Published in final edited form as:

Eur Urol. 2017 October ; 72(4): 499–506. doi:10.1016/j.eururo.2017.03.031.

Co-clinical Analysis of a Genetically Engineered Mouse Model and Human Prostate Cancer Reveals Significance of *NKX3.1* Expression for Response to 5 α -reductase Inhibition

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Statistical analysis: Dutta, Golombos, Mosquera, Mitrofanova.

Obtaining funding: Dutta, Mostaghel, Barbieri, Mitrofanova, Abate-Shen.

Administrative, technical, or material support: Mostaghel.

Supervision: Barbieri, Mitrofanova, Abate-Shen.

Other: None.

Financial disclosures: Cory Abate-Shen certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

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Abstract

Background—Although men on active surveillance for prostate cancer (PCa) may benefit from intervention with 5 α -reductase inhibitors (5-ARIs), it has not been resolved whether 5-ARIs are effective for delaying disease progression and, if so, whether specific patients are more likely to benefit.

Objective—To identify molecular features predictive of patient response to 5-ARIs.

Design, setting, and participants—*Nkx3.1* mutant mice, a model of early-stage PCa, were treated with the 5-ARI finasteride, and histopathological and molecular analyses were performed. Cross-species computational analyses were used to compare expression profiles for treated mice with those of patients who had received 5-ARIs before prostatectomy.

Intervention—Finasteride administered to *Nkx3.1* mutant mice. 5-ARI-treated patient specimens obtained retrospectively.

Outcome measurements and statistical analysis—Endpoints in mice included histopathology, immunohistochemistry, and molecular profiling. GraphPad Prism software, R-studio, and Matlab were used for statistical and data analyses.

Results and limitations—Finasteride treatment of *Nkx3.1* mutant mice resulted in a significant reduction in prostatic intraepithelial neoplasia (PIN), as evident from histopathological and expression profiling analyses. Cross-species computational analysis comparing finasteride-treated mice with two independent 5-ARI-treated patient cohorts showed that reduced *NKX3.1* expression is predictive of response to 5-ARI. A limitation of the study is that these retrospective human cohorts have relatively few patients with limited clinical outcome data. Future prospective clinical trials are needed to validate whether stratifying patients on the basis of *NKX3.1* expression improves the benefit of 5-ARIs during active surveillance.

Conclusions—This co-clinical study implicates *NKX3.1* status as a predictor of response to 5-ARIs, and suggests that molecular features, including *NKX3.1* expression, may help to identify PCa patients most likely to benefit from 5-ARIs during active surveillance.

Patient summary—The aim of precision cancer prevention is to tailor interventions on the basis of individualized patient characteristics. We propose that patients with low *NKX3.1* expression are optimal candidates for intervention with 5 α -reductase inhibitors as an adjunct to active surveillance.

Keywords

5 α -Reductase inhibitors; Active surveillance; Chemoprevention; Dutasteride; Finasteride; *NKX3.1*; Precision cancer prevention; Prostate cancer

1. Introduction

Widespread screening combined with the rise in the aging population has led to increased diagnosis of prostate cancer (PCa), but the majority of men diagnosed are at low risk of disease progression [1,2]. Rather than undergoing invasive procedures, many men are opting for active surveillance, whereby treatment is delayed until signs of overt progression [2,3]. It would be advantageous to better understand the risk of disease progression at the individual patient level and to identify molecular features predictive of response for specific interventions, which is the premise of precision cancer prevention [4].

Because of the critical dependence of PCa on the androgen receptor (AR) [5,6], many interventions for PCa target AR signaling or androgen biosynthesis [7]. Among these are agents that target 5 α -reductase, which catalyzes the conversion of testosterone to dihydrotestosterone (DHT) [8]. Although 5 α -reductase inhibitors (5-ARIs), including finasteride and dutasteride, have been evaluated in prospective clinical trials for both primary prevention [9–12] and secondary prevention for patients on active surveillance [13,14], their benefits in these settings remain controversial.

We performed co-clinical analyses to evaluate the phenotypic and molecular consequences of finasteride treatment in a genetically engineered mouse model (GEMM) that is analogous to low-risk PCa in humans [15,16]. Using cross-species computational analyses to compare finasteride treatment of GEMMs with retrospective cohorts of 5-ARI-treated patients, we show that reduced expression of *NKX3.1* is a predictor of response to 5-ARIs. We propose that expression of *NKX3.1* be evaluated as a means of stratifying men on active surveillance as candidates for intervention with 5-ARIs.

2. Patients and methods

2.1. Preclinical analyses

Experiments using animals were performed according to protocols approved by the Institutional Animal Care and Use Committee at Columbia University Medical Center. *Nkx3.1* wild-type (*Nkx3.1^{+/+}*) and germline homozygous mutant (*Nkx3.1^{-/-}*) mice, and *Pten* germline heterozygous mutant mice (*Pten^{+/-}*) have been described previously [16,17]. Finasteride (Kemprotec, Carnforth, UK) was dissolved in ethanol at a concentration of 20 mg/ml and diluted using sterile phosphate-buffered saline to a working stock of 1 mg/ml. Cohorts of mice were randomly assigned to treatment with finasteride or vehicle. At sacrifice, prostate tissues were fixed in 10% formalin and paraffin-embedded or snap-frozen

in liquid nitrogen. Histopathological grading was performed according to the classification of Park et al [18]. Immunohistochemical staining was as previously described [19]; images were captured using an Olympus VS120 whole-slide scanning microscope. Levels of steroids in serum were determined by extraction with hexane/dichloromethane (3:2 v/v) followed by purification using a XEVO TQS tandem mass spectrometer (Waters, Milford, MA, USA) with a detection limit of 10 pg/ml. Quantitative real-time polymerase chain reaction (PCR) was carried out on RNA prepared using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) with a QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany) [19]. RNA sequencing was performed using a MagMAX-96 total RNA isolation kit (Life Technologies) as previously described [19]. Raw counts for RNA sequencing (RNAseq) data were normalized and the variance was stabilized using the DESeq2 package (Bioconductor) in R-studio 0.99.902, R v3.3.0.

2.2. Patient cohorts

Patient specimens were obtained following protocols approved by the institutional review board of Weill Cornell Medicine (WCM) and Fred Hutchinson Cancer Research Center (FHCRC). Two independent retrospective patient cohorts were used for training and testing/validation (Table 1). The WCM cohort ($n = 9$) included patients with clinically localized PCa who had been receiving finasteride or dutasteride before prostatectomy. The FHCRC cohort ($n = 15$) included samples collected as part of the multicenter ARI40010 study [20] from patients who had received dutasteride for 4 mo before prostatectomy.

Immunohistochemistry was performed on paraffin-embedded tissues with a rabbit polyclonal NKX3.1 antibody (Biocare Medical, Pacheco, CA, USA) using a Leica Bond III automated stainer (Leica Biosystems, Wetzlar, Germany), and quantified using HALO software (Indica Labs, Corrales, NM, USA).

2.3. Statistical analysis

Independent groups were compared using a two-tailed two-sample Welch t test assuming that variances between the populations were not equal. When two phenotypes were compared (ie, finasteride- vs vehicle-treated samples), the Welch t test was applied to estimate the difference in RNAseq counts (ie, differential expression) between these phenotypes for each gene. Differential expression signatures were thus defined as the list of genes ranked by t values from a two-tailed two-sample t test comparing the finasteride- and vehicle-treated samples. For comparison with human gene signatures, mouse genes were mapped to human orthologs using the homoloGene database (www.ncbi.nlm.nih.gov/homologene).

Pathway enrichment was assessed using gene set enrichment analysis (GSEA) [21] to query pathways collected from the C2 database, in which normalized enrichment scores (NES) and p values were estimated using 1000 gene permutations. Master regulator (MR) analysis was performed using the Master Regulator Inference algorithm (MARINA) by interrogating a human PCa interactome [22] using “humanized” mouse signatures and human signatures. The statistical significance of each MR was estimated using 1000 gene permutations, and $p < 0.05$ was considered significant. To estimate clinical relevance, we used a Cox proportional hazards model for univariate analysis and Kaplan-Meier survival analysis for

multivariate analysis, performed with the *surv* and *coxph* functions from the *survcomp* package (Bioconductor; www.bioconductor.org). Before the Kaplan-Meier analysis, *k*-means clustering was used to segregate patients into two groups corresponding to low or high MR activity. The *p* values were estimated using a Wald test for the Cox proportional hazards model, and a log-rank test for Kaplan-Meier survival analysis. Significance for statistical tests was assumed at *p* < 0.05. Statistical analysis was performed using GraphPad Prism software (Version 6.0), R-studio (0.99.902, R v3.3.0), and Matlab (2012a).

2.4. Accession numbers

Raw and normalized expression profiling data are publicly available through the Gene Expression Omnibus database via accession number GSE92999.

3. Results

3.1. Finasteride abrogates PIN in a GEMM of early-stage PCa

Nkx3.1 mutant mice develop PIN as a consequence of aging, and share conserved molecular features with early-stage human PCa [15,16,23]. We reasoned that this GEMM could provide information on the efficacy of finasteride in delaying progression of early-stage PCa and on the molecular programs associated with such a response. Cohorts of littermate wild-type (*Nkx3.1^{+/+}*) and homozygous mutant (*Nkx3.1^{-/-}*) mice were randomly assigned to the vehicle or finasteride arms at age 4 mo (Fig. 1A), which is before the onset of PIN in these mice [16]. Treatment (10 mg/kg/d, five times weekly [24]) was continued for up to 12 mo, by which point untreated *Nkx3.1^{-/-}* mice display PIN [15,16,23]. At the conclusion of the study, mice were euthanized to assess DHT levels (Fig. 1B), histopathological phenotype (Fig. 1C,D), and expression of markers of prostate differentiation, cancer initiation, and androgen response (Figs. 1E and 2A).

Finasteride was highly effective in inhibiting 5 α -reductase, as evidenced by the nearly complete inhibition of serum DHT levels in both *Nkx3.1^{+/+}* and *Nkx3.1^{-/-}* mice (*n* = 5 per group; *p* = 0.024 and *p* = 0.0001, respectively; Fig. 1B). In addition, several AR-regulated genes, including *Tmprss2* and *Foxa1*, were downregulated to a similar extent in *Nkx3.1^{+/+}* and *Nkx3.1^{-/-}* prostates (*n* = 5 per group; *p* < 0.001; Fig. 1E), whereas expression levels of AR itself and a non-AR regulated gene, *Hoxb13*, were not affected (*n* = 5 per group; Fig. 1E). Notably, expression of *Nkx3.1*, a known target of AR, was not affected in finasteride-treated *Nkx3.1^{+/+}* mice, reflecting the complexity of the relationship between AR and NKX3.1 expression [25,26].

However, finasteride had a profound effect on the histological phenotype of *Nkx3.1^{-/-}* but not *Nkx3.1^{+/+}* prostates (Fig. 1C,D). Specifically, the PIN phenotype of *Nkx3.1^{-/-}* prostates was significantly abrogated following treatment, while the normal prostate histology of *Nkx3.1^{+/+}* mice was virtually unaffected (*n* = 25–30 per group, *p* < 0.001; Fig. 1C,D). Notably, abrogation of the PIN phenotype was not observed in finasteride-treated *Pten* heterozygous mutant mice (*Pten^{+/-}*), which develop PIN but are wild type for *Nkx3.1* (Supplementary Fig. 1). The striking reversal of the PIN phenotype in finasteride-treated *Nkx3.1^{-/-}* mice was accompanied by increased expression of markers of prostate

differentiation, including E-cadherin and probasin ($n = 5$ per group; $p < 0.0001$; Fig. 1C,E). RNA sequencing comparing prostate tissues from vehicle- and finasteride-treated *Nkx3.1^{+/+}* or *Nkx3.1^{-/-}* mice further indicated the similar inhibition of 5 α -reductase in *Nkx3.1^{+/+}* and *Nkx3.1^{-/-}* prostates, as evidenced from their comparable reduction in expression of AR-regulated genes ($p < 0.001$; Fig. 2A) [27]. However, GSEA comparing a differential gene expression signature between finasteride- and vehicle-treated *Nkx3.1^{-/-}* prostates with a comparable signature from *Nkx3.1^{+/+}* prostates revealed a strong difference after treatment. Specifically, genes downregulated in finasteride-treated *Nkx3.1^{-/-}* prostates were upregulated in *Nkx3.1^{+/+}* prostates (NES = 8.77; $p < 0.001$; Fig. 2B); conversely, genes upregulated in finasteride-treated *Nkx3.1^{-/-}* prostates were downregulated in *Nkx3.1^{+/+}* prostates (NES = 14.69; $p < 0.001$; Fig. 2B).

We further investigated the molecular consequences of finasteride treatment through GSEA comparison of biological pathways affected after treatment in *Nkx3.1^{-/-}* and *Nkx3.1^{+/+}* prostates, which also revealed strong reversal in both upregulated and downregulated leading edges (NES = 5.72 and 7.26, respectively; $p < 0.001$; Fig. 2C). The pathways significantly reverted in the finasteride- versus vehicle-treated *Nkx3.1^{-/-}* mice include those associated with steroid hormone signaling, such as REACTOME steroid metabolic signaling ($p = 0.002$), and cancer initiation or progression, including REACTOME translation ($p = 0.001$) and REACTOME PI3K-AKT signaling ($p = 0.001$) (Fig. 2D). Taken together, these histopathological and molecular studies demonstrate that the *Nkx3.1^{-/-}* prostate is profoundly affected by finasteride, which is evident from the striking abrogation of the PIN phenotype and accompanied by global reversion of the molecular phenotype towards that of normal prostate.

3.2. Molecular signature of response to 5 α -reductase inhibition in human PCa

We investigated whether the molecular signature of finasteride response in GEMMs may help to predict patient response for human PCa. We performed expression profiling using a cohort of PCa patients who had received 5-ARIs before prostatectomy ($n = 9$, WCM cohort; Table 1). These tumor samples segregated into low or high levels of *NKX3.1* mRNA expression (Fig. 3A), which was confirmed at the protein level by immunohistochemistry (Fig. 3B). Working from the presumption that low *NKX3.1* expressers should be analogous to *Nkx3.1^{-/-}* mice and high *NKX3.1* expressers to *Nkx3.1^{+/+}* mice, we compared gene signatures from 5-ARI-treated low versus high *NKX3.1*-expressing human tumors with finasteride-treated *Nkx3.1^{-/-}* versus *Nkx3.1^{+/+}* prostates. Specifically, we performed cross-species GSEA to compare biological pathways affected in finasteride-treated *Nkx3.1^{-/-}* versus *Nkx3.1^{+/+}* prostates with pathways affected in 5-ARI-treated low versus high *NKX3.1*-expressing human tumors. The findings revealed strong enrichment of pathways in both the upregulated and downregulated leading edges (NES = 4.51 and 5.2, respectively; $p < 0.001$; Fig. 3C), indicative of conservation of response to 5-ARI treatment in mouse and human prostates that is contingent on the status of *NKX3.1* expression.

Given this conservation, we performed cross-species computational analyses of the *NKX3.1* low and high mouse and human treatment groups to identify MRs of treatment response. The rationale follows from our previous work showing that computational analyses of

preclinical data from GEMMs can predict treatment response in human PCa [22,28], and from the broader conceptual framework that GEMMs can be used to infer patient response for precision prevention [29].

First, we used MARINA to identify MRs of 5-ARI response for the human and mouse treatment groups by interrogating a human PCa interactome with corresponding human and mouse signatures (ie, 5-ARI-treated low vs high *NKX3.1*-expressing human prostate and finasteride-treated *Nkx3.1*^{-/-} vs *Nkx3.1*^{+/+} prostate) [22,28]. We then used cross-species GSEA to compare the 5-ARI-responsive “human” and “mouse” MRs, which revealed strong conservation of the signatures in both the upregulated and downregulated leading edges (NES = 6.38 and 6.28, respectively; $p < 0.001$; Fig. 3D).

On the basis of this conservation of the 5-ARI-treated mouse and human prostate contingent on *NKX3.1* expression, combined with the striking phenotypic consequences of finasteride treatment in *Nkx3.1*^{-/-} prostates (Fig. 2), we reasoned that patients with low *NKX3.1* expression may be optimal candidates for intervention with 5-ARIs. Indeed, we found that a signature comprising activated MRs from the positive leading edge in low *NKX3.1*-5-ARI-treated prostates was associated with adverse patient outcomes when tested on an independent cohort of PCa patients described by Glinsky et al [30]. In particular, Kaplan-Meier survival analysis comparing a high versus low level of activity of the MR signature segregated the patients into worse and better outcomes, respectively, on the basis of biochemical recurrence-free survival ($p = 0.035$; Fig. 3E). Furthermore, a subset of the MRs were associated with adverse outcome, as evidenced by univariate analyses using a Cox proportional hazards model on a second cohort described by Sboner et al [31] in which the clinical endpoint was death due to PCa (p values ranging from 0.04 to 0.0001; Fig. 3F). We further validated these findings using a cohort of patients who had received no treatment or a lower (0.5 mg) or higher (3.5 mg) dose of dutasteride daily for 4 mo in a neoadjuvant trial ($n = 15$, FHCRC cohort; Table 1 [20]). Similar to the WCM cohort, these patients could be grouped on the basis of low or high *NKX3.1* expression (Fig. 3G). Notably, six of the MRs associated with adverse outcome (as Fig. 3F) were independently validated in the FHCRC cohort (Fig. 3H). In particular, dutasteride treatment resulted in reduced expression of these MRs in the low but not the high *NKX3.1* patient samples. Taken together, these findings suggest that patients with low *NKX3.1* expression are more likely to benefit from 5-ARI intervention, and further suggest that these treatment-response MRs can be used as a readout to assess the efficacy of such treatment.

4. Discussion

The rise in diagnoses of low-risk, early-stage PCa has led to a surge in active surveillance monitoring in lieu of more invasive procedures. Indeed, since PCa is characteristically indolent, simply delaying progression may be highly advantageous for many patients. In this context, 5-ARIs have been investigated as an adjunct to active surveillance [13,14]; however, considering their potential adverse side effects and variable response, it would be valuable to identify the subset(s) of patients who are most likely to benefit from 5-ARI intervention. Using co-clinical analyses of GEMMs and human PCa to investigate the phenotypic and molecular contexts in which 5-ARIs may be most effective, we found that prostate tumors

with low *NKX3.1* expression are more likely to respond to and benefit from 5-ARI treatment. *NKX3.1* is a prostate-specific homeobox gene that is essential for proper prostate development, differentiation, specification, and stem cell function [15,16,19,23,32]; conversely, loss of *NKX3.1* function leads to defects in prostate differentiation, as well as preinvasive prostate phenotypes that share molecular conservation with early-stage human PCa [15,16,19,23]. Notably, *NKX3.1* is localized to chromosomal region 8p21, which undergoes deletion in a majority (>60%) of PCas at early stages, while *NKX3.1* expression is reduced in cancer progression and coincident with cancer initiation [6,16,33,34]. Thus, given the biological functions of *NKX3.1*, combined with the prevalence and significance of its loss of expression in early-stage PCa, a substantial number of men may benefit from assessment of *NKX3.1* expression as a way of selecting for 5-ARI intervention. However, this will need to be tested in prospective clinical trials in which patients on active surveillance are evaluated to determine whether *NKX3.1* expression correlates with response to 5-ARI intervention and whether such intervention delays progression. We further propose that the efficacy of 5-ARI intervention can be monitored using the MR signature of treatment response identified here. Our study also highlights the importance of incorporating precision medicine for cancer prevention; precision medicine has been widely adopted, albeit primarily for cancer therapeutics [35]. In particular, our findings suggest that the varied response to 5-ARIs among PCa patients on active surveillance may reflect differences in levels of *NKX3.1* expression, which can be readily evaluated at the mRNA or protein level. Moreover, the co-clinical paradigm, with the aim of integrating analyses of GEMMs and human clinical studies to provide information on patient care [36], has largely been limited to therapeutic studies until now. Our study highlights the importance of using a co-clinical approach for cancer prevention. We envision that future studies investigating the long-term benefit of 5-ARIs for patients on active surveillance, and more generally other interventions, can be guided by co-clinical studies in a precision prevention paradigm [29].

5. Conclusions

Our results suggest that patients with low *NKX3.1* expression are likely to benefit from intervention with 5-ARIs, and that the beneficial consequences can be evaluated using a molecular signature of treatment response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support and role of the sponsor: Aditya Dutta was supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health, grant UL1 TR000040. Christopher E. Barbieri received funding from the National Cancer Institute (K08CA187417-01), the Prostate Cancer Foundation, a Urology Care Foundation Rising Star in Urology Research Award, and a Damon Runyon Cancer Research Foundation MetLife Foundation Family Clinical Investigator award. Elahe A. Mostaghel was supported in part by Pacific Northwest Prostate Cancer SPORE grant P50 CA097186. Antonina Mitrofanova is a recipient of a Prostate Cancer Foundation Young Investigator Award. Cory Abate-Shen had access to the HICCC facilities supported by the Cancer Center Support Grant (P30 CA013696-40) and the National Center for Advancing Translational Sciences (UL1TR001873), and received support from the National Cancer Institute (U01 CA141535-05 and P01 CA154293-03) and an American Cancer Society Research Professorship supported in part by a generous gift from the F.M. Kirby Foundation. The sponsors played a role in manuscript approval.

We are grateful to Edward Gelmann M.D. for helping to obtain the patient cohorts for this study and for helpful discussions. Immunohistochemistry was performed at the Translational Research Program, Department of Pathology and Laboratory Medicine at Weill Cornell Medicine. RNA sequencing was carried out at the J.P. Sulzberger Columbia Genome Center at Columbia University Medical Center (mouse data) and the Genomics core facility at Weill Cornell Medicine (human data). Antonina Mitrofanova acknowledges access to the HPC facilities and support of the computational biology scientists of the Office of Advanced Research Computing (OARC) at Rutgers University.

References

1. Smith RA, Andrews K, Brooks D, et al. Cancer screening in the United States, 2016: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin.* 2016; 66:96–114. [PubMed: 26797525]
2. Cooperberg MR, Moul JW, Carroll PR. The changing face of prostate cancer. *J Clin Oncol.* 2005; 23:8146–51. [PubMed: 16278465]
3. Cooperberg MR, Carroll PR, Klotz L. Active surveillance for prostate cancer: progress and promise. *J Clin Oncol.* 2011; 29:3669–76. [PubMed: 21825257]
4. Rebbeck TR. Precision prevention of cancer. *Cancer Epidemiol Biomarkers Prev.* 2014; 23:2713–5. [PubMed: 25362191]
5. Culig Z, Santer FR. Androgen receptor signaling in prostate cancer. *Cancer Metastasis Rev.* 2014; 33:413–27. [PubMed: 24384911]
6. Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev.* 2010; 24:1967–2000. [PubMed: 20844012]
7. Watson PA, Arora VK, Sawyers CL. Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. *Nat Rev Cancer.* 2015; 15:701–11. [PubMed: 26563462]
8. Azzouni F, Mohler J. Role of 5 α -reductase inhibitors in prostate cancer prevention and treatment. *Urology.* 2012; 79:1197–205. [PubMed: 22446342]
9. Andriole GL, Bostwick DG, Brawley OW, et al. Effect of dutasteride on the risk of prostate cancer. *N Engl J Med.* 2010; 362:1192–202. [PubMed: 20357281]
10. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. *N Engl J Med.* 2003; 349:215–24. [PubMed: 12824459]
11. Thompson IM Jr, Goodman PJ, Tangen CM, et al. Long-term survival of participants in the prostate cancer prevention trial. *N Engl J Med.* 2013; 369:603–10. [PubMed: 23944298]
12. Robinson D, Garmo H, Bill-Axelsson A, Mucci L, Holmberg L, Stattin P. Use of 5 α -reductase inhibitors for lower urinary tract symptoms and risk of prostate cancer in Swedish men: nationwide, population based case-control study. *BMJ.* 2013; 346:f3406. [PubMed: 23778271]
13. Finelli A, Trotter G, Lawrentschuk N, et al. Impact of 5 α -reductase inhibitors on men followed by active surveillance for prostate cancer. *Eur Urol.* 2011; 59:509–14. [PubMed: 21211899]
14. Fleshner NE, Lucia MS, Egerdie B, et al. Dutasteride in localised prostate cancer management: the REDEEM randomised, double-blind, placebo-controlled trial. *Lancet.* 2012; 379:1103–11. [PubMed: 22277570]
15. Irshad S, Bansal M, Castillo-Martin M, et al. A molecular signature predictive of indolent prostate cancer. *Sci Transl Med.* 2013; 5:202ra122.
16. Bhatia-Gaur R, Donjacour AA, Sciavolino PJ, et al. Roles for Nkx3.1 in prostate development and cancer. *Genes Dev.* 1999; 13:966–77. [PubMed: 10215624]
17. Kim MJ, Cardiff RD, Desai N, et al. Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. *Proc Natl Acad Sci U S A.* 2002; 99:2884–9. [PubMed: 11854455]
18. Park JH, Walls JE, Galvez JJ, et al. Prostatic intraepithelial neoplasia in genetically engineered mice. *Am J Pathol.* 2002; 161:727–35. [PubMed: 12163397]
19. Dutta A, Le Magnen C, Mitrofanova A, Ouyang X, Califano A, Abate-Shen C. Identification of an NKX3.1-G9a-UTY transcriptional regulatory network that controls prostate differentiation. *Science.* 2016; 352:1576–80. [PubMed: 27339988]

20. Gleave M, Qian J, Andreou C, et al. The effects of the dual 5 α -reductase inhibitor dutasteride on localized prostate cancer—results from a 4-month pre-radical prostatectomy study. *Prostate*. 2006; 66:1674–85. [PubMed: 16927304]
21. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005; 102:15545–50. [PubMed: 16199517]
22. Aytes A, Mitrofanova A, Lefebvre C, et al. Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. *Cancer Cell*. 2014; 25:638–51. [PubMed: 24823640]
23. Kim MJ, Bhatia-Gaur R, Banach-Petrosky WA, et al. Nkx3.1 mutant mice recapitulate early stages of prostate carcinogenesis. *Cancer Res*. 2002; 62:2999–3004. [PubMed: 12036903]
24. Opoku-Acheampong AB, Nelsen MK, Unis D, Lindshield BL. The effect of finasteride and dutasteride on the growth of WPE1-NA22 prostate cancer xenografts in nude mice. *PLoS One*. 2012; 7:e29068. [PubMed: 22242155]
25. Lei Q, Jiao J, Xin L, et al. NKX3.1 stabilizes p53, inhibits AKT activation, and blocks prostate cancer initiation caused by PTEN loss. *Cancer Cell*. 2006; 9:367–78. [PubMed: 16697957]
26. Tan PY, Chang CW, Chng KR, Wansa KD, Sung WK, Cheung E. Integration of regulatory networks by NKX3-1 promotes androgen-dependent prostate cancer survival. *Mol Cell Biol*. 2012; 32:399–414. [PubMed: 22083957]
27. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell*. 2011; 19:575–86. [PubMed: 21575859]
28. Mitrofanova A, Aytes A, Zou M, Shen MM, Abate-Shen C, Califano A. Predicting drug response in human prostate cancer from preclinical analysis of in vivo mouse models. *Cell Rep*. 2015; 12:2060–71. [PubMed: 26387954]
29. Le Magnen C, Dutta A, Abate-Shen C. Optimizing mouse models for precision cancer prevention. *Nat Rev Cancer*. 2016; 16:187–96. [PubMed: 26893066]
30. Glinsky GV, Glinskii AB, Stephenson AJ, Hoffman RM, Gerald WL. Gene expression profiling predicts clinical outcome of prostate cancer. *J Clin Invest*. 2004; 113:913–23. [PubMed: 15067324]
31. Sboner A, Demicheli F, Calza S, et al. Molecular sampling of prostate cancer: a dilemma for predicting disease progression. *BMC Med Genomics*. 2010; 3:8. [PubMed: 20233430]
32. Wang X, Kruithof-de Julio M, Economides KD, et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature*. 2009; 461:495–500. [PubMed: 19741607]
33. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. *Cell*. 2015; 163:1011–25. [PubMed: 26544944]
34. Bowen C, Bubendorf L, Voeller HJ, et al. Loss of NKX3.1 expression in human prostate cancers correlates with tumor progression. *Cancer Res*. 2000; 60:6111–5. [PubMed: 11085535]
35. Friedman AA, Letai A, Fisher DE, Flaherty KT. Precision medicine for cancer with next-generation functional diagnostics. *Nat Rev Cancer*. 2015; 15:747–56. [PubMed: 26536825]
36. Nardella C, Lunardi A, Patnaik A, Cantley LC, Pandolfi PP. The APL paradigm and the “co-clinical trial” project. *Cancer Discov*. 2011; 1:108–16. [PubMed: 22116793]

Fig. 1.

Finasteride abrogates prostatic intraepithelial neoplasia (PIN) in a genetically engineered mouse model of prostate cancer. (A) Preclinical trial design. Cohorts of $Nkx3.1^{+/+}$ and $Nkx3.1^{-/-}$ mice aged 4 mo were randomly assigned to treatment with finasteride (1 mg/ml in phosphate-buffered saline) or vehicle once a day on a schedule of 5 d/wk for 8 mo, until the mice were aged 12 mo. At the conclusion of the study, mice were sacrificed and analysis of the endpoints indicated was performed. PCR = polymerase chain reaction. (B) Levels of dihydrotestosterone (DHT) in serum as indicated ($n = 5$ per group); p values represent comparisons between bracketed groups and were estimated using a two-tailed two-sample t test. Veh = vehicle; Fin = finasteride. (C) Histological analysis. Shown are representative images of hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) staining of anterior prostate from $Nkx3.1^{+/+}$ and $Nkx3.1^{-/-}$ mice treated with finasteride or vehicle, as indicated ($n = 25$ per group). Antibodies were as previously reported [19]. Scale bars represent 100 μ m (H&E low power) or 50 μ m (H&E high power and IHC staining). (D)

Summary of PIN phenotype following treatment. Shown is the percentage area of prostatic tissue that is normal/PIN1, PIN2, PIN3, and PIN4 following treatment with finasteride in *Nkx3.1^{+/+}* ($n = 5$ per group) and *Nkx3.1^{-/-}* ($n = 15$ per group) mice; p values were estimated using a two-tailed two-sample t test. ns = not significant. (E) Quantitative real-time PCR was carried out using total RNA from *Nkx3.1^{+/+}* and *Nkx3.1^{-/-}* prostate treated with vehicle or finasteride, as indicated. Analyses were performed in triplicate and normalized to GAPDH; p values were estimated using a two-tailed two-sample t test.

Fig. 2.

Finasteride leads to reversal of the molecular phenotype of *Nkx3.1* mutant mice. (A) Heat map depicting gene expression levels of AR-regulated genes reported by Carver et al [27] comparing vehicle- or finasteride-treated *Nkx3.1*^{+/+} or *Nkx3.1*^{-/-} prostate, as indicated. (B) Gene set enrichment analysis (GSEA) comparing a reference gene expression signature from prostates of *Nkx3.1*^{+/+} finasteride-treated mice ($n = 5$) versus *Nkx3.1*^{+/+} vehicle-treated mice ($n = 3$) with a query signature ($p < 10^{-7}$) of *Nkx3.1*^{-/-} finasteride-treated mice ($n = 5$) versus *Nkx3.1*^{-/-} vehicle-treated mice ($n = 3$). (C) GSEA comparing a reference pathway signature from prostates of *Nkx3.1*^{+/+} finasteride-treated mice ($n = 5$) versus *Nkx3.1*^{+/+} vehicle-treated mice ($n = 3$) with a query pathway signature (top 50 differentially changed pathways) of *Nkx3.1*^{-/-} finasteride-treated mice ($n = 5$) versus *Nkx3.1*^{-/-} vehicle-treated mice ($n = 3$). (D) Heat maps depicting expression levels of selected leading edge genes from the pathways in (C) from the C2 database, comparing *Nkx3.1*^{-/-} mice treated with vehicle or finasteride.

Fig. 3.

Cross-species analysis identifies a 5 α -reductase inhibitor (5-ARI) response signature with prognostic value. (A) Heat map indicating relative *NKX3.1* expression levels in 5-ARI-treated human prostates from the Weill Cornell Medicine (WCM) cohort (Table 1). (B) Immunohistochemistry images at low (top) and high (bottom) magnification of representative prostate cancer cases with weak (STID31052) and strong (STID31058) *NKX3.1* protein expression. The adjacent bar plot represents automated image analysis of the percentage of cells with strong (blue) or negative (red) staining (original magnification, 20 $\times$ and 200 $\times$). (C) Gene set enrichment analysis (GSEA) comparing a reference mouse pathway signature from prostates of *Nkx3.1*^{-/-} finasteride-treated mice ($n = 5$) versus *Nkx3.1*^{+/+} finasteride-treated mice ($n = 5$) with a query human pathway signature (top 50 differentially changed pathways) of 5-ARI-treated low-*NKX3.1* ($n = 4$) versus high-*NKX3.1* ($n = 5$) human prostates. (D) GSEA comparing a reference mouse master regulator (MR) signature from prostates of *Nkx3.1*^{-/-} finasteride-treated mice ($n = 5$) versus *Nkx3.1*^{+/+} finasteride-treated mice ($n = 5$) with a query human MR signature ($p < 0.001$) of 5-ARI-treated low-*NKX3.1* ($n = 4$) versus high-*NKX3.1* ($n = 5$) human prostates. The green bar indicates positively enriched MRs in the leading edge. (E) Kaplan-Meier survival analysis on the basis of activity levels of MRs of the 5-ARI response signature from (C) using biochemical recurrence (BCR)-free survival as the endpoint as reported by Glinsky et

al [30]. The p value was estimated using a log-rank test to determine the difference in outcomes between patients with higher MR activity levels (red) and those with lower MR activity levels (blue). (F) Selected MRs with function and Cox p value indicated. The Cox proportional hazards model was based on MR activity levels in the data set described by Sboner et al [31], using prostate cancer-specific survival as the endpoint. The Cox p value was estimated using a Wald test. (G) Heat map indicating relative *NKX3.1* expression levels from 5-ARI-treated human prostates from the Fred Hutchinson Cancer Research Center (FHCRC) cohort including prostate samples with no treatment, low dutasteride treatment (0.5 mg), and high dutasteride treatment (3.5 mg) (Table 1). (H) Validation of selected MRs in the FHCRC cohort by qualitative real-time polymerase chain reaction. Experiments were performed using five samples per group and carried out; values are normalized to GAPDH and p values were estimated using two-tailed two-sample t test.

Table 1

Characteristics of the patients from the 5-ARI cohorts

	WCM cohort (training)	FHCRC (validation)
Patients (<i>n</i>)		
5-ARI treated	9	10 ^a
Untreated	18	5
Sample collection period	2012–2015	2003–2005
Age (yr)	64 (59.5–68.5) ^b	61 (46–73) ^c
PSA range (ng/ml)	2.39–14.5	2.5–10
Pathological Gleason score		6 (6–9) ^d
Treated patients	3 + 3 (<i>n</i> = 4), 3 + 4 (<i>n</i> = 4), 4 + 3 (<i>n</i> = 1)	
Untreated patients	3 + 4 (<i>n</i> = 7), 4 + 3 (<i>n</i> = 8), 4 + 4 (<i>n</i> = 3)	

5-ARI = 5 α -reductase inhibitor; WCM = Weill Cornell Medicine; FHCRC = Fred Hutchinson Cancer Research Center; PSA = prostate-specific antigen.

^aOf the ten patients, five received a low (0.5 mg) and five a high ARI dose (3.5 mg).

^bMedian (interquartile range).

^cMean (range).

^dMedian (range).