

cancer cells in culture-modulation by steroid hormones. *Breast Cancer Res Treat* 23: 77–86, 1992.

29. Simard J, Dauvois S, Haagensen DE, Lévesque C, Méréand Y, and Labrie F: Regulation of progesterone-binding breast cyst protein GCDFP-24 secretion by estrogens and androgens in human breast cancer cells: a new marker of steroid action in breast cancer. *Endocrinology* 126: 3223–3231, 1990.

30. Katchen B, and Buxbaum S: Disposition of a new, non-steroid, antiandrogen, α,α,α -trifluoro-2-methyl-4'-nitro-*m*-propionotoluidide (flutamide), in men following a single oral 200 mg dose. *J Clin Endocrinol Metab* 41: 373–379, 1975.

31. Veldscholte J, Berrevoets CA, Brinkmann AO, Grootegoed JA, and Mulder E: Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction and transcription activation. *Biochemistry* 31: 2393–2399, 1992.

32. Fuhrmann U, Bengtson C, Repenthin G, and Schillinger E: Stable transfection of androgen receptor and MMTV-CAT into mammalian cells: inhibition of CAT expression by anti-androgens. *J Steroid Biochem Mol Biol* 42: 787–793, 1992.

33. Kempainen JA, and Wilson EM: Agonist and antagonist activities of hydroxyflutamide and Casodex relate to androgen receptor stabilization. *Urology* 48: 157–163, 1996.

34. Luo S, Martel C, Leblanc G, Candas B, Singh SM, Labrie C, Simard J, Bélanger A, and Labrie F: Relative potencies of flutamide and Casodex: preclinical studies. *Endocrine Rel Cancer* 3: 229–241, 1996.

35. Juniewicz RE, McCarthy M, Lemp BM, Barbolt TA, Shaw C, Hollenbaugh DM, Winneker RC, Reel JR, and Batzold FH: The effect of the steroidal androgen receptor antagonist, Win 49,596, on the prostate and testis of beagle dogs. *Endocrinology* 126: 2625–2634, 1990.

36. Winneker RC, Wagner MM, and Batzold FH: Studies on the mechanism of action of Win 49,596: a steroidal androgen receptor antagonist. *J Steroid Biochem* 33: 1133–1138, 1989.

37. Marchetti B, and Labrie F: Characteristics of flutamide action on prostatic and testicular functions in the rat. *J Steroid Biochem* 29: 691–698, 1988.

38. Snyder BW, Winneker RC, and Batzold FH: Endocrine profile of Win 49,596 in the rat: a novel androgen receptor antagonist. *J Steroid Biochem* 33: 1127–1132, 1989.

39. Chandolia RK, Weinbauer GF, Behre HM, and Nieschlag E: Evaluation of peripherally selective antiandrogen (Casodex) as a tool for studying the relationship between testosterone and spermatogenesis in the rat. *J Steroid Biochem Mol Biol* 38: 367–375, 1991.

40. Furr BJA, and Milsted RAV: LH-RH analogues in cancer treatment, in Stoll B (Ed): *Endocrine Management of Cancer: 2. Contemporary Therapy*. Basel, Karger, 1988, pp 16–29.

41. Lacoste D, St-Arnaud R, Bélanger A, and Labrie F: A pure antiandrogen does not interfere with the LHRH agonist-induced blockade of testicular androgen secretion in the dog. *Mol Cell Endocrinol* 56: 141–147, 1988.

42. Lacoste D, Dubé D, Bélanger A, and Labrie F: Effect of 2-week combination therapy with the LHRH agonist [D-Trp⁶, des-Gly-NH₂]LHRH ethylamide and the antiandrogen flutamide on prostate structure and steroid levels in the dog. *Mol Cell Endocrinol* 67: 131–138, 1989.

43. Bélanger A, Labrie F, Dupont A, Brochu M, and Cusan L: Endocrine effects of combined treatment with an LHRH agonist in association with flutamide in metastatic prostatic carcinoma. *Clin Invest Med* 11: 321–326, 1988.

EDITORIAL COMMENT

One may conceptualize androgen receptors as (docking) sites in prostate cells to which androgens bind to initiate ge-

nome activation; antiandrogens block androgen action by competitively occupying the receptor site. Evidence now shows that these interactions are more diverse and complicated than simple receptor occupation and blockade. In 1989, Wilding *et al.*¹ described the paradoxical agonist response of the LNCaP cell line to the antiandrogens cyproterone acetate, nilutamide, and flutamide, but not to bicalutamide, related to the presence of an androgen receptor mutation (threonine-alanine in condon 186) in the LNCaP cells. Clinical relevance of these findings was shown by significant decreases in prostate-specific antigen (PSA) and objective clinical responses after discontinuation of flutamide.² Similar withdrawal responses were reported after discontinuation of other hormones (Megace, stilbesterol, nilutamide, Casodex).^{3,4} Furthermore, failure of one antiandrogen does not eliminate a response to a different antiandrogen. Casodex, 200 mg, administered for an increasing PSA level after a flutamide withdrawal response produced yet another PSA decrease of more than 50%.⁵ A paradoxical agonist response to an antiandrogen in the absence of a known receptor mutation may also occur through stimulation of the wild-type receptor, as described, *in vitro*, for Eulexin.⁶ Within the class of antiandrogens are individual compounds with unique actions.

Labrie and colleagues make several interesting experimental observations. They question the claim that Casodex is a more potent antiandrogen than Eulexin. In their view, the intact rat model is not an appropriate model for evaluation of the potency of antiandrogens. Eulexin traverses the blood-brain barrier and, as a result of androgen receptor blockade in the hypothalamus, causes increases in LHRH and LH output, which in turn cause a marked increase in serum testosterone. The result is a higher circulating testosterone concentration to compete with the antiandrogen at the receptor. Casodex does not cross the blood-brain barrier in rats.⁷ There is lower circulating testosterone to compete with Casodex and therefore a more effective blockade. However, in humans, both Casodex and Eulexin cross the blood-brain barrier, therefore, this differential does not exist. Nevertheless, in many animal models studied thus far, Casodex has demonstrated potency equivalent to or greater than that of Eulexin. The Casodex used by Labrie and colleagues was synthesized in their laboratories. Because nothing is known about the purity or proportion of enantiomers, bioequivalence to the Zeneca commercial product must be questioned.

Labrie and colleagues rightly observe that no rat model findings can be totally translated to humans with regard to relative effect of receptor blockade. Relative potencies of drugs can vary with the animal species used, the duration of dosing, the nature of the challenge, and the experimental model chosen. This observation underscores the necessity to conduct clinical trials in humans. In humans, both Eulexin and Casodex are less effective as monotherapy than is medical or surgical castration at doses tested so far.^{8,9} The optimal dose of either antiandrogen in conjunction with LHRH agonist therapy that eliminates testicular androgen and leaves only adrenal androgen to counteract is unknown. Only the 50-mg Casodex dose has been used as part of combined androgen blockade; Eulexin is dosed at 250 mg three times daily for combination therapy.

The only clinical trial available comparing Casodex 50 mg daily with Eulexin 250 mg three times daily each in combination with an LHRH agonist analyzed the end points of time to treatment failure, time to clinical progression, and overall survival and showed them to be equivalent after a median follow-up of 95 weeks (Fig. 4); namely, there is no statistically significant difference between the two regimens based on these three end points.^{10,11}

Studies as reported by Labrie and colleagues are of importance in continuing to dissect and clarify the role of the an-

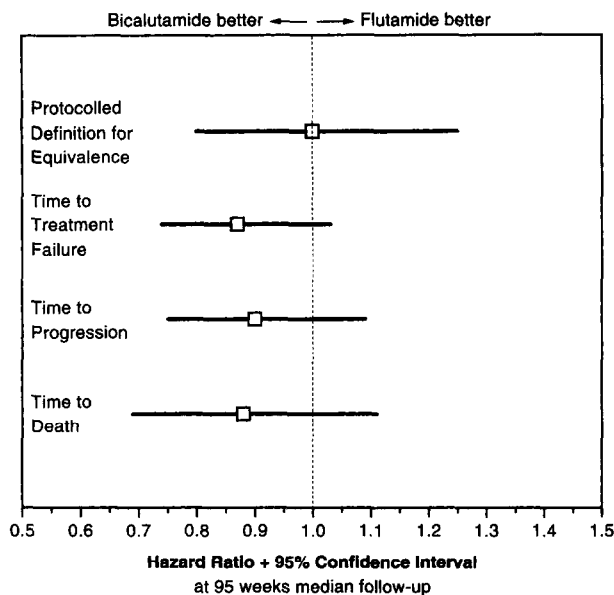


FIGURE 4. Hazard ratios and 95% confidence intervals for bicalutamide plus luteinizing hormone-releasing hormone-A (LHRH-A) and flutamide plus LHRH-A groups for time to treatment failure, time to progression, and time to death.

drogen receptor in prostate cancer treatment and will further clarify the physiology of androgen receptor activity and lead to new generations of antiandrogens. It is hoped that this information will improve treatment strategies. However, prospective double-blind randomized clinical trials like the one yielding the results summarized in Figure 4 will continue to provide the pertinent human clinical evidence necessary for physicians and patients to make informed treatment decisions.

Paul F. Schellhammer, M.D.
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REFERENCES

1. Wilding G, Chen M, and Gelmann EP: Aberrant response in vitro of hormone-responsive prostate cancer cells to antiandrogens. *Prostate* 14: 103-114, 1989.
2. Scher HI, and Kelly WK: The flutamide withdrawal syndrome: its impact on clinical trials in hormone-refractory prostatic cancer. *J Clin Oncol* 11: 1566-1572, 1993.
3. Schellhammer P, Venner P, Haas G, Small E, Nieh P, Seabaugh R, Patterson L, Klein E, Wajzman Z, Furr B, Chen Y, and Kolvenbag G: Prostate-specific antigen decreases after withdrawal of antiandrogen therapy with bicalutamide or flutamide in patients receiving combined androgen blockade. *Urology* (in press).
4. Small EJ, and Srinivas S: The antiandrogen withdrawal syndrome. *Cancer* 76: 1428-1434, 1995.
5. Liebertz C, Kelly WK, Theodoulou M, Curley T, Dean L, Mazumdar M, Ylamis V, and Scher HI: High dose Casodex for prostate cancer (PC): PSA declines in patients (PTS) with flutamide withdrawal responses (abstract). *Proc ASCO* 14: 232, 1995.
6. Kempainen JA, and Wilson EM: Agonist and antagonist activities of hydroxyflutamide and Casodex relate to androgen receptor stabilization. *Urology* 48: 157-163, 1996.

7. Furr BJA, and Tucker H: The preclinical development of bicalutamide: pharmacodynamics and mechanism of action. *Urology* 47: 13-25, 1996.

8. Bales GT, and Chodak GW: A controlled trial of bicalutamide versus castration in patients with advanced prostate cancer. *Urology* 47 (suppl 1A): 38-43, 1996.

9. Chang A, Yeap B, Davis T, Blum R, Hahn K, Khanna O, Fisher H, Rosenthal J, Witte R, Schinella R, et al: Double-blind, randomized study of primary hormonal treatment of stage D2 prostate carcinoma, flutamide vs. diethylstilbestrol. *J Clin Oncol* 14: 2250-2257, 1996.

10. Schellhammer P, Sharifi R, Block N, Soloway M, Venner P, Patterson AL, Sarosdy M, Vogelzang N, Jones J, and Kolvenbag G: Maximal androgen blockade for patients with metastatic prostate cancer: outcome of a controlled trial of bicalutamide versus flutamide, each in combination with luteinizing hormone-releasing hormone analogue therapy. *Urology* 47(suppl 1A): 54-60, 1996.

11. Schellhammer P, Sharifi R, Block N, Soloway M, Venner P, Patterson L, Sarosdy M, Vogelzang N, Chen Y, and Kolvenbag G: A controlled trial of bicalutamide versus flutamide, each in combination with luteinizing hormone-releasing hormone analogue therapy, in patients with advanced prostate carcinoma. *Cancer* 78: 2164-2169, 1996.

REPLY BY THE AUTHORS

A few points mentioned by Dr. Schellhammer need some clarifications. The paradoxical stimulation of LNCaP cell proliferation by antiandrogens as well as by estrogens and progestins is an interesting observation. However, it should be realized that the LNCaP cell line is an exception rather than a rule, and the findings obtained with this cell line cannot be extrapolated to prostate cancer in general. Indeed, the decrease in serum PSA and the positive clinical responses observed upon discontinuation of flutamide and other antiandrogens in a significant proportion of patients showing progression after a positive response to combination therapy^{1,2} is still an unexplained phenomenon that is very rarely accompanied by a mutation of the androgen receptor. This second clinical response observed upon discontinuation of an antiandrogen is apparently an additional benefit of combination therapy.

It is of interest that Schellhammer makes reference to the study of Kempainen and Wilson,³ since these investigators describe not only one (mentioned by Schellhammer *et al.*⁷), but two relevant findings: first, the study of Kempainen and Wilson shows that OH-flutamide (OH-FLU) is an androgen antagonist that is about 50 times more potent than Casodex; second, concerning the agonistic activity shown by both OH-FLU and Casodex in the special in vitro system used, the apparent 10-fold higher agonistic activity of OH-FLU is more than compensated by the 50-fold higher antagonistic action of the same compound, thus yielding an advantage of approximately five in favor of OH-FLU versus Casodex in terms of antagonistic/agonistic ratios of activities. In another comparable in vitro study, Casodex was shown to be 10 times less potent than OH-FLU.⁴

Concerning the Casodex synthesized in our laboratory and used for in vitro and animal studies, its GLP (good laboratory practice) certificate of analysis showed a 99.7% degree of purity. Our understanding, however, is that the same question could be asked about the flutamide used by Zeneca in both their fundamental and especially their clinical studies, in which bioequivalence is a potential concern and should have been unequivocally demonstrated. Moreover, contrary to Schellhammer's statement, Casodex has been shown to be approximately three times less potent than flutamide in the appropriate animal model,⁵ whereas the use of the intact rat model by Furr *et al.*⁶ led to the erroneous suggestion that

Casodex was 5 to 10 times more potent than flutamide. Schellhammer *et al.*,⁷ however, acknowledge the problems of the intact rat model, which apparently played an important role in the choice of the low 50-mg dose of Casodex for clinical studies. However, the statement that Casodex does not cross the blood barrier in rats is not accurate since serum testosterone clearly increases in this animal species at the high doses required for useful antiandrogenic efficacy.^{5,6}

Because the results of the clinical trial of Casodex (Schellhammer study^{7,11}) are mentioned, it seems appropriate to indicate the following: Because flutamide adds 19.7% of survival to the effect of the LHRH agonist on survival,⁸ the minimum 25% difference needed to demonstrate the nonequivalence of Casodex and flutamide in the Schellhammer study⁷ clearly demonstrates that this study does not have the statistical power to evaluate any activity of Casodex.⁹ Indeed, removing the 19.7% effect of flutamide by not administering flutamide or by administering a placebo in combination with an LHRH agonist would have led to a result within the 25% difference; that is, a placebo would have met the criteria of equivalence with flutamide in the Schellhammer study and such a placebo (instead of Casodex) could have been approved for marketing in combination with an LHRH agonist for the treatment of prostate cancer.

Despite these irreparable defects of the design of the Schellhammer study, early data are presented⁷ without acknowledgment of these limitations. Indeed, as a result of the use of the inappropriate treatment failure as end point, 34% more patients were at risk of not receiving treatment in the flutamide arm at 49 weeks.⁷ It is thus not too surprising that no difference is evident with regard to time to progression or time to death at later time intervals since so many patients were not treated in the flutamide group. The most efficacious drug cannot show efficacy if the patient does not take it. It is also important to mention that an interim analysis at 49 or 95 weeks is one that is much too early to show significant differences even if the design of the clinical trial had been correct. It is of interest that the senior author of the Casodex study recently stated the pitfalls of drawing conclusions from interim analyses of study data.¹⁰ Then, with reference to the EORTC 30853 trial, no significant advantage of combination therapy (Zoladex + flutamide versus orchiectomy) was apparent at 2.5 years, whereas a 15.1-month cancer-specific survival advantage was observed in favor of combination therapy at 5 years.¹⁰ Schellhammer¹⁰ states: "Early or interim analysis of data, even from trials with sufficient sample sizes to achieve adequate statistical power at maturity, can reach invalid conclusions." Obedience to these fundamental principles would certainly have precluded the premature analyses at 49 and 95 weeks^{7,11} in the Casodex trial.

An even more important problem is the large number of patients removed from flutamide treatment very early in the trial. By simply assuming that the patients already removed from the flutamide arm at 49 weeks for noncancer reasons would show an evolution similar to those who remained under treatment, the following figure (Fig. 5) is obtained. We have simply corrected for the bias in early treatment failures by applying the correction factor of 1.34 calculated at 49 weeks⁷ to the hazard ratios obtained at 95 weeks¹¹ to estimate the ratios expected if the same proportion of patients had remained under treatment in the two groups. As is apparent, not only hazard ratios for time to progression and time to death move to the right in favor of Eulexin but, and of interest, the values achieved are almost superimposable on those obtained in the NCI 0036 study, in which the LHRH agonist + Eulexin combination was compared with LHRH agonist + placebo. Although the study is still in too early a stage to allow conclusions to be reached, such estimates suggest little impact of the 50-mg dose of Casodex on these clinically important parameters.

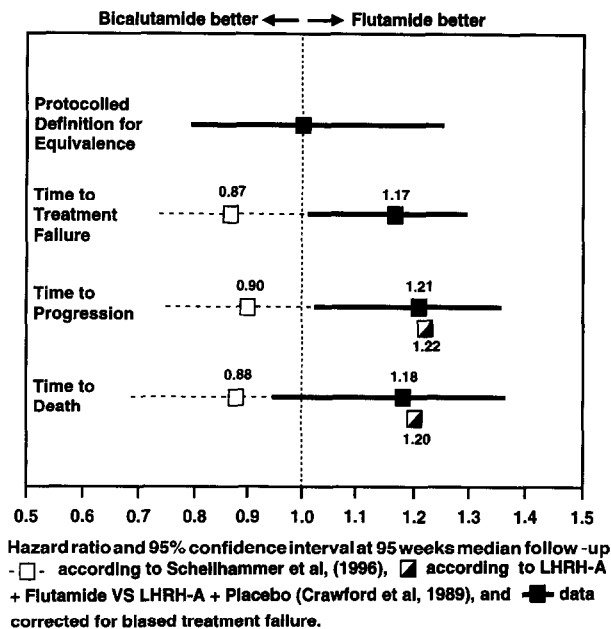


FIGURE 5. Hazard ratio and 95% confidence interval at 95 weeks' median follow-up.

As correctly stated by Schellhammer, prospective clinical trials are essential to prove the efficacy of a drug, but the design of such studies must be able "to provide the pertinent human clinical evidence necessary for physicians and patients to make informed treatment decisions." We have no reason to believe that Casodex will not be as efficient as Eulexin when used at a proper dose, but it is equally clear that the clinical dose of Casodex is still unknown and that no significant information on this subject can be obtained from the Schellhammer study. Until such data are obtained, only Eulexin and Nilandron have shown prolongation of survival.¹⁰ Therefore, we consider it urgent to initiate a clinical trial to determine the efficacious dose of Casodex to be used in association with an LHRH agonist for the treatment of prostate cancer. The finding that Casodex 200 mg, administered for an increasing PSA level after flutamide withdrawal response, produced yet another PSA decrease of more than 50%¹² is encouraging and clearly suggests that a dose of Casodex much higher than 50 mg daily is needed. Different pure antiandrogens are likely to induce different conformational changes in the androgen receptor, with the possibility of added benefits to patients by the sequential or even combined use of these powerful compounds. Because prostate cancer is the most sensitive of all hormone-sensitive cancers to hormone deprivation, we should take full advantage of this unique property of prostate cancer and use the best available therapies shown to prolong life for the optimal benefit of the prostate cancer patients.

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REFERENCES

- Dupont A, Gomez JL, Cusan L, Koutsilieris M, and Labrie F: Response to flutamide withdrawal in advanced pros-

- tate cancer in progression under combination therapy. *J Urol* 150: 908–913, 1993.
2. Scher HI, and Kelly WK: The flutamide withdrawal syndrome: its impact on clinical trials in hormone-refractory prostatic cancer. *J Clin Oncol* 11: 1566–1572, 1993.
 3. Kempainen JA, and Wilson EM: Agonist and antagonist activities of hydroxyflutamide and Casodex relate to androgen receptor stabilization. *Urology* 48: 157–163, 1996.
 4. Veldscholte J, Berrevoets CA, Brinkmann AO, Grootegoed JA, and Mulder E: Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction, and transcription activation. *Biochemistry* 31: 2393–2399, 1992.
 5. Luo S, Martel C, Leblanc G, Candas B, Singh SM, Labrie C, Simard J, Bélanger A, and Labrie F: Relative potencies of flutamide and Casodex: preclinical studies. *Endocrine Rel Cancer* 3: 229–241, 1996.
 6. Furr BJA, Valcaccia B, Curry B, Woodburn JR, Chesterson G, and Tucker H: ICI176,334: a novel nonsteroidal peripherally selective antiandrogen. *J Endocrinol* 113: R7–R9, 1987.
 7. Schellhammer P, Sharifi R, Block N, Soloway M, Venner P, Patterson AL, Sarosdy M, Vogelzang N, Jones J, and Kolvenbag G: A controlled trial of bicalutamide versus flutamide, each in combination with luteinizing hormone-releasing hormone analogue therapy, in patients with advanced prostate cancer. *Urology* 45: 745–752, 1995.
 8. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, Blumenstein DA, Davis MA, and Goodman PJ: A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N Engl J Med* 321: 419–424, 1989 [erratum: *N Engl J Med* 321: 1420, 1989].
 9. Labrie F: The estimated potency of Casodex and the problematic design of Schellhammer et al. (letter). *Urology* (in press).
 10. Schellhammer PF: Combined androgen blockade for the treatment of metastatic cancer of the prostate. *Urology* 47: 622–629, 1996.
 11. Schellhammer P, Sharifi R, Block N, Soloway M, Venner P, Patterson AL, Sarosdy M, Vogelzang N, Jones J, and Kolvenbag G: Maximal androgen blockade for patients with metastatic prostate cancer: outcome of a controlled trial of bicalutamide versus flutamide, each in combination with luteinizing hormone-releasing hormone analogue therapy. *Urology* 47(suppl 1A): 54–60, 1996.
 12. Liebertz C, Kelly WK, Theodoulou M, Curley T, Dean L, Mazumdar M, Ylami V, and Scher HI: High dose Casodex for prostate cancer (PC): PSA declines in patients (PTS) with flutamide withdrawal responses (abstract). *Proc ASCO* 14: 232, 1995.