RESEARCH BRIEF

Opposing Effects of Androgen Deprivation and Targeted Therapy on Prostate Cancer Prevention

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ABSTRACT
Prostate cancer is an ideal target for chemoprevention. To date, chemoprevention clinical trials with 5α-reductase inhibitors have yielded encouraging yet ultimately confounding results. Using a preclinical mouse model of high-grade prostatic intraepithelial neoplasia (HG-PIN) induced by PTEN loss, we observed unprecedented deteriorating effects of androgen deprivation, in which surgical castration or MDV3100 treatment accelerated disease progression of the otherwise stable HG-PIN to invasive castration-resistant prostate cancer (CRPC). As an alternative, targeting the phosphoinositide 3-kinase (PI3K) signaling pathway via either genetic ablation of genes encoding PI3K components or pharmacologic inhibition of the PI3K pathway reversed the PTEN loss–induced HG-PIN phenotype. Finally, concurrent inhibition of the PI3K and mitogen-activated protein kinase (MAPK) pathways was effective in blocking the growth of PTEN-null CRPC. Together, these data have revealed the potential adverse effects of antiandrogen chemoprevention in certain genetic contexts (such as PTEN loss) while showing the promise of targeted therapy in the clinical management of this complex and prevalent disease.

SIGNIFICANCE: Chemoprevention with antiandrogen therapies is attractive for prostate cancer, given its prevalence and established hormonally mediated pathogenesis. However, because PTEN loss has been found in 9% to 45% of HG-PIN in the clinic, the current findings suggest that patients with PTEN-deficient prostate tumors might be better treated with PI3K-targeted therapies. Cancer Discov; 3(1): 44–51. ©2012 AACR.

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Note: Supplementary data for this article are available at Cancer Discovery Online (http://cancerdiscovery.aacrjournals.org/).

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doi: 10.1158/2159-8290.CD-12-0262
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INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer in men and second only to lung cancer in the number of cancer deaths, with a total of 241,740 new cases and 28,170 deaths from prostate cancer projected to occur in 2012 (1). Despite early detection, currently no cure exists for the advanced stage of the disease. Prostate cancer is an age-associated disease, the incidence of which dramatically increases in men older than 65 years. The fact that a 76% increase in men older than 65 years is projected to occur by the year 2050 (World Health Organization report) has called for effective management of this deadly disease.

Prostate cancer seems to be an ideal target for chemoprevention because of its prevalence and established hormonally mediated pathogenesis. Androgen deprivation with 5α-reductase inhibitors (5-ARI), which function to decrease serum levels of dihydrotestosterone (DHT), reduced the overall risk of low-grade prostate cancer in 2 landmark randomized, placebo-controlled prostate cancer chemoprevention trials: the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial and the Prostate Cancer Prevention Trial (PCPT) with Finasteride (2, 3). However, the cumulative risk of high-grade prostate cancers at the end of both trials has generated widespread debates and concern, partly due to the intrinsic limitations of clinical trials (such as time frame, patient selection, and flaws in methodology) and the genetic heterogeneity of prostate cancer (4).

RESULTS

High-grade prostatic intraepithelial neoplasia (HG-PIN) is considered a major precursor to prostate cancer. To reevaluate the effects of androgen deprivation on prostate cancer prevention, we conducted a preclinical trial using a genetically engineered mouse model (GEMM) in which HG-PIN induced by PTEN loss recapitulates the features of its human counterpart (5). In the mouse strain used in this study, an HG-PIN phenotype is induced by 8 weeks of age at nearly 100% penetrance in all 3 mouse prostate lobes, namely the ventral prostate (VP), anterior prostate (AP), and dorsal lateral prostate (DLP; Fig. 1A, left, and Supplementary Fig. S1). This HG-PIN phenotype features an intact smooth muscle layer and remains stable with no noticeable invasiveness up to 1 year of age (Fig. 1A, right, and data not shown). To study the biologic effects of androgen deprivation in the preclinical setting, we surgically castrated mice with HG-PIN at 8 weeks of age and monitored tumor growth over time. Consistent with previous reports (5–7), androgen deprivation induced extensive apoptosis (Fig. 1B, left), rapidly shrinking the HG-PIN in all lobes of the prostate gland (Fig. 1C). However, a subpopulation of the PTEN-deficient prostate tumor cells displayed castration-resistant growth (Fig. 1B, right) and repopulated the shrunken glands by 4 to 8 weeks after castration (Fig. 1C and data not shown), a phenotype mostly evident in the VP. Strikingly, in contrast to the sham operation group, we found an unprecedented deteriorating effect of androgen deprivation within 16 to 18 weeks after castration, in which surgical castration accelerated progression of the otherwise stable HG-PIN to invasive castration-resistant prostate cancer (CRPC), characterized by broken layers of smooth muscle (Fig. 1D, and Supplementary Fig. S2 and S3). Paralleling androgen deprivation in men, the circulating and intraprostatic testosterone levels in the CRPC mice dropped significantly to 5% to 15% of those seen in intact mice (Supplementary Fig. S2).

Notably, these invasive CRPC lesions carry the common features of hormone-refractory recurrent prostate cancer in the clinic. We and others have reported that human CRPC retains widespread androgen receptor (AR) positivity within the tumor cell nuclei (8). Consistently, here we observed widespread nuclear AR staining in the epithelial lesions in both castration-naïve HG-PIN and CRPC (Fig. 1E), accompanied by slightly increased AR expression as measured by Western blot analysis (Fig. 1F and Supplementary Fig. S4). In line with its function as a safeguard in cancer (9), p53 protein loss (Fig. 1F and Supplementary Fig. S5), inactivating somatic point mutations, and gain-of-function mutation (Supplementary Fig. S6 and Supplementary Table S1), occurred during the development of invasive CRPC, a process accompanied by downregulation of the antimetastatic protein SMAD4 (ref. 10; Supplementary Fig. S5). Of note, androgen deprivation enriched tumor cells with positive p63 nuclear staining, especially in the invasive lesions (Supplementary Fig. S7 and S8), which is consistent with the recently suggested concepts of a basal cell of origin for human prostate cancer (11). In addition, chemical castration with MDV3100 (12), an AR antagonist that blocks the binding of DHT to the AR, also accelerated the progression of HG-PIN to invasive CRPC in the VP at 16 weeks after castration, even though treatment did decrease the size of both the AP and DLP as compared with mock-treated tumors (Fig. 1G, Supplementary Fig. S9, and data not shown). Together, our studies show that, despite initial shrinkage of the HG-PIN tumor mass, androgen deprivation profoundly influenced the outcome of HG-PIN by accelerating disease progression (primarily in the VP), a result that is consistent with the observed increased risk of progression to high-grade prostate cancer seen in clinical trials. A comparison between clinical and preclinical studies is summarized in Fig. 1H.

To explore the preventive potential of targeted therapy, we evaluated the effects of genetically ablating the genes encoding phosphoinositide 3-kinase (PI3K) isoforms (13) on PTEN in the VP, the gland that is the most responsive to androgen deprivation. Interestingly, we found lobe-specific and PI3K isoform-dependent effects on prostate tumorigenesis. Unlike the dominant role of p110β in the tumorigenesis of PTEN-deficient AP (ref. 13; Fig. 2A–E, and Supplementary Fig. S10), ablation of either p110β or p110α was insufficient to block PTEN-controlled tumorigenesis in the VP (Fig. 2F–I). Instead, concurrent ablation of both p110β and p110α restored a normal glandular appearance to the PTEN-null VP, accompanied by a concomitant diminution of AKT phosphorylation (Fig. 2J, Supplementary Fig. S11, and data not shown). A similar result was also observed in the DLP (data not shown). These data suggest that ablation of particular PI3K isoforms is effective in preventing prostate tumor initiation and continued development. However, we cannot rule out the involvement of p110γ and p110δ, the other class I PI3K isoforms, in this process (14).

To evaluate the therapeutic effects of pharmacologically blocking PI3K activity on established HG-PIN, we treated HG-PIN–bearing mice at the age of 8 weeks with placebo or...
Androgen deprivation potentiated the disease progression from HG-PIN to invasive CRPC. A, genetic ablation of PTEN in prostatic epithelium caused HG-PIN. p-AKT, phospho-AKT, SMA, smooth muscle actin. B, surgical castration induced extensive apoptosis in HG-PIN lesions [left, IF, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL)], whereas a subpopulation of tumor cells continued to proliferate [right, immunohistochemistry (IHC), anti-BrdUrd]; IF, immunofluorescence staining. C, PTEN-null prostate tumor masses initially shrank in response to surgical castration but gradually grew back. D, androgen deprivation accelerated progression of PTEN-null HG-PIN to invasive CRPC; arrows indicate invasive lesions. Shown are representative lesions observed in 30 of 32 (93.75%) mice. IHC, anti-SMA. E, AR staining in CRPC vs castration-naïve HG-PIN. IHC, anti-AR. F, Western blot analysis of p53 and AR in age-matched wild-type prostate (WT), HG-PIN, and CRPC. G, chemical castration accelerated progression of PTEN-null HG-PIN to invasive CRPC; arrows indicate invasive lesions. Shown are representative lesions observed in 8 of 10 (80%) mice. IHC, anti-SMA. Mice harboring HG-PIN at 8 weeks of age were surgically or chemically castrated for another 16 to 18 weeks; representative data are shown in D–G. H, comparison between the clinical and preclinical trials over time. High-grade cancer is seen in human trials, whereas invasive CRPC is evident in the preclinical mouse studies. DAPI, 4',6-diamidino-2-phenylindole; H&E, hematoxylin and eosin.

BEZ235, an orally delivered pan-PI3K/mTOR dual inhibitor (Fig. 3A, top). At the end of 4 weeks of daily oral gavage, BEZ235-treated PTEN-null prostate tumors were easily distinguished from their placebo-treated counterparts because of their smaller size, dramatically reduced weight, and gross translucent appearance, resembling wild-type prostates (Fig. 3B and data not shown). Histologically, BEZ235 treatment resulted in an almost normal luminal architecture in PTEN-null VP compared with the multilayer HG-PIN and activated stroma in the placebo group (Fig. 3A, bottom). Such therapeutic effects of PI3K inhibition were maintained upon prolonged treatment for up to 8 weeks (data not shown). Mechanistically, BEZ235 potently blocked tumor growth (Fig. 3C) and induced apoptosis (Fig. 3D). At the signaling level, BEZ235 blocked the phosphorylation of AKT and ribosomal protein S6 (RPS6), the molecular surrogates for signaling downstream of PI3K, in PTEN-null prostate tumors (Fig. 3E), although mitogen-activated protein kinase (MAPK) signaling was not affected (data not shown). Consistent with the effects of BEZ235, BKM120, a small-molecule targeting pan-class I PI3Ks only, also reversed the HG-PIN phenotype (Fig. 3F). Thus, PI3K-targeted therapy could be useful in the prevention of HG-PIN with PTEN deficiency.
We were curious whether BEZ235 could also reverse the more severe tumor phenotype of CRPC. To determine whether this is the case, we surgically castrated HG-PIN–harboring mice at the age of 8 weeks and allowed the CRPC tumor cells to grow for 16 weeks. We then treated animals with either BEZ235 or placebo. Surprisingly, we found that PTEN-null CRPC tumors were not as responsive to BEZ235 treatment as the PTEN-null castration-naïve primary tumors had been (Supplementary Fig. S12). This finding led us to wonder whether CRPC had an altered signaling profile. In addition to the slightly increased AR expression (Fig. 1F and Supplementary Fig. S4), we consistently found a signaling shift in CRPC featuring modestly increased MAPK/extracellular signal-regulated kinase (ERK) activation and a compromised activation of PI3K signaling (Fig 4A and Supplementary Fig. S13), a result in line with previous findings that activation of MAPK is associated with clinical prostate cancer progression (15), and combinatorial activities of AKT and B-RAF/ERK signaling conferred androgen resistance (16). Notably, this alteration of the PI3K–MAPK–AR signaling axis differs slightly from the recently reported PI3K–AR signaling cross-talk in PTEN-null prostate tumors (6, 7), a discrepancy that may arise from the change in predominant cell type in our model or from differences in the genetic background of GEMMs used in various studies (17). To ask whether inhibition of such altered signaling would affect the growth of CRPC, we first tested the status of bromodeoxyuridine (BrdUrd)-labeled cell proliferation after pharmacologically blocking PI3K signaling with BEZ235 and/or MAPK/ERK signaling with AZD6224, a MAPK/ERK (MEK) inhibitor that is currently being tested in the clinic. In line with an earlier report (18), our results show that concurrent inhibition of PI3K and MAPK signaling pathways substantially suppressed cell division of CRPC, in addition to the partial effects with single-agent activity (Fig. 4B and Supplementary Fig. S14), although no effects on apoptosis were seen (data not shown). To assess the effects of combination therapy on the development of CRPC, we surgically castrated 8-week-old mice harboring HG-PIN and let them recover for 5 days before treatment with BEZ235 and/or AZD6224. At the end of 2 months of treatment, the combined use of BEZ235 and AZD6224 effectively suppressed the growth of CRPC as compared with placebo treatment (Fig. 4C). Thus, a combination of targeted therapies aimed at the PI3K and/or RAF–MAPK pathways is effective in the management of PTEN-deficient CRPC.

**DISCUSSION**

It has long been thought that androgen deprivation would impair the progression of primary prostate diseases, a concept extensively tested in preventive clinical trials. Our data suggest that androgen deprivation could inadvertently accelerate the progression of stable HG-PIN to invasiveness in certain genetic contexts (such as PTEN loss).

These findings may have clinical relevance for several reasons. First, PTEN loss has been found in 9% to 45% of HG-PIN (19–21), the occurrence of which is associated with disease progression in clinical studies. For instance, one third of men diagnosed with HG-PIN eventually developed prostate cancer (22). Second, the levels of circulating androgen and the effects of antiandrogen treatments on testosterone levels are comparable in mice and humans (Supplementary Fig. S2). Finally, it is intriguing that, in mice, treatment of HG-PIN with compounds targeting the PI3K pathway effectively reduces tumor size without causing invasiveness during the period studied (Fig. 3). However, the clinical translation of this result would require finding effective doses of a PI3K-targeting drug that could be tolerated over long periods of time.
There are caveats to the interpretation of the current results. Obviously, mice are not men, and it remains to be seen whether similar processes might be occurring in humans. Notably, both of the antiandrogen treatments used in the current work affect the levels or actions of both testosterone and DHT, whereas the antiandrogen drugs (5-ARI) used in the clinical trials only altered DHT. It would also be interesting to measure androgen levels in patients on dutasteride or finasteride for cancer prevention and compare these results with those from the PTEN-null animal model used here. Moreover, it remains unclear how the dynamics of hormone levels (i.e., hormone flare) might affect the clinical responsiveness to a luteinizing hormone-releasing hormone (LHRH) agonist, despite the ultimate androgen deprivation by 5-ARI and LHRH agonists in patients with prostate cancer. Of note, the model used here behaves differently from the one described in an earlier report, in which the F2 generation of offspring from a cross of 129/Balb/c and C57BL/6 mice displayed an invasive phenotype as early as 9 weeks and died by 29 weeks (5). In the current study, Ptenflox/flox;PbCre4+ compound mice in a C57BL/6 background displayed an HG-PIN phenotype but did not progress beyond HG-PIN even in mice up to 60 weeks old. This discrepancy in tumor phenotype between the current and previous studies might be attributed to these differences in genetic background (17). Finally, it remains to be seen whether changes in the relative strength of signaling in the ERK and PI3K pathways observed in CRPC in the PTEN-null mice are also seen in the clinic.

Prostate cancer therapy is poised to enter a new era of personalized medicine. The recent advent of diagnostic technology has enabled clinical detection of PTEN status for patients with HG-PIN. Our findings raise a note of caution about the potential risk of chemoprevention for these patients while providing proof-of-principle demonstration of targeted therapy in the clinical management of this complex and prevalent disease (Fig. 4D). Further identification of the genetic, epigenetic, or clinical features that predict patients prone to developing high-grade prostate cancer in clinical trials would help stratify patients for future chemoprevention and provide a rationale for targeted therapy.
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Figure 4. Combined inhibition of PI3K and MAPK signaling suppressed the growth of CRPC. A, tissue Western blot analysis. Mice harboring HG-PIN at 8 weeks were surgically castrated, and prostate tissues were harvested 16 weeks later. CRPC, PTEN-null CRPC; HG-PIN, PTEN-null HG-PIN; WT, wild-type prostate tumor. B, proliferation index of CRPC tumors after treatment. Mice harboring PTEN-null HG-PIN at 8 weeks of age underwent surgical castration for 16 weeks. BrdUrd was administered 1 hour after drug treatment, and prostate tissues were harvested 5 hours later. Red arrows indicate the proliferating cells. IHC, anti-BrdUrd. C, the effects of drug treatment on the weight of CRPC prostate tissues. D, targeted therapy in the clinical management of prostate diseases with PTEN deficiency. MEKi, MEK inhibitor; PI3Ki, PI3K inhibitor. *P < 0.05; **P < 0.01.

METHODS

Mice

Pten<sup>fl ox/fl ox</sup> (Pten<sup>f/f</sup>) mice, Pk3cb<sup>fl ox/fl ox</sup> (Pk3cb<sup>f/f</sup>) mice, Pk3dc<sup>fl ox/fl ox</sup> (Pkdcb<sup>f/f</sup>) mice, and PbCre4 transgenic mice (PbCre4; ref. 13) were used in this study. All protocols for mouse experiments were approved by the Institutional Animal Care and Use Committee of the Dana-Farber Cancer Institute (Boston, MA).

Drug Treatment

We used BEZ235 (dual PI3K/mTOR inhibitor, 45 mg/kg/d), AZD6244 (MEK inhibitor, also named ARRY-142886, 25 mg/kg/d), BKM120 (PI3K inhibitor, 50 mg/kg/d), and MDV3100 (AR inhibitor, 30 mg/kg/d) as previously described (12, 23, 24). The doses used here do not affect the body weight or the growth of normal prostates in wild-type mice. BEZ235 and BKM were obtained from Novartis Institutes for BioMedical Research (Basel, Switzerland), whereas MDV3100 and ARRY were obtained from a commercial source. Unless otherwise indicated, the drugs were administered by oral gavage on a daily basis, 7 days a week.

Surgical Castration

Surgical castration was conducted as previously described (5).

BrdUrd Labeling

BrdUrd labeling and staining were done according to the manufacturer’s instructions (Invitrogen). Mice were treated with drug for 1 hour followed by a 5-hour BrdUrd labeling period.

Western Blotting and Quantification

Western blot assays were done as described previously (13) with antibodies against AR PG-21 (06–680, Upstate), AR c-19 (sc-815;
Santa Cruz) p53 (sc-126; Santa Cruz), tubulin (T9026; Sigma Aldrich), vinculin (V9131; Sigma-Aldrich), and antibodies from Cell Signaling Technology: phospho-AKT (9271), AKT (9272), phospho-p44/42 MAPK (9101), p44/42 MAPK (9102), phospho-S6 ribosomal protein (2211), and S6 ribosomal protein (2217). The quantification of Western blots was done with Odyssey software (LI-COR Biosciences). The values refer to control samples after they were normalized to reference proteins, either tubulin or vinculin.

**Testosterone Measurement**

The androgen levels both in circulation and intraprostate were measured with a mouse testosterone ELISA kit (Calbiotech) based on the manufacturer’s instructions. Basically, mouse blood samples were extracted from the retro-orbital sinus, and serum was prepared with the use of serum separator tubes. For intraprostate measurement, mouse prostates were microdissected in cold PBS and lysed in NP-40 buffer. Androgen levels were calculated as the total amount per milliliter or per gram.

**Histology and Immunohistochemistry**

Prostate tissues were processed and stained as described previously (8). Formalin-fixed, paraffin-embedded blocks were sectioned (5 μm) and stained with hematoxylin and eosin at the Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute. We conducted immunohistochemical staining using the antibodies against PTEN (138G6; Cell Signaling), p-AKT (3787; Cell Signaling), p63 (sc-8431; Santa Cruz Biotechnology), CK8 (MMS-162P; Covance), AR (06–680; Cell Signaling), p-AKT (3787; Cell Signaling), p44/42 MAPK (9101), p44/42 MAPK (9102), phospho-S6 ribosomal protein (2211), and S6 ribosomal protein (2217). We conducted apoptosis terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay using the Roche (13513068001; Roche) kit and stained with hematoxylin and eosin at the Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute. We conducted apoptosis terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay using the Roche In Situ Cell Death Detection Kit according to the manufacturer’s instructions.

**Statistical Analyses**

All statistical analyses were conducted with the Student t test and are represented as mean ± SD unless otherwise stated. In the figures, one asterisk indicates P < 0.05, and two asterisks indicate P < 0.01.

**Disclosure of Potential Conflicts of Interest**

T.M. Roberts has a commercial research grant and is a consultant/advisory board member of Novartis. J.J. Zhao has a commercial research grant from Novartis, receives honoraria for serving on the advisory board member of the National Brain Tumor Society. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**


**Acknowledgments**

The authors thank Drs. P. Kantoff (Dana-Farber Cancer Institute; Boston, MA) and W. Sellers (Novartis Institutes for Biomedical Research; Cambridge, MA) for insightful discussions, H. Wu (from University of California, Los Angeles), and P. Roy-Burman (from University of Southern California, Los Angeles) for animals, and I. Aspalter and J. Kye for technical assistance. The authors also thank C. Priolo, J. Wang, P. Bayliss, Z. Ding, S. Xie, Y. Geng (all from Dana-Farber), members of the Roberts/Zhao/Loda/Signoretti labs, and the Novartis Oncology Decision Board for helpful discussions. The authors apologize to colleagues whose primary papers were not cited due to space constraints.

**Grant Support**

This work was supported by grants from the Dana-Farber/Harvard Cancer Center Specialized Program of Research Excellence (P.A.R.T Investigatorship Award to S. Jia), the NIH (CA030002 and CA089021 to T.M. Roberts; CA089021 to M. Loda; CA134502 to J.J. Zhao; and CA148164-01 to T.M. Roberts and J.J. Zhao).

Received June 7, 2012; revised November 14, 2012; accepted November 15, 2012; published OnlineFirst December 20, 2012.

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