated by the findings that it modulated classic markers of AD neuropathology (amyloid-β [Aβ]; tau phosphorylation) and prevented AD-related cognitive decline in mouse models [12,14,99]. Specifically, leuprolide acetate has been shown to reduce total brain $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ concentrations by 3.5-fold and 1.5-fold, respectively, after 2 months of treatment in C57BL/6 mice [12]. Leuprolide acetate may modulate Aβ levels via its suppression of serum gonadotropins [12] as *in vitro* studies indicate that LH promotes processing of the Aβ precursor protein (AβPP) towards the amyloidogenic pathway in neuroblastoma cells, as evidenced by the increased generation and secretion of Aβ, decreased secretion of AβPP and increased AβPPCT100 production [12]. It has also been demonstrated that LH dose-dependently alters tau phosphorylation, increases oxidative stress and induces cytotoxicity in neuroblastoma cells [99]. Importantly, it was demonstrated in a mouse model of amyloidosis (Tg2576 mice carrying the Swedish AβPP mutation) that leuprolide acetate halted Aβ deposition in these aged transgenic mice and improved cognitive performance [14]. Although these effects seem to be mediated by LH, it is also possible that leuprolide acetate is mediating its effect directly via GnRH receptors, recently identified on neurons in the human brain [50].

Based on these and another study [100], leuprolide acetate has been tested for its efficacy and safety for the treatment of AD in Phase II clinical trials [206]. Subgroup analysis of cognitive performance in women with mild-to-moderate AD receiving acetylcholinesterase inhibitors and implanted subcutaneously at 0, 12, 24 and 36 weeks with leuprolide acetate showed a stabilization in cognitive decline (Alzheimer's Disease Assessment Scale Cognitive Subscale; Alzheimer's Disease Cooperative Study–Clinical Global Impression of Change) and activities of daily living (Alzheimer's Disease Cooperative Study–Activities of Daily Living Scale) [207] at 48 weeks. Further clinical trials are underway to determine the use of leuprolide acetate for the treatment of neurologic disorders.

4.2 Functional bowel disease

Functional bowel disease, also known as irritable bowel syndrome, is characterized by spasms within the colon wall that are recognized with symptoms such as abdominal pain

and cramping, nausea, constipation or diarrhea. Ultimately, the goal for treating this disorder is to minimize the symptoms associated with it. Present treatments for irritable bowel syndrome include antispasmodics to control muscular spasms and other such muscle relaxants (**Table 2**). In 1989, leuprolide acetate was first assessed as a possible symptom-relieving medication in an informal pilot study [19]. A few years later, a double-blind, placebo-controlled study was conducted with a long-term follow up [101,102]. After the initial 12-week study period using the 3.75-mg monthly depot, patients were allowed to continue treatment for an additional 40 weeks. With 89% of patients completing the entire 52-week treatment plan, the conclusions indicated successful and significant reductions in nausea, abdominal pain, early satiety, anorexia and abdominal distension [101,102].

4.3 Polycystic ovary syndrome

PCOS is a common disorder of unknown etiology that affects women during their reproductive years. This disease is characterized by elevated androgen production that in turn affects menstrual cycle control and the process of ovulation [103]. Typically, therapies involve re-establishing the menstrual cycle (using hormonal contraception and progesterone) and suppression of ovarian steroidogenesis using GnRH agonists. Thus, leuprolide acetate has been investigated as a possible therapy option [103,104] and studies indicate that leuprolide acetate is equally as effective as laparoscopic laser diathermy [15], another PCOS treatment option. However, the focus, in the use of leuprolide acetate for PCOS, has been on combination therapies for improving ovulation and IVF [93,94].

4.4 Premenstrual syndrome

Leuprolide acetate has been investigated as a therapy for reducing the symptoms associated with PMS, including but not limited to water retention, pain and psychologic function (depression) [21,105-107]. Pilot studies conducted in the early 1990s indicated improvements for these symptoms using two different doses: 3.75- and 7.5-mg monthly injections [21,105]. The former study included 'add-back' therapy of estrogen and progesterone in addition to the GnRH agonist treatment, for long-term use and showed significant improvements in managing symptoms associated with PMS [21]. Together, these studies suggest that PMS may be regulated by the suppression of gonadotropins more so than by sex steroids.

4.5 Short stature

Although controversial [108,109], leuprolide acetate is also used in combination with growth hormone (GH) [20,110] or other anabolic drugs [111] to treat short stature (**Table 2**). One particular study investigated the effects of combined GH and GnRH analog therapy to treat normal, short girls, also referred to as familial short stature. These adolescent girls had neither GH deficiency nor an abnormal onset of puberty. Using a leuprolide acetate dose of 3.75 mg every 25 days with daily injections of GH at a dose of 0.1 IU/kg, the subjects underwent a total treatment period of ~ 28 months. Results indicated a significant improvement of predicted height after 12 months of treatment, as well as at the conclusion of the treatment protocol. However, on withdrawal of the GH plus leuprolide acetate therapy for 2 years, the final height of these girls was not significantly different than their height at the start of the treatment. Overall, no significant increase in adult stature was observed once the treatment period ceased [20].

Leuprolide acetate was also investigated for the treatment of short stature in adult patients with congenital adrenal hyperplasia and compromised height prediction [110]. Patients were treated for a total of 2 years with a daily injection of GH (0.3 mg/kg) and a monthly injection of leuprolide acetate (300 µg/kg every 28 days). In general, both the GH treatment alone and the combined GH treatment with leuprolide acetate showed significant improvement in growth rate and height prediction [110]. Finally, leuprolide acetate has also been combined with anabolic steroids to address the problem of short stature. Two different case studies using this therapy indicated an increase in pubertal height gain for these two children that entered puberty with short stature for different reasons [111].

4.6 Paraphilias and autism

Leuprolide acetate has also been considered as a possible treatment for paraphilia [112] and perhaps for autism [113].

5. Conclusions

The diversity of diseases and conditions treated by GnRH agonists such as leuprolide acetate illustrates the centrality and importance of GnRH signaling in health and disease. Aside from their affects on the HPG axis, continued studies on i) the signaling of GnRH and its analogs via GnRHR; ii) of different GnRHR variants; iii) of potential non-receptor–mediated affects; iv) of receptor distribution; and v) of ligand distribution, concentration and affinities, may explain their different therapeutic effects. Irrespective of the mechanism of action, the clinical uses of GnRH agonists and antagonists are likely to increase even further considering how changes in reproductive hormones modulate diseases of the elderly [114]. Indeed, GnRH analogs have been suggested as an alternative for modulating the HPG axis for extending longevity [114].

6. Expert opinion

The early observation that acute GnRH agonist administration caused a marked and prolonged increase in gonadotropins, but paradoxically, the chronic administration of GnRH agonists such as leuprolide acetate resulted in chemical castration (decreased 17β-estradiol and atrophy of ovaries in female rats, decreased testosterone, decreased

plasma testosterone, weights of testes and accessory sex organs in male rats [115,116]) opened the way for the use of GnRH analogs in various reproductive scenarios, as well as their use in treating cancer [117-120].

An initial mechanism of action invoked to explain GnRH agonist activity in prostate cancer was the decrease in serum plasma testosterone levels, which has also been observed following castration, with both treatments leading to the regression of the prostate gland/tumor. More recent studies in other diseases have indicated that GnRH agonist activity may be explained by the suppression of plasma gonadotropins as much as the decline in plasma sex steroid levels [13,105,114,121,122]. Most recently, data have indicated that direct mechanisms of action (i.e., GnRH signaling via GnRHRs) rather than indirect mechanisms of action (i.e., suppression of plasma sex steroids and gonadotropins) are central to GnRH agonist action [50,123]. Indeed, GnRH receptors have been localized to the placenta, uterus, ovary, prostate gland, breast, liver, heart, skeletal muscle, kidney and the brain. The ubiquitous nature of GnRHR expression indicates not only the importance of this hormonal signaling pathway for normal tissue function, but also the potential for regulating this pathway in numerous tissues. Therefore, it is expected that the list of clinical applications for leuprolide acetate based on its signaling via GnRHRs will increase in the next 10 years.

The clinical use of leuprolide acetate for approved applications is expected to remain strong. Leuprolide acetate is the most sought GnRH agonist based on its efficacy and ease of administration. In some cases, patient non-compliance with intranasal spray counterparts, together with patient convenience and cost has led to the leuprolide acetate depot becoming a preferred option for reproductive applications [98]. Leuprolide acetate is also the only treatment option available for CPP. There are few drugs that have the diversity of application that leuprolide acetate provides, an attribute that is only expected to increase over the next decade.

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Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Chemical structure of leuprolide acetate.

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