

# The Role of the FSH System in the Development and Progression of Prostate Cancer

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## Abstract

This article describes relationships between follicle-stimulating hormone (FSH), vascular endothelial growth factor (VEGF), and other modulators of prostatic cancer, in order to help optimize treatment decisions. A comprehensive literature search of PubMed and relevant congress abstract databases was conducted using combinations of the key words *prostate cancer, follicle-stimulating hormone, vascular endothelial growth factor, inhibins/activins, gonadotropin-releasing hormone (GnRH)/luteinizing hormone-releasing hormone (LHRH) receptor agonists/antagonists, and angiogenesis/neogenesis*. This was followed by a consensus meeting of prostate cancer experts to discuss current knowledge surrounding FSH and the relevant evidence for its role in the development and progression of prostate cancer.

Our understanding of prostate cancer and its progression indicate an increase in levels of FSH and the FSH receptor, along with alterations in key modulatory proteins that regulate FSH synthesis and receptor signaling. Elevations in VEGF and differential modulation of testosterone with GnRH/LHRH agonists and antagonists may contribute to treatment outcomes. Considerable evidence supports the hypothesis that dysregulation of the FSH system plays a role in both the development and progression of prostate cancer. Data indicate that FSH can be modulated by the choice of treatment intervention, especially when androgen-deprivation therapy is used.

**Key Words:** FSH, GnRH/LHRH receptors, androgen-deprivation therapy, prostate cancer, PSA, angiogenesis

## Introduction

It is likely that dysregulation of the follicle-stimulating hormone (FSH) system plays a significant role in the progression of abnormal prostate growth from benign prostatic hyperplasia (BPH) to hormone-dependent prostate cancer to castration-resistant prostate cancer (CRPC). This review will focus on the data supporting this role of FSH in prostate cancer, and begin to draw together evidence of the mechanisms by which the FSH system may be dysregulated. Finally, we will discuss the impact that pharmacologic androgen-deprivation therapy (ADT) with gonadotropin-releasing hormone, also called luteinizing hormone-releasing hormone (GnRH/LHRH), receptor agonists and antagonists may have on the FSH system.<sup>1-5</sup> Throughout this review, we use

the term *FSH system* to encompass all aspects of FSH, including the synthesis, release, and circulating levels of FSH itself, as well as its receptor and receptor signaling.

## Follicle-Stimulating Hormone

FSH is a 30 kDa heterodimeric glycoprotein that belongs to a class of proteins that includes luteinizing hormone (LH), thyroid-stimulating hormone, and human chorionic gonadotropin. Structurally, these glycoproteins share a common alpha subunit, but have unique beta subunits that confer receptor specificity.<sup>6</sup> FSH binds to the FSH receptor, which belongs to the G-protein coupled superfamily characterized by their 7 hydrophobic transmembrane domains comprising intracellular and extracellular helices. The FSH receptor is coupled to the G<sub>s</sub> subtype, which activates cyclic AMP (cAMP) when the receptor is activated by FSH.<sup>7,8</sup>

FSH was traditionally thought to be synthesized and secreted solely from the anterior pituitary in response to the binding of GnRH/LHRH to its receptor. GnRH/LHRH is released from the hypothalamus in a pulsatile manner and stimulates the production and secretion of both FSH and LH. However, additional research indicates that there are extrapituitary sources of FSH such as the prostate,<sup>9,10</sup> testes, gastrointestinal tract, and breast.<sup>11</sup> The normal physiologic targets and functions of FSH are numerous. In females, FSH stimulates the maturation of germ cells, maintains ovarian follicle development by augmenting growth of granulosa cells of the ovarian follicle, and synergizes with LH to increase the production of ovarian estrogen. In males, FSH stimulates the Sertoli cells in the seminiferous tubules of the testes to produce androgen-binding protein.

## Prostate Cancer

Prostate cancer is the most common noncutaneous-related cancer in men, and the American Cancer Society estimated over 230,000 new cases of prostate cancer and nearly 30,000 deaths in the United States in 2014.<sup>12</sup> Early research demonstrated a pivotal role for androgens (dihydrotestosterone, testosterone) and estrogens in the regulation of prostatic growth and function. However, recent data additionally indicate an important role for nonandrogenic hormones, in both the normal physiology and

pathophysiology of the human prostate. In addition, the prostate gland itself synthesizes FSH<sup>10</sup> and expresses FSH receptors in pathologic states (BPH and prostate cancer).<sup>13,14</sup>

The National Comprehensive Cancer Network (NCCN) Guidelines recommend ADT as the primary systemic therapy for advanced disease, or as neoadjuvant/concomitant/adjuvant therapy in combination with radiation in localized or locally advanced prostate cancers. While low levels of androgens were traditionally achieved using bilateral orchiectomy or estrogen treatment, the most common current methods of ADT are the use of GnRH/LHRH receptor agonists, antagonists, and combined androgen blockade (CAB). Most prostate carcinomas will respond initially to ADT, though a castration-resistant state invariably emerges with ongoing therapy. This conversion is typically associated with an acceleration of the disease and requires treatment with alternative therapies, such as agents that inhibit angiogenesis, androgen receptors, insulin-like growth factor, endothelin receptors, and Src family kinases.<sup>15</sup>

### Literature Search

A colloquium of prostate cancer experts was convened in 2011 to discuss current knowledge surrounding FSH and the relevant evidence for its role in the progression of prostate cancer. A comprehensive literature search of PubMed and relevant congress abstract databases was conducted using combinations of the key words *prostate cancer*, *follicle-stimulating hormone*, *vascular endothelial growth factor*, *inhibins/activins*, *gonadotropin-releasing hormone (GnRH)/luteinizing hormone-releasing hormone (LHRH) agonists/antagonists*, and *angiogenesis/neogenesis*. Basic science and clinical studies that reported an association between FSH, its regulatory pathways, and downstream effects and prostate cancer were selected for further review. Data from selected studies were presented, reviewed, and discussed by the authors. During the writing of this review, an updated search of the literature was conducted.

### Findings

#### *Levels of FSH and FSH Receptors and Prostate Cancer*

In addition to its synthesis and secretion from the anterior pituitary, it has been apparent for some time that FSH and its receptor are also generated in benign and malignant human prostate cells.<sup>10,13,14,16,17</sup> Data also demonstrate a correlation between levels of FSH and its receptor and the malignancy status of prostate cancer. For example, Heracek et al<sup>18</sup> analyzed serum levels of FSH in 250 men who underwent radical retropubic prostatectomy for histologically confirmed prostate cancer, and reported significantly higher levels of serum FSH in patients with locally advanced prostate cancer as compared with localized cancer ( $7.07 \pm 0.65$  U/L vs  $5.63 \pm 0.31$  U/L). Mariani et al<sup>14</sup> found that FSH receptor expression was either low or nondetectable in normal prostate tissue and BPH, while higher FSH receptor gene expression was consistently observed in samples from prostate cancer

tumors, suggesting that receptor gene expression may increase with the progression of cancer.

Additionally, recent data demonstrate that, in general, the majority of metastatic tumors also express FSH receptors. Siraj et al<sup>19</sup> investigated the density of FSH receptors in metastatic tumors in 6 different tissues (liver, lymph node, bone, lung, pleura, and brain) that originated from 6 different primary tumors in lung, breast, prostate, colon, kidney, and leiomyosarcoma, respectively. The authors reported that approximately 60% to 70% of blood vessels associated with prostate cancer metastases in the brain and lymph nodes stained positively for FSH receptors. This is an important finding because metastatic tumors are primarily responsible for the terminal illnesses that cause nearly 90% of human cancer deaths.<sup>20</sup> Elevated expression of FSH receptors has been shown to be specific to tumoral tissue; Siraj et al<sup>19</sup> reported an absence of FSH receptor expression in nontumoral tissue taken from patients with no known history of cancer, and Radu et al<sup>17</sup> reported a similar finding in other nonmalignant inflammatory, regenerative, or proliferative tissues.

Converging evidence indicates a direct role for FSH in the development of metastatic disease. The receptor is located on the luminal endothelial surface,<sup>17</sup> which suggests FSH may play a role in tumor intravasation, a key component in the metastatic process by which malignant cells penetrate the endothelium and enter the circulation. In addition, the dense expression of FSH receptors in vessels at the periphery of tumors,<sup>17,19</sup> where the tumor interacts with the stroma, further suggests that these receptors may be relevant to the metastatic process.<sup>14</sup> Prostate cell growth can be stimulated by exogenous FSH in castration-resistant cell lines,<sup>13</sup> and stimulation of FSH receptors in these cell lines was associated with an increase in cAMP levels,<sup>14</sup> indicating that these receptors are functional.

#### *Modulators of the FSH System*

The regulatory pathways that control the synthesis and release of FSH from the anterior pituitary and the periphery are extremely complex. Thus, an in-depth review of every modulating protein that could influence the FSH system and their potential role in the development and progression of prostate cancer is beyond the scope of this review. There are, however, a few proteins that are of particular interest with respect to prostate cancer.

#### *Prostatic Inhibin Peptide*

Prostatic inhibin peptide (PIP) is an FSH-regulating peptide produced by both the prostate and testes. PIP potently inhibits the synthesis and secretion of FSH from the anterior pituitary and prostate,<sup>21</sup> and, in vitro, inhibits the tropic effects of FSH on the growth of PC3 cells.<sup>22</sup> Moreover, in vivo, when anaplastic castration-resistant cells (Mat-LyLu) were injected into rats, daily PIP treatment over 14 days significantly decreased tumor growth by up to 38%, while concurrently lowering levels of FSH by up to 60%,<sup>22</sup> indicating the ability of PIP to inhibit FSH and its

physiologic effects. Zhang et al<sup>23</sup> conducted a study investigating the level of PIP immunorexpression in healthy human (control) prostate tissue, BPH, prostatic intraepithelial neoplasia (PIN), and prostate cancer. Strong PIP immunorexpression was detected in the epithelium and stroma of normal prostate tissue and BPH, but was significantly lower in the PIN and prostate cancer samples. Moreover, there was a gradation in the density of PIP expression in accordance with the grade of cancer; high-grade PIN tended to lack PIP more than low-grade PIN, and PIP immunorexpression was significantly lower in prostate cancer tissue compared with PIN. In contrast, prostate cancer tissue exhibited the highest PSA reactivity in a greater proportion of tumor cells, though there was no correlation between levels of PIP and PSA, likely due to the relatively small sample number. Given the critical role for PIP in the negative regulation of FSH release and the manner in which PIP levels decrease in conjunction with the progression of hyperplasia, these data add further weight to the hypothesis that dysregulation of the FSH system plays a significant role in the development and progression of prostate cancer.

#### *Regulators of G-Protein Signaling*

As we have alluded to thus far in this review, much basic and clinical research has shown that excessive stimulation of the FSH system is likely to play an important role in the transition toward advanced prostate cancer, whether it is due to unregulated FSH release or overexpression of the FSH receptor. Important regulators in G-protein coupled receptor signaling that may underpin aberrant receptor signaling are regulators of G-protein signaling (RGS) proteins, which accelerate the rate of guanosine-5'-triphosphate (GTP) hydrolysis of the receptor, thus inactivating the G-protein and terminating the signaling cascade. Recent research has indicated that key RGS proteins are decreased in prostate cancer, and one in particular, RGS2, has been suggested to play a direct role in the progression to CRPC. Cao et al<sup>24</sup> demonstrated a 30-fold decrease in RGS2 expression in aggressively growing CRPC cells (LNCaP-C81) when compared with slower-growing androgen-dependent cells (LNCaP-C33), a finding that the authors repeated with another CRPC cell line, CWR22Rv1.<sup>24</sup>

Additionally, exogenously added RGS2 functioned as a potent cell growth inhibitor, and suppressed colony growth of LNCaP-C81 cells by 70% and reduced castration-resistant PSA secretion by 90%.<sup>24</sup> Given that RGS2 proteins have been shown to profoundly inhibit G<sub>s</sub>-coupled signaling<sup>25</sup> and FSH receptors rely on G<sub>s</sub> proteins for their signaling, substantial decreases in the concentration of RGS2 proteins represent yet another mechanism by which FSH receptor-mediated signaling may become dysregulated. Of note is a recent study by Sethakorn and Dulin.<sup>26</sup> These authors performed an analysis of publicly available gene expression datasets and discovered that alterations in the expression of the RGS gene was associated with a multitude of other cancer types, suggesting that RGS proteins may contribute to development and progression of a wide variety of cancers.

#### *Other Modulators*

Activins, together with inhibins, are members of the transforming-growth factor  $\beta$  superfamily. Activins are multifunctional cytokines that regulate critical phases of development, play a role in reproduction, counterbalance the effects of inhibins, and play a role in the regulation of FSH synthesis and release from the anterior pituitary.<sup>27,28</sup> Four mammalian activin subunits have been identified ( $\beta_A$ ,  $\beta_B$ ,  $\beta_C$ ,  $\beta_E$ ), and individual subunits are able to dimerize to produce functional homo- or heteromers.<sup>29</sup> Prostate epithelium of malignant and nonmalignant tissue in men with prostate cancer is a site for activin A and B subunit expression.<sup>30</sup> Activin A (composed of  $\beta_A\beta_A$ ) possesses potent growth-inhibiting properties in LNCaP and DU145 cells<sup>31</sup>; however, PC3 cells have been shown to be insensitive to the effects of activin A, leading some researchers to propose that progression to CRPC may be due, in part, to the acquisition of activin A insensitivity.<sup>32</sup> Follistatins are proteins made and secreted by folliculostellate cells in the anterior pituitary. They function to regulate FSH secretion and have affinity for activins via their  $\beta$  subunit,<sup>33</sup> to which they bind and inhibit. Evidence suggests follistatins may also play a separate role in angiogenesis<sup>34</sup> and in the pathogenesis of bone metastasis. A recent study reported higher levels of follistatin in patients with prostate cancer compared with BPH patients.<sup>35</sup> Moreover, there was a positive correlation in cancer patients between levels of follistatin and PSA serum concentrations and the presence of bone metastasis, possibly through the ability of follistatin to modulate bone morphogenic protein-7 activity.<sup>36</sup>

Other important modulators that may have relevance in the control of FSH secretion from the anterior pituitary include kisspeptin, neurokinin B, and dynorphin. Originally described as a suppressor of metastasis, kisspeptin, along with neurokinin B and dynorphin, is expressed in hypothalamic neurons, and all 3 play a pivotal role in controlling the hypothalamic-pituitary-gonadal axis through the regulation of GnRH/LHRH secretion.<sup>37,41</sup> The influence of these peptides in the development and progression of prostate cancer is uncertain, but given their ability to modulate the anterior pituitary and FSH, more research is needed.

#### *Downstream Effects of FSH Signaling*

Angiogenesis is a pivotal process in the growth, progression, and metastasis of solid tumors.<sup>42</sup> In the past few years it has been increasingly recognized that FSH acts as an important mitogen and a positive tropic signal of tumor angiogenesis through its influence on vascular endothelial growth factor (VEGF).<sup>43,47</sup> Following binding to its receptor, FSH initiates an extensive signaling cascade that is central to the activation of its target genes. Briefly, G<sub>s</sub> activates cAMP, which leads to increased levels of protein kinase A, stimulation of cAMP response element-binding protein (CREB), and upregulation of the PI3-kinase/AKT pathway.<sup>48</sup> One of the factors upregulated by FSH-induced CREB is hypoxia inducible-factor (HIF)-1 $\alpha$ , which can be activated under

normoxic conditions to enhance protein synthesis via the PI3-kinase pathway.<sup>49</sup> HIF-1 $\alpha$  activates genes that promote vascularity, of which the VEGF receptor is one. While there are 5 different isoforms of VEGF obtained through alternative splicing (VEGF-A to E), VEGF-A is considered the most important form in tumoral tissue,<sup>50</sup> though not all studies have precisely identified which isoform they quantified.

VEGF-A can induce vascularization around growing tumor cells, and is overexpressed in prostatic tumors such as BPH and prostate cancer.<sup>51</sup> A strong positive correlation between plasma levels of VEGF-A and Gleason scores has been reported in patients following radical prostatectomy,<sup>52</sup> and George et al<sup>53</sup> found that plasma VEGF levels were negatively correlated with duration of survival in patients with CRPC. Patients with VEGF levels  $\leq 64$  pg/mL had a longer median survival compared with patients with levels  $> 64$  pg/mL; indeed, with every combination evaluated, including the median level, plasma VEGF was significantly prognostic for duration of survival. Moreover, plasma levels of VEGF have been shown to be increased in patients with metastatic prostate cancer, and levels of VEGF were positively correlated with advanced stages of the disease.<sup>52,54,56</sup>

In a recent study measuring plasma and tumor levels of VEGF, Tomic et al<sup>57</sup> showed that CRPC was associated with higher intratumor levels of VEGF compared with hormone-naïve tumors before the initiation of ADT.<sup>57</sup> After 3 months of ADT, patients had an increase in intratumoral VEGF. However, levels of VEGF in the plasma decreased after the 3-month course of ADT, and began to rise again at time of tumor relapse. One hypothesis that may explain these results is that patients with CRPC have rising FSH levels leading to VEGF expression, likely mediated by the elevated density of FSH receptors, which have been shown to be expressed on prostate tumors.<sup>14,17</sup>

Targeting angiogenesis has always been a potential approach in the treatment of advanced prostate cancer. Recently Anai et al<sup>58</sup> applied a dual approach by treating PC3 cells with both antisense *Bcl-2* (an oncogene overexpressed in cancer cells) and bevacizumab (a monoclonal antibody that inhibits angiogenesis by targeting VEGF-A). While antisense *Bcl-2* alone reduced tumor size by 41% and bevacizumab alone reduced growth by 50%, the combination of the 2 resulted in a reduction of tumor size by more than 95%. Moreover, reduction in size was associated with the induction of apoptosis, inhibition of angiogenesis, and reduced chemokine production.<sup>58</sup> Iacobelli<sup>59</sup> demonstrated the effectiveness of bevacizumab treatment in a patient with hormone-refractory prostate cancer. PSA levels fell by about 80% after 6 months, from 14 ng/mL to 4 ng/mL. Interestingly, when bevacizumab treatment was discontinued to allow the patient to undergo dental surgery, PSA levels returned to around 6 ng/mL, suggesting continuous treatment was necessary to maintain low PSA levels.

However, despite these promising data, many recent phase 3 clinical trials investigating inhibitors of either VEGF or angiogenesis in patients with metastatic CRPC have failed to meet their primary end points. For example, patients in the CALGB 90401

study receiving bevacizumab, in addition to standard docetaxel and prednisone, failed to show significant improvement in overall survival (OS) when compared with patients receiving placebo in addition to docetaxel and prednisone.<sup>60</sup> These data were similar to those from the VENICE study, where the combination of aflibercept (also known as VEGF-Trap, a recombinant fusion protein that binds to and inhibits VEGF-A, VEGF-B, and platelet-derived growth factors) with docetaxel resulted in no improvement in OS and a higher incidence of toxicity, as compared with placebo and docetaxel.<sup>61</sup> An endothelin A receptor antagonist, zibotentan, also recently failed to demonstrate a significant improvement in OS when combined with docetaxel compared with placebo,<sup>62</sup> despite previous evidence of potential efficacy in an earlier phase 2 trial.<sup>63</sup>

#### *Impact of Pharmacologic ADT on FSH*

Currently, the mainstay of ADT in the treatment of advanced prostate cancer is pharmacologic treatment with GnRH/LHRH agonists or antagonists to achieve castration levels of testosterone, defined as serum levels  $< 50$  ng/dL. Effects on testosterone levels are downstream to the reduced release of gonadotropins (LH and FSH) from the anterior pituitary.

#### *Effects of GnRH/LHRH Agonists on FSH*

While chronic treatment with agonists eventually results in downregulation of the GnRH/LHRH receptor, this process takes a considerable amount of time, and the clinical benefit of chronic GnRH/LHRH receptor agonist treatment on levels of LH, FSH, and testosterone is sometimes not seen for several weeks. One explanation for this lag in therapeutic benefit may be the manner in which GnRH/LHRH receptors are regulated by agonists. G-protein coupled receptors are typically desensitized and downregulated in response to sustained agonist stimulation through phosphorylation of key sites on the intracellular carboxy-terminal tail, leading to binding of  $\beta$ -arrestin, thereby targeting them for internalization. GnRH/LHRH receptors, however, are unique because they are the only G-protein coupled receptor known to lack carboxy-terminal tails, and therefore do not undergo rapid desensitization.<sup>64</sup> Hence, internalization occurs at an exceptionally slow rate,<sup>65</sup> which may account for the time it takes for GnRH/LHRH agonists to produce therapeutically low levels of serum LH, FSH, and testosterone.

Prior to the downregulation of the receptor, in response to the supraphysiological activation by the agonist, a counterproductive surge in levels of LH, FSH, and testosterone occurs in around 80% of patients.<sup>70</sup> This can result in significant adverse consequences (clinically termed “flare”), such as acute spinal cord compression, bone pain, and ureteral/urethral obstruction.<sup>66,68</sup> In an attempt to reduce flare, patients are often prescribed antiandrogens such as bicalutamide (termed *combined androgen blockade*). Antiandrogens do not block the initial surge in testosterone per se, but rather block the binding of testosterone to the androgen receptor and consequent signaling. However, despite the combined androgen blockade, surges still occur during the



initial stages of agonist treatment,<sup>69,70</sup> and microsurgies in the levels of testosterone occur with GnRH/LHRH agonist treatment in approximately 6% of patients.<sup>70</sup>

Once FSH nadir is achieved, however, levels of FSH begin to rise steadily throughout the course of treatment. The precise mechanism underlying this gradual increase is not known, but it could be due to either residual signaling of the GnRH/LHRH pathway (for example, receptor reserve) or changes in the expression of other proteins involved in regulation of FSH such as PIP, as discussed earlier. Porter et al<sup>71</sup> hypothesized that the reduced FSH secretion may cause an involution of the Sertoli cells and seminiferous tubules, leading to a reduction in both testosterone and testicular PIP. The disinhibition of the anterior pituitary from reduced PIP may then result in unregulated elevations in FSH.

Clinically, this multidirectional regulation of FSH levels with GnRH/LHRH agonist treatment is well characterized. For example, a study by Santen et al<sup>72</sup> of 22 patients with prostate cancer treated with D-Leu6- GnRH/LHRH proethylamide reported an initial decrease of around 66% in FSH levels compared with baseline, after 10 to 11 weeks of treatment. After reaching nadir, FSH levels began rising between weeks 25 to 97, and by study end (week 97) were only between 10% to 20% lower than baseline. Similarly, a study by Huhtaniemi et al<sup>73</sup> measured serum bioactive (B) and immunoreactive (I) FSH levels in 5 patients with prostate cancer during treatment for 6 months with the GnRH/LHRH agonist analog buserelin, and for up to 12 weeks after subsequent orchidectomy. After the initiation of treatment with buserelin, FSH bio- and immunoactivities both transiently increased two- to threefold for 1 to 3 days. The increase in bioactivity was greater and more prolonged than the changes in FSH immunoactivity, and the B/I ratio increased nearly 7-fold in 2 weeks. Serum FSH immunoreactivity declined to below the pretreatment level by day 5 and remained suppressed for the rest of the treatment period. In contrast, serum FSH bioactivity did not decrease significantly below baseline during the 6-month treatment period, although the B/I ratio returned slowly toward baseline values. After bilateral orchidectomy, both FSH activities increased dramatically, presumably due to removal of negative feedback, and the ratio between bioactive and immunoreactive FSH also rose from 1.5 to 7 in 2 weeks.

McLeod et al<sup>74</sup> conducted a large clinical trial comparing the effects of leuprolide (agonist) and abarelix (antagonist). These investigators noted that median levels of FSH increased 300% above baseline in the leuprolide group on day 2 of treatment, returned back to baseline by day 4, and were reduced by 72% by day 15. Following nadir, FSH levels then gradually rose through day 85 of the study. Parallel findings were noted by Trachtenberg et al<sup>75</sup> for depot GnRH/LHRH formulations. A phase 3 randomized controlled trial by Klotz et al<sup>70</sup> reported a similar pattern of agonist-induced regulation of FSH levels. Serum levels of FSH were 146% above baseline on day 1, and then reduced by 76% on day 14 of leuprolide treatment. Following nadir, FSH then increased until day 56 and remained elevated until day 364, pla-

teating at 55% of baseline.

One of the controversial issues in the field of prostate cancer has been the apparently opposing outcomes from 2 large phase 3 double-blind, randomized, controlled trials that investigated the effectiveness of flutamide (an antiandrogen), administered either concurrently with a GnRH/LHRH agonist or following bilateral orchiectomy, on improving survival in patients with advanced prostate cancer. The first study by Crawford and colleagues,<sup>76</sup> in which patients received leuprolide only or leuprolide plus flutamide, reported significantly longer progression-free survival (PFS) and improved median length of survival in the leuprolide/flutamide group. In contrast, the second study, conducted by Eisenberger et al,<sup>77</sup> reported that flutamide following bilateral orchiectomy failed to provide any additional benefit in PFS or length of survival, compared with orchiectomy and placebo. The rationale behind these studies was that residual testosterone, produced by either the prostate cancer cells themselves or the adrenal glands,<sup>78-80</sup> may stimulate tumor growth. Inhibiting its actions directly at the receptor, therefore, would improve survival. While the lack of therapeutic benefit in the latter study would seem to argue that the advantage of combined androgen blockade in patients with metastatic prostate cancer is negligible, these disparate outcomes could be explained in light of FSH serum levels. Bilateral orchiectomy is well known to substantially increase circulating levels of FSH due to removal of the negative feedback regulating secretion from the anterior pituitary.<sup>73</sup> Therefore, any therapeutic benefit from the flutamide in the orchiectomized/flutamide arm may have been masked by the physiological overdrive resulting from the mitogenic and angiogenic effects of FSH.

#### *Effects of GnRH/LHRH Antagonists on FSH*

In contrast to agonists, GnRH/LHRH receptor antagonists directly block the GnRH/LHRH receptor, and therefore do not rely on downregulation to attenuate the physiologic actions of GnRH/LHRH. As a result, levels of LH, FSH, and testosterone are lowered rapidly and to a significant degree. For example, abarelix reduced median FSH levels 38% below baseline by day 2, 63% by day 4, and 75% by day 15.<sup>74</sup> Degarelix, a third-generation GnRH/LHRH antagonist, reduced FSH levels by 80% on day 1 and maintained profound FSH suppression (89%) through day 364 in the phase 3 clinical trial CS21.<sup>70</sup> Patients who participated in CS21 were given the opportunity to enter the US Food and Drug Administration-mandated extension trial, CS21A, in which they either continued to receive degarelix or were "crossed over" from leuprolide to degarelix. Interestingly, FSH levels remained very low in patients who continued treatment with degarelix, while patients who crossed over from leuprolide to degarelix experienced further FSH suppression (63% reduction).<sup>81</sup>

Finally, the development of degarelix has resulted not only in an effective treatment for advanced prostate cancer, but also a valuable clinical tool for assessing the potential influence of FSH. For example, patients on degarelix had a lower risk (34%) of PSA failure ( $P = .05$ ) compared with leuprolide, and the risk

of PSA failure decreased in patients who switched from leuprolide to degarelix.<sup>81</sup> Moreover, degarelix significantly prolonged PSA PFS compared with leuprolide during the initial treatment year.<sup>82</sup> PSA PFS also improved in patients who switched from leuprolide to degarelix in the extension trial.<sup>81</sup> A recent analysis of 6 phase 3 prospective randomized trials reported that the risk of developing adverse cardiac events was significantly lower in patients receiving degarelix when compared with those receiving leuprolide.<sup>83</sup> Since one of the main differences between chronic agonist and chronic antagonist treatment is their effect on FSH, it is plausible that the long-term benefits from antagonists may be due, at least in part, to their profound suppression of the FSH system. However, more clinical and basic science data are needed in order to address and confirm these hypotheses.

### Conclusions

A growing body of evidence now strongly supports a role for the FSH system in the development and progression of prostate cancer. Studies have shown elevated levels of FSH and FSH receptors in benign and malignant prostate cells, and there is a correlation between levels of FSH and the progression of prostate cancer. Proteins involved in the regulation of the FSH system, such as inhibins, activins, follistatins, and RGS proteins, are also dysregulated, and in many cases their levels are proportionate to the malignancy status of the cancer. There is evidence of the pathophysiological consequences of aberrant FSH signaling with downstream effector molecules such as increases in VEGF, resulting in increased angiogenesis and tumor growth. One of the main differences between GnRH/LHRH receptor agonists and antagonists is their long-term effect on FSH levels. ADT treatment with antagonists results in profound, rapid, and consistent suppression of FSH, while treatment with agonists results in biphasic FSH levels that neither reach the same nadir as antagonists nor the same level of continued suppression.

Finally, given the overwhelming evidence of a role of the FSH system in the development and progression of prostate cancer, many researchers are beginning to investigate targeting the FSH receptor directly in order to develop novel, effective medications.<sup>43</sup> This may be achievable in the near future using negative allosteric modulators and antagonists, such as ADX61623,<sup>84-86</sup> as well as targeted antibodies or drug immunoconjugates. Since FSH appears to play a mitogenic role in nearly every form of cancer, continued biochemical and clinical research will help guide today's interventions as well as guide the next generation of cancer treatments.

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**Disclosures:** Research was partially funded by Ferring Pharmaceuticals Inc. Dr Crawford serves on advisory boards for Ferring, Genomic Health GTX, Dendreon, and Bayer. Dr Rove serves on an advisory board for GTx. Dr Rick has no financial relationships to disclose. Dr Block is founder and board member of Biscayne Pharmaceuticals Inc. Dr Schally receives research grant funding from Ferring for experimental studies with degarelix in prostate cancer. Drs Beveridge, Dahdal, and Marshall are employees of Ferring Pharmaceuticals Inc.

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