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Maximal Testosterone Suppression in Prostate Cancer—Free vs Total Testosterone

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Abstract

Testosterone remains a key target in the treatment of advanced prostate cancer. The relationship of free testosterone to prostate cancer treatment and outcomes remains largely unexplored. A consensus of prostate cancer experts was convened in 2013 to review current knowledge surrounding relationship of total and free testosterone to prostate cancer, discuss the free hormone hypothesis, and highlight future avenues for therapeutics. Free testosterone may better reflect prostate cancer tissue androgen levels than serum total testosterone concentration. Free testosterone deserves more research regarding its relation to clinical outcomes.

Prostate cancer is an androgen receptor (AR)–dependent disease. The current treatment of men with metastatic disease entails hormonal manipulations, referred to as androgen deprivation therapy (ADT), which is designed to reduce androgen levels and/or inhibit AR signaling. Extensive preclinical data indicate that prostate cancer maintains expression and function of AR despite initial therapeutic reduction of serum androgen levels.

Current therapeutic strategies aim to inhibit various known mechanisms of AR activation through further inhibition of androgen synthesis, enhancement of binding specificity of AR antagonists that effectively reduces deoxyribonucleic acid binding, and prevention of AR nuclear translocation. Although efficacy of primary ADT is ultimately lost in all patients, we now understand that AR activity remains intact and very active despite low levels of serum testosterone. With this understanding, maintaining lower testosterone levels continues to be the mantra of clinicians, who use serum testosterone and prostate-specific antigen (PSA) as

the primary clinical day-to-day measures of efficacy in designing and delivering treatments for advanced prostate cancer.

With the advent of luteinizing hormone-releasing hormone (LHRH) agonists more than 30 years ago, the post-treatment goal was to achieve serum testosterone levels that were comparable with orchiectomy. Early on, however, the lower limit of detection (using an older double-isotope dilution technique) was only 50 ng/dL, and various guidelines, including those by the National Comprehensive Cancer Network, the American Urological Association, and the U.S. Food and Drug Administration deem serum testosterone levels that are <50 ng/dL as castrate, despite modern testing demonstrating mean values for surgically castrate patients approximately 15 ng/dL.¹⁻³ Furthermore, older assays were only capable of measuring total serum testosterone, not the unbound (free) active form. Free testosterone levels, although challenging to measure, are more reflective of ongoing androgenic signaling within prostate cancer cells. Despite better understanding of testosterone and its relationship to prostate cancer, current guidelines remain vague regarding ongoing monitoring of total or free testosterone primarily because of the lack of well-designed clinical trials. There are suggestions, however, that lower levels of testosterone may provide clinical benefit and that perhaps by focusing on free testosterone (the active fraction of testosterone), we may better affect the biology of prostate cancer.^{1,4,5}

A consensus of prostate cancer experts was convened in 2013 to discuss current knowledge surrounding relationship of total and free testosterone to prostate cancer. We examined data supporting the maximal suppression of testosterone, delved into how testosterone is best measured and considered the merits of these tests and whether they are clinically meaningful. We further debated the application of the free hormone hypothesis to prostate cancer and discussed the merits of lowering free testosterone, side effects of ADT, and estrogen-related therapies.⁶⁻⁸

MATERIALS AND METHODS

A comprehensive literature search of PubMed and relevant congress abstract databases was conducted using combinations of the key words prostate cancer, androgen receptor, testosterone, free testosterone, androgen deprivation therapy, estrogen, and castration resistant prostate cancer. Clinical studies that reported androgen suppression (serum total testosterone or free testosterone) to clinical outcomes such as progression-free survival (PFS) and overall survival (OS) were selected for further review. Data from the selected studies were presented, reviewed, and discussed by the authors during an expert panel round table meeting held in Memphis, TN, on February 2, 2013.

COMMENT

The validation and use of serum testosterone as a surrogate for clinical benefit has been time tested.⁹ Intensive research in AR biology and prostate cancer progression has provided strong evidence supporting the enduring link between testosterone suppression and successful treatment of hormone-naïve advanced prostate cancer and, more recently, castration-resistant prostate cancer (CRPC).¹⁰ Clinical data suggest that the optimal level of

testosterone after ADT should be the lowest achievable level of testosterone, although rigorous testing of this hypothesis remains to be completed.^{4,6} We know, for example, that using current commercially available liquid chromatography-tandem mass spectrometry assays, surgical castration, and LHRH agents do in fact achieve total testosterone levels below the established <50 ng/dL cutoff and that there may be a relationship between prostate cancer progression and incomplete castration.^{11,12}

Most research in this area focuses on lowering total testosterone, but there are other determinants of male hormone activity such as free testosterone that have not yet been tested as prognostic or therapeutic biomarkers in prostate cancer. Circulating testosterone remains the main source of androgens for prostate cancer cells. Up to 95% of testosterone is produced by the Leydig cells in the testes in response to luteinizing hormone (LH) released from the anterior pituitary, and the remainder of testosterone is derived from the adrenal glands.¹³ However, these values largely reflect the concentration of testosterone bound to plasma proteins, including sex hormone binding globulin (SHBG) and albumin. The fraction bound to SHBG (about 30%-44% of total testosterone) is considered to be unavailable for androgenic signaling. Only about 1%-2% circulates as free testosterone, constituting the active form of the hormone able to diffuse into cells and bind AR (free hormone hypothesis).^{14,15} The remaining testosterone is loosely bound to albumin in a reversible fashion and may be biologically available in some tissues. For the purposes of this article, we primarily focus on total and free testosterone.

Orchiectomy and LHRH analogs only affect testicular production of testosterone and are limited in the degree to which they can lower testosterone. Oefelein et al² reported a median level of 15 ng/dL after surgical castration and recommended that a castration level of <20 ng/dL should be the goal of primary ADT. Given the difficulty in designing clinical trials, there is a lack of level 1 evidence to support this. Adrenal and intratumoral androgen biosynthesis contribute to circulating testosterone levels and the availability of free testosterone. This limitation can lead to ineffective or incomplete testosterone suppression for some patients even if serum testosterone is found to be <50 ng/dL.

LHRH agonists have been reported to result in noncastrate levels of testosterone (using the more permissible cutoff of 50 ng/dL) in 2%-35% of men with prostate cancer.^{4,8,16-18} These numbers rise even further to 13%-68% of patients for the stricter definition of castrate levels of testosterone <20 ng/dL.^{4,8,16-19} Interestingly, even with bilateral orchiectomy, up to 25% of patients do not achieve <20 ng/dL of testosterone.^{2,20}

Although we know that some patients may not reach castrate levels of testosterone with ADT (medical or surgical), there are few studies that examine the clinical impact of these measures. Perachino et al²¹ retrospectively reviewed 129 consecutive hormone-naïve patients with bone metastases to assess the relationship of total testosterone levels during ADT and prostate cancer outcomes. The study reported a statistically significant correlation between the risk of death and total testosterone levels achieved during ADT (hazard ratio 1.32, $P < .05$). In another study, Morote et al⁸ assessed the relationship between survival free of prostate cancer progression and total testosterone breakthrough levels during ADT. Mean survival free of prostate cancer progression was 88 months (95% confidence interval

55-121) if serum total testosterone levels were >32 ng/dL and 137 months (95% confidence interval 104-170) if serum total testosterone levels were <32 ng/dL. The studies by Perachino and Morote remain small and retrospective. A large study of 2290 patients, however, who received combination of ADT and radiotherapy demonstrated PFS rates that were lower with testosterone breakthroughs of 32-50 ng/dL compared with other patients in whom it remained <32 ng/dL.²² These studies do not define optimal timing of testosterone testing and may not account for some treatment failures or breakthroughs. Intermittent primary ADT using serum total testosterone levels to determine treatment schedule had lower risk of treatment failure and castration resistance (hazard ratio 0.65, $P = .02$) compared with calendar-based treatment, suggesting that lower serum testosterone levels were more important for better cancer outcome.²³ In contrast, recent data suggest that one cannot definitively rule out a 20% greater risk of death with intermittent therapy than with continuous therapy, mainly for metastatic disease, supporting the rationale that inadequate testosterone suppression may be hazardous.²⁴ Analysis of 626 patients in the continuous arm of the PR7 randomized intermittent ADT trial confirmed that median serum total testosterone in the first year of ADT treatment had an inverse correlation with time to androgen-independent progression.²⁵

Still, the studies by Perachino and Morote are retrospective and modest in size. As such, they should be considered hypothesis generating. There is an absence of large, prospective studies with robust end points addressing the relationship between levels of testosterone achieved with ADT and time to progression and prostate cancer mortality. This is a major unmet need of current evidence. The importance of intracrine synthesis of androgen in promoting castrate resistance also means that some skepticism about the critical role of ultralow levels of testosterone on ADT may be warranted.

Many experts now recommended that maximal suppression of total and free testosterone should be the goal of primary ADT.^{2,6,8,12,19,26} The evidence to support this consensus opinion is compelling, including data showing that primary ADT patients who achieve lower serum testosterone levels and men with CRPC who undergo secondary hormonal therapy to further reduce testosterone experience better prostate cancer outcomes.^{4,6,8,11,21,26,27} However, robust study and prospectively designed end points correlating free testosterone suppression with clinical prostate cancer outcomes are still needed.

Classical ADT (LHRH agonists) prostate cancer cells inevitably resume growth and result in cancer progression in almost all patients, giving rise to CRPC through the AR signaling axis.¹⁰ CRPC cells have adapted to the low testosterone environment and are hypersensitive such that they are stimulated by lower concentrations of testosterone and other androgen precursors.^{11,28} Secondary hormonal therapies that block AR or further reduce testosterone and dihydrotestosterone (DHT) production are able to rescue these ADT failures and increase PFS and OS, further demonstrating that ongoing focus on lowering testosterone benefits patients.^{29,30}

Secondary hormonal manipulation remains possible because advanced prostate cancer maintains a functioning AR signaling axis throughout disease progression.^{31,32} These observations suggest that current methods for primary ADT may not be adequately reducing

testosterone levels in some patients from the onset, particularly in patients with unrecognized breakthroughs. Alternatively, we may not be focusing on the biologically active fraction of testosterone, free testosterone.

There are 3 classes of drugs that have been used for secondary hormone therapy, that is, further lowering testosterone or blocking AR in men who develop CRPC on ADT:

- 1) Estrogens (diethylstilbestrol, estradiol, premarin, and estramustine)—lower free testosterone indirectly by suppressing LH production (when used as a single agent),³³⁻³⁷
- 2) Antiandrogens (flutamide, bicalutamide, and enzalutamide)—block AR, and
- 3) CYP17 (17 α -hydroxylase)/c17,20-lyase inhibitors (ketoconazole and abiraterone)—lower circulating total testosterone by inhibiting steroidogenesis, thus decreasing adrenal and testicular androgens and eliminating intratumoral testosterone and DHT levels.^{28,30,38,39}

We will briefly review these drugs, their mechanisms of action, and supporting evidence of their use in prostate cancer.

Abiraterone/prednisone is approved in patients with CRPC before and after the initiation of cytotoxic chemotherapy, and enzalutamide is approved for use in patients with chemotherapy-resistant CRPC. Both these agents have shown in phase III clinical studies that further suppressing the AR signaling axis resulted in significant and clinically meaningful improvements in symptoms, PSA responses, PFS, and OS.^{29,30} Enzalutamide is a potent inhibitor of the AR (up to 5 times greater affinity for AR than bicalutamide and no agonist activity). It also blocks nuclear translocation and prevents binding of androgens and coactivator proteins. In a phase I clinical study of abiraterone in men with CRPC, baseline total testosterone levels on LHRH agonist alone were 7 ng/dL (median; range, <1-34 ng/dL) and fell to <1 ng/dL after abiraterone and prednisone.⁴⁰ Therefore, maximal suppression of testosterone does rescue patients who develop CRPC on primary ADT.

Estrogens, including diethylstilbestrol, capesaris (GTx-758), and estramustine, are also able to maximize testosterone suppression. These drugs suppress LH production when given alone and markedly increase SHBG production by acting on the liver. This has the added effect of decreasing free testosterone even further and reducing adrenal androgens and intratumoral testosterone.^{35,41-43} In a small study, Levell et al³⁴ assessed the relationship of total and free testosterone and survival in men with advanced prostate cancer after orchiectomy or treatment with estrogens. Although total testosterone levels were not different among groups, free testosterone levels were lower in estrogen-treated patients. All the estrogen-treated patients had free testosterone levels that fell into the lower third of the range achieved by orchiectomized patients. Patients who had the lowest free testosterone levels had a greater 2-year OS. Indeed, estramustine was approved because it also increased survival in CRPC.⁴⁴

For the reasons stated previously, estrogens have important attributes considered by many to be one of the best ways in which to treat prostate cancer with the exception of the side effects, principally, thromboembolic events. We now recognize that adverse events

associated with traditional ADT use (eg, osteoporosis and hot flashes) are in fact related to estrogen deficiency. Capesaris (GTx-758), a selective ER α agonist, is being developed as a more selective estrogen-based therapy to reduce free testosterone as combination primary ADT and secondary hormonal therapy for men with CRPC.⁴⁵

In the hormone-naïve prostate cancer microenvironment for men before the initiation of ADT, serum testosterone levels show no correlation with tissue levels of DHT.⁴⁶ After 6 months of primary ADT, although testosterone levels decline and correlate with intraprostatic levels, absolute levels achieved are likely still sufficient to activate the AR.^{9,13} Serum PSA levels also correlate strongly with total testosterone levels during primary ADT, suggesting that PSA levels reflect the androgen milieu during ADT.⁴⁶ Recent data showed no linear correlation between total testosterone and PSA levels in patients using LHRH analogs, contesting the equivalence of different pharmacologic castrations regarding total testosterone and PSA levels. Total testosterone may not represent the real exposure to active free testosterone.⁴⁷ The consensus panel grappled with knowing that total testosterone levels do not necessary correlate with free testosterone given varied and unknown levels of albumin and SHBG, thus making inference to ongoing androgenic signaling at the cellular level very difficult using just a serum testosterone measurement. To complicate matters further, our review of the literature revealed that free testosterone is not always reported in ADT studies. We know, however, that lower free testosterone levels have been previously correlated with improved survival.^{30,34} Although measurement of free testosterone can, at times, be complicated for a variety of reasons, lowering the levels of free testosterone remains the ultimate goal.^{15,48}

Total but not free testosterone concentrations are affected by alterations in SHBG. In men whose LH secretion is suppressed (eg, by LHRH agonists or estrogens), free testosterone levels also depend on SHBG levels (eg, relatively greater free testosterone suppression because of increased SHBG levels associated with estrogen administration). Equilibrium dialysis or ultrafiltration to determine the free fraction of testosterone combined with liquid chromatography-tandem mass spectrometry to measure total testosterone is the gold standard method to measure free testosterone.¹⁵ Until such time as a reference standard becomes available for clinical laboratories to standardize measurements of testosterone, reliable testing for patients in the community by clinicians should be performed and interpreted with caution.⁴⁸

CONCLUSION

Serum testosterone levels, despite the difficulties in selecting definitions for castrate levels and creating assay standardization and harmonization, remain an important clinical surrogate marker and therapeutic target in the treatment of advanced and metastatic prostate cancer. Clinical data suggest the optimal level of testosterone after ADT should be the lowest achievable level of free testosterone.^{4,6} There is scientific rationale to strive for maximal testosterone suppression with throughout all stages of prostate cancer:

- 1) Current methods for ADT may provide inadequate suppression of serum testosterone for some patients and may not fully ablate the free fraction of testosterone that is

responsible for ongoing androgenic signaling despite castrate levels of total serum testosterone.

2) Secondary hormonal therapy rescues patients who have incomplete castration on primary ADT and/or who develop CRPC.

Maximal suppression of the AR signaling axis by secondary hormone therapy has demonstrated clinical benefit in large, well-controlled phase III clinical trials (abiraterone and enzalutamide) with improvements in PSA progression, PFS, and OS. Thus, the goal of newer ADT modalities should be to maximally lower the free fraction of serum testosterone, which may better reflect prostate cancer tissue androgen levels than serum total testosterone concentration. This ongoing focus on testosterone and in particular, free testosterone deserves more research regarding its relation to clinical outcomes. With better understanding of the clinical significance of maximal suppression of testosterone and the importance of free testosterone, the definition of optimal testosterone suppression for ADT should be updated by both regulatory and clinical consensus groups.²⁰ This will require more studies in which both total and free testosterone levels are measured and correlated with clinically important outcomes.

Finally, considering currently available accurate testosterone assays and new approaches able to further decrease testosterone levels with clinical benefits, the refinement of the surrogate marker in prostate cancer therapy is a matter of time, and free testosterone might be the main candidate.

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