# Phase I-II Trial of Weekly Bicalutamide in Men with Elevated Prostate-Specific Antigen and Negative Prostate Biopsies

Silvia Zanardi,[1] Matteo Puntoni,[1,5] Massimo Maffezzini,[2] Roberto Bandelloni,[3] Marco Mori,[4] Alessandra Argusti,[1] Fabio Campodonico,[2] Laura Turbino,[3] Daniela Branchi,[1] Rodolfo Montironi[6] and Andrea Decensi[1,7]

**Abstract**

**Background:** Men with elevated prostate-specific antigen (PSA) and negative prostate biopsies are at risk for prostate cancer. The antiandrogen bicalutamide has a prolonged half-life, thus potentially allowing an intermittent administration to retain activity while reducing toxicity. We conducted a phase I-II trial of weekly bicalutamide in men with PSA >4 ng/mL and negative biopsies.

**Methods:** Eighty subjects were nonrandomly assigned to a three-arm trial to either bicalutamide 50 mg/wk ($n$ = 26) or 100 mg/wk ($n$ = 28) or no treatment ($n$ = 26) for 6 months. Blood samples were obtained at 0, 3, and 6 months, and prostate biopsies were repeated after 6 months. The outcome measures were 6-month changes of tissue Ki-67 (primary end point), high-grade prostatic intraepithelial neoplasia (HG-PIN), proliferative inflammatory atrophy, circulating PSA, and sex hormones.

**Results:** Ki-67 expression was higher in HG-PIN than in normal tissue (10% versus 3%; $P < 0.01$) but was not modulated by bicalutamide in normal luminal cells. A trend toward an improvement of HG-PIN status was found in treated subjects (26% improved, 60% had no change, 15% worsened) as compared with the no-treatment arm (4% improved, 83% had no change, 13% worsened; $P = 0.07$). Proliferative inflammatory atrophy prevalence was not reduced by bicalutamide. Bicalutamide reduced PSA by 50% in both arms and raised testosterone and estradiol levels. Asymptomatic breast swelling was noted in 40% of the treated cases.

**Conclusions:** A weekly administration of bicalutamide seems to be reasonably safe and shows an encouraging signal of activity on HG-PIN prevalence, supporting further studies of this schedule in men at high risk despite the negative primary end-point findings on Ki-67.



Chemoprevention may be a practical approach to reduce prostate cancer incidence and mortality (1–3). Finasteride, a 5-α-reductase inhibitor, reduced prostate cancer by 25% in average-risk men (4), although the original finding of a higher proportion of high-grade cancers raised debate over its long-term benefit (5). Recent bias-adjusted analyses of updated Prostate Cancer Prevention Trial results convincingly show that finasteride did not increase high-grade cancer rates (6, 7), but treatment side effects, including gynecomastia and reduced sexual function (4), have so far lessened the attractiveness of finasteride as a preventive agent for prostate cancer.

An alternative targeted approach for prostate cancer prevention is the use of androgen receptor antagonists. Bicalutamide as a single agent at 150 mg/d is effective in locally advanced prostate cancer (8), but its administration is associated with sexual side effects and grade 3 gynecomastia in most subjects (8), which have thus far prevented its assessment in chemoprevention. However, bicalutamide has a prolonged absorption and half-life (~7 days; ref. 9) and its serum concentration after a single 50 mg dose is nearly 0.8 μg/mL, a value in the range of inhibiting concentration for several prostate cancer cell lines (10), thus providing the rationale for a weekly schedule in an attempt to retain activity while reducing toxicity.

Men with elevated serum prostate-specific antigen (PSA) and negative prostate biopsies have a 20% to 25% risk of being diagnosed with cancer at subsequent biopsies (11, 12). Because no standard treatment is available, chemoprevention represents a promising approach for these high-risk individuals (1, 13, 14). Whereas no surrogate end-point biomarkers have been validated for prostate cancer, a number of tissue-based and non–tissue-based markers may be potential targets for

**Authors' Affiliations:** [1]Medical Oncology Unit, [2]Urology Unit, [3]Pathology Unit, and [4]Clinical Chemistry and Microbiology Laboratory, Galliera Hospital; [5]Biostatistics Unit, Department of Health Sciences, University of Genoa, Genoa, Italy; [6]Section of Pathological Anatomy, Polytechnic University of the Marche Region, Ancona, Italy; and [7]Division of Cancer Prevention and Genetics, European Institute of Oncology, Milan, Italy

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**Requests for reprints:** Andrea Decensi, Medical Oncology Unit, Galliera Hospital, Mura delle Cappuccine 14, 16128 Genova, Italy. Phone: 39-010-5634501; Fax: 39-010-57481090; E-mail: andrea.decensi@galliera.it.



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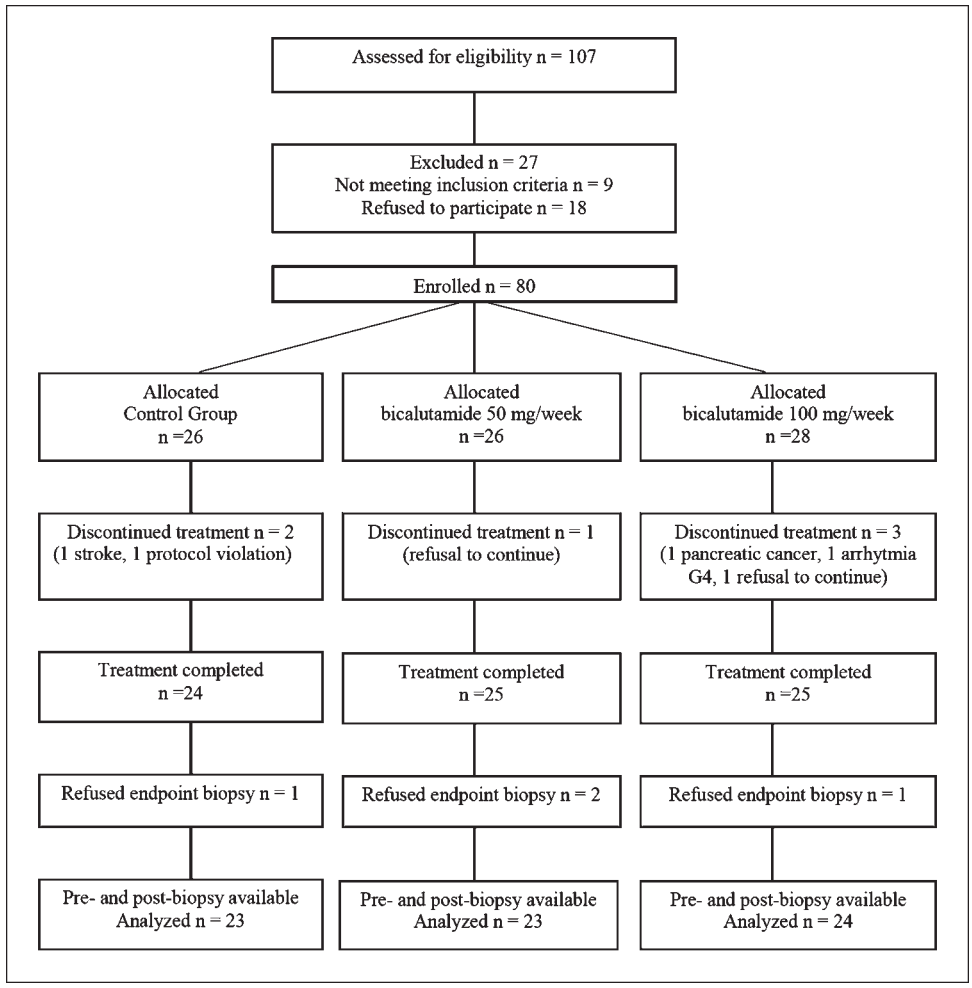


**Fig. 1.** Participant flow diagram.

chemoprevention, including high-grade prostatic intraepithelial neoplasia (HG-PIN); Ki-67, an immunohistochemically detectable antigen of proliferation; and proliferative inflammatory atrophy (PIA), another putative biomarker in prostate cancer prevention (1, 15, 16). Among circulating biomarkers, PSA is also being used to assess the activity of chemopreventive agents in at-risk cohorts (13, 14).

The present phase I-II trial evaluated the feasibility and toxicity and explored the activity of a weekly administration of bicalutamide in subjects with PSA levels >4 ng/mL and noncancer findings on prostate biopsies using immunohistochemical, morphologic, and biochemical parameters as activity end points.

## Subjects and Methods

### Eligibility criteria and study design

Men ages 50 to 80 y and with PSA levels >4 ng/mL and at least one negative biopsy (8-10 cores) within 90 d from study entry were eligible. Exclusion criteria were prior malignancy within 5 y; grade >2 alterations of liver, renal, metabolic, and cardiac functions; and use of antiandrogens or 5-α-reductase inhibitors within 3 mo.

This study was a two-step, nonrandomized, open-label, dose-escalation trial. The first 25 subjects were treated with a single 50 mg bicalutamide dose once a week with a stopping rule of 30% in the upper confidence interval (exact method) of G3 gynecomastia incidence (i.e., less than one half the incidence reported with 150 mg/d; ref. 8). Having checked the safety of 50 mg/wk, a second phase including a 100 mg/wk arm and a no-treatment arm was implemented. Inclusion in either arm was based on patient preference after discussion of potential risks and benefits. The primary end point was the change of Ki-67 expression in normal prostate luminal cells. Secondary end points were the changes of HG-PIN and PIA prevalence and of circulating PSA and sex hormone levels. The study was approved by the hospital review board and a written informed consent was obtained by each subject.

### Treatment plan and study procedures

The study started on June 1, 2004 and the accrual was completed on June 22, 2006. Subjects received bicalutamide (Casodex, AstraZeneca) at the dose of 50 mg (1 tablet) or 100 mg (2 tablets at once) at lunchtime on every Sunday for 6 mo. The drug was purchased through the hospital Pharmacy, then packaged and labeled for the study. A third group of subjects did not receive any treatment and served as controls. Fasting blood samples were taken between 8 and 10 a.m. at baseline and at 3 and 6 mo visits for blood hematology, total and free PSA, gonadotropins, sex hormones, and sex hormone binding globulin. After 6 mo, an end-of-study prostate biopsy (8-10 cores) was done to assess changes in tissue biomarkers and morphology. Compliance was assessed by pill count. Toxicity was evaluated using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE; ref. 17), except for gynecomastia, which in the NCI-CTCAE is not graded quantitatively and does not include minimal asymptomatic breast gland enlargements. Thus, we

**Table 1.** Baseline subject characteristics by treatment arm

| | Control group (*n* = 26) | Bicalutamide 50 mg/wk (*n* = 26) | Bicalutamide 100 mg/wk (*n* = 28) |
|---|---|---|---|
| Age, y (mean ± SD) | 66 ± 7.6 | 64 ± 7.0 | 64 ± 6.8 |
| Body mass index, kg/m$^2$ (mean ± SD) | 25.8 ± 2.5 | 25.5 ± 3.1 | 25.9 ± 4.1 |
| Smoking habit (smoker/nonsmoker/former) | 5/10/11 | 4/15/7 | 7/14/7 |
| Alcohol consumption (0/1-14/15+ glasses/wk) | 7/10/9 | 11/10/5 | 6/16/6 |
| No. of subjects with 1st/2nd degree family history for prostate cancer | 7 | 5 | 1 |
| No. of cores per biopsy before entry biopsy (median, range) | 8 (6-10) | 6 (5-10) | 6 (5-8) |
| Total PSA, ng/mL (median, IQR) | 7.2 (6.2-9.9) | 8.8 (5.4-13.5) | 9.6 (7.6-12.0) |
| Free PSA, ng/mL (median, IQR) | 1.1 (0.7-1.7) | 1.8 (0.8-2.3) | 1.4 (1.1-2.0) |
| LH, mIU/mL (median, IQR) | 4.5 (3.4-6.1) | 4.6 (3.3-5.6) | 4.1 (3.6-7.0) |
| Testosterone, ng/mL (median, IQR) | 3.9 (3.1-5.2) | 3.8 (3.4-4.8) | 4.1 (3.3-5.3) |
| Free testosterone, % (median, IQR) | 33.3 (29.1-46.1) | 32.1 (28.9-36.2) | 35.8 (29.8-40.6) |
| 17β-Estradiol, pg/mL (median, IQR)* | 28.9 (22.4-32.7) | 20.5 (17.5-26.5) | 22.5 (18.7-29.8) |

Abbreviations: LH, luteinizing hormone; IQR, interquartile range.
*$P$ = 0.02 among arms.

graded G1 gynecomastia for asymptomatic breast gland swelling limited to the areolar perimeter, G2 gynecomastia for asymptomatic breast gland swelling extended beyond the areolar perimeter, and G3 gynecomastia for symptomatic breast swelling or gynecomastia requiring treatment.

### Analytic methods

***Histology and immunohistochemistry.*** Baseline and end-of-study biopsy samples were stained with H&E and examined under light microscopy. Six prespecified morphologic features were identified and ranked orderly: normal tissue, inflammation, simple atrophy, PIA, HG-PIN, and adenocarcinoma (18, 19), and each specimen was recorded according to type and location (apex, peripheral zone, transitional and central zones of both lobes, seminal vesicles). Specifically, PIA was identified according to the criteria described as postatrophic hyperplasia in a Working Group Classification of Focal Atrophy of the Prostate (19), which also includes detailed description of simple atrophy features. When two or more morphologic features were detected, the most severe was chosen to rank the specimen. All samples were analyzed blinded as to treatment arm and pretreatment versus posttreatment status.

Immunohistochemical detection of Ki-67 was done on normal acinar cells, where most prostate cancers are thought to arise, and on HG-PIN lesions, using the Benchmark XT, an automated slide preparation system (Ventana Medical Systems). Ki-67 expression was scored by a single pathologist on high-power (40× HPF) light microscopy on at least 300 nuclei as the percentage of luminal cells with positive nuclear staining in both normal tissue and HG-PIN.

***Circulating biomarkers.*** Blood samples were centrifuged at 1,000 × *g*, and sera were kept at −20°C until processing. Total PSA, free PSA, luteinizing hormone, follicle-stimulating hormone, testosterone, estradiol, and sex hormone binding globulin concentrations were assessed using electrochemiluminescence immunometric assays designed for the Elecsys automated analyzer (Roche Diagnostics SpA). Sensitivity of the assays was 0.002 ng/mL, <0.01 ng/mL, 0.10 mIU/mL, <0.10 mIU/mL, 0.069 ng/mL, 5.0 pg/mL and 0.35 nmol/l, respectively, and the intra-assay and interassay coefficients of variation were <2% and 5%, respectively. Free testosterone was calculated as the total testosterone/sex hormone binding globulin ratio.

### Study power and statistical analysis

We anticipated a linear dose-response effect on Ki-67 with relative reductions of 35%, 20%, and 0% on bicalutamide 100 mg/wk, bicalutamide 50 mg/wk, and no-treatment arm, respectively. Assuming a SD of 30% for the Ki-67 percent change after two serial biopsies (20) and a 10% drop-out, 25 subjects per arm were necessary to have 80% power to detect those differences. Bonferroni correction was used to compare each antiandrogen dose with the control group.

Data were analyzed using ANOVA and ANCOVA for baseline and end-point biomarker values, respectively. To achieve normality,

**Table 2.** HG-PIN status at baseline and at 6 mo in the three study arms

| Baseline* | No treatment, 6 mo (*n* = 23) | | Bicalutamide 50 mg/wk, 6 mo (*n* = 23) | | Bicalutamide 100 mg/wk, 6 mo (*n* = 24) | |
|---|---|---|---|---|---|---|
| | HG-PIN (–) | HG-PIN (+) | HG-PIN (–) | HG-PIN (+) | HG-PIN (–) | HG-PIN (+) |
| HG-PIN (–) | 14 | 3 | 12 | 3 | 12 | 4 |
| HG-PIN (+) | 1 | 5 | 5 | 3 | 7 | 1 |

NOTE: Data include samples with matched pretreatment and posttreatment data.
*Absence (–) or presence (+) of HG-PIN.

**Table 3.** Repeated measure analysis of total and free PSA at 3 and 6 mo by treatment group with median percent changes from baseline values

| Biomarker | Least square means | 95% CI | Median % change | Contrast | *P* for contrast | *P* for treatment |
|---|---|---|---|---|---|---|
| Total PSA, ng/mL | | | | | | |
| 3 mo | | | | | | |
| Control | 9.4 | 7.2-11.7 | 1.4 | Treated vs control | **<0.001** | **<0.001** |
| 50 mg/wk | 7.7 | 5.4-9.9 | -33.1 | 50 mg/wk vs 100 mg/wk | 0.8 | |
| 100 mg/wk | 6.9 | 4.7-9.1 | -30.7 | | | |
| 6 mo | | | | | | |
| Control | 9.7 | 7.5-12.0 | 7.4 | Treated vs control | **0.001** | |
| 50 mg/wk | 8.1 | 5.8-10.3 | -29.4 | 50 mg/wk vs 100 mg/wk | 0.4 | |
| 100 mg/wk | 8.0 | 5.8-10.2 | -25.0 | | | |
| Free PSA, ng/mL | | | | | | |
| 3 mo | | | | | | |
| Control | 1.3 | 1.0-1.6 | 2.4 | Treated vs control | **<0.001** | **<0.001** |
| 50 mg/wk | 1.2 | 0.9-1.5 | -29.0 | 50 mg/wk vs 100 mg/wk | 0.3 | |
| 100 mg/wk | 1.1 | 0.8-1.4 | -24.1 | | | |
| 6 mo | | | | | | |
| Control | 1.4 | 1.1-1.7 | 20.3 | Treated vs control | **<0.001** | |
| 50 mg/wk | 1.3 | 1.0-1.6 | -28.2 | 50 mg/wk vs 100 mg/wk | 0.5 | |
| 100 mg/wk | 1.2 | 0.9-1.5 | -24.4 | | | |

NOTE: *P* values for contrast were compared with *P* values adjusted for multiple comparisons. Bold *P* values remain statistically significant after Benjamini adjustment.

logarithmic transformation of Ki-67 was used and model assumptions were assessed using residual plots. $\chi^2$ or Fisher exact tests were used to compare arms for the prevalence of HG-PIN, PIA, and adverse event incidence. Cancer incidence was an exploratory measure given the short treatment period. Repeated measure analysis using mixed-effect modeling was adopted to test differences in mean circulating biomarker levels over time. Two linear orthogonal contrasts were used to compare the three treatments groups: any bicalutamide dose versus no treatment and 50 mg/wk versus 100 mg/wk. Two-tailed *P* value of 0.05 was used to define nominal statistical significance, with Benjamini-Hochberg adjustment (21) for multiple comparisons. Analyses were done using SAS software (SAS Institute, Inc.).

## Results

### Subject characteristics

From June 1, 2004 to June 22, 2006, 107 subjects were assessed for eligibility, 18 refused to participate, and 9 were not eligible, thus leaving 80 subjects for analysis (Fig. 1). The baseline subject characteristics and serum biomarkers are summarized in Table 1. There were no differences among arms except for estradiol, the levels of which were higher in the no-treatment arm ($P = 0.02$).

### Tissue biomarkers

***Immunohistochemistry.*** Treatment with bicalutamide did not significantly affect Ki-67 expression in luminal cells in either intervention group. The median (range) Ki-67 expression at baseline was low, being 3% (1-20%) in all three groups, and remained unchanged after 6 months in the no-treatment and 50 mg/wk groups, whereas it decreased to 2% (1-10%) in the 100 mg/wk group. Ki-67 expression was significantly higher in HG-PIN lesions (median, 10%; range, 1-35%) compared with normal tissue (median, 3%; range, 1-20%; $P < 0.01$). Any reliable analysis of Ki-67 change in HG-PIN was precluded because only 9 of 22 subjects with HG-PIN at baseline had HG-PIN foci on end-of-study biopsy.

***Prevalence of HG-PIN, PIA, and cancer.*** The median (range) number of cores on both baseline and end-of-study biopsy was 8 (8–10). Table 2 depicts HG-PIN status at baseline and at 6 months. HG-PIN status did not change at 6 months in 19 of 23 no-treatment subjects, 15 of 23 subjects treated with bicalutamide 50 mg/wk, and 13 of 24 subjects treated with bicalutamide 100 mg/wk. HG-PIN status worsened in three no-treatment, three 50 mg/wk, and four 100 mg/wk subjects; this status improved in one no-treatment, five 50 mg/wk, and seven 100 mg/wk subjects. The preventive effect was similar in the high-dose and low-dose arms, and so we combined these arms to increase statistical power. In the combined drug arms, 15% of subjects worsened, 60% had no change, and 26% improved, versus 13% worsened, 83% had no change, and 4% improved in the no-treatment group (Fisher's exact test, $P = 0.076$). This favorable trend on HG-PIN was more evident when the analysis was restricted to the small subset of subjects with HG-PIN at baseline, as HG-PIN foci were found on end-of-study biopsy in 5 of 6 no-treatment subjects, 3 of 8 subjects treated with 50 mg/wk, and 1 of 8 subjects treated with 100 mg/wk ($P = 0.03$ versus controls). Likewise, when subjects with multifocal HG-PIN at baseline were considered (four in each group), the prevalence of multifocal HG-PIN lesions at end-of-study biopsy was 3, 2, and 0 in the no-treatment, 50 mg, and 100 mg groups, respectively.

At baseline, PIA was found in 24 (30%) subjects (4, 12, and 6 subjects in the control, 50 mg/wk, and 100 mg/wk bicalutamide arms, respectively; $P < 0.03$). There was no effect of

bicalutamide on PIA prevalence change, as 5, 10, and 4 subjects exhibited PIA on end-of-study biopsy in the control, 50 mg, and 100 mg arms, respectively.

Eleven prostate cancers were found on end-of-study biopsy, 5 (22%) in the control arm and 3 (13%) in each bicalutamide arm. They were all Gleason score 4 to 6, except for two Gleason score 7 lesions (one in the control and one in the 50 mg/wk arm). Among subjects with baseline HG-PIN, 3 (50%) cancers were found in the control arm and 1 (13%) in each bicalutamide arm.

### Circulating PSA and hormones

Changes in total and free PSA during treatment are shown in Table 3. Compared with baseline, total and free PSA declined by ~30% after 3 months of bicalutamide, regardless of the dose, and plateaued thereafter. Conversely, total and free PSA increased up to 7% and 20% in the control arm ($P < 0.001$).

The changes of circulating hormones are shown in Table 4. Luteinizing hormone levels increased by 30% on 50 mg/wk and by 50% on 100 mg/wk and were unchanged in the control arm. Total and free testosterone levels increased by 30% to 40%, without differences between doses, and were unchanged in the control arm ($P < 0.001$). Compared with the control group, estradiol levels increased on bicalutamide ($P = 0.025$), although the trend was different in the two doses. No changes in follicle-stimulating hormone or SHBG levels were noted (data not shown).

**Table 4.** Repeated measure analysis of hormones at 3 and 6 mo by treatment group with median percent changes from baseline values

| Biomarker | Least square means | 95% CI | Median % change | Contrast | *P* for contrast | *P* for treatment |
|---|---|---|---|---|---|---|
| LH, mIU/mL | | | | | | |
| 3 mo | | | | | | |
| Control | 5.8 | 4.3-7.3 | 0 | Treated vs control | **0.003** | 0.04 |
| 50 mg/wk | 6.8 | 5.3-8.3 | 29.6 | 50 mg/wk vs 100 mg/wk | 0.5 | |
| 100 mg/wk | 8.6 | 7.1-10.1 | 53.6 | | | |
| 6 mo | | | | | | |
| Control | 5.7 | 4.1-7.2 | 2.6 | Treated vs control | 0.06 | |
| 50 mg/wk | 6.0 | 4.5-7.5 | 28.6 | 50 mg/wk vs 100 mg/wk | 0.3 | |
| 100 mg/wk | 8.3 | 6.8-9.8 | 50.6 | | | |
| Testosterone, ng/mL | | | | | | |
| 3 mo | | | | | | |
| Control | 4.6 | 4.0-5.2 | 8.5 | Treated vs control | **<0.001** | **<0.001** |
| 50 mg/wk | 5.7 | 5.1-6.3 | 42.3 | 50 mg/wk vs 100 mg/wk | 0.3 | |
| 100 mg/wk | 6.2 | 5.6-6.8 | 54.8 | | | |
| 6 mo | | | | | | |
| Control | 4.5 | 3.9-5.1 | 1.5 | Treated vs control | **<0.001** | |
| 50 mg/wk | 5.4 | 4.8-6.0 | 42.1 | 50 mg/wk vs 100 mg/wk | 0.4 | |
| 100 mg/wk | 6.0 | 5.4-6.6 | 43.7 | | | |
| Free testosterone, % | | | | | | |
| 3 mo | | | | | | |
| Control | 38.5 | 34.1-42.8 | 10.2 | Treated vs control | **<0.001** | **<0.001** |
| 50 mg/wk | 49.9 | 45.4-54.3 | 32.7 | 50 mg/wk vs 100 mg/wk | 0.9 | |
| 100 mg/wk | 49.5 | 45.0-53.9 | 34.2 | | | |
| 6 mo | | | | | | |
| Control | 38.3 | 33.8-42.8 | 3.1 | Treated vs control | **0.01** | |
| 50 mg/wk | 44.5 | 39.9-49.2 | 31.0 | 50 mg/wk vs 100 mg/wk | 0.4 | |
| 100 mg/wk | 47.6 | 43.3-52.0 | 36.2 | | | |
| 17β-Estradiol, pg/mL | | | | | | |
| 3 mo | | | | | | |
| Control | 30.8 | 27.1-34.4 | 11.5 | Treated vs control | 0.03 | **0.025** |
| 50 mg/wk | 30.2 | 26.5-33.9 | 33.3 | 50 mg/wk vs 100 mg/wk | 0.8 | |
| 100 mg/wk | 31.2 | 27.3-35.2 | 36.4 | | | |
| 6 mo | | | | | | |
| Control | 29.6 | 25.8-33.3 | -7.9 | Treated vs control | **0.01** | |
| 50 mg/wk | 33.8 | 30.1-37.5 | 58.2 | 50 mg/wk vs 100 mg/wk | 0.06 | |
| 100 mg/wk | 28.8 | 25.1-32.6 | 11.3 | | | |

NOTE: *P* values for contrast were compared with *P* values adjusted for multiple comparisons. Bold *P* values remain statistically significant after Benjamini adjustment.

**Table 5.** Number of subjects (%) with adverse events by treatment arm

| | Control group ($n$ = 26) | | Bicalutamide 50 mg/wk ($n$ = 26) | | Bicalutamide 100 mg/wk ($n$ = 28) | |
|---|---|---|---|---|---|---|
| | G1 | G2 | G1 | G2 | G1 | G2 |
| Fatigue | 2 (7.7) | — | 5 (19.2) | — | 3 (10.7) | 1 (3.6) |
| Weight gain | — | — | 1 (3.8) | — | 3 (10.7) | — |
| Hot flashes | — | — | — | — | 1 (3.6) | 1 (3.6) |
| Pollakiuria/nicturia | 4 (15.4) | — | 1 (3.8) | 1 (3.8) | 1 (3.6) | — |
| Gynecomastia* | — | — | 6 (23.1) | 6 (23.1) | — | 12 (42.9) |
| Decreased libido* | — | — | 6 (23.1) | 1 (3.8) | 7 (25.0) | 1 (3.6) |
| Impotence* | — | — | 5 (19.2) | — | 6 (21.4) | 1 (3.6) |

*Pearson $\chi^2$ $P < 0.05$.

### Compliance and toxicity

Compliance to bicalutamide was very high, the mean pill count being 99% on 50 mg/wk and 98% on 100 mg/wk. The most frequent adverse events are reported in Table 5. Serious adverse events included one stroke in the control arm, one pancreatic adenocarcinoma in the 100 mg bicalutamide arm, and one posttraumatic subdural hematoma in the 50 mg arm. There were no G3-G4 adverse events, including gynecomastia. Sexual complaints and breast symptoms were more frequent in treated subjects. Grade 2 gynecomastia (i.e., asymptomatic breast enlargement extending beyond the areola according to the grading system adopted) was noted in 6 (23%) subjects in the 50 mg/wk arm and in 12 (43%) subjects in the 100 mg/wk arm.

## Discussion

Bicalutamide was selected in this trial chiefly because of its proven efficacy and long-term safety profile in the treatment setting. Differently from finasteride, bicalutamide directly interacts with the androgen receptor, thereby neutralizing the potentially detrimental testosterone surge that is observed during treatment. Moreover, at the daily dose of 50 to 150 mg, bicalutamide was found to decrease the incidence and extent of HG-PIN in pilot trials (22, 23), thus predicting a beneficial modulation of prostate carcinogenesis in at-risk subjects. Finally, its pharmacodynamic and pharmacokinetic properties (9, 10) justified a once-a-week administration with 50 to 100 mg in an attempt to retain activity while substantially reducing toxicity. Subject selection was based on the notion that men with elevated circulating PSA and negative, noncancer biopsy findings constitute a suitable and relatively large population at higher risk of developing prostate cancer, for whom no intervention is currently available (11, 12).

We found that a low-dose, once-a-week schedule of bicalutamide is reasonably safe as compared with the daily administration of higher doses used in the therapeutic setting. Compliance was remarkably high and toxicity was mild, especially at the dose of 50 mg/wk, although this finding must be taken cautiously, given the lack of a placebo arm and quantitative measurement of breast gland enlargement. Nevertheless, gynecomastia was much lower in incidence (20-40%) and severity (≤G2) compared with that reported with daily bicalutamide monotherapy (>70% G3 gynecomastia; ref. 8).

Ki-67 expression in normal luminal cells was unaffected by bicalutamide. Normal luminal cells consistently expressed the proliferation marker Ki-67. However, the low levels of expression suggest that Ki-67 in normal luminal cells may not be the right surrogate biomarker for prostate cancer prevention. Although Ki-67 expression was significantly higher in HG-PIN lesions than in normal cells, most HG-PIN at baseline were not detected after 6 months of bicalutamide (12 of 16 lesions). Thus, we were unable to determine whether the observed change in HG-PIN prevalence was associated with a drop of Ki-67 expression in HG-PIN. Expression of Ki-67 in prostate cancer has prognostic value (24, 25), and its decrease after androgen withdrawal represents an early event associated with tumor growth inhibition in animal models of prostate cancer (26, 27). Whereas in normal prostate tissue Ki-67 is mainly expressed in basal cells, progression of prostate carcinogenesis is associated with a shift in proliferation from the basal to the luminal epithelial layer, with Ki-67 expression increasing from 1% to 2% in normal luminal cells to ~10% in HG-PIN (28), in line with our findings.

A secondary analysis of the changes induced by a 6-month treatment with weekly, low-dose bicalutamide on prostate morphology revealed a tendency to a favorable modulation of high-risk proliferative lesions such as HG-PIN. Namely, HG-PIN status improved in 26% of treated subjects as compared with 4% of subjects in the no-treatment arm. These findings must be interpreted with extreme caution due to the inherent limitations of prostate sampling with needle biopsy. However, our analysis including all changes in HG-PIN status (i.e., both HG-PIN resolution and HG-PIN development after 6 months of treatment) may account, at least in part, for sampling errors, supporting the worth of our findings although they derive from a post hoc analysis. The 28% incidence of HG-PIN found in our study was unusually high compared with that reported in the general population, which ranges from 0.7% to 25% in noncancerous prostate biopsies (24). HG-PIN incidence increases with age and is higher in men seen in clinical practice compared with men participating in screening programs (29). As most subjects in our study were

in their seventies and as all were urological practice outpatients, this could partly explain our findings. Moreover, whereas HG-PIN by itself does not seem to increase PSA (29), the incidence of HG-PIN in homogeneous cohorts of men with elevated PSA is largely unknown. A trend toward a reduction of prostate cancer prevalence with bicalutamide versus no treatment (13% versus 22%) was also observed in our study, especially considering subjects with HG-PIN at baseline (13% versus 50%). We did not collect data on changes in prostate volume, which is known to be reduced by bicalutamide at the dose of 50 mg/d (22). Because prostate volume shrinkage under antiandrogen manipulation increases the likelihood of detecting prostate cancer or HG-PIN on end-of-study biopsy (7), the effect of weekly bicalutamide in treated subjects could even be underestimated in our study.

PIA prevalence was unaffected by bicalutamide. Because this lesion seems to arise from regenerative epithelial proliferation in response to injury caused by inflammatory oxidants (16), our findings imply that anti-inflammatory or antioxidant agents are more likely than hormonal maneuvers to favorably affect this different carcinogenic pathway.

Compared with controls, who exhibited a 20% increase of PSA, subjects on bicalutamide showed a 50% decrease of total and free PSA. Changes of serum hormones were comparable to those reported with higher daily doses of the drug (30). Whereas the role of PSA decline as a surrogate biomarker of prostate cancer risk reduction is still unclear, and may partly be the result of the drug antiandrogenic activity (31), risk assessment models clearly indicate that prostate cancer risk increases along with the increase in PSA levels (32). More importantly, in the Prostate Cancer Prevention Trial, a nearly 50% decrease of PSA during finasteride in men with a baseline PSA value of <3 ng/mL was associated with a 25% risk reduction of prostate cancer (32), suggesting a potential role for PSA as a surrogate biomarker for prostate cancer risk prevention. Altogether, our findings on PSA and hormone changes indicate that a dose as low as 50 mg/wk is able to exert meaningful biological changes that might translate into favorable clinical effects.

An important limitation of our study is the lack of randomization and of a blinded placebo arm. However, safety considerations guided our study design in the first place. Given the likely occurrence of dose-response gynecomastia, allocation to increased bicalutamide dose through randomization was not deemed appropriate and was therefore based on patient's preference after thorough discussion of potential benefits and risks. Another weakness of our study turned out to be the choice of the primary end-point Ki-67 in normal luminal cells, whose low levels of expression may prevent detection of meaningful changes. However, the study was exploratory in nature, given the lack of established biomarkers for phase II chemoprevention trials of prostate cancer. Another limitation comes from the observed favorable trend on HG-PIN, which emerged from a secondary post hoc analysis on a small number of subjects.

Nevertheless, to our knowledge, this is the first study assessing an intermittent (weekly) low-dose administration of bicalutamide in men at risk for prostate cancer. These results as well as those recently achieved by low-dose flutamide (i.e., 250 mg daily; ref. 33) further support the contention that androgen receptor antagonists at doses lower than the therapeutic setting may be reasonably safe and effective in this high-risk setting.

In conclusion, we showed that a once-a-week administration of bicalutamide in men at risk for prostate cancer is feasible and reasonably safe. This finding, coupled with the encouraging signal of activity emerging from the analysis of HG-PIN changes, supports further studies of this schedule at the lowest dose for prostate cancer prevention in men at high risk despite the negative primary end-point findings on Ki-67.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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