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# Discovery of BMS-641988, a Novel and Potent Inhibitor of Androgen Receptor Signaling for the Treatment of Prostate Cancer

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## Abstract

**Despite an excellent initial response to first-line hormonal treatment, most patients with metastatic prostate cancer will succumb to a hormone-refractory form of the disease. Because these tumors are still dependent on a functional androgen receptor (AR), there is a need to find novel and more potent antiandrogens. While searching for small molecules that bind to the AR and inhibit its transcriptional activity, BMS-641988 was discovered. This novel antiandrogen showed an increased (>1 log) potency compared with the standard antiandrogen, bicalutamide, in both binding affinity to the AR and inhibition of AR-mediated transactivation in cell-based reporter assays. In mature rats, BMS-641988 strongly inhibited androgen-dependent growth of the ventral prostate and seminal vesicles. In the CWR-22-BMSLD1 human prostate cancer xenograft model, BMS-641988 showed increased efficacy over bicalutamide (average percent tumor growth inhibition >90% versus <50%), even at exposure levels of bicalutamide 3-fold greater than what can be attained in humans. Furthermore, BMS-641988 was efficacious in CWR-22-BMSLD1 tumors initially refractory to treatment with bicalutamide. BMS-641988 was highly efficacious in the LuCaP 23.1 human prostate xenograft model, inducing stasis throughout the ~30-day dosing. To explore the functional mechanisms of BMS-641988, gene expression profiling analysis was done on CWR-22-BMSLD1 xenograft models in mice. Treatment with BMS-641988 resulted in a global gene expression profile more similar to castration compared with that of bicalutamide. Overall, these data highlight that the unique preclinical profile of BMS-641988 may provide additional understanding for the hormonal treatment of prostate cancer.** [Cancer Res 2009;69(16):6522–30]

## Introduction

Carcinoma of the prostate is the second leading cause of cancer-related death in men, with an estimated 186,320 new cases diagnosed and 28,660 deaths expected in the United States for 2008 (1). The androgen receptor (AR), a ligand-regulated transcription factor member of the nuclear hormone receptor superfamily (2, 3), plays an important role in both the etiology and the progression of prostate carcinoma. The binding of its specific steroid hormones, testosterone and the more potent dihydrotestosterone (DHT), to the ligand-binding domain of the AR, promotes its disassociation from heat shock proteins and related chaperones, allowing its dimerization, phosphorylation, and subsequent translocation into the nucleus. Once inside the nucleus, the AR binds to the androgen response elements present in the regulatory regions of a variety of genes, many of which are involved in the growth, survival, and differentiation of prostate cells (4). Complete androgen blockade was pursued in the clinic by the use of antiandrogens, such as bicalutamide (AstraZeneca), flutamide (Schering Plough), or nilutamide (Sanofi-Aventis), in combination with chemical castration achieved by the use of luteinizing hormone–releasing hormone agonists, such as leuprolide acetate (Takeda). Despite an impressive initial response, in most cases, the tumors will progress through treatment to a hormone-refractory prostate cancer (HRPC) or castration-resistant prostate cancer stage in an average of 18 months (5). The first designation is a misnomer, because these tumors are by no means AR-independent, as shown by a significant body of evidence recently accumulated; there is a clear dependence on AR signaling, even in the most advanced HRPC. Analysis of tumor samples from patients with HRPC has revealed several mechanisms used by tumor cells to reactivate the AR signaling at subphysiologic serum concentration of androgens or even in the presence of AR antagonists. These mechanisms include (*a*) gene amplification and increased expression of the AR protein (6); (*b*) selection of point mutations in the AR ligand binding that can result in activation by nonandrogenic ligands (7), or mutations in other regions, such as the amino terminus (8) or the DNA-binding domain that confer oncogenic properties to the AR (9); (*c*) expression of alternatively spliced variants of the AR that lack the ligand-binding domain and are constitutive active (10–12); the mRNA of two of the most abundant isoforms, AR-V1 and AR-V7, showed an average 20-fold higher expression in HRPC specimens ($n$ = 25) when compared with hormone-naive prostate cancer ($n$ = 82; $P$ < 0.0001; ref. 11); (*d*) changes in the expression ratios between AR, coactivators, and corepressors (13–15); (*e*) changes in the expression of enzymes involved in steroidogenesis (16); and (*f*) changes in signal transduction pathways (epidermal growth factor, insulin-like growth factor, interleukin-6, Wnt signaling, Stat5a/b, Ras/Raf/mitogen-activated protein kinase, phosphatidylinositol 3-kinase/Akt, etc.) that promote post-translational modifications (e.g., phosphorylation) of the AR and potentiate its activity under androgen-depleted conditions (17–21). Consistent with these observations, recent data have shown that many patients will respond to a second round of

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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hormone therapy, confirming the critical role of the AR in HRPC and suggesting that targeting the AR with novel agents could have additional clinical benefits.

In this report, the preclinical characterization of BMS-641988, a new-generation small-molecule inhibitor of androgen signaling, is described. Our data show that BMS-641988 is a potent antiandrogen both *in vitro* and *in vivo*. Furthermore, gene expression analysis indicates that BMS-641988 functions more similar to castration than does bicalutamide.

## Materials and Methods

**AR binding assay.** A cell-based radioligand AR-competitive binding assay was performed using the human breast adenocarcinoma MDA-MB-453 cells as reported previously (22). For the saturation analysis, medium containing [$^3$H]DHT (0.1-16 nmol/L) without (total binding) or with (nonspecific binding) 500-fold molar excess unlabeled DHT was used. Specific binding was evaluated by Scatchard analysis; the $K_d$ for [$^3$H]DHT was 0.4 nmol/L. For the competition studies, medium containing 2 nmol/L [$^3$H]DHT and test compounds (two replicates/sample) in concentrations ranging from $10^{-10}$ to $10^{-5}$ nmol/L was used. $K_i$ was determined by using the Cheng-Prusoff equation.

**Cell-based AR-dependent transactivation assay.** The human breast MDA-MB-453 (expressing wild-type AR) and prostate LNCaP (American Type Culture Collection) or 22rv1 (ref. 23; American Type Culture Collection; both expressing mutant endogenous AR; refs. 12, 24) cell lines were used. Transient transfections by electroporation were optimized accordingly (25) with either pSEAP2/PSA540/Enhancer or pGL3/PSA540/Enhancer luciferase reporter plasmid. The secreted alkaline phosphatase reporter plasmid is composed of the human prostate-specific antigen (PSA) promoter (position 5284-5831; accession no. U37672; ref. 26) and a PSA upstream enhancer containing additional androgen response elements (position 503-1952), which were generated by PCR and subcloned into the *Bgl*II/*Hind*III and *Xho*I/*Hind*III sites of pSEAP2/basic (Clontech), respectively. The firefly luciferase reporter plasmid (PSA-Luc) was constructed in a similar manner, using the pGL3 vector (Promega).

Cells were collected in medium containing 10% charcoal-stripped fetal bovine serum. LNCaP and MDA-MB-453 cells were distributed into cuvettes and electroporated with 8 μg reporter construct using a Bio-Rad Gene Pulser at 210 V and 960 μF. 22rv1 cells were transfected using Lipofectamine 2000 (Invitrogen), 50 ng of the reporter plasmid, and 25 ng of a *Renilla* luciferase control plasmid (Promega). Cells were assayed in the absence (blank) or presence (control) of 1 nmol/L DHT (Sigma) and BMS-641988 or bicalutamide ($10^{-10}$–$10^{-5}$ mol/L). After 24 or 48 h, supernatants from duplicate samples were assayed individually for secreted alkaline phosphatase or luciferase activity using the Phospha-Light Chemiluminescent Reporter Gene Assay System (Tropix) or the Dual-Glo Luciferase Assay System (Promega), respectively.

**Proliferation assays.** Cell proliferation was determined by using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega), by [$^3$H]thymidine incorporation, and by direct cell counting. $IC_{50}$ was defined as the concentration that inhibited 50% of cellular growth.

**Animal studies.** All animal studies were done according to the USPHS Policy on Humane Care and Use of Laboratory Animals in an American Association for Laboratory Animal Care–approved site.

**Mature rat prostate weight model.** Matched sets of Harlan-Sprague-Dawley rats (40-42 days old, 180-220 g) were treated orally by gavage (p.o.) once a day for 14 days with the test compounds in dissolved/suspensions of 80% PEG-400 and 20% Tween 20. Intact and castrated animals (five per cohort) were dosed at 0.5 mL vehicle/100 g body weight. Two additional groups of intact animals were dosed with BMS-641988 or bicalutamide (AstraZeneca). Animals were sacrificed and the ventral prostate, seminal vesicles, and liver were removed surgically and weighed. Immediately after euthanasia, serum was collected and promptly frozen (−80°C). Serum rat luteinizing hormone was quantified using the rLH-[$^{125}$I] Biotrak assay (Amersham). Total testosterone was quantified using Coat-A-Count (Diagnostic Products; PITKTT-3).

**Immunohistochemistry.** Tissue specimens were prepared by standard techniques. Staining optimization was automated in a BioGenex i6000. For antigen retrieval, Citra Plus (BioGenex) was used at 95°C for 15 min. Cellular proliferation was determined with primary rabbit monoclonal antibody Ki-67 at 1:100 dilution (Thermo Scientific). For detection, an EnVision kit (DAKO) was used. Slides were then scanned with the Aperio Scanning System and analyzed using the nuclear quantification algorithm.

**Efficacy studies in s.c. human prostate cancer xenograft models.** Male immunodeficient mice [BALB/c athymic (*nu/nu*); Harlan; 8-10 weeks old] were used. CWR-22-BMSLD1 and LuCaP 23.1 human xenografts were implanted s.c. Compounds were prepared in 80% PEG-400 and 20% Tween-80 and administered orally by gavage needle. Treatments were initiated when tumors reached a median size between 75 and 200 mm$^3$. Each treatment regimen was tested in cohorts of eight mice. Tumors were measured weekly for LuCaP 23.1 and twice-weekly for CWR-22-BMSLD1. Tumor size (mm$^3$) was calculated as (length × width$^2$) / 2. Efficacy was defined as ≥50% median tumor growth inhibition (%TGI) for longer than one tumor volume doubling time (TVDT) compared with controls. Efficacy was determined by time to tumor progression to target size (T-C), where the time (days) for the median tumor size of control (C) mice to reach target size was subtracted from that of treated (T) mice. A delay of more than one TVDT in reaching target size was considered an active result.

To determine whether BMS-641988 could exhibit antitumor activity in tumors that failed bicalutamide treatment, animals bearing CWR-22-BMSLD1 tumors (median tumor size of 80 mm$^3$) were treated daily, starting at day 21 post-implant with bicalutamide (150 mg/kg). Once the median tumor size of the group tripled, animals were randomized into two groups: one continued receiving bicalutamide (150 mg/kg) and the other group switched to BMS-641988 (90 mg/kg). BMS-641988 was also evaluated as a single agent at 90 mg/kg.

**PSA determination.** Mouse blood samples were collected from the retro-orbital plexus and serum PSA levels were determined weekly in duplicate 5 μL samples using an EIA kit (Sierra).

**Gene expression analysis of CWR-22-BMSLD1 tumors.** CWR-22-BMSLD1 human prostate tumors were implanted into nude mice at an average volume of 150 mm$^3$ and treated with vehicle (p.o.; 80% PEG-400 and 20% Tween 20), BMS-641988 (p.o.; 90 mg/kg), castration, or bicalutamide (p.o.; 150 mg/kg). RNA samples were isolated (one tumor per animal, three mice per group per time point) at 1, 2, and 7 days. Tumors were profiled on Affymetrix U133A DNA microarray chips, containing sequences of ~22,000 different genes (36 microarrays). The resulting microarray data were analyzed using Rosetta Resolver.

**Quantitative reverse transcription-PCR.** cDNA was prepared with SuperScript II (Invitrogen) and random primers. Quantitative PCR analysis was done on a 7900HT SDS with SYBR Green PCR Master Mix (Applied Biosystems). Gene-specific primers (Supplementary Table S1) were designed using Primer Express software. cDNA equivalent to 25 ng RNA was used as PCR template. Sample variation was normalized by 18S RNA. Samples were run in duplicate using the default thermal profile. Dissociation curve analysis was done after completion of PCR to confirm the specificity of amplified products.

**Statistical methods.** Analysis of covariance on the tumor volume at specific time points was applied for the tumor efficacy studies. *P* values of the multiple comparisons at each time point were adjusted by Hochberg's method. Two-sample *t* tests were used to analyze results from the Mature Prostate Weight Model and PSA serum levels.

## Results

**BMS-641988 has potent *in vitro* antiandrogen activity.** BMS-641988 was identified as a novel and highly potent series of [2.2.1]-oxobicyclo-imide–based AR antagonists (ref. 27; Fig. 1*A*). BMS-641988 binds to the endogenous wild-type AR with 20-fold higher affinity compared with the standard antiandrogen bicalutamide as determined by [$^3$H]DHT competition assays done with MDA-MB-453 cells (Table 1). BMS-641988 binds with high selectivity to

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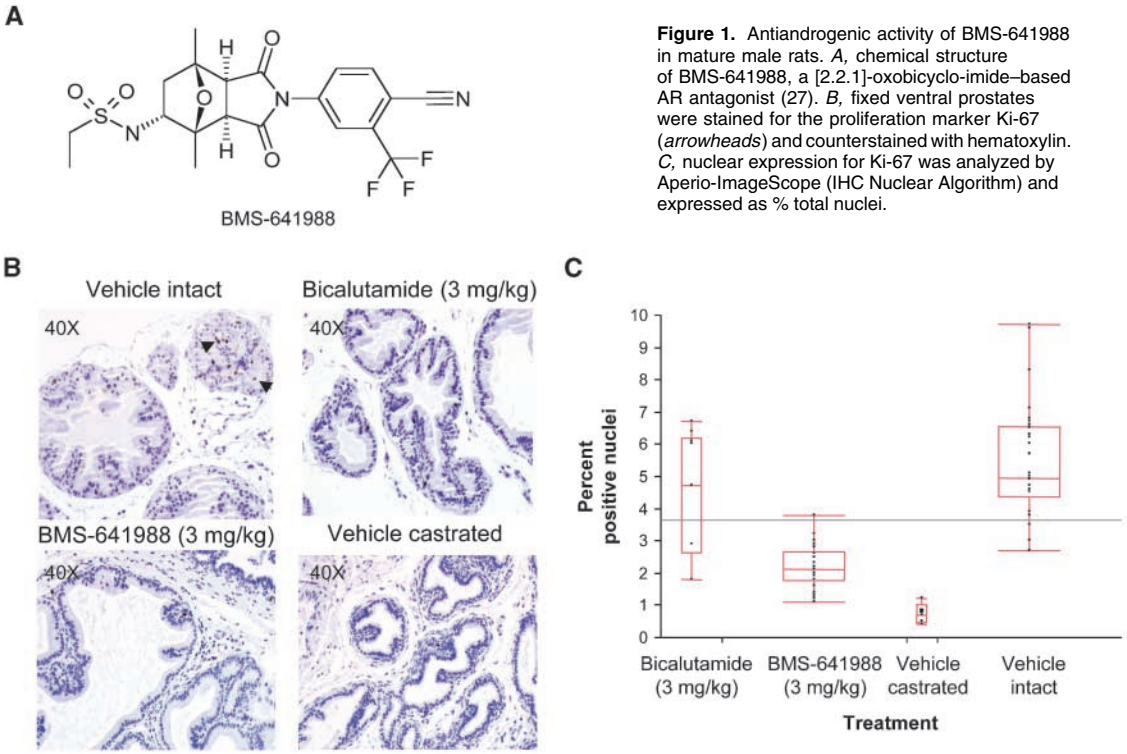


**Figure 1.** Antiandrogenic activity of BMS-641988 in mature male rats. *A,* chemical structure of BMS-641988, a [2.2.1]-oxobicyclo-imide–based AR antagonist (27). *B,* fixed ventral prostates were stained for the proliferation marker Ki-67 (*arrowheads*) and counterstained with hematoxylin. *C,* nuclear expression for Ki-67 was analyzed by Aperio-ImageScope (IHC Nuclear Algorithm) and expressed as % total nuclei.

the AR compared with the glucocorticoid and progesterone receptors (data not shown). To determine the effect of BMS-641988 on the transcriptional activity of the AR, cell lines expressing either endogenous wild-type (MDA-MB-453) or mutant (LNCaP or 22rv1) forms of the AR were transiently transfected with a construct consisting of the secreted alkaline phosphatase or the luciferase reporter genes driven by both the immediate promoter and the enhancer of the human PSA gene. In these assays, BMS-641988 displayed between 3- and 7-fold increased antagonist activity of AR transactivation compared with bicalutamide (Table 1). Interestingly, as observed with other potent antiandrogens (24, 28), BMS-641988 stimulated the proliferation of LNCaP cells.

**BMS-641988 exhibits potent antiandrogen activity in mature rats.** The effect of BMS-641988 on the ventral prostate and seminal vesicles, two organs normally regulated by androgens, was tested in sexually mature male rats. As shown in Table 1, castration promoted a dramatic reduction in the weights of ventral prostate [5 ± 3% with respect to the vehicle-treated controls (%C)] and seminal vesicles (10 ± 1%C). In addition, BMS-641988 produced a significant reduction in the weight of ventral prostate compared with bicalutamide (52 ± 7%C versus 70 ± 24%C; $P = 0.013$) at 1 mg/kg and in the weight of the seminal vesicles (32 ± 3%C versus 50 ± 5%C; $P = 0.0035$) when administered at 1 mg/kg as well as when dosed at 3 mg/kg (26 ± 6%C versus 33 ± 15%C; $P = 0.020$).

To determine the effect of BMS-641988 and bicalutamide on the rate of cellular proliferation in the prostate, tissue sections were subjected to immunohistochemistry staining for the detection of Ki-67 expression. As shown in Fig. 1*B*, prostates isolated from control animals treated with vehicle alone showed nuclei positive for Ki-67 (5.1% of all nuclei showed brown staining; Fig. 1*C*). Prostates from animals treated with BMS-641988 or bicalutamide at 3 mg/kg showed 2.1% and 4.7% of the nuclei stained positive, respectively ($P < 0.0001$, *t* test). These results suggest a more marked antiproliferative effect of BMS-641988 at that dose. Prostates from castrated animals showed the lowest levels of proliferation (0.7%), which is expected because prostates do not develop in the absence of androgen signaling.

The use of sexually mature rats allowed the assessment of the effects of the compounds on the androgen-controlled hypothalamic-pituitary-endocrine axis, specifically on the changes in the serum levels of luteinizing hormone and testosterone. As the result of its potent antiandrogen activity, BMS-641988, at its highest dose, significantly increased the serum levels of serum luteinizing hormone and testosterone compared with vehicle-treated controls (Table 1).

**BMS-641988 has potent antitumor activity in the human prostate cancer model CWR-22-BMSLD1.** The CWR-22 human tumor xenograft model was derived from a primary adenocarcinoma of the prostate of a patient undergoing prostatectomy (29, 30). The AR in this tumor line exhibits a H874Y substitution in the ligand-binding domain (31). CWR-22 cells grow in athymic male mice without testosterone supplementation, unlike the original tumor line developed by Pretlow and colleagues (29).

Paradoxically, CWR-22-BMSLD1 cells retain sensitivity to endogenous circulating levels of androgens, because marked TGI occurs on castration. With regard to antiandrogen sensitivity, CWR-22-BMSLD1 tumors are marginally responsive to flutamide (data not shown) or bicalutamide (Fig. 2*A*). Thus, this model is appropriate to investigate the antitumor activity of BMS-641988 (10, 30, and 90 mg/kg) compared with bicalutamide (50 and

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**Table 1.** BMS-641988 *in vitro* and *in vivo* antiandrogen activity

| *In vitro* pharmacology* | Cell line | BMS-641988 | Bicalutamide |
|---|---|---|---|
| AR binding, $K_i$ (nmol/L) | MDA-MB-453 | 1.8 ± 0.2 | 37 ± 3 |
| AR transactivation, $IC_{50}$ (nmol/L) | MDA-MB-453 | 16 ± 3 | 173 ± 67 |
| | LNCaP | 153 ± 77 | 935 ± 257 |
| | CWR-22rv1 | 273 ± 160 | 3,543 ± 648 |
| Proliferation, $IC_{50}$ (μmol/L) | LNCaP | Agonist | >5 |

Mature rat prostate weight model †

| Treatment | Castration | Dose (mg/kg) | VP | SV | LH | T |
|---|---|---|---|---|---|---|
| | | | %C ± SD | %C ± SD | %C ± SD | %C ± SD |
| Vehicle | | NA | 100 ± 31 | 100 ± 24 | 100 ± 17 | 100 ± 50 |
| | Y | NA | 5 ± 3 | 10 ± 1 | 2,991 ± 815 | ND |
| BMS-641988 | | 0.3 | 77 ± 20 | 49 ± 7 | 154 ± 39 | 84 ± 46 |
| | | 1 | 52 ± 7 | 32 ± 3 | 164 ± 58 | 76 ± 24 |
| | | 3 | 24 ± 12 | 26 ± 6 | 191 ± 59 | 152 ± 55 |
| | | 10 | 35 ± 9 | 15 ± 4 | 291 ± 31 | 188 ± 97 |
| | | 30 | 27 ± 6 | 15 ± 3 | 782 ± 172 | 464 ± 60 |
| Bicalutamide | | 0.3 | 106 ± 9 | 56 ± 12 | 127 ± 28 | 96 ± 51 |
| | | 1 | 70 ± 24 | 50 ± 5 | 109 ± 37 | 88 ± 57 |
| | | 3 | 57 ± 8 | 33 ± 15 | 109 ± 50 | 212 ± 74 |
| | | 10 | 32 ± 6 | 21 ± 3 | 291 ± 102 | 100 ± 39 |
| | | 30 | 35 ± 8 | 16 ± 4 | 291 ± 66 | 144 ± 101 |

*All indicated values are the result of multiple assays done in duplicate.
†Ventral prostate (VP) and seminal vesicles (SV) were normalized as mg/100 g body weight and expressed as % intact control (%C). Serum luteinizing hormone (LH) and testosterone (T) values are also expressed as %C.

150 mg/kg). Both compounds were administered p.o. once a day × 37. BMS-641988 (90 mg/kg) showed a markedly superior, dose-dependent antitumor effect with significant ($P < 0.05$) activity at all doses tested compared with vehicle-treated mice (Fig. 2*A*; Table 2*A*). Furthermore, at 90 mg/kg, minimal increases in tumor growth were observed over time: the median tumor size at initiation and end of treatment was of 106 and 144 $mm^3$, respectively. Conversely, animals treated with 150 mg/kg bicalutamide had tumors that grew progressively (Fig. 2*A*).

On cessation of treatment, tumors restored their capacity to grow. However, treatment with 90 mg/kg BMS-641988 delayed progression of tumor growth to target size (1,000 $mm^3$), as indicated by a T-C of 36 days (approximately three TVDT), whereas 150 mg/kg bicalutamide only delayed tumor growth to target size by 12 days (approximately one TVDT). All doses of BMS-641988 and bicalutamide were well tolerated throughout the study (data not shown).

It was also investigated whether mice treated for 30 days with BMS-641988 had decreased serum PSA levels, a surrogate marker for tumor burden in humans (32) and, in this case, an *in vivo* pharmacodynamic endpoint of antiandrogen activity. BMS-641988 was evaluated at its optimal dose of 90 mg/kg compared with bicalutamide at 150 mg/kg (Table 2*B*). BMS-641988 treatment resulted in almost complete stasis of tumor growth (%TGI = 95%), whereas bicalutamide showed marginal antitumor activity (%TGI = 33%). Similar to its antitumor activity, BMS-641988 reduced PSA levels >80% compared with vehicle controls, whereas bicalutamide reduced PSA levels by only 20% to 50%.

**BMS-641988 shows antitumor activity in CWR-22-BMSLD1 tumors that progress during bicalutamide treatment.** BMS-641988 was tested in CWR-22-BMSLD1 xenografts that failed bicalutamide treatment. Fourteen days after initiation of treatment (day 21), the group receiving daily bicalutamide 150 mg/kg showed a 3-fold increase in median tumor size. At this point, the animals were randomized into two groups (Fig. 2*B, arrow*). The first group continued to be treated with bicalutamide at 150 mg/kg, and the second group was switched to treatment with BMS-641988 at 90 mg/kg. There was a significant delay of tumor growth in the group switched to BMS-641988 compared with the bicalutamide-only group (Fig. 2*B*, days 43-56; Table 2*C*; $P < 0.05$). Clearly, tumors from animals switched to BMS-641988 showed minimal growth, whereas tumors from animals treated with bicalutamide alone grew progressively. As seen in previous studies, BMS-641988 as a single agent showed efficacy at 90 mg/kg with a T-C of 35 days. Conversely, bicalutamide (150 mg/kg) in mice bearing CWR-22-BMSLD1 tumors that failed a suboptimal dose of BMS-641988 did not inhibit tumor growth (data not shown).

**Antitumor activity of BMS-641988 in the LuCaP 23.1 human prostate carcinoma model.** BMS-641988 antitumor activity was evaluated in LuCaP 23.1 cells (33), a human prostate tumor line that presents two copies of the wild-type AR gene, expresses high levels of PSA, responds to bicalutamide, and is androgen dependent (34). BMS-641988 at doses of 10, 30, and 90 mg/kg and bicalutamide at doses of 16.7 and 150 mg/kg produced significant inhibition of tumor growth ($P < 0.01$; Fig. 3; Table 2*D*). Once treatments ended, tumors treated with both bicalutamide and

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BMS-641988 began to grow at similar rates. These data suggest that, in a tumor model sensitive to bicalutamide, BMS-641988 produced significant antitumor activity within a wide range of doses.

**BMS-641988 generates a transcriptomic profile that more closely resembles to castration than does bicalutamide.** To elucidate mechanistic differences, the global gene expression of CWR-22-BMSLD1 human prostate xenografts in nude mice treated was compared with vehicle control, castration and 90 mg/kg BMS-641988 or 150 mg/kg bicalutamide, the doses with maximal antitumor activity. Gene expression was profiled on Affymetrix U133A DNA microarray chips. Three hundred fifty-six transcripts significantly changing among the treatments (defined as $P < 0.01$ and $\geq$2-fold change for at least two of three time points in a treatment group) showed a significantly higher correlation between BMS-641988 and castration than between bicalutamide and castration (a correlation of 92% versus 67% following 7 days of treatment; Fig. 4*A*).

More than 50 PCR probe sets that displayed high confidence changes on the DNA microarray analysis were selected, and the responses to 7 days of castration were confirmed by quantitative reverse transcription-PCR. The fold changes (castration versus control) from the Affymetrix chips analysis correlated well with those from quantitative reverse transcription-PCR (Supplementary Table S2). In addition, the profile for several of the genes tested (*IGFBP3*, *FKBP5*, and *ODC1*) correlated with previous reports (35–37). The similarity between BMS-641988 treatment and castration on several genes was further confirmed by ANOVA of the microarray data (Fig. 4*B*; Supplementary Fig. S1). The similarities between the global effects exhibited by treatment with BMS-641988 and castration are striking and grant further investigation of BMS-641988.

## Discussion

Prostate cancer is the second leading cause of cancer death in men in the United States, and although androgen ablation modalities have been used effectively as palliative therapies over the past 60 years, they are almost never curative. The AR plays a key role in the etiology and progression of prostate cancer (7, 38). Highlighting its importance, the expression of the AR is preserved in most prostate cancer specimens independently of the stage and grade. Unlike the ER in breast cancer, the AR is rarely lost; for example, Sasaki and colleagues reported that only 3 of 38 (8%) HRPC patient specimens presented methylation of the AR promoter; similarly, all normal tissues studied were unmethylated (39). The understanding of the molecular mechanisms responsible for the prostate cancer transition to a hormone-refractory stage remains a challenge, both therapeutically and experimentally. However, a large amount of evidence supports the notion that functional AR signaling is required for the growth of castration-resistant prostate cancer and that more potent antiandrogens could address many of the proposed resistance mechanisms (7). In this report, data are presented on the preclinical characterization of BMS-641988, a member of a novel and highly potent series of [2.2.1]-oxobicycloimide–based AR antagonists (27).

In most studies presented herein, BMS-641988 was compared directly with bicalutamide (Casodex; AstraZeneca), the most widely used antiandrogen in the clinic. BMS-641988 is an androgen-competitive inhibitor that binds to the endogenous AR with 20-fold higher affinity compared with bicalutamide. It exhibits between 3- and 7-fold increased potency as an antagonist of both wild-4type and mutant AR transcriptional activity compared with the standard antiandrogen. Although BMS-641988 showed potent antagonist activity in transactivation assays done with LNCaP cells transiently

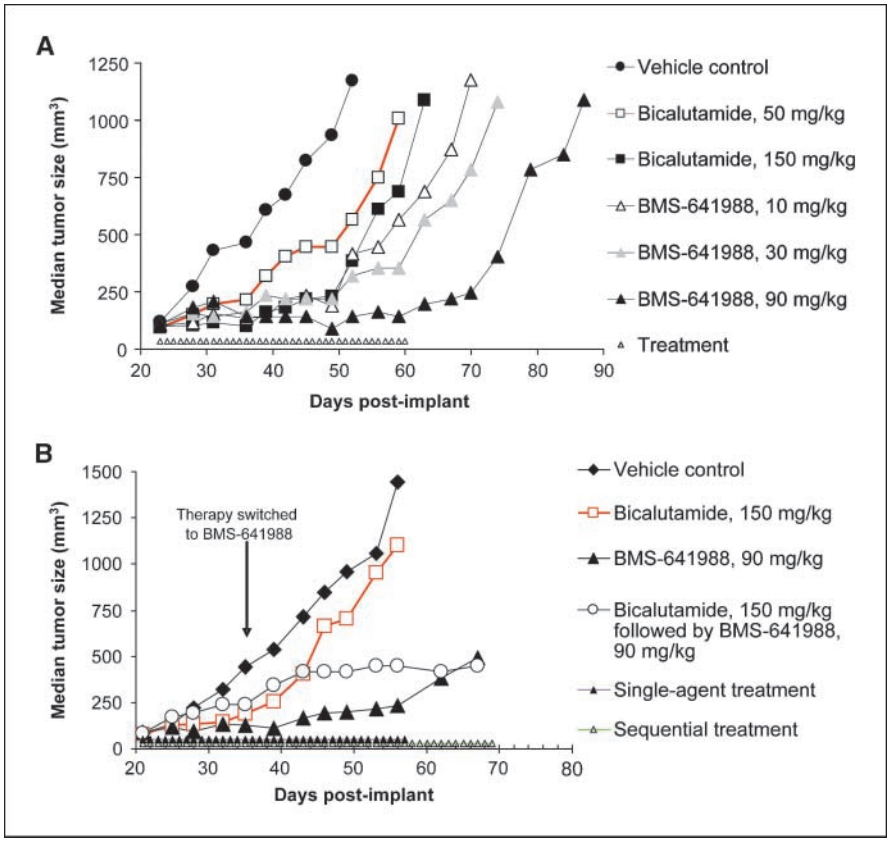


**Figure 2.** BMS-641988 has potent antitumor activity in the CWR-22-BMSLD1 model including in progression during bicalutamide treatment. *A,* human CWR-22-BMSLD1 tumors were s.c. implanted in male nude mice and treated p.o. once a day × 37. *B,* human CWR-22-BMSLD1 tumors were s.c. implanted in male nude mice. Treatments were administered p.o. once a day (*small triangles*). Mice treated with bicalutamide were randomized (*arrow*) to remain on bicalutamide (*open squares*) or switched to BMS-641988 (*open circles*).

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**Table 2.** BMS-641988 antiandrogen activity in human prostate cancer models

(A) BMS-641988 antitumor activity in the human prostate cancer model CWR-22-BMSLD1

| Treatment | Dose (mg/kg) | %TGI | T-C (1,000 $mm^3$) |
|---|---|---|---|
| Vehicle | | | |
| BMS-641988 | 10 | 60* | 18 |
| | 30 | 81* | 22 |
| | 90 | 97*, † | 36 |
| Bicalutamide | 50 | 10 | 9 |
| | 150 | 47 | 12 |

(B) BMS-641988 antitumor activity in the human prostate cancer model CWR-22-BMSLD1

| Treatment | Dose (mg/kg) | %TGI | PSA (ng/mL) ± SD | | | |
|---|---|---|---|---|---|---|
| | | | Day 0 | Day 7 | Day 15 | Day 22 |
| Vehicle | | | 197 ± 58 | 362 ± 66 | 471 ± 35 | 475 ± 40 |
| BMS-641988 | 90 | 95 | 206 ± 88 | 120 ± 100*, † | 77 ± 19*, † | 112 ± 41*, † |
| Bicalutamide | 150 | 33 | 179 ± 33 | 208 ± 60* | 254 ± 72* | 369 ± 83 |

(C) Antitumor activity in CWR-22-BMSLD1 tumors that progress during bicalutamide treatment

| Treatment (schedule) | Dose (mg/kg) | — | T-C (1,000 $mm^3$) |
|---|---|---|---|
| Vehicle | — | — | — |
| Bicalutamide (once a day × 36) | 150 | — | 4 |
| BMS-641988 (once a day × 36) | 90 | — | 35 |
| Bicalutamide (once a day × 14) + BMS-641988 | 150 + 90 | — | 42 † |

(D) BMS-641988 antitumor activity in the LuCaP 23.1 human prostate carcinoma model

| Treatment | Dose (mg/kg) | %TGI ‡ | T-C§ (800 $mm^3$) |
|---|---|---|---|
| Vehicle control | | | |
| BMS-641988 | 10 | 80‖ | 56 |
| | 30 | 97‖ | 72 |
| | 90 | 91‖ | 56 |
| Bicalutamide | 16.7 | 85‖ | 52 |
| | 150 | 90‖ | 72 |

NOTE: (A) Antitumor activity (%TGI and T-C) was determined. TVDT = 13 d; target size = 1,000 $mm^3$. (B) TVDT = 13 d; target size = 1,000 $mm^3$. PSA were the result of five duplicate samples per treatment group. (C) Antitumor activity (T-C) was determined. Target size = 1,000 $mm^3$; TVDT = 15 d. (D) Compounds were administered for 70 d. TVDT = 16 d.

*$P < 0.05$ versus control.

†$P < 0.05$ versus bicalutamide.

‡%TGI = TGI of treated groups over control group (day 50, post-treatment).

§T-C = time (in days) for median tumor size of treated group (T) to reach 800 $mm^3$ - time for control (C) to reach 800 $mm^3$.

‖$P < 0.01$ at the end of treatment versus control.

transfected with the artificial reporter constructs, it promoted the proliferation of these cells. This agonist activity has been observed with other potent antiandrogens, such as nilutamide and flutamide. This effect was attributed to the altered ligand specificity of a mutant AR (40). Although the real incidence of AR mutations in prostate cancer is not known, it has been reported to be between 10% and 40% (7). Interestingly, exposure to antiandrogens could promote the selection for certain mutant forms of the AR; for example, sequencing of the AR expressed in bone marrow samples of histologically prostate cancer–positive biopsies showed that 5 of 16 patients previously treated with long-term hydroxyflutamide presented mutations in codon 877 compared with only 1 (in codon 890) of 17 patients who did not receive this drug (41). Two additional AR mutants (W741C or W741L) were isolated from prostate cells, the growth of which is induced by bicalutamide (42, 43), and BMS-641988 is a strong antagonist of both of them (Supplementary Fig. S2). Unfortunately, further understanding of the clinical relevance of these mutations has been hampered by the difficulty in accessing large numbers of metastatic prostate cancer samples (7, 44).

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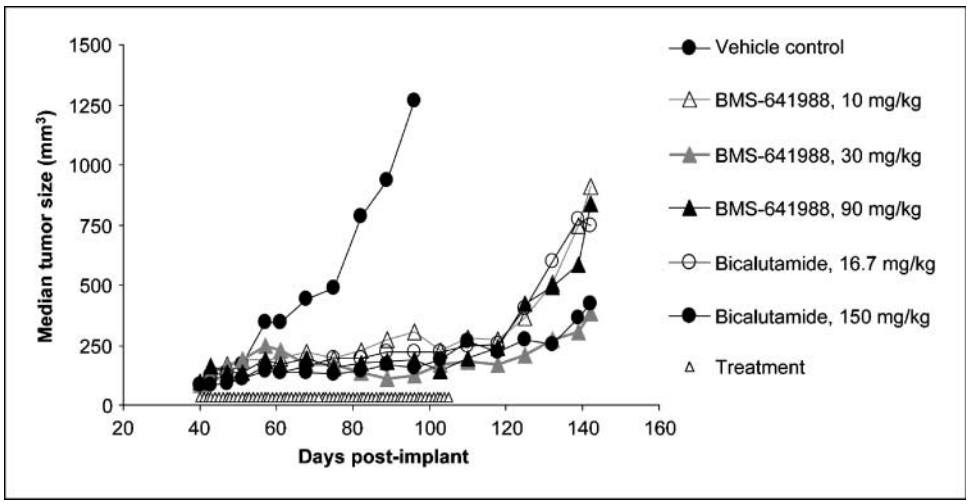


**Figure 3.** BMS-641988 promotes antitumor activity in the androgen-responsive LuCaP 23.1 model. Human LuCaP 23.1 tumors were s.c. implanted in male nude mice. Treatments were administered p.o. once a day × 70 (*empty triangles*).

The potent *in vitro* antiandrogenic activity of BMS-641988 was evident by its effect on the secondary sexual organs of mature male rats after 14 days of treatment. BMS-641988 produced a significant reduction in the weights of the ventral prostate and the seminal vesicles as well as in the percent of nuclei stained positively for the proliferation marker Ki-67 in prostate sections compared with the values derived from animals treated with vehicle control. In addition, the highest dose of BMS-641988 yielded a significant increase in serum levels of luteinizing hormone and testosterone compared with the levels attained with the same dose of bicalutamide.

BMS-641988 had significant antitumor activity in the human xenograft model CWR-22-BMSLD1, a tumor model derived from advanced prostate cancer and established in our laboratory in the absence of testosterone supplementation. CWR-22-BMSLD1 was

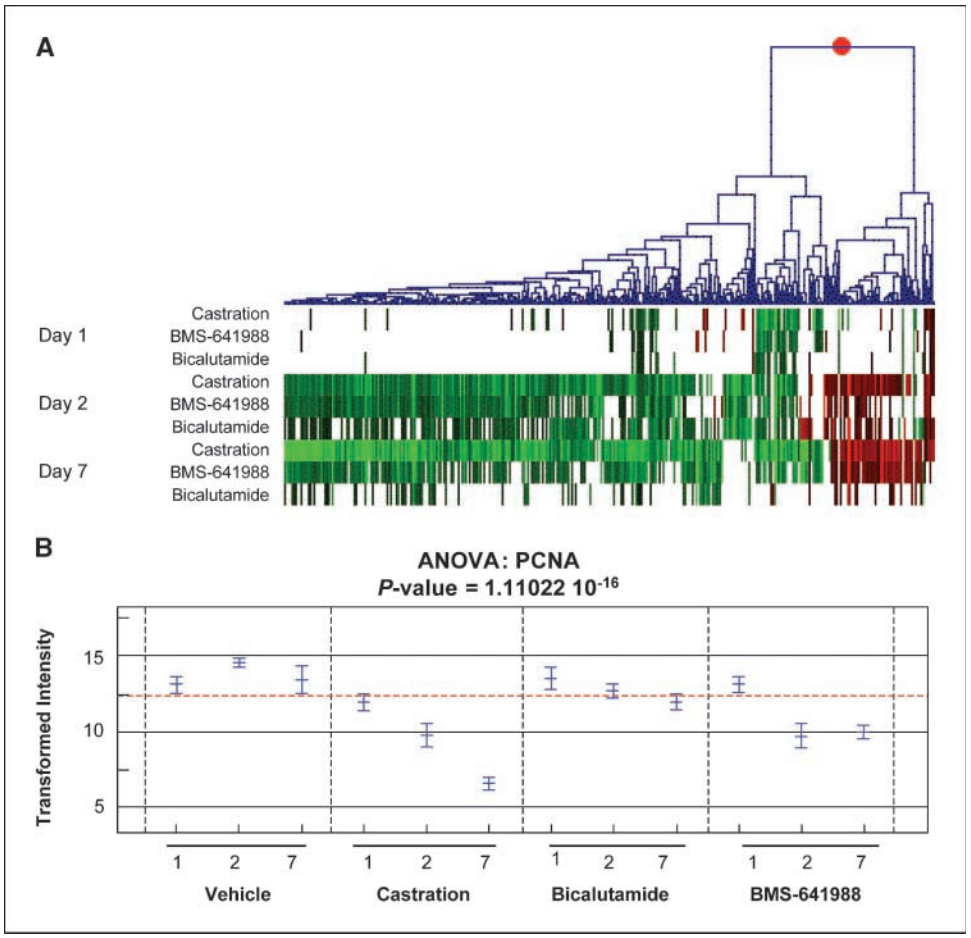


**Figure 4.** Expression of more genes in CWR-22-BMSLD1 tumors was commonly affected by the treatment of mice with castration and BMS-641988 than with bicalutamide. Nude mice bearing CWR-22-BMSLD1 human prostate tumors were castrated or treated orally with daily doses of vehicle, BMS-641988 (90 mg/kg), or bicalutamide (150 mg/kg) for 1, 2, and 7 d. *A,* Affymetrix transcript analysis showing markers that significantly changed among treatments groups. *B,* time and treatment response of proliferating cell nuclear antigen (*PCNA*) gene expression evaluated by ANOVA of microarray data.

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marginally sensitive to 150 mg/kg bicalutamide, which yields 3-fold higher steady-state exposures in mice than in humans (150 versus 52 μmol/L; ref. 45). Furthermore, tumors treated with bicalutamide grew progressively during treatment. Conversely, only minimal increases in tumor growth were observed over time in the group treated with the highest dose of BMS-641988. On cessation of treatments, tumors recovered their growth capacity; however, animals receiving the highest dose of BMS-641988 showed a 3-fold delay in target tumor progression compared with those receiving the highest dose of bicalutamide. In addition, BMS-641988 was active in the androgen-dependent LuCaP 23.1 xenograft model.

The effectiveness of BMS-641988 as a second-line hormonal therapy was explored. Several studies have shown the benefits of such approaches in the clinic; for example, nilutamide treatment promoted a 50% reduction in PSA in 29% of patients whose disease had progressed on flutamide or bicalutamide (46). A retrospective data analysis showed that 40% of patients who received nilutamide as second-line hormonal therapy experienced >50% reduction in PSA with a median time to progression of 4.4 months (47). More recently, a multicenter trial followed the clinical outcome of 232 patients with advanced prostate cancer who relapsed to first-line therapy (surgical or medical castration combined with a nonsteroidal antiandrogen) after switching to a second antiandrogen. These studies showed that 61.2% of the patients showed a PSA decrease (>50%) in response to an alternative antiandrogen and that these responders had significantly better survival than nonresponders (48). Our studies show that BMS-641988 therapy promoted stasis of CWR-22-BMSLD1 tumor growth that had tripled in size under bicalutamide treatment, whereas tumors from animals continuing under bicalutamide alone grew progressively. On the other hand, bicalutamide did not delay the growth of the tumors progressing in animals receiving suboptimal doses of BMS-641988 (data not shown). These results support the testing of BMS-641988 as a second-line hormonal therapy in the clinic.

It has been reported for the estrogen receptor that different ligands can promote the recruitment of different peptides corresponding to coactivators and corepressors (49). Therefore, it is conceivable that BMS-641988 and bicalutamide, two distinct chemical entities, could promote, on binding to the AR, different conformations with different capacities to recruit coactivators and/or corepressors, ultimately mediating distinct effects on AR-dependent gene expression. Those potential differences were explored by comparing the global gene expression profile in CWR-22-BMSLD1 tumors from mice subjected with the comparison of treatments. This approach suggests that BMS-641988 promotes qualitative and quantitative changes in the mRNA expression profiles more similar to the changes promoted by castration when compared with bicalutamide. These results need to be further validated at the protein level. This supports the hypothesis that, based on their chemical differences, BMS-641988 and bicalutamide could promote distinct ligand-AR conformations with distinct transcriptional activity. Furthermore, the closer similarities between BMS-641988 treatment and surgical castration are compelling and could imply therapeutic benefits, because it has been shown that castration promotes apoptosis in this tumor model (50). On the other hand, BMS-641988 faces, as other antiandrogens, two main challenges. The first challenge is the plasticity of cancer cells in general and of the AR in particular; the resistance mechanisms already mentioned to be involved in castration-resistant prostate cancer could also apply to BMS-641988. The second challenge is the existence of constitutive active variants of the AR that lack the ligand-binding domain and therefore would not be affected by BMS-641988; patients expressing these isoforms should not be treated with antiandrogens. In summary, BMS-641988 is a novel and potent antiandrogen that is active in human prostate xenograft models, including CWR-22-BMSLD1 failing bicalutamide treatment. Furthermore, BMS-641988 presents a gene expression signature similar to surgical castration in the preclinical experimental model tested. These data offer opportunities to further understand the biology of antiandrogens and their utility for the treatment of prostate cancer.

## Disclosure of Potential Conflicts of Interest

R.M. Attar, L. Schweizer, J.S. Platero, W. Shan, W.R. Foster, J. Dinchuk, and G. Vite: employment, Bristol-Myers Squibb. M. Jure-Kunkel: ownership interest, Bristol-Myers Squibb. The other authors disclosed no potential conflicts of interest.

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# Discovery of BMS-641988, a Novel and Potent Inhibitor of Androgen Receptor Signaling for the Treatment of Prostate Cancer

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