

the bicalutamide used was not Casodex as currently marketed, and the relative potency observed is contradictory to evidence from other laboratories.

Luo *et al.*¹ comment that their findings are in agreement with previous *in vitro* and *in vivo* data from their laboratory.^{2,3} This is certainly true, but although they report that 2-hydroxyflutamide, the active metabolite of flutamide, and "Casodex" have equivalent affinities for both wild-type rat and human prostate androgen receptors,³ other studies agree that bicalutamide has between two and four times higher affinity for these receptors.⁴⁻⁸ Furthermore, the Labrie laboratory obtained IC₅₀ values (IC₅₀ = concentration of drug required to produce 50% displacement of [³H]R1881) for both 2-hydroxyflutamide and bicalutamide that were over threefold higher than those seen in other studies.^{4,6-8} In intact rats, despite evidence that bicalutamide has a higher potency than flutamide in reducing ventral prostate and seminal vesicle weights,^{4,9,10} the Labrie laboratory found similar potencies.³

The anomalous findings of the Labrie laboratory may be due to inappropriate assay conditions, the formulation of bicalutamide used, and the proportion of bicalutamide enantiomers.¹¹ The *R* enantiomer of bicalutamide shows higher androgen receptor binding than the *S* enantiomer, and whereas for most other studies Casodex was supplied by Zeneca Pharmaceuticals,^{4,6-10} the Labrie laboratory performed the synthesis of "Casodex" and reported no analytical data.

Although animal studies are a guide and not an absolute determinant of clinical activity, they are useful for determining the starting dose for clinical trials. The only clinical trial comparing Eulexin and Casodex was a large (*n* = 813), double-blind, randomized evaluation of their use as components of CAB in patients with advanced (Stage D2) prostate cancer.¹² When Eulexin (250 mg three times daily) and Casodex (50 mg once daily) were given with luteinizing hormone-releasing hormone analogue (LHRH-A) therapy, tumor responses were similarly high, with a 99% decrease in prostate-specific antigen concentrations after 3 months of therapy. At a median follow-up of 160 weeks, Casodex plus LHRH-A achieved longer progression-free survival than Eulexin plus LHRH-A (median 97 versus 77 weeks, respectively) and longer overall survival (median 180 versus 148 weeks, respectively), although hazard ratios indicated no significant difference.

An oversimplistic reliance has been placed by Labrie and colleagues on the relative potencies of drugs in animals compared with those in humans. If their data were accepted and taken at face value, and if the dose of Eulexin used clinically was optimal, then Casodex would be underdosed by 45-fold (15 times lower dose used and alleged 3-fold lower potency). This is obviously an absurd conclusion based on the clinical data generated in the comparative clinical trial.

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REPLY BY THE AUTHORS:

To further help the scientific community to discriminate between scientifically based facts and fallacies, we are pleased to provide some additional clarifications concerning the points mentioned in the above Letter to the Editor.

As previously explained in great detail,¹⁻⁴ the intact rat model used as the basis for the choice of the dose of bicalutamide in men^{5,6} has no relevance to the situation in human male subjects because the large increase in luteinizing hormone (LH) secretion induced by flutamide in this model is not found in men.¹⁻⁴ In fact, the use of the intact rat model by Furr *et al.*⁵ and Furr and Tucker⁶ led to the erroneous estimate that bicalutamide was 5 to 10 times more potent than flutamide. Thus, definitively, the castrated and not the intact rat model should be used to assess the potency of pure antiandrogens. The anomalous findings are thus those using the intact rat model^{5,6} and not ours.^{1,3}

As previously indicated,⁴ the bicalutamide used for our studies^{1,3,4} showed 99.7% purity.⁴ Our understanding, however, is that the same question could be asked about the flut-

TABLE I. IC_{50} values of displacement of 5 nM [3H]R1881 from the rat prostate androgen receptor by the unlabeled ligand R1881, OH-flutamide, or bicalutamide for two homogenization buffers after various incubation periods of 0 to 4°C*

Time (hr)	Method 1 (nM)			Method 2 (nM)		
	R1881	Bicalutamide	OH-Flu	R1881	Bicalutamide	OH-Flu
0.5	5.9 ± 0.53	1871 ± 156	2182 ± 158	6.6 ± 0.34	620 ± 82	1264 ± 445
2	4.3 ± 0.43	3820 ± 220	3485 ± 334	7.8 ± 0.77	1860 ± 162	3169 ± 309
18	5.7 ± 0.45	6247 ± 570	8220 ± 1290	7.1 ± 0.75	5670 ± 1033	17,400 ± 5400

Key: Method 1 = homogenization buffer of Luo et al.³; Method 2 = homogenization buffer of Furr et al.⁵; Flu = flutamide. Data presented are mean value ± SEM of triplicate measurements.

* Prostates from adult Sprague-Dawley rats castrated 24 to 48 hours before sacrifice. After the indicated incubation periods, 300 μL of dextran-coated charcoal (5% Norite A charcoal, 0.5% dextran T-70 wt/vol) was used to remove free ligand, and incubation continued for another 15 minutes at 0 to 4°C.

amide used by Zeneca in both their fundamental and clinical studies where no such information has been provided. The bicalutamide used in our studies showed two equivalent peak areas of 50.5% and 49.5%, as determined by chiral high-performance liquid chromatographic analysis, thus providing the proof that this compound was indeed, as expected, a racemate of the two enantiomers.

Because of the experimental differences between our previously reported experiment³ and that of Furr *et al.*,⁵ we have compared the affinity of R1881, bicalutamide, and OH-flutamide when using our homogenization buffer (method 1)³ or the Furr *et al.*⁵ homogenization buffer (method 2) on rat prostate cytosol after various incubation periods at 0 to 4°C (Table I). Using method 1, the relative potencies of OH-flutamide and bicalutamide were not significantly different, in agreement with the data of others.⁷ The androgen receptor binding capacity of [3H]R1881 was much higher when method 1 was used, which is most likely due to an increased stability of androgen receptors in the presence of glycerol, a well-known stabilizing agent for the androgen receptor that has been used during the past two decades by us^{3,8} and several other laboratories.⁹ It is somewhat surprising that Furr and coworkers did not use experimental conditions offering the optimal androgen receptor stability.

The intact cell situation is, however, much more important than binding data. Indeed, our recent *in vitro* data^{3,4} demonstrated that the antiandrogenic activity of OH-flutamide is 3.0 to 7.8-fold higher than that of bicalutamide. Even more convincingly, using androgen-regulated reporter genes, the antiandrogenic potency of OH-flutamide was reported to be 10 and 50 times higher than that of bicalutamide.^{7,10}

The apparent misinterpretation of preclinical data by Furr *et al.*⁵ and Furr and Tucker⁶ provides a possible explanation for the choice of the 50-mg dose of bicalutamide (Casodex) for clinical trials and for the fact that both the 50- and 150-mg doses of Casodex used alone are still too low and show a level of efficacy inferior to castration, as discussed previously,^{3,4} and the search still continues to find a dose equivalent to castration. The only clinical trial where bicalutamide has been evaluated as part of combination therapy suffers from flaws in its statistical design that do not permit an acceptable estimate of the efficacy of bicalutamide. Because a 25% worse effect was required before a difference could be found, and flutamide itself gives only a 19.7% difference (Labrie *et al.*² and references therein), a 25% difference could never be reached. In fact, the statistical design of that study¹¹ does not have the power to reach a conclusion, even in the complete absence of activity of

bicalutamide or the use of a placebo. An additional problem that occurred during the course of the study was that 34% more patients were at risk of not receiving treatment in the flutamide arm as early as 49 weeks. It was indicated in the final report that 61% more patients (41 versus 66) were removed from the flutamide group.¹² Obviously, the most efficacious drug cannot show efficacy if the patient does not take it. There is no reason to believe that bicalutamide is not an efficacious pure antiandrogen, but the clinical dose remains a question that cannot be answered by the Schellhammer *et al.* study.^{11,12}

We hope that these clarifications will help both scientists and clinicians to have a clear image of the facts concerning the relative potencies of flutamide and bicalutamide and stimulate further research on the optimal dosage of bicalutamide.

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