REVIEW

GnRH antagonists in the treatment of advanced prostate cancer

Peter J. Pommerville, MD,[1] Johan G. de Boer, PhD[2]

[1]Can-Med Clinical Research Inc, Victoria, British Columbia, Canada

[2]De Boer Consulting, Victoria, British Columbia, Canada

POMMERVILLE PJ, DE BOER JG. GnRH antagonists in the treatment of advanced prostate cancer. The Canadian Journal of Urology. 2010;17(2):5063-5070.

Analogues of the gonadotropin releasing hormone (GnRH) inhibit the hypothalamic-pituitary-gonadal axis. This has provided treatment modalities for advanced and metastatic prostate cancer. The latest group of analogues, the GnRH antagonists, make promising treatments available that avoid the transient surge in testosterone that occurs with the use of GnRH agonists. Such surges may stimulate tumor growth, causing patients to experience new or worsening cancer symptoms and potential serious adverse effects, including increased bone pain, urinary retention, and spinal cord compression and consequently delay the therapeutic benefits of agonist therapy. Degarelix, an antagonist, recently approved in the United States and Europe, achieves faster, more profound and sustained testosterone suppression and with fewer adverse effects when compared with agonists and other antagonists. This review discusses and compares the compounds degarelix, abarelix, and cetrorelix.

Key Words: prostate cancer, GnRH antagonists, testosterone, treatment modality

Introduction

According to the Canadian Cancer Society, there will be an estimated 25500 new cases of prostate cancer in Canada in 2009, or 28.6% of all cancer types in men with 4400 deaths.[1] This constitutes by far the largest cancer group in men. In addition to mortality, the impact of prostate cancer on life is significant, as for example urinary incontinence and impotence seriously compromise social activities and personal life. An important avenue for treatment was opened nearly 70 years ago when Huggins and Hodges[2] realized that prostate cancer cells require the androgen testosterone for growth. As testosterone is produced in the testes, bilateral orchidectomy, therefore, has been the surgical gold standard of treatment for advanced sex hormone dependent cancer. In addition, antiandrogenic compounds (including nonsteroidals such as flutamide, nilutamide, and cyproterone) block the testosterone receptor on prostate cells. Several studies have shown that this ablation technique has similar efficacy and is generally preferred over orchidectomy.[3]

However, the physiology of hormone cascades offers alternatives. GnRH (gonadotropin releasing hormone or luteinizing hormone releasing hormone, LHRH) is released by the hypothalamus. Cells in the anterior pituitary respond to binding GnRH by releasing

Accepted for publication January 2010

Address correspondence to Dr. Peter J. Pommerville, Can-Med Clinical Research Inc, 330-1641 Hillside Avenue, Victoria, BC, V8T 5G1 Canada

luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the bloodstream. LH subsequently binds to receptors in the Leydig cells in the testes and stimulates testicular steroidogenesis, including the production of testosterone. GnRH also stimulates the release of FSH, which was shown in mice to stimulate binding of LH to the Leydig cells, resulting in an increased response to LH.[4] Testosterone subsequently binds to androgen receptors in the prostate and alters the expression of many genes. In particular, androgenic stimulation is essential for the development and progression of prostate cancer cells, and stimulation of the fibroblast growth factor gene in human prostate cancer cells by the androgen receptor was shown by Gnanapragasam et al.[5] Depriving prostate cancer cells of testosterone results in cell death and tissue atrophy. Prostate cells, as well as androgen dependent and androgen independent prostate cancer cells also have receptors for FSH[6,7] and FSH binding stimulates cancer cell growth. Lowering FSH levels is therefore expected to be beneficial. After 8 weeks of therapy with antiandrogens (bicalutamide, flutamide) or after surgical castration, pituitary and serum FSH levels (as measured by radioimmunoassay and *in vitro* bioassays in rats) were elevated as compared to controls.[8]

This places GnRH at the top of a hormonal signal cascade and has offered a targeted approach for intervention for several decades. Synthesized from a 92 amino acid precursor, GnRH is a decapeptide produced by neurons in the mediobasal hypothalamus. It is secreted in a pulsatile manner as sustained secretion desensitizes its receptor. This short peptide is bound by receptors in several tissues, including in the anterior pituitary, the hypothalamus, and in testes in males and ovaries and placenta in females. The receptor is also expressed in a wide range of other tissue types, including liver, heart, skeletal muscle, and kidney, as well as in the breast tumor cell line MCF-7 and in an ovarian tumor.[9] GnRH has also been shown to be expressed by either androgen dependent or androgen independent prostate cancer cells.[10]

This hormonal cascade has been exploited in several treatment modalities, as reversible chemical castration can be accomplished by interfering in the signal pathway, with fewer irreversible and psychological consequences than orchidectomy.

GnRH analogues

Modification of the GnRH decapeptide molecule with unnatural amino acids yielded a number of analogues, Table 1. The first group of these analogues were agonists that strongly bind to and stimulate the GnRH receptor. These compounds have been a major treatment option for over 2 decades, however, they have several shortcomings, as their binding causes a sharp initial increase in LH level and a subsequent 'surge' in testosterone production. The initial increase in testosterone concentration and concomitant clinical flare as well as microflares is a concern in patients with advanced disease, and may be of particular concern in case of intermittent therapy. The incidence of clinical flare has been estimated at between 4% and 33% of treated patients (reviewed in[11,12]). This testosterone 'surge', which can last 3 to 21 days, can stimulate advanced or metastatic steroid sensitive cancer cells, and is a particular concern in patients with bone metastases, causing severe pain and possible compression of the spinal cord. Agonist treatment, therefore, requires the additional temporary use of antiandrogens. Continued stimulation of the receptor, however, eventually desensitizes pituitary release of LH, which then drops to very low levels after 30 days, resulting in castrate levels of testosterone, defined as ≤ 0.5 ng/mL. A number of

TABLE 1. **Amino acid sequence of GnRH and selected analogues**

GnRH	**pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2**
Agonists	
Buserelin	pGlu-His-Trp-Ser-Tyr-DLeu(tBu)-Leu-Arg-Pro-NHEt
Leuprolide	pGlu-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NHEt
Goserelin	pGlu-His-Trp-Ser-Tyr-Dleu-Leu-Arg-Pro-(Aza)Gly-NH2
Triptorelin	pGlu-His-Trp-Ser-Tyr-DTrp-Leu-Arg-Pro-Gly-NH2
Antagonists	
Abarelix	Ac-DNal-DCpa-DPal-Ser-NaMeTyr-DAsp-Leu-Ilys-Pro-DAla
Cetrorelix	Ac-DNal-DCpa-DPal-Ser-Tyr-DCit-Leu-Arg-Pro-DAla
Degarelix	Ac-DNal-DCpa-DPal-Ser-Aph(Hor)-D4Aph(Cbm)-Leu-Ilys-Pro-DAla
Ganirelix	Ac-DNal-DCpa-DPal-Ser-Tyr-DHar(Et2)-Leu-Har(Et2)-Pro-DAla

studies have voiced concern regarding testosterone levels not reaching castrate levels (< 0.5 ng/mL). For example, in two studies, 34% to 37.5% of patients receiving leuprolide, serum testosterone remained ≥ 2 ng/mL.[13,14] Breakthrough events (≥ 5 ng/mL) have also been reported in 4% of patients receiving goserelin.[15] Tombal[16] and Oefelein et al[17] investigated the failure of achieving castration levels in patients receiving 1 and 3 month depots. Approximately 5% and 13% of patients did not achieve castration levels of 50 ng/mL and 20 ng/mL, respectively.

Modifications at different amino acids positions in the decapeptide affect different properties, including receptor binding and protease sensitivity.[18] Introducing additional amino acid changes, therefore, resulted in a second group of analogues, the antagonists. These blocking compounds occupy GnRH receptors but do not stimulate them, resulting in an immediate and sustained drop in LH levels, thus avoiding the testosterone surge. GnRH antagonists not only block the GnRH receptor, but the antagonist cetrorelix also down-regulates expression of the pituitary GnRH receptor gene in rats.[19-21] Three major antagonists will be discussed in this review.

Cetrorelix

Cetrorelix (Serono, ASTA) is better known for its role in fertility issues, but early studies with GnRH antagonists were performed with cetrorelix in patients with advanced prostate cancer. In a couple of small studies, Gonzalez-Barcena et al,[22,23] observed significant improvements in bladder function, prostate volume, and PSA value, as well as disease-related neurologic symptoms, after twice daily 0.5 mg injections. Five patients with advanced prostate cancer, and rendered paraplegic due to metastatic invasion of the spinal cord, all became ambulatory after 3 to 6 months. However, castration levels of testosterone were only achieved after 6 weeks of treatment. A front-loading regimen was subsequently developed, starting with 10 mg for 2-5 days, followed by 1 mg-2 mg daily, in order to achieve immediate testosterone ablation.[24]

Abarelix

A number of trials have compared the clinical efficacy of abarelix (Praecis) with GnRH agonists in patients with advanced or metastatic disease.[13,25-30] Abarelix was the first antagonist approved by the Food and Drug Administration and was significantly more effective in reducing testosterone levels early on in the treatment compared with agonist treatment. Specifically, no testosterone surge has been observed in any of the treated patients. In a seminal open label study by Koch et al,[26] 81 patients in a multicenter study received 100 mg injections each month for up to a year. An objective improvement rate of 88% was achieved after 85 days. In addition, a reduction in PSA value of 75% and 95% was observed after 15 and 57 days, respectively. A phase III study with 255 patients compared 100 mg abarelix against 7.5 mg leuprolide in an 24 week open label randomized multicenter study.[27] Abarelix was significantly more successful in a rapid lowering of testosterone levels and completely avoided a testosterone surge ($p < 0.001$ for both). Castrate levels were achieved in 68% of the patients on day 8 but in none of the leuprolide patients at that time point. Both treatments resulted in similar levels of adverse effects, frequently related to fatigue. A few studies specifically evaluated the effect of abarelix on FSH levels. Garnick and Campion[29] compared abarelix depot against GnRH "superagonists" and found that abarelix immediately reduced FSH to low sustained levels, while a surge is seen after agonist administration. Beer et al[25,30] concluded that with injections given every 2 weeks for 4 weeks and every 4 weeks for 24 weeks, FSH levels were reduced by approximately 50%.

Degarelix

Degarelix (marketed as Firmagon from Ferring Pharmaceuticals) highlights the latest generation of GnRH antagonists.[31] Studies using animal models demonstrated that degarelix effectively lowered testosterone levels in rats.[32] However, in comparison with abarelix, there was a significantly longer lasting suppression of LH levels after degarelix administration. Concentrations of plasma testosterone were fully suppressed to castration levels for up to 56 days after administration of 2 mg/kg degarelix, significantly lower and longer lasting than with equal doses of abarelix, ganirelix, or azaline B. Increasing the dose of degarelix did not increase the efficacy, but rather increased the duration of suppression. This suggested that its physicochemical properties allowed subcutaneous gel like depot formation after injection and a sustained release of the compound into the blood stream.[32] In fact, plasma concentrations of degarelix in rats remained above 5 ng/mL for up to 41 days after injection before dropping to below 3 ng/mL after 84 days.[31]

Degarelix has undergone three published clinical trials to date, two phase II dose optimization trials and a phase III efficacy trial. In two 1 year phase II trials, 127 and 187 patients, respectively, with confirmed prostate adenocarcinoma were treated with degarelix

using different dosing regimens. Gittelman et al[33] started with 200 mg degarelix followed by either 60 mg or 80 mg monthly injections during the 1 year study. Van Poppel et al[34] treated patients with a starting dose of either 200 mg or 240 mg, and subsequently with 80 mg, 120 mg, or 160 mg monthly maintenance injections. In both these studies, degarelix lowered the testosterone level at day 3 to below 0.5 ng/mL in 89% of patients on 200 mg,[33] and in 88% and 92% in patients on 200 mg and 240 mg, respectively.[34] Testosterone levels remained below 0.5 ng/mL throughout the studies. In both studies, PSA levels were reduced by 90% of the baseline value in 56 days and had decreased by 96% to 98% after 1 year. No testosterone surge or clinical flares were detected in any of the patients.

An optimized dosing regime was used in a 1 year, three-armed, randomized, open label phase III study[35] for a comparison of degarelix (240 mg s.c. starting dose, followed by monthly maintenance injections of 80 mg or 160 mg s.c.) against monthly 7.5 mg i.m. injections of leuprolide. All 610 patients had histologically confirmed adenocarcinoma of the prostate, including localized, advanced, metastatic, and other stages of the disease, and had not received hormonal therapy for at least 6 months prior to the start of the trial. Baseline testosterone and PSA levels were ≥ 1.5 ng/mL and ≥ 2.0 ng/mL, respectively. Three days into the treatment, testosterone levels were ≤ 0.5 ng/mL in 96.1% and 95.5% of the patients in the 240 mg/80 mg and 240 mg/160 mg degarelix groups, respectively, but in none of the patients in the leuprolide arm. Characteristic of a testosterone surge, the median testosterone level in the leuprolide patient group was 65% higher than baseline after 3 days, and did not reach 0.5 ng/mL until 28 days after the start of the treatment. From 28 days till the end of the 1 year study all treatments achieved median testosterone levels between 0.078 ng/mL-0.088 ng/mL.

In the leuprolide group, the antiandrogen bicalutamide could be administered at the start of treatment for clinical flare protection at the discretion of the investigator. A surge in testosterone (defined as a testosterone increase of ≥ 15% from baseline, on any 2 days during the first 2 weeks) occurred in 81% of patients receiving leuprolide alone and in 74% of those receiving leuprolide plus antiandrogen (11% of all leuprolide patients received concomitant bicalutamide). Testosterone increases > 0.25ng/mL (microsurges) occurred in eight patients (4%) in the leuprolide group, and testosterone breakthrough (> 0.5ng/mL) occurring in four of these patients (2%). Neither dosing schedule of degarelix resulted in a testosterone surge or microsurge, both of which were observed with leuprolide.[35]

A significant difference was also seen in the rate of reduction in PSA levels in the three study arms. Leuprolide did not change the median PSA level during the first 7 days and reduced it by 18% after 14 days, compared to 64% and 65% after treatment initiation with 240 mg/80 mg and 240 mg/160 mg degarelix, respectively, after 14 days. All levels approached 90% after 56 days. Concomitant bicalutamide administration, however, made leuprolide induced PSA decline comparative to that seen with degarelix. Concentrations of LH and FSH increased and spiked immediately after commencement of treatment with leuprolide, whereas they dropped immediately in the degarelix treated patients. At the end of the study, FSH levels were never reduced by the same amount in the leuprolide group (54.8%) as compared to that in the degarelix group (88.5% and 89.0% in the 240 mg/80 mg and 240 mg/160 mg groups, respectively). PSA failure rates were similar in all three groups. In a recent study, however, patients with advanced cancer treated with 240 mg/80 mg degarelix experienced an average of 8.9% PSA failure, compared with 14.1% of patients treated with 7.5 mg leuprolide.[36]

A comparison of degarelix and abarelix

A comparison can be made between the 100 mg *(i.m.)* abarelix and 240 mg/60 mg degarelix results from two phase III trial reports.[27,35] Both studies were open label, randomized comparisons in patients with confirmed local or advanced prostate adenocarcinoma. When comparing time points of the treatment, degarelix reduced the testosterone level faster than abarelix. On day 3, only 54% of patients treated with abarelix reached castration levels (0.5 ng/mL), compared to 96.1% of degarelix treated patients. When compared over a longer treatment period, the fraction of abarelix treated patients that remained at castration levels gradually decreased from at least 95% to 92.9% to 90.4 % from day 28 to day 85 to day 169, respectively, after the start of the treatment. In the degarelix treated group, this remained constant at 97.2% over this same time period. A comparison of PSA values shows that 240 mg/80 mg degarelix reduces PSA values by 64% and 83% after 14 and 28 days, respectively. Abarelix, on the other hand, lowered PSA only by 50% after 169 days. Intermediate time points were not reported.

Adverse effects of GnRH antagonists

Most adverse effects associated with GnRH antagonists and related therapies such as GnRH agonists and

orchidectomy, are similar to those seen with androgen deprivation.[37] These include hot flashes, sexual dysfunction, and sequelae of comorbid disorders. In addition, androgen deprivation therapy by orchidectomy results in a decline in lean body mass.[38] However, the reported incidence of various adverse effects differ between studies. Mild to moderate adverse effects occur in a significant number of patients; in 81% in the combined 240 mg/80 mg and 240 mg/160 mg degarelix groups,[35] and in 93% in the abarelix treatment study.[27] Trachtenberg et al[27] reported fatigue as the most common spontaneously reported effect in patients on abarelix (14% of patients). Gittelman et al[33] reported that overall 43% of patients treated with 200 mg/80 mg and 200 mg/160 mg degarelix experienced hot flashes, followed by 20% that reported fatigue. Klotz et al[35] reported that hot flashes occurred in 26% of patients.

Klotz et al[35] also reported that 40% of patients treated with degarelix experienced injection site reactions (e.g. erythema, swelling, pain) compared to less than 1% of patients treated with leuprolide. The majority of injection site reactions with degarelix occurred with the first dose and only 4% with subsequent maintenance doses (240 mg/80 mg). Gittelman et al[33] only reported on injection site pain (7%).

Other AEs reported by Klotz et al[35] included weight increase, back pain, and fatigue (< 10%). Nonfatal adverse effects resulting in discontinuations occurred in 5% and 7% of the patients in the 240 mg/80 mg and 240 mg/160 mg groups, respectively, similar to that seen in the leuprolide control group. Only 1% of patients withdrew from the study due to injection site reactions. More patients died in the leuprolide group (4%) versus both degarelix groups (2%); none were considered related to study drug.

GnRH antagonists promote the release of histamine from mast cells. This results in significant systemic or local anaphylactic adverse effects in many patients. However, modifications at positions 5 and 6 in the decapeptide have significant effect on immunostimulatory activity.[39] Comparison of the release of histamine after administration of antagonists in rats showed that the EC_{50} (causing half maximal stimulation) for degarelix is 1.7 and 130-fold higher than for abarelix and cetrorelix, respectively.[32] No allergic reactions have thus far been reported in degarelix studies, in contrast to experiences with other antagonists.

Androgen deprivation therapy has also been associated with a number of neurological problems, including depression, memory difficulties, and cognitive functioning.[40,41] Two recent studies suggest that GnRH agonist treatment for prostate cancer[42] and for ovarian hormone suppression[43] resulted in a decline in the scores on a number of neuropsychological tests and in effects on the cholinergic system. Neurological effects, however, have also been attributed to an older population of prostate cancer patients.[44] Whether these effects with agonists translate into similar effects with antagonists is not known.

The occurrence of vasomotor hot flashes in patients treated with androgen deprivation is a common and significant adverse effect[45] with a complicated aetiology. Treatment options for managing hot flashes include estrogen, fluvoxamine, gabapentin and clonidine.[45,46]

Bone demineralization and androgen deprivation therapy

Androgen deprivation therapy is associated with significant bone loss during the first year of treatment with subsequent osteoporotic fractures,[47-50] requiring the administration of bisphosphonates to inhibit bone resorption. Results from recent studies in FSH-transgenic mice have suggested that high concentrations of FSH may cause loss of bone mass and that mice without FSH receptor protein do not lose bone mass.[51] Estrogen therapy in women also correlates with decreased FSH levels and increases in bone mass.[52] These findings are of interest as GnRH antagonists achieve significantly lower FSH serum concentration in patients compared with agonists. For example, leuprolide causes an initial spike in FSH concentration which subsequently drops by only 55% compared to 89% after treatment with degarelix.[35]

GnRH antagonists for the treatment of benign prostate hyperplasia

Cetrorelix has been assessed for the treatment of benign prostate hyperplasia (BPH). Comrau-Schally et al[53] treated 13 patients with symptomatic BPH with cetrorelix (5 mg, sc, twice daily for 2 days, followed by 1 mg/day for 2 months) in a phase I/II trial. Evaluation of the international prostate symptom score (IPSS), peak urinary flow rate, quality of life, and prostate volume indicated a significant improvement in all parameters, which continued for at least 85 weeks. Subsequently, 140 patients were treated in a phase II trial by Debruyne et al.[54] Rapid improvements in IPSS score (18.7-19.3 at baseline; 13.0-14.5 after 12 weeks) and in urinary flow rate were observed using a 4 week regimen with 5 mg/wk and 10 mg/wk. Prostate volume also decreased. These improvements lasted

TABLE 2. **Summary of GnRH analogues**

Compound	Name	Manufacturer	Clinical flare observed	Anaphylactic reaction observed
Leuprolide	Several	Generic	Yes	Yes
Cetrorelix	Cetrotide	Serono, ASTA	No	Yes
Abarelix	Plenaxis	Praecis Pharmaceuticals	No	Yes
Degarelix	Firmagon	Ferring Pharmaceuticals	No	No

for at least 12 weeks, even though the concentration of testosterone already returned to normal levels after 4 weeks. These results indicated that cetrorelix is safe and effective in patients with symptomatic BPH. Dose finding trials are currently investigating the use of degarelix for the treatment of BPH.

GnRH antagonists and tumour volume

Reductions in prostate tumor volume in the Dunning rat model were observed after administration of degarelix.[55,56] Using this model, which involves transplantation of the Dunning R-3327H rat carcinoma into the Copenhagen rat, allows evaluation of treatment directly on tumor growth and volume. In a study by Princivalle et al[56] degarelix suppressed testosterone levels in this rat model to below castration levels within 2 days. A sustained inhibition of growth and tumor volume, similar to that seen in castrated rats, was observed, while inhibition by leuprolide depot was intermediate between degarelix and mannitol control. After 223 days, mean tumor volume was 10.6%, 39.7% and 13.1% of controls rats for castrated rats, leuprolide-, and degarelix-treated rats, respectively. This data provides a direct correlation between degaralix treatment and tumor growth, and this is currently being investigated in a phase IIIB clinical trial (ClinicalTrials.gov Identifier: NCT00884273).

Summary

GnRH antagonists provide promising novel treatments for patients with advanced or metastatic prostate carcinoma, because of immediate suppression of testosterone and FSH levels. This avoids the clinical flare associated with the use of GnRH agonists which can be potentially dangerous in some patients with advanced stage cancer. A clinical flare can stimulate cancer cells and result in cord compression and increased pain. Degarelix achieves a castrate level in a higher number of patients and for a longer period. Adverse effects are mostly those associated with androgen deprivation, or are injection site related which occur more frequently with the initial dose and decrease substantially with maintenance doses. Systemic allergic reactions, seen with abarelix and cetrorelix, have not been observed with degarelix. A summary is provided in Table 2. As of December 2009, 26 clinical trials involving degarelix and prostate cancer were registered at ClinicalTrials.gov.

Disclosure

Peter J. Pommerville has received funding from and/or is a clinical investigator for Abbott Laboratories, Æterna Zentaris Inc, Allergan Inc., Amgen Inc., American Medical Systems, Astellas Pharma Inc., Astra Zeneca Inc., Dendreon Corp., Eli Lilly, Ferring Pharmaceuticals, GlaxoSmithKlein Inc., Pfizer Inc, Protox Therapeutics, Spectrum Pharmaceuticals, Inc., and Uromedica Inc. Johan G. de Boer is a consultant for CanMed Clinical Research, Inc. □

References

1. Canadian Cancer Society. Canadian Cancer Statistics 2009. ISSN 0835-2976. 2009. Toronto.
2. Huggins C, Hodges CV. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941;1:293-297.
3. Seidenfeld J, Samson DJ, Hasselblad V, Aronson N, Albertsen PC, Bennett CL, Wilt TJ. Single-therapy androgen suppression in men with advanced prostate cancer: a systematic review and meta-analysis. *Ann Intern Med* 2000;132(7):566-577.
4. Takase M, Tsutsui K, Kawashima S. Effects of PRL and FSH on LH binding and number of Leydig cells in hypophysectomized mice. *Endocrinol Jpn* 1990;37(2):193-203.
5. Gnanapragasam VJ, Robson CN, Neal DE, Leung HY. Regulation of FGF8 expression by the androgen receptor in human prostate cancer. *Oncogene* 2002;21(33):5069-5080.
6. Ben Josef E, Yang SY, Ji TH, Bidart JM, Garde SV, Chopra DP, Porter AT, Tang DG. Hormone-refractory prostate cancer cells express functional follicle-stimulating hormone receptor (FSHR). *J Urol* 1999;161(3):970-976.

7. Mariani S, Salvatori L, Basciani S, Arizzi M, Franco G, Petrangeli E, Spera G, Gnessi L. Expression and cellular localization of follicle-stimulating hormone receptor in normal human prostate, benign prostatic hyperplasia and prostate cancer. *J Urol* 2006;175(6):2072-2077.
8. Simoni M, Weinbauer GF, Chandolia RK, Nieschlag E. Microheterogeneity of pituitary follicle-stimulating hormone in male rats: differential effects of the chronic androgen deprivation induced by castration or androgen blockade. *J Mol Endocrinol* 1992;9(2):175-182.
9. Kakar SS, Jennes L. Expression of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor mRNAs in various non-reproductive human tissues. *Cancer Lett* 1995;98(1):57-62.
10. Wormald PJ, Eidne KA, Millar RP. Gonadotropin-releasing hormone receptors in human pituitary: ligand structural requirements, molecular size, and cationic effects. *J Clin Endocrinol Metab* 1985;61(6):1190-1194.
11. Bubley GJ. Is the flare phenomenon clinically significant? *Urology* 2001;58(2 Suppl 1):5-9.
12. Mahler C. Is disease flare a problem? *Cancer* 1993;72(12 Suppl): 3799-3802.
13. McLeod D, Zinner N, Tomera K, Gleason D, Fotheringham N, Campion M, Garnick MB. A phase 3, multicenter, open-label, randomized study of abarelix versus leuprolide acetate in men with prostate cancer. *Urology* 2001;58(5):756-761.
14. Morote J, Esquena S, Abascal JM, Trilla E, Cecchini L, Raventos CX, Catalan R, Reventos J. Failure to maintain a suppressed level of serum testosterone during long-acting depot luteinizing hormone-releasing hormone agonist therapy in patients with advanced prostate cancer. *Urol Int* 2006;77(2):135-138.
15. Fontana D, Mari M, Martinelli A, Boccafoschi C, Magno C, Turriziani M, Maymone SS, Cunico SC, Zanollo A, Montagna G, Frongia M, Jacobellis U. 3-month formulation of goserelin acetate ('Zoladex' 10.8-mg depot) in advanced prostate cancer: results from an Italian, open, multicenter trial. *Urol Int* 2003; 70(4):316-320.
16. Tombal B. Appropriate castration with luteinising hormone releasing hormone (LHRH) agonists: what is the optimal level of testosterone? *Eur Urol Suppl* 2005;4:14-19.
17. Oefelein MG, Cornum R. Failure to achieve castrate levels of testosterone during luteinizing hormone releasing hormone agonist therapy: the case for monitoring serum testosterone and a treatment decision algorithm. *J Urol* 2000;164(3 Pt 1): 726-729.
18. Fluker MR. Gonadotropin-releasing hormone antagonists. *Curr Opin Endocrinol and Diabetes* 2000;7(6):350-356.
19. Horvath JE, Bajo AM, Schally AV, Kovacs M, Herbert F, Groot K. Effects of long-term treatment with the luteinizing hormone-releasing hormone (LHRH) agonist Decapeptyl and the LHRH antagonist Cetrorelix on the levels of pituitary LHRH receptors and their mRNA expression in rats. *Proc Natl Acad Sci USA* 2002;99(23):15048-15053.
20. Kovacs M, Schally AV. Comparison of mechanisms of action of luteinizing hormone-releasing hormone (LHRH) antagonist cetrorelix and LHRH agonist triptorelin on the gene expression of pituitary LHRH receptors in rats. *Proc Natl Acad Sci USA* 2001; 98(21):12197-12202.
21. Kovacs M, Schally AV, Csernus B, Rekasi Z. Luteinizing hormone-releasing hormone (LH-RH) antagonist Cetrorelix down-regulates the mRNA expression of pituitary receptors for LH-RH by counteracting the stimulatory effect of endogenous LH-RH. *Proc Natl Acad Sci USA* 2001;98(4):1829-1834.
22. Gonzalez-Barcena D, Vadillo-Buenfil M, Gomez-Orta F, Fuentes GM, Cardenas-Cornejo I, Graef-Sanchez A, Comaru-Schally AM, Schally AV. Responses to the antagonistic analog of LH-RH (SB-75, Cetrorelix) in patients with benign prostatic hyperplasia and prostatic cancer. *Prostate* 1994;24(2):84-92.
23. Gonzalez-Barcena D, Vadillo-Buenfil M, Cortez-Morales A, Fuentes-Garcia M, Cardenas-Cornejo I, Comaru-Schally AM, Schally AV. Luteinizing hormone-releasing hormone antagonist cetrorelix as primary single therapy in patients with advanced prostatic cancer and paraplegia due to metastatic invasion of spinal cord. *Urology* 1995;45(2):275-281.
24. Gonzalez-Barcena D, Schally AV, Comaru-Schally AM. Treatment of patients with advanced prostate cancer with LHRH antagonist Cetrorelix. In *Treatment with LHRH analogs: Controversies and Perspectives* (Filicori, M. and Flamigni, C., eds). 1996 pp. 139-145, Parthenon Publishing group, London, New York.
25. Beer TM, Ryan C, Bhat G, Garnick M. Dose-escalated abarelix in androgen-independent prostate cancer: a phase I study. *Anticancer Drugs* 2006;17(9):1075-1079.
26. Koch M, Steidle C, Brosman S, Centeno A, Gaylis F, Campion M, Garnick MB. An open-label study of abarelix in men with symptomatic prostate cancer at risk of treatment with LHRH agonists. *Urology* 2003;62(5):877-882.
27. Trachtenberg J, Gittleman M, Steidle C, Barzell W, Friedel W, Pessis D, Fotheringham N, Campion M, Garnick MB. A phase 3, multicenter, open label, randomized study of abarelix versus leuprolide plus daily antiandrogen in men with prostate cancer. *J Urol* 2002;167(4):1670-1674.
28. Tomera K, Gleason D, Gittelman M, Moseley W, Zinner N, Murdoch M, Menon M, Campion M, Garnick MB. The gonadotropin-releasing hormone antagonist abarelix depot versus luteinizing hormone releasing hormone agonists leuprolide or goserelin: initial results of endocrinological and biochemical efficacies in patients with prostate cancer. *J Urol* 2001;165(5):1585-1589.
29. Garnick MB, Campion M. Abarelix Depot, a GnRH antagonist, v LHRH superagonists in prostate cancer: differential effects on follicle-stimulating hormone. Abarelix Depot study group. *Mol Urol* 2000;4(3):275-277.
30. Beer TM, Garzotto M, Eilers KM, Lemmon D. Phase II study of abarelix depot for androgen independent prostate cancer progression during gonadotropin-releasing hormone agonist therapy. *J Urol* 2003;169(5):1738-1741.
31. Jiang G, Stalewski J, Galyean R, Dykert J, Schteingart C, Broqua P, Aebi A, Aubert ML, Semple G, Robson P, Akinsanya K, Haigh R, Riviere P, Trojnar J, Junien JL, Rivier JE. GnRH antagonists: a new generation of long acting analogues incorporating p-ureido-phenylalanines at positions 5 and 6. *J Med Chem* 2001; 44(3):453-467.
32. Broqua P, Riviere PJ, Conn PM, Rivier JE, Aubert ML, Junien JL. Pharmacological profile of a new, potent, and long-acting gonadotropin-releasing hormone antagonist: degarelix. *J Pharmacol Exp Ther* 2002;301(1):95-102.
33. Gittelman M, Pommerville PJ, Persson BE, Jensen JK, Olesen TK. A 1-year, open label, randomized phase II dose finding study of degarelix for the treatment of prostate cancer in North America. *J Urol* 2008;180(5):1986-1992.
34. Van Poppel H, Tombal B, de la Rosette JJ, Persson BE, Jensen JK, Kold OT. Degarelix: a novel gonadotropin-releasing hormone (GnRH) receptor blocker--results from a 1-yr, multicentre, randomised, phase 2 dosage-finding study in the treatment of prostate cancer. *Eur Urol* 2008;54(4):805-813.
35. Klotz L, Boccon-Gibod L, Shore ND, Andreou C, Persson BE, Cantor P, Jensen JK, Olesen TK, Schroder FH. The efficacy and safety of degarelix: a 12-month, comparative, randomized, open-label, parallel-group phase III study in patients with prostate cancer. *BJU Int* 2008;102(11):1531-1538.
36. Tombal B, Miller K, Boccon-Gibod L, Schroder FH, Jensen JK, Olesen TK, Person B-E. Degarelix vs. Leuprolide treatment in patients with advanced prostate cancer: PSA failures during a randomised, phase III trial (CS21). *Eur Urol Suppl* 2009;8(4):130 (abstract).

37. Higano CS. Side effects of androgen deprivation therapy: monitoring and minimizing toxicity. *Urology* 2003;61(2 Suppl 1): 32-38.
38. Smith MR. Changes in fat and lean body mass during androgen-deprivation therapy for prostate cancer. *Urology* 2004;63(4): 742-745.
39. Rivier JE, Porter J, Rivier CL, Perrin M, Corrigan A, Hook WA, Siraganian RP, Vale WW. New effective gonadotropin releasing hormone antagonists with minimal potency for histamine release in vitro. *J Med Chem* 1986;29(10):1846-1851.
40. Rosenblatt DE, Mellow A. Depression during hormonal treatment of prostate cancer. *J Am Board Fam Pract* 1995;8(4):317-320.
41. Green HJ, Pakenham KI, Headley BC, Yaxley J, Nicol DL, Mactaggart PN, Swanson C, Watson RB, Gardiner RA. Altered cognitive function in men treated for prostate cancer with luteinizing hormone-releasing hormone analogues and cyproterone acetate: a randomized controlled trial. *BJU Int* 2002; 90(4):427-432.
42. Jim HS, Small BJ, Patterson S, Salup R, Jacobsen PB. Cognitive impairment in men treated with luteinizing hormone-releasing hormone agonists for prostate cancer: a controlled comparison. *Support Care Cancer* 2009 Apr 3.
43. Craig MC, Fletcher PC, Daly EM, Rymer J, Brammer M, Giampietro V, Stahl D, Maki PM, Murphy DG. The interactive effect of the cholinergic system and acute ovarian suppression on the brain: an fMRI study. *Horm Behav* 2009;55(1):41-49.
44. Shahinian VB, Kuo YF, Freeman JL, Goodwin JS. Risk of the "androgen deprivation syndrome" in men receiving androgen deprivation for prostate cancer. *Arch Intern Med* 2006;166(4): 465-471.
45. Nishiyama T, Kanazawa S, Watanabe R, Terunuma M, Takahashi K. Influence of hot flashes on quality of life in patients with prostate cancer treated with androgen deprivation therapy. *Int J Urol* 2004; 11(9):735-741.
46. Vilar GS, Montana PF, Aguayo MM, Villas Sanchez MV, Sevillano Capellan MM, Sabater M. S. [Review of current treatment for hot flushes induced by androgen deprivation in prostate carcinoma]. *Actas Urol Esp* 2009;33(4):337-343.
47. Daniell HW. Osteoporosis due to androgen deprivation therapy in men with prostate cancer. *Urology* 2001;58(2 Suppl 1): 101-107.
48. Eastham JA. Bone health in men receiving androgen deprivation therapy for prostate cancer. *J Urol* 2007;177(1):17-24.
49. Israeli RS, Ryan CW, Jung LL. Managing bone loss in men with locally advanced prostate cancer receiving androgen deprivation therapy. *J Urol* 2008;179(2):414-423.
50. Greenspan SL, Coates P, Sereika SM, Nelson JB, Trump DL, Resnick NM. Bone loss after initiation of androgen deprivation therapy in patients with prostate cancer. *J Clin Endocrinol Metab* 2005;90(12):6410-6417.
51. Sun L, Peng Y, Sharrow AC, Iqbal J, Zhang Z, Papachristou DJ, Zaidi S, Zhu LL, Yaroslavskiy BB, Zhou H, Zallone A, Sairam MR, Kumar TR, Bo W, Braun J, Cardoso-Landa L, Schaffler MB, Moonga BS, Blair HC, Zaidi M. FSH directly regulates bone mass. *Cell* 2006;125(2):247-260.
52. Kawai H, Furuhashi M, Suganuma N. Serum follicle-stimulating hormone level is a predictor of bone mineral density in patients with hormone replacement therapy. *Arch Gynecol Obstet* 2004;269(3):192-195.
53. Comaru-Schally AM, Brannan W, Schally AV, Colcolough M, Monga M. Efficacy and safety of luteinizing hormone-releasing hormone antagonist cetrorelix in the treatment of symptomatic benign prostatic hyperplasia. *J Clin Endocrinol Metab* 1998;83(11):3826-3831.
54. Debruyne F, Gres AA, Arustamov DL. Placebo-controlled dose-ranging phase 2 study of subcutaneously administered LHRH antagonist cetrorelix in patients with symptomatic benign prostatic hyperplasia. *Eur Urol* 2008;54(1):170-177.
55. Pinski J, Reile H, Halmos G, Groot K, Schally AV. Inhibitory effects of analogs of luteinizing hormone-releasing hormone on the growth of the androgen-independent Dunning R-3327-AT-1 rat prostate cancer. *Int J Cancer* 1994;59(1):51-55.
56. Princivalle M, Broqua P, White R, Meyer J, Mayer G, Elliott L, Bjarnason K, Haigh R, Yea C. Rapid suppression of plasma testosterone levels and tumor growth in the dunning rat model treated with degarelix, a new gonadotropin-releasing hormone antagonist. *J Pharmacol Exp Ther* 2007;320(3):1113-1118.