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14. ABSTRACT

Prostate cancer screening remains the subject of much controversy, largely because there are unacceptable levels of *over-treatment of low-risk, indolent prostate cancer, which* incurs significant morbidity and costs with minimal impact on life expectancy. Over-treatment leads not only to *avoidable morbidity and cost*, but also to *decisional regret*, *low satisfaction*, and much avoidable suffering associated with prostate cancer diagnosis. We will address both **incomplete information and poor understanding** among men diagnosed with low-risk prostate cancer. *We propose that an integrated risk prediction model, and implementation of a decision support intervention to help patients understand their disease risk and management options, we will reduce anxiety and uncertainty, improve decision quality and satisfaction, and increase acceptance of initial active surveillance for low-risk prostate cancer.* **We will evaluate this hypothesis through the following specific aims**: **Aim 1**: We will *develop* and *validate* a novel integrated risk prediction model, incorporating clinical, lifestyle, tumor genomic, and germline genetic variables, to provide *better information* to men about their risk of having more aggressive disease. **Aim 2**: We will *implement* and *evaluate (in a randomized controlled trial)* a decision support intervention, based on the risk model from Aim 1, to improve *understanding* of one's disease risks, and the pros and cons of different management options.

15. SUBJECT TERMS

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1. INTRODUCTION

Despite ongoing declines in prostate cancer mortality, prostate cancer screening remains the subject of much controversy. There is consensus that present *levels of over-treatment of low-risk, indolent prostate cancer are unacceptable*, given significant morbidity and costs—and minimal impact on life expectancy—associated with such treatment. Several factors underlie the over-treatment of low-risk disease, including the *concern that a biopsy may not fully reflect tumor heterogeneity and aggressiveness*. The possibility of under-estimation of risk of disease progression due to this incomplete information creates *anxiety* for patients and their clinicians. Such concerns coupled with poor understanding among patients regarding the risks and benefits of treatments drive many men to opt for treatment rather than pursue active surveillance, even for clinically low-risk disease. Such over-treatment leads not only to *avoidable morbidity and cost*, but also to *decisional regret*, *low satisfaction*, and much avoidable suffering associated with prostate cancer diagnosis. Our **goal** was to address both incomplete information and poor understanding among men diagnosed with low-risk prostate cancer. *We proposed that through development of an integrated risk prediction model, and implementation of a decision support intervention to help patients understand their disease risk and treatment options, we will reduce anxiety and uncertainty, improve decision quality and satisfaction, and increase acceptance of initial active surveillance for low-risk prostate cancer.*
We evaluated this hypothesis through the following specific aims:
Aim 1: We *developed* and *validated* a novel integrated risk prediction model, incorporating clinical, lifestyle, and molecular variables, to provide *better information* to men with clinically low-risk prostate cancer.
Aim 2: We *implemented* and *evaluated (in a randomized controlled trial)* a decision support intervention, based *in part* on the risk model developed in Aim 1, through which patients received a highly personalized summary of their cancer risk and management options, to support *better understanding* of the risks and benefits of treatments and of active surveillance.

2. KEYWORDS

Prostate cancer, biomarker, decision aid, early-stage disease, prognosis, body mass index, smoking, genetics, tumor genomics, quality-of-life, anxiety, treatment satisfaction, decision quality, web portal, decision-support, health coaching, tumor expression

3. Accomplishments

Below we provide an overall update on **Accomplishments**, organized by aim.

(original) Aim 1 - We will develop an integrated risk prediction model that will inform men with clinically low-risk prostate cancer of the likelihood that their cancer will be upstaged or upgraded on subsequent clinical or pathologic evaluation (repeat biopsy, imaging, etc).

Update: Our primary accomplishments of Aim 1 are summarized in the following abstract, which is part of a manuscript, currently under review at the journal Urology. The risk model

described in this abstract was subsequently used as part of the pilot and multi-site clinical trial in Aim 2 (see below).

The Development and Application of a Detailed Clinical Risk Prediction Model for Upgrading/Upstaging among Men with Low-risk Prostate Cancer

Chan JM, Neuhaus J, Cowan JE, Kenfield SA, Van Blarigan EL, Tenggara I, Broering JM, Witte JS, Simko J, Belkora J, Carroll PR[1], Cooperberg MR[1] ([1]shared last authors)

Purpose: Active surveillance is increasingly utilized for men with low risk prostate cancer, yet there are limited data on what clinical factors best predict risk of upgrading or upstaging (UG/US). We aimed to develop a risk prediction model to guide treatment decision-making or intensity of surveillance at diagnosis of localized disease.

Materials & Methods: We used multivariate logistic regression and receiver operating characteristic (ROC) curves to develop and test a prediction model for UG/US in men with prostate cancer who were potential candidates for active surveillance. The model was developed among 864 men with low-risk disease, who had surgery within 12 months of diagnosis (cohort 1). We considered biopsy grade, T-stage, prostate specific antigen (PSA), percent positive cores, number of positive cores, total number of cores taken, prostate volume as assessed by TRUS (transrectal ultrasound), PSA-density, race, and age as predictors. To test the model's predictive ability, we used the logistic model developed in the first sample to estimate the predicted probability of UG/US in 2,267 distinct men with similar prostate cancers (cohort 2) and computed area under the ROC curve (AUC) from these probabilities.

Results: The prediction model for UG/US developed using the first cohort included diagnostic grade, PSA, percent positive cores, TRUS prostate volume, and age ($AUC_{average}$ 0.72). When applied to a second independent population, the AUC was 0.69.

Conclusion: A model incorporating clinical variables can be applied to improve prediction of UG/US and guide management or the intensity of follow up among men who choose active surveillance.

Other related publications developed under this Aim include:

- Kornberg Z, Cooperberg MR, Cowan JE, Chan JM, Shinohara K, Simko JP, Tenggara I, Carroll PR. A 17-Gene Genomic Prostate Score as a Predictor of Adverse Pathology for Men on Active Surveillance. J Urol. 2019 Apr 26. Epub ahead of print PMID: 31026214
- Cedars BE, Washington SL 3rd, Cowan JE, Leapman M, Tenggara I, Chan JM, Cooperberg MR, Carroll PR. Stability of a 17-gene genomic prostate score in serial testing on men on active surveillance for early stage prostate cancer. J Urol. 2019 Apr 8. Epub ahead of print PMID: 30958742
- Cooperberg MR, Erho N, Chan JM, Feng FY, Fishbane N, Zhao SG, Simko JP, Cowan JE, Lehrer J, Alshalalfa M, Kolisnik T, Chelliserry J, Margrave J, Aranes, M, Plessis MD, Buerki

C, Tenggara I, Davicioni E, Carroll PR. The diverse genomic landscape of clinically low-risk prostate cancer. Eur Urol. 2018 Oct;74(4):444-452. PMID: 29853306

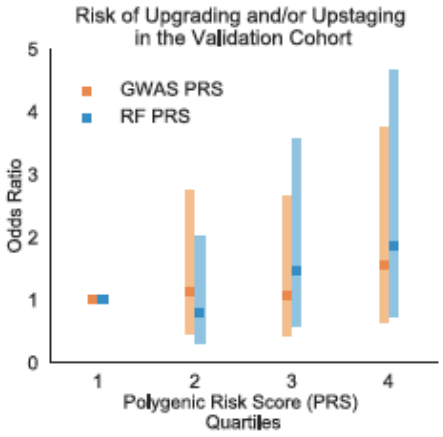
- Also presented at "Best of Abstracts" session at the national American Urological Association Meeting in May 2017, Boston MA.

- Knudsen BS, Kim HL, Erho N, Shin H, Alshalalfa M, Lam LL, Tenggara I, Chadwich K, Van Der Kwast T, Fleshner N, Davicioni E, Carroll PR, Cooperberg MR, Chan JM, Simko JP. Application of a Clinical Whole-Transcriptome Assay for Staging and Prognosis of Prostate Cancer Diagnosed in Needle Core Biopsy Specimens. J Mol Diagn. 2016 May;18(3):395-406. PMID: 26945428

- Emami NC, Leong L, Wan E, Van Blarigan EL, Cooperberg MR, Tenggara I, Carroll PR, Chan JM, Witte JS, Simko JP. Tissue Sources for Accurate Measurement of Germline DNA Genotypes in Prostate Cancer Patients Treated With Radical Prostatectomy. Prostate. 2017 Mar;77(4):425-434. doi: 10.1002/pros.23283. Epub 2016 Nov 30. https://www.ncbi.nlm.nih.gov/pubmed/?term=27900799

Other Ongoing Work/Updates related to Aim 1

- We continue to conduct separate analyses examining the germline DNA array data from the UCSF and CaPSURE populations (from Aim 1b) in relation to the short-term up-grading/up-staging outcome, and considering exploratory analyses examining other longer-term outcomes in this set (e.g., recurrence risk, etc). Our team recently presented an abstract at the American Society for Human Genetics conference on this work in progress (2018, Cavazos T et al, *Integrating Genetic Information with Machine Learning to Predict Which Prostate Cancer Cases Should not be Immediately Treated*, See Appendix). The goal of this project was to explore genetic predictors (polygenic risk score, PRS) of UG/US and use random forest (RF) inference to prioritize patients for active surveillance. The figure below shows initial results for two PRS, one computed using Random Forest, another using GWAS. We are continuing to optimize this model for potential future use in predicting who is the best candidate for active surveillance.



    - Preliminary work on this project was presented at American Society for Human Genetics, 2018.

- As described previously, due to initial analyses indicating that the genomic tests did not enhance the clinical risk prediction model substantively, we redirected efforts to Aim 2b and are no longer pursuing running additional genomics in the CaPSURE population. Individual analysis on the DNA from CaPSURE are being analyzed and reported with the UCSF data, as described above.
- We have also collected additional saliva/DNA specimens from the remaining participants in CaPSURE, irrespective of their risk profile, in recognition that this cohort is aging and there may be broader further uses for examining their DNA profiles. In total, between the original low-risk population (supported by this grant) and our subsequent specimen collection (supported intramurally by the investigator team), we have collected and sent ~960 saliva specimens to the genetics lab for analysis from CaPSURE (the last 4 batches were sent between Sept-Dec 2019). Approximately 5 were repeat samples on the same man, thus this reflects data on ~955 individual men with prostate cancer. Final DNA processing and array assessments are underway. These data will be examined with regards to other types of prostate cancer outcomes, such as disease progression.

AIM 2

Aim 2 - We will implement and evaluate (in a randomized controlled trial) a decision support intervention, based in part on the risk model developed in Aim 1, through which patients received a highly personalized summary of their cancer risk and management options, to support better understanding of the risks and benefits of treatments and of active surveillance. We approached this aim in two phases, first with a single arm pilot trial, next using a site-randomized cross-over design with multiple clinical enrollment sites.

Update: We completed the pilot study, which found that we could feasibly deliver our online decision aid using student interns as health coaches. Pilot results showed promising improvement in patient knowledge of key facts about active surveillance. We summarized the pilot study in a manuscript published in Cancer Medicine. The manuscript was recently published:

- Belkora J, Chan J, Cooperberg M, Neuhaus J, Stupar L, Weinberg T, Broering J, Tenggara I, Cowan J, Rosenfeld S, van Blarigan E, Simko J, Witte J, Carroll P. Development and Pilot Evaluation of a Personalized Decision Support Intervention for Low Risk Prostate Cancer Patients. Cancer Med. 2019; 00: 1– 8. https://doi.org/10.1002/cam4.2685.
    - An abstract for the manuscript was presented at ASCO Genitourinary Cancer Symposium Conference in Feb. 2018.

A brief synopsis of the study design for our multi-site trial is described below:

- Primary outcome: to increase the occurrence of *informed* decision-making among men with early-stage prostate cancer. This will be assessed via 2 questions on knowledge from the Decision Quality Index, Prostate.
- Secondary Outcomes: anxiety, decision self-efficacy, and decision quality, as measured by validated survey instruments, and management choice.
- Site-crossover study, with each site administering 2 phases - Usual Care or Coaching with Decision Aid – in sequence; starting sequence is randomly assigned and the randomization schema has been provided by our biostatistician to achieve balance across sites at any given time.
- Project has been named ***Pioneering Advances in Care and Education*** (***PACE***) and a study specific e-mail was created: pace@ucsf.edu
- We are using a randomized crossover design. Sites were originally expected to recruit 20 patients for the intervention arm and 20 for the usual care arm.
- The participants will complete the following instruments at the following timepoints:
  - **T1- Baseline**
    - Control Preferences Scale (CPS)
    - Decision Self-Efficacy (DSE)
    - Choice Predisposition (CP)
    - Decision Quality Instrument (DQI) – sections 1 & 2
    - Risk Reclassification Understanding
    - Memorial Anxiety Scale for Prostate Cancer (MaxPC)
    - Participant Demographics
    - Short Form-12
    - Expanded Prostate Cancer Index Composite (EPIC-26)
  - **T2: Decision Support Intervention (DSI) – intervention arm only**
  - **T3: Before Consultation with Urologist**
    - Control Preferences Scale (CPS)
    - Decision Self-Efficacy (DSE)
    - Choice Predisposition (CP)
    - Decision Quality Instrument (DQI) – sections 1 & 2
    - Risk Reclassification Understanding
    - Memorial Anxiety Scale for Prostate Cancer (MaxPC)
    - Access to Patient Education Materials
    - Access to Other Diagnostic Tests and External Interventions
    - Other Medical Visits
  - **T4: After Consultation Visit with Urologist**
    - Control Preferences Scale (CPS)
    - Decision Self-Efficacy (DSE)
    - Choice Predisposition (CP)

- Decision Quality Instrument (DQI) – sections 1, 2, & 3
- Risk Reclassification Understanding
- Memorial Anxiety Scale for Prostate Cancer (MaxPC)
- The **participating urologist's** will be asked to rate their experience with the intervention or usual care along with the opportunity for them to provide qualitative comments about their experience.

- **T5: Two Weeks After Consultation Visit**
  - Service Satisfaction w/ Cancer Care
  - Total Illness Burden Index for Prostate Cancer – TIBI-CaP subset of key questions

This multi-site trial is ongoing and close to completing enrollment. Highlights of the trials progress are summarized below:

- As sites come onboard, they work with the UCSF team to insert test fictitious patients into the REDCap application and work on local workflow screening processes. We have 5 sites opened and accruing: UCSF, San Francisco Veterans Affairs Medical Center, CentraCare Urology (Minnesota), Palo Alto Medical Foundation, and Lancaster Urology (Michigan). Enrollment numbers as of 11/20/19 is shown in Table 1. *Total enrollment as of 12/21/19 is slightly higher, with 1,221 screened and 112 consented (Fig.2).* .
- We initiated calls with sites coordinators in April of 2017. We held these this calls every other week from April through June 2018, and monthly since August 2018. These calls are audio recorded with screen capture in the event the onsite coordinator cannot attend the call. We have conducted 46 group calls since trial inception.

Table 1. Screening by Site, for PACE (Aim 2) Clinical Trial (as of 11/20/19)

Site	Screened	Pending	Intervention	Usual Care	Total
UCSF	531	5	35	33	68
SF-VA	15	0	0	0	0
Lancaster Urology	156	0	8	0	8
CentraCare	390	2	2	22	24
PAMF	53	3	3	0	3
	1145	10	48	55	103

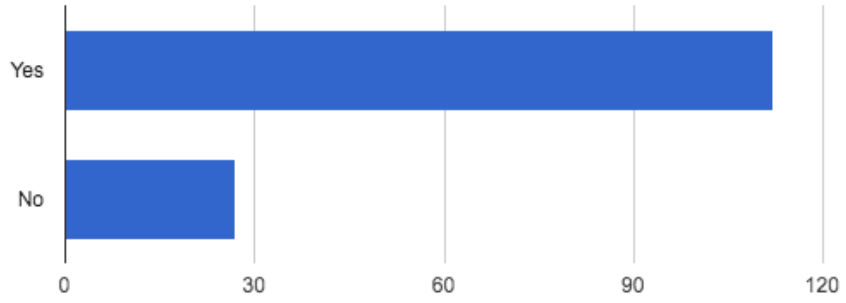


Figure 2. Total Consented (112 Yes, 27 No) for PACE

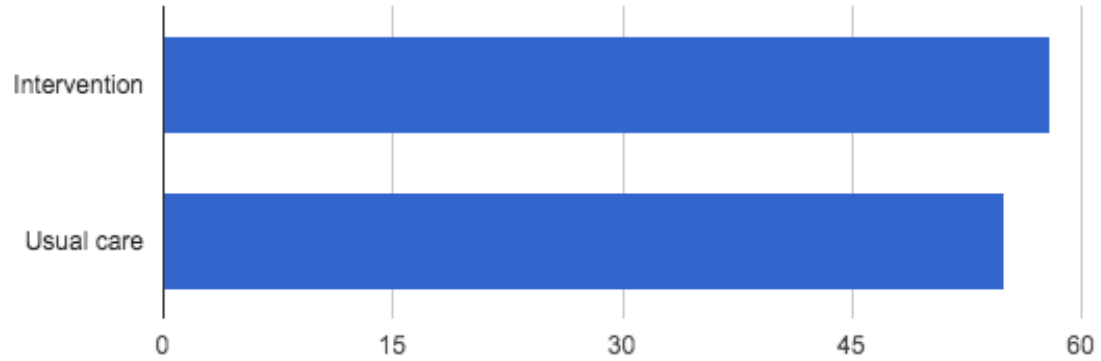


Figure 3. Distribution of Assignment to Intervention or Usual Care for PACE

In summary, we are close to completing enrollment for PACE. Our current goal is to enroll 116 individuals and we anticipate completing enrollment in the next 4-5 weeks (based on current rate and allowing for the holiday slow-down). Once completed, we will conduct statistical analyses and report the results.

4. Impact

Impact on the Principal Disciplines: Over the course of this project, our publications and presentations have had two major impacts – we have increased the adoption and acceptance of active surveillance for low-risk prostate cancer; and we have improved the way in which active surveillance can be refined and personalized for the individual (both through usage of tailored clinical risk models and decision support tools). These are impacts on clinical care, patient education/knowledge, and hopefully public health (e.g., by reducing morbidity associated with over-treatment of prostate cancer).

Impact on Other Disciplines: As a central part of this effort, this grant helped support two large prostate cancer databases – UODB and CaPSURE. Thus, indirectly, it has helped to support our understanding about prostate cancer in several other areas, not part of our original aims, including other biomarkers, imaging, diet factors, and quality of life. We have provided a complete list of publications from these data resources since 2012 in Section 6 and indicated those that were directly part of the aims with an asterisk.

Impact on Technology Transfer: Nothing to Report

Impact on Society beyond Science and Technology: While not directly measured, as alluded to above, these projects have supported the adoption of more conservative management of prostate cancer (i.e., active surveillance), which in turn may improve morbidity from this

disease. Our projects have also raised awareness regarding the potential usage of health coaches and online digital decision aids, to improve informed decision making

5. Changes & Problems

For both Aims, the theme of our primary challenges was related to changing patterns of standards of care. In Aim 1, we had focused on examining men who would be eligible for active surveillance but were going to surgery, such that we could analyze up-grading and up-staging in their full prostate specimen, compared to biopsy. However, since the inception of this grant, active surveillance picked up throughout the country and at our clinical site. Thus, we found it harder and harder to identify men who met our original eligibility criteria of "low-risk" and who went on to surgery for our Aim 1 analysis (i.e., patients going to surgery were generally higher risk). Additionally, magnetic resonance imaging (MRI) techniques emerged strongly during this time-frame, which influenced the number of cores taken at biopsy, further pushing incoming patients outside our original eligibility criteria. Another challenge encountered in Aim 1 was that for our short-term outcome of up-grading and up-staging, our initial analyses of the molecular tumor signatures did not markedly improve prognostication and dropped out of the model. Thus, we proceeded with the clinical model only for Aim 2. We hypothesize that perhaps up-grading and up-staging was not sufficiently indicative of truly bad or aggressive prostate cancer, and more follow-up time is needed to distinguish indolent from lethal phenotypes. While we successfully executed the original aims, we plan to maximize data collected and leverage resources supported by Aim 1, and are focusing on collecting data to examine longer-term outcomes, such as prostate cancer progression, in the future.

For Aim 2, we experienced a similar type of impact. In 2012, the USPSTF gave a D rating against PSA screening for prostate cancer. Consequently, some clinical sites experienced an increase in the grade and stage of men presenting with prostate cancer, and we found it harder to identify eligible men for our trial. While the rating was updated in 2017/2018 to a C, we continued to observe challenges identifying low-risk men at initial diagnosis who met our eligibility criteria. Thus, enrollment for the trial went slower than anticipated at most sites.

Our initial pilot trial indicated that the intervention was feasible and acceptable, and we have a very productive data collection team and standard operating processes (as one might expect from CAPSURE sites and the UCSF coordinating site). However, we ran into several issues that led to lower than anticipated enrollment, especially at the community sites. These included:

- Some community sites were unable to screen continuously due to high personnel turnover and other personnel issues.
- When the site staff were present, they told us that they were not always able to prioritize recruitment for this study.

- When they were able to screen, they found that many patients were not eligible (for reasons described above).
- When they found eligible patients, often the window of opportunity to recruit was tight, because we needed to enroll people after they had been told their diagnosis but before their next appointment. Many patients eluded us due to this tight window.
- The decline rate was higher than we expected.

Thus, we did not meet our accrual goals at community sites, and this led us to open at UCSF, where recruitment has been much higher volume. However, the original design was to randomize sites and then cross them over. In the end, we will have sufficient patients from one crossover site (UCSF) but not from the others, obviating the effects of randomization. Thus, we plan to report on the data overall, but acknowledge that the great majority will be from UCSF, rather than community sites. These experiences indicate that implementing this kind of interventional research at community sites is infeasible at this time. If our results show that the intervention is effective at our academic site, the question will remain regarding how to spread it to community sites.

6. Products

PUBLICATIONS – As mentioned above, this grant helped support two large prostate cancer databases – UODB and CaPSURE. Thus, it has supported the science of the original aims, as well as helped to support broader research that leveraged these databases during the timeframe. We have provided a complete list of publications from these data resources (2013-2019) below and indicated those that were directly related to the aims with an asterisk. These are presented by data source and by year.

PUBLICATIONS 2013-2019

Urologic Outcomes Database (UODB)

2013

Bauer SR, Richman EL, Sosa E, Weinberg V, Song X, Witte JS, Carroll PR, Chan JM. Antioxidant and vitamin E transport genes and risk of high-grade prostate cancer and prostate cancer recurrence. Prostate. 2013 Dec;73(16):1786-95. PMID: 24038157

Cary KC, Cowan JE, Sanford M, Shinohara K, Perez N, Chan JM, Meng MV, Carroll PR. Predictors of pathologic progression on biopsy among men on active surveillance for localized prostate cancer: the value of the pattern of surveillance biopsies. Eur Urol. 2014 Aug 66(2):337-42. Epub 2013 Sep 9. PMID: 24035632

Cooperberg MR, Simko JP, Cowan JE, Reid JE, Djalilvand A, Bhatnagar S, Gutin A, Lanchbury JS, Swanson GP, Stone S, Carroll PR. Validation of a cell-cycle progression gene panel to improve risk stratification in a contemporary prostatectomy cohort. J Clin Oncol. 2013 Apr 10;31(11):1428-34. PMID: 23460710

Glass AS, Porten SP, Bonham M, Tran TC, Cowan JE, Punnen S, Chan JM, Carroll PR. Active surveillance: Does serial prostate biopsy increase histological inflammation? Prostate Cancer Prostatic Dis. 2013;16(2):165-9. PMID: 23318528

Hawley S, Fazli L, McKenney JK, Simko J, Troyer D, Nicolas M, Newcomb LF, Cowan JE, Crouch L, Ferrari M, Hernandez J, Hurtado-Coll A, Kuchinsky K, Liew J, Mendez-Meza R, Smith E, Tenggara I, Zhang X, Carroll PR, Chan JM, Gleave M, Lance R, Lin DW, Nelson PS, Thompson IM, Feng Z, True LD, Brooks JD. A model for the design and construction of a resource for the validation of prognostic prostate cancer biomakers: the Canary Prostate Cancer Tissue Microarray. Adv Anat Pathol. 2013 Jan;20(1):39-44. PMID: 23232570

Lin DW, Newcomb LF, Brown EC, Brooks JD, Carroll PR, Feng Z, Gleave ME, Lance RS, Sanda MG, Thompson IM, Wei JT, Nelson PS; Canary Prostate Active Surveillance Study Investigators. Urinary TMPRSS2:ERG and PCA3 in an active surveillance cohort: results from a baseline analysis in the Canary Prostate Active Surveillance Study. Clin Cancer Res. 2013 May 1:19(9):2442-50. PMID: 23515404

Odisho AY, Washington SL 3rd, Meng MV, Cowan JE, Simko JP, Carroll PR. Benign prostate glandular tissue at radical prostatectomy surgical margins. Urology. 2013;82(1):154-9. PMID: 23522995

Ornish D, Lin J, Chan JM, Epel E, Kemp C, Weidner G, Marlin R, Fenda SJ, Magbanua MJ, Daubenmier J, Estay I, Hills NK, Chainani-Wu N, Carroll PR, Blackburn EH. Effect of comprehensive lifestyle changes on telemorase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study. Lancet Oncol. 2013 Oct;14(11):1112-20. PMID: 24051140

Punnen S, Meng MV, Cooperberg MR, Greene KL, Cowan JE, Carroll PR. How does robot-assisted radical prostatectomy (RARP) compare with open surgery in men with high-risk prostate cancer? BJU Int. 2013 Aug;112(4):E314-20. PMID: 23451984

Punnen S, Cowan JE, Dunn LB, Shumay DM, Carroll PR, Cooperberg MR. A longitudinal study of anxiety, depression and distress as predictors of sexual and urinary quality of life in men with prostate cancer. BJU Int. 2013. Jul;112(2):E67-75. PMID: 23795800

Whitson JM, Porten SP, Cowan JE, Simko JP, Cooperberg MR, Carroll PR. Factors associated with downgrading in patients with high grade prostate cancer. Urol Oncol. 2013 May;31(4):442-7. Epub 2011 Apr 8. PMID: 21478037

2014

Carroll PR, Parsons JK, Andriole G, Bahnson RR, Barocas DA, Catalona WJ, Dahl DM, Davis JW, Epstein JI, Etzioni RB, Giri VN, Hemstreet GP 3rd, Kawachi MH, Lange PH, Loughlin KR, Lowrance W, Maroni P, Mohler J, Morgan TM, Nadler RB, Poch M, Scales C, Shanefelt TM, Vickers AJ, Wake R, Shead DA, Ho M. Prostate cancer early detection, version 1.2014. J Natl Compr Canc Netw. 2014 Sep;12(9):1211-9. PMID: 25190691

Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, Chan JM, Li J, Cowan JE, Tsiatis AC, Cherbavaz DB, Pelham RJ, Tenggara-Hunter I, Baehner FL, Knezevic D,

Febbo PG, Shak S, Kattan MW, Lee M, Carroll PR. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol. 2014 Sep;66(3):550-60. PMID: 24836057 Reply to letter to the editor: PMID: 25150174

Glass AS, Hilton JF, Cowan JE, Washington SL, Carroll PR. Serial prostate biopsy and risk of lower urinary tract symptoms: results from a large, single-institution active surveillance cohort. Urology. 2014 Jan;83(1):33-9. Epub 2013 Nov 15. PMID: 24246319

Punnen S, Cary KC, Glass AS, Cowan JE, Carroll PR. Autologous retro-pubic urethral sling: a novel, quick, intra-operative technique to improve continence after robotic-assisted radical prostatectomy. J Robotic Surg. 2014;8:99–104 PMID: None

Wang SY, Cowan JE, Cary KC, Chan JM, Carroll PR, Cooperberg MR. Limited ability of existing nomograms to predict outcomes in men undergoing active surveillance for prostate cancer. BJU Int. 2014 Dec;114(6b):E18-24. PMID: 24712895

Wang SY, Shiboski S, Belair CD, Cooperberg MR, Simko JP, Stoppler H, Cowan J, Carroll PR, Blelloch R. miR-19, miR-345, miR-519c-5p serum levels predict adverse pathology in prostate cancer patients eligible for active surveillance. PLoS One. 2014 Jun 3;9(6):e98597. PMID: 2489317

2015

*Filippou P, Welty CJ, Cowan JE, Perez N, Shinohara K, Carroll PR. Immediate Versus Delayed Radical Prostatectomy: Updated Outcomes Following Active Surveillance of Prostate Cancer. Eur Urol. 2015 Sep;68(3):458-63. PMID: 26138041

Hussein AA, Welty CJ, Ameli N, Cowan JE, Leapman M, Porten SP, Shinohara K, Carroll PR. Untreated Gleason grade progression on serial biopsies during prostate cancer active surveillance: clinical course and pathological outcomes. J Urol. 2015 Jul;194(1):85-90. PMID: 25623742

Jalloh M, Leapman MS, Cowan JE, Shinohara K, Greene KL, Roach M 3rd, Chang AJ, Chan JM, Simko JP, Carroll PR. Patterns of local failure following radiation therapy for prostate cancer. J Urol. 2015 Oct;194(4):977-82. PMID: 25983194

Cancer Genome Atlas Research Network (TCGA). The molecular taxonomy of primary prostate cancer. Cell. 2015 Nov 5;163(4):1011-25. PMID: 26544944

*Welty CJ, Cowan JE, Nguyen H, Shinohara K, Perez N, Greene KL, Chan JM, Meng MV, Simko JP, Cooperberg MR, Carroll PR. Extended follow-up and risk factors for disease reclassification from a large active surveillance cohort for localized prostate cancer. J Urol. 2015 Mar;193(3):807-11. Epub 2014 Sep 24. PMID: 25261803

2016

Eltemamy MM, Leapman MS, Cowan JE, Westphalen A, Shinohara K, Carroll PR. Serial anatomic prostate ultrasound imaging during prostate cancer active surveillance. J Urol. 2016 Sep;196(3):727-33. PMID: 27117443

Kenfield SA, Batista JL, Jahn JL, Downer MK, Van Blarigan EL, Sesso HD, Giovannucci EL, Stampfer MJ, Chan JM. Development and application of a lifestyle score for prevention of lethal prostate cancer. J Natl Cancer Inst. 2016;108(3):djv329. PMID: 26577654

*Knudsen BS, Kim HL, Erho N, Shin H, Alshalalfa M, Lam LL, Tenggara I, Chadwich K, Van Der Kwast T, Fleshner N, Davicioni E, Carroll PR, Cooperberg MR, Chan JM, Simko JP. Application of a Clinical Whole-Transcriptome Assay for Staging and Prognosis of Prostate Cancer Diagnosed in Needle Core Biopsy Specimens. J Mol Diagn. 2016 May;18(3):395-406. PMID: 26945428

Leapman MS, Ameli N, Cooperberg MR, Chu C, Hussein A, Shinohara K, Carroll PR. Quantified clinical risk change as an end point during prostate cancer active surveillance. Eur Urol. 2017 Sep;72(3):329-332. Epub 2016 May 3. PMID: 27157998

Newcomb LF, Thompson IM Jr, Boyer HD, Brooks JD, Carroll PR, Cooperberg MR, Dash A, Ellis WJ, Fazli L, Feng Z, Gleave ME, Kunju P, Lance RS, McKenney JK, Meng MV, Nicolas MM, Sanda MG, Simko J, So A, Tretiakova MS, Troyer DA, True LD, Vakar-Lopez F, Virgin J, Wagner AA, Wei JT, Zheng Y, Nelson PS, Lin DW; Canary Prostate Active Surveillance Study Investigators. Outcomes of active surveillance for the management of clinically localized prostate cancer in the prospective, multi-institutional Canary PASS cohort. J Urol. 2016 Feb;195(2):313-20. Epub 2015 Aug 29. PMID: 26327354

*Emami NC, Leong L, Wan E, Van Blarigan EL, Cooperberg MR, Tenggara I, Carroll PR, Chan JM, Witte JS, Simko JP. Tissue Sources for Accurate Measurement of Germline DNA Genotypes in Prostate Cancer Patients Treated With Radical Prostatectomy. Prostate. 2017 Mar;77(4):425-434. doi: 10.1002/pros.23283. Epub 2016 Nov 30. https://www.ncbi.nlm.nih.gov/pubmed/?term=27900799

2017

Hope TA, Aggarwal R, Chee B, Tao D, Greene KL, Cooperberg MR, Feng F, Chang A, Ryan CJ, Small EJ, Carroll PR. Impact of 68Ga-PSMA-11 PET on management in patients with biochemically recurrent prostate cancer. J Nucl Med. 2017 Dec;58(12):1956-61. PMID: 28522741

Lake ST, Greene KL, Westphalen AC, Behr SC, Zagoria R, Small EJ, Carroll PR, Hope TA. Optimal MRI sequences for 68Ga-PSMA-11 PET/MRI in evaluation of biochemically recurrence prostate cancer. EJNMMI Res. 2017 Sep 19;7(1):77. PMID: 28929350

Leapman MS, Ameli N, Shinohara K, Nguyen HG, Meng MV, Cooperberg MR, Carroll PR. Validity of the Cancer of the Prostate Risk Assessment Score Derived from Targeted Biopsy: Modeling Evidence from Ultrasound Lesion-Directed Biopsy. Clin Genitourin Cancer. 2017 Feb;15(1):93-99. Epub 2016 Jul 21. PMID: 27522449

Leapman MS, Cowan JE, Nguyen HG, Shinohara K, Perez N, Cooperberg MR, Catalona WJ, Carroll PR. Active surveillance in younger men with prostate cancer. J Clin Oncol. 2017. Jun 10;35(17):1898-1904. PMID: 28346806

*Leapman MS, Westphalen AC, Ameli N, Lawrence HJ, Febbo PG, Cooperberg MR, Carroll PR. Association between a 17-gene prostate score and multi-parametric prostate MRI in men with low and intermediate risk prostate cancer (PCa). PLoS One. 2017 Oct 10;12(10). PMID: 29016610

Lin DW, Newcomb LF, Brown MD, Sjoberg DD, Dong Y, Brooks JD, Carroll PR, Cooperberg M, Dash A, Ellis WJ, Fabrizio M, Gleave ME, Morgan TM, Nelson PS, Thompson IM, Wagner AA, Zheng Y; Canary Prostate Active Surveillance Study Investigators. Evaluating the Four Kallikrein Panel of the 4K score for prediction of high-grade prostate cancer in men in the Canary Prostate Active Surveillance Study. Eur Urol. 2017 Sep;72(3):448-454. Epub 2016 Nov 23. PMID: 27889277

Nguyen HG, Punnen S, Cowan JE, Leapman M, Cary C, Welty K, Weinberg V, Cooperberg MR, Meng MV, Greene KL, Garcia M, Carroll PR. A randomized study of intra-operative autologous retropubic urethral sling on urinary control after robotic-assisted radical prostatectomy. J Urol. 2017 Feb;197(2):369-375. Epub 2016 Sep 27. PMID: 27693447

Tran GN, Leapman MS, Nguyen HG, Cowan JE, Shinohara S, Westphalen AC, Carroll PR. Magnetic resonance imaging-ultrasound fusion biopsy during prostate cancer active surveillance. Eur Urol. 2017 Aug;72(2):275-281. Epub 2016 Aug 29. PMID: 27595378

Welty CJ, Sanford TH, Wright JL, Carroll PR, Cooperberg MR, Meng, MV, Porten SP. The Cancer of the Bladder Risk Assessment (COBRA) score: Estimating mortality after radical cystectomy. Cancer. 2017 Dec 1;123(23):4574-4582. PMID: 28881475

2018

Cooperberg MR, Brooks JD, Faino AV, Newcomb LF, Kearns JT, Carroll PR, Dash A, Etzioni R, Fabrizio MD, Gleave ME, Morgan TM, Nelson PS, Thompson IM, Wagner AA, Lin DW, Zheng Y. Refined analysis of prostate-specific antigen kinetics to predict prostate cancer active surveillance outcomes. Eur Urol. 2018 Aug;74(2):211-217. PMID: 29433975

*Cooperberg MR, Erho N, Chan JM, Feng FY, Fishbane N, Zhao SG, Simko JP, Cowan JE, Lehrer J, Alshalalfa M, Kolisnik T, Chelliserry J, Margrave J, Aranes, M, Plessis MD, Buerki C, Tenggara I, Davicioni E, Carroll PR. The diverse genomic landscape of clinically low-risk prostate cancer. Eur Urol. 2018 Oct;74(4):444-452. PMID: 29853306

Inoue LYT, Lin DW, Newcomb LF, Leonardson AS, Ankerst D, Gulati R, Carter HB, Trock BJ, Carroll PR, Cooperberg MR, Cowan JE, Klotz LH, Mamedov A, Penson DF, Etzioni R. Comparative analysis of biopsy upgrading in four prostate cancer active surveillance cohorts. Annals of Internal Med. 2018 Jan 2;168(1):1-9. Epub 2017 Nov 28. PMID: 29181514

Kearns JT, Faino AV, Newcomb LF, Brooks JD, Carroll PR, Dash A, Ellis WJ, Fabrizio M, Gleave ME, Morgan TM, Nelson PS, Thompson IM, Wagner AA, Zheng Y, Lin DW. Role of surveillance

biopsy with no cancer as a prognostic marker for reclassification: results from the Canary Prostate Active Surveillance study Eur Urol. 2018 May;73(5):706-712. PMID: 29433973

Lange JM, Gulati R, Leonardson AS, Lin DW, Newcomb LF, Trock BJ, Carter HB, Cooperberg MR, Cowan JE, Klotz LH, Etzioni R. Estimating and comparing cancer progression risks under varying surveillance protocols. Ann Appl Stat. 2018 Sep;12(3):1773-1795. PMID: 30627300

Leapman MS, Nguyen HG, Cowan JE, Xue L, Stohr B, Simko J, Cooperberg MR, Carroll PR. Comparing Prognostic Utility of a Single-marker Immunohistochemistry Approach with Commercial Gene Expression Profiling Following Radical Prostatectomy. Eur Urol. 2018 Nov;74(5):668-675. PMID: 30181067

Masic S, Cowan JE, Washington SL, Nguyen HG, Shinohara K, Cooperberg MR, Carroll PR. Effects of Initial Gleason Grade on Outcomes during Active Surveillance for Prostate Cancer. Eur Urol Oncol. 2018 Oct;1(5):386-394. PMID: 31158077

Nguyen HG, Welty C, Lindquist K, Ngo V, Gilbert E, Bengtsson H, Magi-Galluzzi C, Jean-Gilles J, Yao J, Cooperberg M, Messing E, Klein EA, Carroll PR, Paris PL. Validation of GEMCaP as a DNA based biomarker to predict prostate cancer recurrence after radical prostatectomy. J Urol. 2018 Mar;199(3):719-725. Epub 2017 Sep 20. PMID: 28941923

Nguyen HG, Conn CS, Kye Y, Xue L, Forester CM, Cowan JE, Hsieh AC, Cunningham JT, Truillet C, Tameire F, Evans MJ, Evans CP, Yang JC, Hann B, Koumenis C, Walter P, Carroll PR, Ruggero D. Development of a stress response therapy targeting aggressive prostate cancer. Sci Transl Med. 2018 May 2;10(439). PMID: 29720449

Winters-Stone KM, Kenfield SA, Van Blarigan EL, Moe EL, Ramsdill JW, Daniel K, Macaire G, Paich K, Kessler ER, Kucuk O, Gillespie TW, Lyons KS, Beer TM, Broering JM, Carroll PR, Chan JM. Effect of increasing levels of web-based behavioral support on changes in physical activity, diet, and symptoms in men with prostate cancer: protocol for a randomized controlled trial. JMIR Res Protoc. 2018 Nov 15;7(11):e11257. PMID: 30442638

2019

Balakrishnan AS, Cowan JE, Cooperberg MR, Shinohara K, Nguyen HG, Carroll PR. Evaluating the safety of active surveillance: outcomes of deferred radical prostatectomy after an initial period of surveillance. J Urol. 2019 Sep;202(3):506-510. PMID: 30958738

*Belkora J, Chan JM, Chan JM, Cooperberg MR, Neuhaus J, Stupar L, Weinberg T, Broering JM, Tenggara I, Cowan JE, Rosenfeld S, Kenfield SA, Van Blarigan EL, Simko JP, Witte J, Carroll PR. Development and pilot evaluation of a personalized decision support intervention for low risk prostate cancer patients. Cancer Med. 2019 Nov 12. Epub ahead of print PMID: 31714037

*Cedars BE, Washington SL 3rd, Cowan JE, Leapman M, Tenggara I, Chan JM, Cooperberg MR, Carroll PR. Stability of a 17-gene genomic prostate score in serial testing on men on active surveillance for early stage prostate cancer. J Urol. 2019 Apr 8. Epub ahead of print PMID: 30958742

Greenland NY, Zhang L, Cowan JE, Carroll PR, Stohr BA, Simko JP. Correlation of a commercial genomic risk classifier with histologic patterns in prostate cancer. J Urol. 2019 Jul;202(1):90-95. PMID: 30810466

Herlemann A, Huang HC, Alam R, Tosoian JJ, Kim HL, Klein EA, Simko JP, Chan JM, Lane BR, Davis JW, Davicioni E, Feng FY, McCue P, Kim H, Den RB, Bismar TA, Carroll PR, Cooperberg MR. Decipher identifies men with otherwise clinically favorable intermediate-risk disease who many not be good candidates for active surveillance. Prostate Cancer Prostatic Dis. 2019 Aug 27. Epub ahead of print PMID: 31455846

Kenfield SA, Van Blarigan EL, Ameli N, Lavaki E, Cedars B, Paciorek AT, Monroy C, Tantum LK, Newton RU, Signorell C, Suh JH, Zhang L, Cooperberg MR, Chan JM. Feasibility, acceptability, and behavioral outcomes from a technology-enhanced behavioral change intervention (Prostate 8): A pilot randomized controlled trial in men with prostate cancer. Eur Urol. 2019 Jun;75(6):950-958. PMID: 30638635

Kornberg Z, Cowan JE, Westphalen AC, Cooperberg MR, Chan JM, Zhao S, Shinohara K, Carroll PR. Genomic Prostate Score, PI-RADSv2, and progression in men with prostate cancer on active surveillance. J Urol. 2019 Feb;201(2):300-307. PMID: 30179620 Epub 2018 Sep 1.

*Kornberg Z, Cooperberg MR, Cowan JE, Chan JM, Shinohara K, Simko JP, Tenggara I, Carroll PR. A 17-Gene Genomic Prostate Score as a Predictor of Adverse Pathology for Men on Active Surveillance. J Urol. 2019 Apr 26. Epub ahead of print PMID: 31026214

Newcomb LF, Zheng Y, Faino AV, Bianchi-Frias D, Cooperberg MR, Brown MD, Brooks JD, Dash A, Fabrizio MD, Gleave ME, Liss M, Morgan TM, Thompson IM, Wagner AA, Carroll PR, Nelson PS, Lin DW. Performance of PCA3 and TMPRSS2:ERG urinary biomarkers in prediction of biopsy outcome in the Canary Prostate Active Surveillance Study (PASS). Prostate Cancer Prostatic Dis. 2019 Sep;22(3):438-44. PMID: 30664734

Odisho AY, Bridge M, Webb M, Ameli N, Eapen RS, Stauf F, Cowan JE, Washington SL 3rd, Herlemann A, Carroll PR, Cooperberg MR. Automating the Capture of Structured Pathology Data for Prostate Cancer Care and Research. JCO Clin Informatics 2019 Jul;(3):1-8. PMID: 31314550

Xu MJ, Kornberg Z, Gadzinski AJ, Diao D, Cowan JE, Wu SY, Boreta L, Spratt DE, Behr SC, Nguyen HG, Cooperberg MR, Davicioni E, Roach M 3rd, Hope TA, Carroll PR, Feng FY. Genomic risk predicts molecular imaging-detected metastatic nodal disease in prostate cancer. Eur Urol Oncol. 2019 Jan 14. Epub ahead of print PMID: 31411984

Zhao SG, Lehrer J, Chang SL, Das R, Erho N, Liu Y, Sjöström M, Den RB, Freedland SJ, Klein EA, Karnes RJ, Schaeffer EM, Xu M, Speers C, Nguyen PL, Ross AE, Chan JM, Cooperberg MR, Carroll PR, Davicioni E, Fong L, Spratt DE, Feng FY. The immune landscape of prostate cancer and nomination of PD-L2 as a potential therapeutic target. J Natl Cancer Inst. 2019 Mar 1;111(3):301-310. Epub 2018 Oct 13. PMID: 30321406

CaPSURE

2013

Akaza H, Hinotsu S, Cooperberg MR, Chung BH, Youl Lee J, Umbas R, Tsukamoto T, Namiki M, Carroll PR. Sixth Joint Meeting of J-CaP and CaPSURE: a multinational perspective on prostate cancer management and patient outcomes. Jpn J Clin Oncol. 2013 Jul;43(7):756-66. PMID: 23723314

Barocas DA, Chen V, Cooperberg M, Goodman M, Graff JJ, Greenfield S, Hamilton A, Hoffman K, Kaplan S, Koyama T, Morgans A, Paddock LE, Phillips S, Resnick MJ, Stroup A, Wu XC, Penson DF. Using a population-based observational cohort study to address difficult comparative effectiveness research questions: the CEASAR study. J Comp Eff Res. 2013 Jul;2(4):445-60. PMID: 24236685

Bauer SR, Richman EL, Sosa E, Weinberg V, Song X, Witte JS, Carroll PR, Chan JM. Antioxidant and vitamin E transport genes and risk of high-grade prostate cancer and prostate cancer recurrence. Prostate. 2013 Dec;73(16):1786-95. PMID: 24038157

Glass AS, Cowan JE, Fuldeore MJ, Cooperberg MR, Carroll PR, Kenfield SA, Greene KL. Patient Demographics, Quality of Life, and Disease Features of Men With Newly Diagnosed Prostate Cancer: Trends in the PSA Era. Urology. 2013 Jul;82(1):60-6. PMID: 23706257

Harris CR, Punnen S, Carroll PR. Men with low preoperative sexual function may benefit from nerve-sparing radical prostatectomy. J Urol. 2013 Sep;190(3):981-6. PMID: 23410984

Resnick MJ, Guzzo TJ, Cowan JE, Knight SJ, Carroll PR, Penson DF. Factors associated with satisfaction with prostate cancer care: results from Cancer of the Prostate Strategic Urologic Research Endeavor (CaPSURE). BJU Int. 2013;111(2):213-20. Epub 2012 Aug 29. PMID: 22928860

Sonn GA, Sadetsky N, Presti JC, Litwin MS. Differing perceptions of quality of life in patients with prostate cancer and their doctors. Urol. 2013 Jan;189(1 Suppl):S59-65. PMID: 23234635

2014

Brajtbord JS, Punnen S, Cowan JE, Welty CJ, Carroll PR. Age and baseline Quality of Life at Radical Prostatectomy: Who Has the Most to Lose? J Urol. 2014 Aug;192(2):396-401. PMID: 24582539

Broering JM, Paciorek A, Carroll PR, Wilson LS, Litwin MS, Miaskowski C. Measurement equivalence using a mixed-mode approach to administer health-related quality of life instruments. Qual Life Res. 2014 Mar;23(2):495-508. Epub Aug 13 2013. PMID: 23943258

Cary KC, Singla N, Cowan JE, Carroll PR, Cooperberg MR. Impact of androgen deprivation on mental and emotional well-being in men with prostate cancer: Analysis from the CaPSURE (Cancer of the Prostate Strategic Urologic Research Endeavor) registry. JUrol. 2014 Apr;191(4):964-70. Epub Oct 29 2013. PMID: 24184370 Reply to letter to the editor PMID: 25194545

Cary KC, Paciorek A, Fuldeore MJ, Carroll PR, Cooperberg MR. Temporal trends and predictors of salvage cancer treatment after failure following radical prostatectomy or radiation therapy: an analysis from the CaPSURE registry. Cancer. 2014 Feb 15;120(4):507-12. Epub 2013 Oct 25. PMID: 24496867

Magbanua MJ, Richman EL, Sosa EV, Jones LW, Simko J, Shinohara K, Haqq CM, Carroll PR, Chan JM. Physical activity and prostate gene expression in men with low-risk prostate cancer. Cancer Causes Control. 2014 Apr;25(4):515-23. PMID: 24504435

Morgan TM, Meng MV, Cooperberg MR, Cowan JE, Weinberg V, Carroll PR, Lin DW. A risk-adjusted definition of biochemical recurrence after radical prostatectomy. Prostate Cancer Prostatic Dis. 2014 Jun;17(2):174-9. PMID: 24614692

Tomaszewski JJ, Richman EL, Sadetsky N, O'Keefe DS, Carroll PR, Davies BJ, Chan JM. Impact of Folate Intake on Prostate Cancer Recurrence Following Definitive Therapy: Data from CaPSURE. J Urol. 2014 Apr;191(4):971-6. Epub Oct 3 2013. PMID: 24095905

Ten Ham RM, Wilson LS, Broering JM, Cooperberg MR, Carroll PR. Sustainable measurement of response shift in prostate cancer patients: adjusting health related quality of life with the Then-test. Value Health. 2014 Nov;17(7):A651. PMID: 27202352

Tseng YD, Paciorek AT, Martin NE, D'Amico AV, Cooperberg MR, Nguyen PL. Impact of national guidelines in brachytherapy monotherapy practice patterns for prostate cancer. Cancer. 2014 Mar 15;120(6):824-32. Epub Dec 2 2013. PMID: 24301555

Xia J, Trock BJ, Gulati R, Mallinger L, Cooperberg MR, Carroll PR, Carter HB, Etzioni R. Overdetection of recurrence after radical prostatectomy: estimates based on patient and tumor characteristics. Clin Cancer Res. 2014 Oct 15;20(20):5302-10. PMID: 25320374

2015

Cooperberg MR, Carroll PR. Trends in Management for Patients with Localized Prostate Cancer, 1990-2013. JAMA. 2015 Jul 7;314(1):80-2. PMID: 26151271 Reply to letter to the editor PMID: 26547474

Garcia-Albeniz X, Chan JM, Paciorek A, Logan RW, Kenfield SA, Cooperberg MR, Carrol PR, Hernan MA. Immediate versus deferred initiation of androgen deprivation therapy in prostate cancer patients with PSA-only relapse. An Observation follow-up study. Eur J Cancer. 2015 May;51(7):817-24. PMID: 25794605

Hampson LA, Cowan JE, Zhao S, Carroll PR, Cooperberg MR. Impact of age on quality-of-life outcomes after treatment for localized prostate cancer. Eur Urol. 2015 Sep;68(3):480-6. PMID: 25656807

Hsu CC, Paciorek AT, Cooperberg MR, Roach M 3rd, Hsu IC, Carroll PR. Post-operative radiation therapy for patients at high-risk of recurrence after radical prostatectomy: does timing matter? BJU Int. 2015 Nov;116(5):713-20. PMID: 25600860

Hussein AA, Punnen S, Zhao S, Cowan JE, Leapman M, Tran TC, Washington SL, Truesdale MD, Carroll PR, Cooperberg MR. Current use of imaging after primary treatment of prostate cancer. J Urol. 2015 Jul;194(1):98-104. PMID: 25640648

Jalloh M, Myers F, Cowan JE, Carroll PR, Cooperberg MR. Racial variation in prostate cancer upgrading and upstaging among men with low-risk clinical characteristics. Eur Urol. 2015 Mar;67(3):451-7. Epub 2014 Apr 5. PMID: 24746973

Punnen S, Cowan JE, Chan JM, Carroll PR, Cooperberg MR. Long-term health-related quality of life after primary treatment for localized prostate cancer: Results from the CaPSURE Registry. Eur Urol. 2015 Oct;68(4):600-8. Epub 2014 Sep 18. PMID: 25242555

2016

Cooperberg MR, Hinotsu S, Namiki M, Carroll PR, Akaza H. Trans-Pacific variation in outcomes for men treated with primary androgen-deprivation therapy (ADT) for prostate cancer. BJU Int. 2016 Jan;117(1):102-9. Epub 2015 May 14. PMID: 25238114

2017

Leapman MS, Cowan JE, Simko J, Roberge G, Stohr BA, Carroll PR, Cooperberg MR. Application of a Prognostic Gleason Grade Grouping System to Assess Distant Prostate Cancer Outcomes. Eur Urol. 2017 May;71(5):750-759. Epub 2016 Dec 8. PMID: 27940155

Vertosick EA, Vickers AJ, Cowan JE, Broering JM, Carroll PR, Cooperberg MR. Interpreting patient-reported urinary and sexual function outcomes across multiple validated instruments. J Urol. 2017 Sep;198(3):671-677. PMID: 28342935

2018

Herlemann A, Cowan JE,Carroll PR, Cooperberg MR. Community-Based Outcomes of Open versus Robot-Assisted Radical Prostatectomy. Eur Urol. 2018 Feb;73(2):215-223. Epub 2017 May 9. PMID: 28499617

Tat D, Kenfield SA, Cowan JE, Broering JM, Carroll PR, Van Blarigan EL, Chan JM. Milk and other dairy foods in relation to prostate cancer recurrence: data from the Cancer of the Prostate Strategic Urologic Research Endeavor (CaPSURETM). The Prostate. 2018 Jan;78(1):32-39. Epub 2017 Nov 6. PMID: 29105845

2019

Balakrishnan AS, Zhao SJ, Cowan JE, Broering JM, Cooperberg MR, Carroll PR. Trends and Predictors of Adjuvant Therapy for Adverse Features Following Radical Prostatectomy: An Analysis from CaPSURE. Urology. 2019 Sep;131:157-165. PMID: 31150694

Jeong CW, Cowan JE, Broering JM, Ten Ham RMT, Wilson LS, Carroll PR, Cooperberg MR. Robust health utility assessment among long-term survivors of prostate cancer: Results from the

Cancer of the Prostate Strategic Urologic Research Endeavor Registry. Eur Urol. 2019 Jul 22. Epub ahead of print PMID: 31345635

*Langlais CS, Cowan JE, Neuhaus J, Kenfield SA, Van Blarigan EL, Broering JM, Cooperberg MR, Carroll P, Chan JM. Obesity at diagnosis and prostate cancer prognosis and recurrence risk following primary treatment by radical prostatectomy. Cancer Epidemiol Biomarkers Prev. 2019 Aug 28. Epub ahead of print PMID: 31462398

Schmidt B, Eapen R, Cowan J, Broering J, Greene K, Carroll R, Cooperberg M. Practice patterns of primary EBRT with and without ADT in prostate cancer treatment. Prostate Cancer Prostatic Dis. 2019 Mar;22(1):117-124. Epub 2018 Aug 31. PMID: 30171230

Tang J, Zhong L, Paoli C, Paciorek A, Carroll P, Wilson L. Longitudinal comparison of patient-level outcomes and costs across prostate cancer treatments with urinary problems. Am J Men's Health. 2019 Mar-Apr;13(2):1557988319835326. PMID: 30836832

Zuniga KB, Zhao S, Kenfield SA, Cedars B, Cowan JE, Van Blarigan EL, Broering JM, Carroll PR, Chan JM. Trends in complementary and alternative medicine use among patients with prostate cancer. J Urol. 2019 May 15. Epub ahead of print PMID: 31091175

ABSTRACTS OR PRESENTATIONS

UCSF Prostate Program Retreat, September 9, 2014 Drs. Chan and Cooperberg presented 20 min talk during the Multi-Investigator Grant Updates portion titled. "**Department of Defense: Predicting Prostate Cancer Progression at Time of Diagnosis**"

Prostate Cancer Foundation Annual Scientific Retreat, Oct. 24, 2014, Carlsbad, CA. Dr. Chan to present, "**Development, Validation, and Dissemination of an Integrated Risk Prediction Model and Decision Aid to Discern Aggressive versus Indolent Prostate Cancer**".

Western Section American Urological Association Annual Conference, Maui HI, October 26, 2014. "**Development, Validation, and Dissemination of an Integrated Risk Prediction Model and Decision Aid to Discern Aggressive versus Indolent Prostate Cancer**" Poster presentation, E. Van Blarigan on behalf of JM Chan.

Western Section American Urological Association Annual Conference, Maui HI, October 31, 2014. "**Risk assessment for upgrading and upstaging among prostate cancer patients with low-risk disease receiving prostatectomy**" Podium presentation, SA Kenfield.

DEVELOPMENT, VALIDATION, AND DISSEMINATION OF AN INTEGRATED RISK PREDICTION MODEL AND DECISION AID TO DISCERN AGGRESSIVE VERSUS INDOLENT PROSTATE CANCER. JM Chan[1] ScD, MR Cooperberg[1] MD MPH, SA Kenfield ScD, J Neuhaus PhD, J Simko MD PhD, EL Van Blarigan ScD, J Belkora PhD, J Witte PhD, L Dunn MD, I Tenggara, JM Broering PhD, JE Cowan, PR Carroll MPH MD, ([1]shared first author). Poster to be presented by: Van Blarigan EL 10/26/14, W. Section American Urologic Association, Maui, HI.

RISK ASSESSMENT FOR UPGRADING AND UPSTAGING AMONG PROSTATE CANCER PATIENTS WITH LOW-RISK DISEASE RECEIVING RADICAL PROSTATECTOMY. Stacey A. Kenfield ScD, Matthew R. Cooperberg MD MPH, John Neuhaus PhD, Janet E. Cowan, Erin L. Van Blarigan ScD, Jeanette M. Broering PhD, Peter R. Carroll MPH MD, June M. Chan ScD, San Francisco, CA. Podium presentation to be made by: SA Kenfield. 10/31/14, W. Section American Urologic Association, Maui, HI.

Pioneering Advances in Care and Patient Information MH Bridge, MR Cooperberg MD MPH, JM Chan ScD, J Belkora PhD, SA Kenfield ScD, EL Van Blarigan ScD, JM Broering PhD, JE Cowan, PR Carroll MPH MD (UCSF Prostate Cancer Retreat 2015)

Assessing Germline DNA Genotyping from Radical Prostatectomy Tissue: A Comparison of Seminal Vesicle and Urethra FFPE Tissue to Blood. Nima Emami, Erin Van Blarigan, Jeffry Simko, Matthew Cooperberg, Peter Carroll, June Chan, John Witte. (UCSF Prostate Cancer Retreat 2015)

THE HETEROGENEOUS GENOMIC LANDSCAPE OF LOW–RISK PROSTATE CANCER Matthew Cooperberg MD, MPH[1], Nicholas Erho[2], June Chan[3], Felix Feng[3], Janet Cowan[3], Jeffry Simko[3], Christine Buerki[2], Imelda Tenggara[3], Elai Davicioni[3] and Peter Carroll[3], [1]UCSF, San Francisco, CA; [2]GenomeDx; [3]UCSF (Presented in "Best of Abstracts" plenary at AUA 2017)

Risk Prediction for Upgrading/Upstaging among Men with Low-risk Prostate Cancer June M. Chan, ScD, John M. Neuhaus, PhD*, Stacey A. Kenfield, ScD, Erin L. Van Blarigan, ScD, Janet E. Cowan, MA*, Mark Bridge*, Imelda Tenggara*, Jenny M. Broering RN, MPH, PhD*, John S. Witte, PhD*, Jeffry Simko, MD, PhD*, Jeffrey K. Belkora, PhD*, Peter R. Carroll, MD, MPH[1], Matthew R. Cooperberg MD, MPH[1] ([1]shared last authors), San Francisco, CA) (Presented at Western Section AUA 2016, and at international sub-meeting hosted by Movember, at the AUA 2017)

Development and Pilot Testing of a Decision Support Intervention for Men with Prostate Cancer. Chan JM, Cooperberg MR, Neuhaus J, Bridge M, Stupar L, Weinberg T, Broering JM, Tenggara I, Lavaki E, Cowan JE, Rosenfeld S, Kenfield SA, Van Blarigan EL, Simko J, Witte J, Carroll PR*, Belkora J* (*denotes shared last authorship) (Presented at international sub-meeting hosted by Movember, at the AUA 2017; and GU ASCO 2018).

Integrating Genetic Information with Machine Learning to Predict Which Prostate Cancer Cases Should not be Immediately Treated. Cavazos T, Emami N, Cowan JE, Cooperberg MR, Chan JM, Carroll PR, Witte J. (Presented at American Society for Human Genetics, 2018).

Technology - For the execution of Aim 2, we developed an online decision support tool. This is currently still being used as part of the mutli-site clinical trial, and thus is not publicly available.

However, upon completion of the study and reporting, we will consider what may be updated and implemented from this website.

Databases – As alluded to above, this grant has supported UODB and CaPSURE databases which continue to provide research opportunities for many faculty, residents, fellows, students, and other trainees at the Dept. of Urology, UCSF.

Grants & New Projects – Several members of the research team have successfully competed for grants or programs since the inception of this award. These are briefly summarized below.

- Dr. Chan received a R01 from the NCI to conduct a RCT of aerobic exercise vs. usual care among men with localized prostate cancer going on active surveillance, to examine changes in cardiopulmonary fitness and prostate cancer biomarkers. (2013)
- Dr. Kenfield received a R21 from the NCI to conduct a RCT of aerobic exercise vs. strength training vs. usual care among men with metastatic prostate cancer, to assess feasibility, acceptability, and changes in risk profiles. (2015)
- Drs. Chan, Kenfield, and Van Blarigan were supported by Movember to execute the TrueNth (TN) Community of Wellness trial focused on helping men with prostate cancer adopt healthier lifestyle habits using high-tech solutions. Drs. Cooperberg and Carroll were supported by Movember to provide backend data from CaPSURE for the TN patient portal. (2016)
- Dr. Kenfield received a new R01 in Aug. 2016, entitled "Web-based Lifestyle Interventions after Prostate Cancer: Prognosis and Symptoms". Drs. Chan, Van Blarigan, Carroll, and Cooperberg are also on this grant.
- Dr. Cooperberg received a new R01 in Aug. 2016 "Improving prostate cancer outcome prediction through noninvasive exRNA assessment." Drs. Chan and Neuhaus are also on this grant.
- Dr. Van Blarigan received an independent K07 from the NIH in July 2016 for her project, "Diet after Colorectal Cancer: Observational Studies to Behavioral Interventions".
- Erin Van Blarigan ScD received intrumural funding from UCSF to conduct updated diet and exercise data collection within CaPSURE and examine additional risk factors, including potential interactions with genetics (partly funded by this award). 2016
- Rebecca Graff, ScD successfully applied for intramural/American Cancer Society funding to collect and analyze additional DNA samples from intermediate/high-risk CaPSURE participants, to complement those originally collected as part of this award, on low-risk men. 2019
- Dr. Chan received intramural funding from the UCSF Osher Center for Integrative Medicine to start a new digital cohort of diverse individuals with prostate, colorectal, or bladder cancer, as a "next generation" version of CaPSURE, with an emphasis on diet, exercise, integrative medicine, and sleep data collection. (2019)
- Dr. Chan received intramural funding from the Cancer League (foundation) to examine features of metabolic health (e.g., metabolic syndrome, diabetes), metabolomics and

prostate cancer aggressiveness, using UODB and the Urology biobank (both of which were partly supported by this award). (2019)

- Drs. Kenfield, Chan, and Van Blarigan received funding to participate in the NCI Speeding Research-tested Interventions (May 2019)
- Drs. Carroll and Chan are on a sub-contract to the University of WA, which recently received a NCI infrastructure award for the Prostate Cancer Active Surveillance Study (PASS); UCSF contributes data on prostate cancer patients to this study from UODB, which was partly funded by this award. Pending 2020

7. Participants & Other Collaborating Organizations

Below reflects personnel supported during 4/1/17 – 11/30/17. During our second and third NCE, no salaries were charged against this grant. Since our last report, there are not any new partner organizations.

Personnel	Role	Percent Effort
Carroll, Peter, R.	PI/PD	4%
Chan, June , M.	Co-PI	15%
Cooperberg, Matthew	Co-PI	4%
Witte, John	Co-Investigator	3%
Broering, Jeanette	Co-Investigator	15%
Tenggara Imelda	Project Director	15%
Stupar, Laura	CRC	19%
Lavaki, Emil	CRC	67%
Weinberg, Tia Haart	Lab Tech	46%

8. Special Reporting Requirements – Please see Appendix for final Quad Chart and publications to date.

8. APPENDICES:

- Quad Chart
- Filippou P, Welty CJ, Cowan JE, Perez N, Shinohara K, Carroll PR. Immediate Versus Delayed Radical Prostatectomy: Updated Outcomes Following Active Surveillance of Prostate Cancer. Eur Urol. 2015 Sep;68(3):458-63. PMID: 26138041
- Welty CJ, Cowan JE, Nguyen H, Shinohara K, Perez N, Greene KL, Chan JM, Meng MV, Simko JP, Cooperberg MR, Carroll PR. Extended follow-up and risk factors for disease reclassification from a large active surveillance cohort for localized prostate cancer. J Urol. 2015 Mar;193(3):807-11. Epub 2014 Sep 24. PMID: 25261803
- Knudsen BS, Kim HL, Erho N, Shin H, Alshalalfa M, Lam LL, Tenggara I, Chadwich K, Van Der Kwast T, Fleshner N, Davicioni E, Carroll PR, Cooperberg MR, Chan JM, Simko JP. Application of a Clinical Whole-Transcriptome Assay for Staging and Prognosis of Prostate Cancer Diagnosed in Needle Core Biopsy Specimens. J Mol Diagn. 2016 May;18(3):395-406. PMID: 26945428
- Emami NC, Leong L, Wan E, Van Blarigan EL, Cooperberg MR, Tenggara I, Carroll PR, Chan JM, Witte JS, Simko JP. Tissue Sources for Accurate Measurement of Germline DNA Genotypes in Prostate Cancer Patients Treated With Radical Prostatectomy. Prostate. 2017 Mar;77(4):425-434. doi: 10.1002/pros.23283. Epub 2016 Nov 30. https://www.ncbi.nlm.nih.gov/pubmed/?term=27900799
- Leapman MS, Westphalen AC, Ameli N, Lawrence HJ, Febbo PG, Cooperberg MR, Carroll PR. Association between a 17-gene prostate score and multi-parametric prostate MRI in men with low and intermediate risk prostate cancer (PCa). PLoS One. 2017 Oct 10;12(10). PMID: 29016610
- Cooperberg MR, Erho N, Chan JM, Feng FY, Fishbane N, Zhao SG, Simko JP, Cowan JE, Lehrer J, Alshalalfa M, Kolisnik T, Chelliserry J, Margrave J, Aranes, M, Plessis MD, Buerki C, Tenggara I, Davicioni E, Carroll PR. The diverse genomic landscape of clinically low-risk prostate cancer. Eur Urol. 2018 Oct;74(4):444-452. PMID: 29853306
- Belkora J, Chan JM, Chan JM, Cooperberg MR, Neuhaus J, Stupar L, Weinberg T, Broering JM, Tenggara I, Cowan JE, Rosenfeld S, Kenfield SA, Van Blarigan EL, Simko JP, Witte J, Carroll PR. Development and pilot evaluation of a personalized decision support intervention for low risk prostate cancer patients. Cancer Med. 2019 Nov 12. Epub ahead of print PMID: 31714037
- Cedars BE, Washington SL 3rd, Cowan JE, Leapman M, Tenggara I, Chan JM, Cooperberg MR, Carroll PR. Stability of a 17-gene genomic prostate score in serial testing on men on active surveillance for early stage prostate cancer. J Urol. 2019 Apr 8. Epub ahead of print PMID: 30958742
- Kornberg Z, Cooperberg MR, Cowan JE, Chan JM, Shinohara K, Simko JP, Tenggara I, Carroll PR. A 17-Gene Genomic Prostate Score as a Predictor of Adverse Pathology for Men on Active Surveillance. J Urol. 2019 Apr 26. Epub ahead of print PMID: 31026214
- Langlais CS, Cowan JE, Neuhaus J, Kenfield SA, Van Blarigan EL, Broering JM, Cooperberg MR, Carroll P, Chan JM. Obesity at diagnosis and prostate cancer prognosis and

recurrence risk following primary treatment by radical prostatectomy. Cancer Epidemiol Biomarkers Prev. 2019 Aug 28. Epub ahead of print PMID: 31462398

Development, Validation, and Dissemination of an Integrated Risk Prediction Model and Decision Aid to Discern Aggressive Versus Indolent Prostate Cancer
Award number W81XWH-13-2-0074 Log #PC121236

PI: Drs. PR Carroll, JM Chan, & MR Cooperberg **Org:** UCSF **Award Amount:** $9.45 M **Updated:** December 21, 2019

Study Objective:

Our goal is to address both incomplete information and poor understanding among men diagnosed with low-risk prostate cancer. We propose that through development of an integrated risk prediction model, and implementation of a decision support intervention to help patients understand their disease risk and treatment options, we will reduce anxiety and uncertainty, improve decision quality and satisfaction, and increase acceptance of initial active surveillance for low-risk prostate cancer.

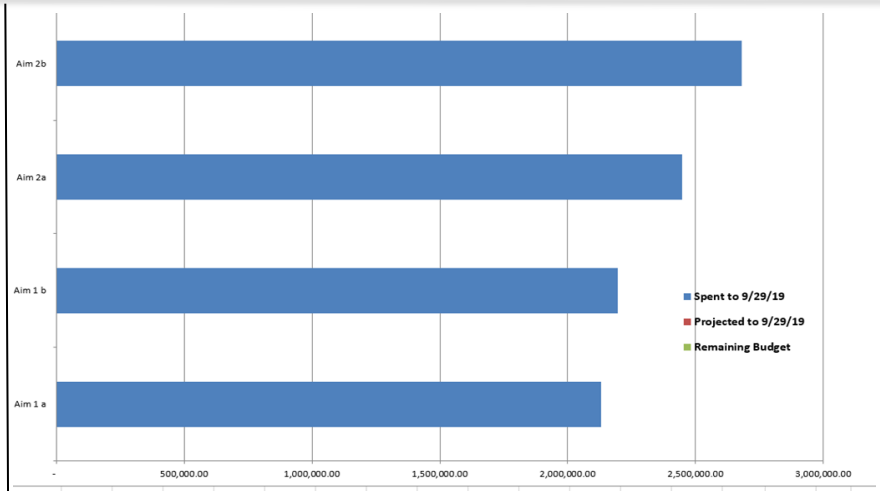
Study Aims

- Major Aim 1a: Develop integrated risk prediction model
- Major Aim 1b: Validate integrated risk prediction model
- Major Aim 2a: Implement and test decision support intervention
- Major Aim 2b: Conduct randomized trial for decision making

Study Approach

For Aim 1, we are conducting two retrospective cohort studies (one among UCSF participants for development; another in CaPSURE participants for validation) to build and test the integrated risk prediction model.

For Aim 2, we are conducting three phases of pilot studies and then a community-based randomized clinical trial (RCT) to test the impact of the novel risk prediction model + decision support intervention on patient management choices and self-reported outcomes.



	Spent to 9/29/19	Projected to 9/29/19	Remaining Budget
Aim 1 a	2,129,865.34	-	-
Aim 1 b	2,196,757.77	-	-
Aim 2a	2,448,529.57	-	-
Aim 2b	2,680,729.32	-	-
	9,455,882.00	-	-

Overall Goals/Milestones

Accomplishments– Completed retrospective cohort study in UCSF cohort to develop novel risk prediction model (Aim 1a); Completed validation analysis in CaPSURE (Aim 1b); Completed "base model" risk prediction pilot study in UCSF clinic (Aim 2a); Enrolled 112 of 116 participants for multi-site decision support clinical trial in 5 sites (Aim 2b)

- ☑ Completed protocols, obtained approvals for 2 protocols for Aim 1 & 3 for Aim 2
- ☑ Built novel website+coaching decision aid, with patient input, now testing in community-based multi-site RCT (Aim 2b)
- ☑ Analyzed >1000 DNA specimens for Aim 1; QC and analyses ongoing.
- ☑ Completed procurement and RNA assessment of tumor specimens for Aim 1
- ☑ Finalized risk prediction model (Aim 1) and deployed novel model in Decision Aid RCT
- ☑ Pilot Decision Support Trial, published in Cancer Medicine 2019
- ☑ 97% complete enrollment for Aim 2b trial
- ☑ Budget is fully spent as of 9/29/19. Project progress/update and accomplishments are referenced in the final progress report along with the publications in appendices.

Pioneering Advances in Care & Education (PACE)

PACE@ucsf.edu

The multi-site national RCT is open at five sites (including UCSF), and evaluating our Decision Aid intervention (Aim 2b). Below are the logos for our 4 community partners.

TABLE 2 Patient performance on first two knowledge items before and after intervention

	After Intervention	
Before Intervention	**At least 1 incorrect**	**Both correct**
At least one incorrect	7	7
Both correct	1	29

Table 2 from Belkora et al, Cancer Medicine 2019, depicting the increase in knowledge regarding early-stage prostate cancer associated with the decision-support intervention.

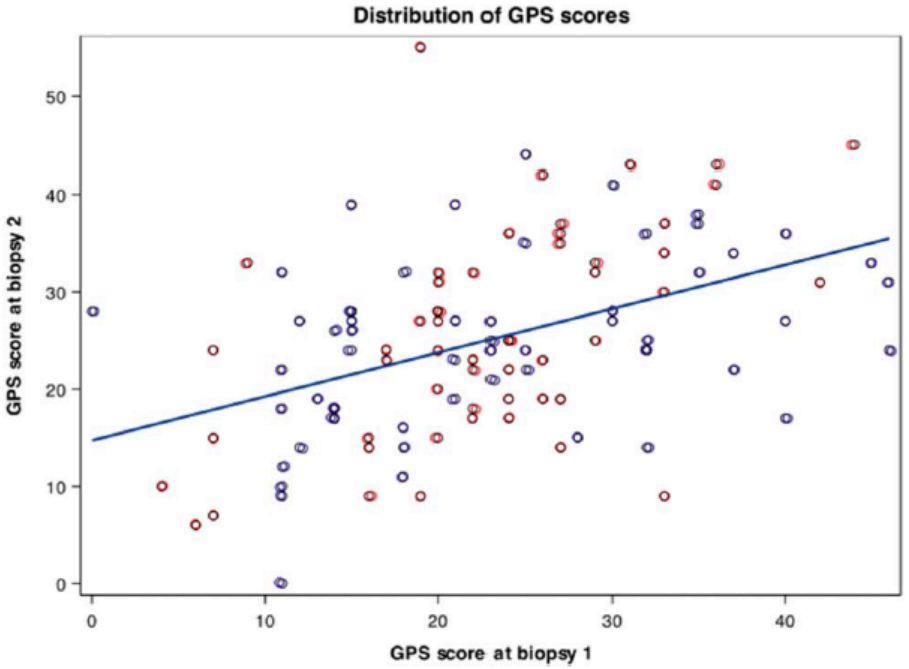


Figure 2. GPS distribution at first (blue) and second (red) biopsies (Pearson correlation coefficient 0.45) in 111 men enrolled on AS of prostate cancer at UCSF.

From Cedars et al, J. of Urol 2019

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Prostate Cancer

Immediate Versus Delayed Radical Prostatectomy: Updated Outcomes Following Active Surveillance of Prostate Cancer

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Abstract

Background: Biopsy progression on active surveillance (AS) for prostate cancer (PCa) often reflects failure of the initial biopsy to detect cancer present at enrollment. The risks for delayed treatment among men who progress on AS are not well defined.
Objective: To report outcomes for men who underwent surgery after AS compared to men who underwent immediate surgery and the influence of selection bias on this outcome.
Design, setting, and participants: AS-eligible (ASE) men who underwent radical prostatectomy (RP) after a median of 20 mo of AS were compared to ASE men who underwent RP within 6 mo of diagnosis. A subset of men on AS who underwent RP after upgrade to Gleason 3 + 4 was compared to matched controls with similar pretreatment biopsy features who underwent immediate RP.
Outcome measurement and statistical analysis: Rates of adverse pathology (upstaging, positive surgical margin, or Gleason upgrading) were examined. Logistic regression was used to determine associations between treatment subgroup and adverse pathology.
Results and limitations: Of 157 ASE men who underwent delayed RP after AS, 54 were upgraded to Gleason 3 + 4 before surgery. ASE men who underwent immediate RP had lower probability of adverse pathology than ASE men who underwent delayed RP (hazard ratio [HR] 0.34, 95% confidence interval [CI] 0.21–0.55). The rate of adverse pathology did not differ between immediate and delayed RP patients matched for pretreatment characteristics (HR 0.79, 95% CI 0.27–2.28). The observational design of this study is its main limitation.
Conclusions: When compared to men with similar pretreatment biopsy features, those who underwent delayed RP were not at higher risk of adverse pathology.
Patient summary: The oncologic safety of delayed treatment when indicated for men enrolled in active surveillance for prostate cancer is important. We found that men who underwent delayed surgery had similar outcomes to men who underwent immediate prostatectomy.

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1. Introduction

The widespread availability of prostate-specific antigen (PSA) testing has led to an increase in the detection of low risk prostate cancer (PCa) over the last two decades [1]. Active surveillance (AS), a management option for low-risk PCa that involves careful observation and treatment of men who appear to have more aggressive disease

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0302-2838/

during follow-up, is currently a recommended treatment option for men with low-risk PCa in Europe and the USA [2,3]. However, widespread uptake of AS has been slow. Reported community usage rates range from 10% to 50% for men with PCa with very low clinical risk [4,5]. This may be because of both patient and provider fear of unrecognized aggressive disease that will progress if not treated immediately. The grade and extent of cancer are probably undersampled in a substantial proportion of men on surveillance, as 20–40% of men will have a higher Gleason score (GS) or cancer volume after enrolling in AS [6–9]. Whether a window for cure is missed during time spent on AS is controversial. Most prior reports have compared the surgical outcomes for patients who underwent radical prostatectomy (RP) after a period of AS (AS + RP) to those who met AS criteria and underwent immediate RP (IRP). This study design leads to a selection bias favoring the IRP group since the goal of AS is to avoid treatment until evidence of progression warrants intervention, and therefore patients with higher risk are selected for surgery. This was well demonstrated in a recent study using this design in which patients who underwent AS + RP had a higher risk of adverse pathologic features when compared to those who met AS criteria and underwent IRP [10].

Here we report updated outcomes for patients who underwent AS + RP in a large, prospectively followed AS cohort and compare them to two separate IRP cohorts. First, we compared those who underwent AS + RP to those who met AS criteria and underwent IRP. Second, to address the issue of selection bias among those undergoing AS + RP, we conducted a matched pair analysis of men who underwent AS + RP and men who underwent IRP, matched for their pretreatment biopsy features.

2. Patients and methods

2.1. Patient population

Men who underwent RP for PCa in the University of California, San Francisco (UCSF) Department of Urology between 1990 and May 2014 formed the study cohort. All patients consented to prospective data collection under supervision by the institutional review board. Patients who underwent surgery within 6 mo of diagnosis (first positive biopsy) made up the IRP group. The delayed RP group comprised those who enrolled in AS and subsequently underwent RP $\geq$6 mo after diagnosis. Strict criteria for AS eligibility were PSA $\leq$10 ng/ml, clinical stage T1 or T2 cancer, biopsy GS 2–6, biopsy cores $\leq$33% positive, and single core $\leq$50% positive.

2.2. Independent variables

Demographic data (age, race/ethnicity, relationship status), diagnostic PSA, prostate volume, PSA density, clinical T stage, GS, number of cores taken and percentage positive at diagnostic biopsy, Cancer of the Prostate Risk Assessment (CAPRA) clinical risk score (0–10), and surgical CAPRA-S (0–12) were collected [11,12]. Validated risk groups for both clinical CAPRA and CAPRA-S scores are low (0–2), intermediate (3–5), and high ($\geq$6).

2.3. Outcomes

The primary outcome was any adverse feature on surgical pathology, defined as Gleason upgrade to primary pattern 4 or 5 since last biopsy, extraprostatic extension (EPE), seminal vesicle invasion (SVI), presence of positive lymph nodes, or positive surgical margin (pSM). The secondary outcome was recurrence-free survival after RP. Recurrence events were biochemical failure, defined as two consecutive PSA increases $\geq$0.2 ng/ml or additional treatment at least 6 mo after RP.

2.4. Selection of controls

Two separate approaches were used to evaluate the immediate and delayed RP groups to ensure accurate findings. The first cohort was selected to evaluate patients with similar clinical characteristics at diagnosis. We updated the previous UCSF study by Dall'Era et al [13] with a larger cohort, comparing groups of men meeting strict eligibility criteria for AS who underwent immediate versus delayed RP (the ASE (active surveillance eligible) analysis) [13]. We compared a second set of men with similar characteristics right before surgery. A sub-group of surgical patients was matched on diagnostic year, age, PSA, and clinical T-stage ("matched pairs"). Matching techniques typically are used to pair cases with controls based on outcome. Instead, we used pair-matching to ensure that the exposure groups were as similar as possible. We then sought to eliminate progression bias between the treatment groups by matching men in the IRP group, who presented with biopsy GS 3 + 4 and underwent RP within 6 months, to the delayed RP group, who were diagnosed with Gleason 3 + 3, progressed to Gleason 3 + 4 upon repeat biopsy, and then underwent RP within 6 months of UG (the Matched analysis). Three IRP patients were matched to one delayed RP patient using the Greedy method [14].

2.5. Statistical analysis

Patient characteristics were compared between RP groups using t-test for continuous variables and Pearson chi-square for categorical variables. Logistic regression was used to evaluate associations between RP group and the outcome of any adverse pathology, adjusting for age, race, relationship status, clinical CAPRA score at diagnosis, PSA at diagnosis, PSAD (log) at diagnosis, and percentage of positive cores at last biopsy before surgery. Conditional logistic regression based on pair ID was used for the analysis of matched pairs, adjusting only for non-matching variables (race, relationship status, prostate volume, and percentage of positive cores at last biopsy before surgery.) Rates of recurrence-free survival were computed as Kaplan-Meier probabilities and compared by RP group with the log-rank test. Model covariates were assessed for inter-item correlations. A p-value $<$0.05 was considered significant. All analyses were performed using SAS 9.2 for Windows (SAS Institute, Cary, NC).

3. Results

Of 3372 men treated with RP during the study period, 241 men underwent delayed RP and 3131 underwent IRP. The cohort for the ASE analysis comprised 678 who met AS eligibility criteria, 521 (77%) from the IRP group and 157 (23%) from the delayed RP group (Fig. 1). Among the 157 men who initially met the AS criteria and underwent delayed RP, the median time on AS before surgery was 20 mo (range 6–148). The mean age at diagnosis was 60.6 yr (standard deviation 6.9) and median PSA at diagnosis was 4.9 ng/ml (interquartile range [IQR] 4.0–6.1). The median number of biopsy cores taken at diagnosis was 14 (IQR 12–16), and a median 13% of those cores contained cancer (IQR 8–21%). A median of two surveillance biopsies (range 1–10) were performed before RP. The median time from the last surveillance biopsy to RP was 4 mo (IQR 3–15) and the

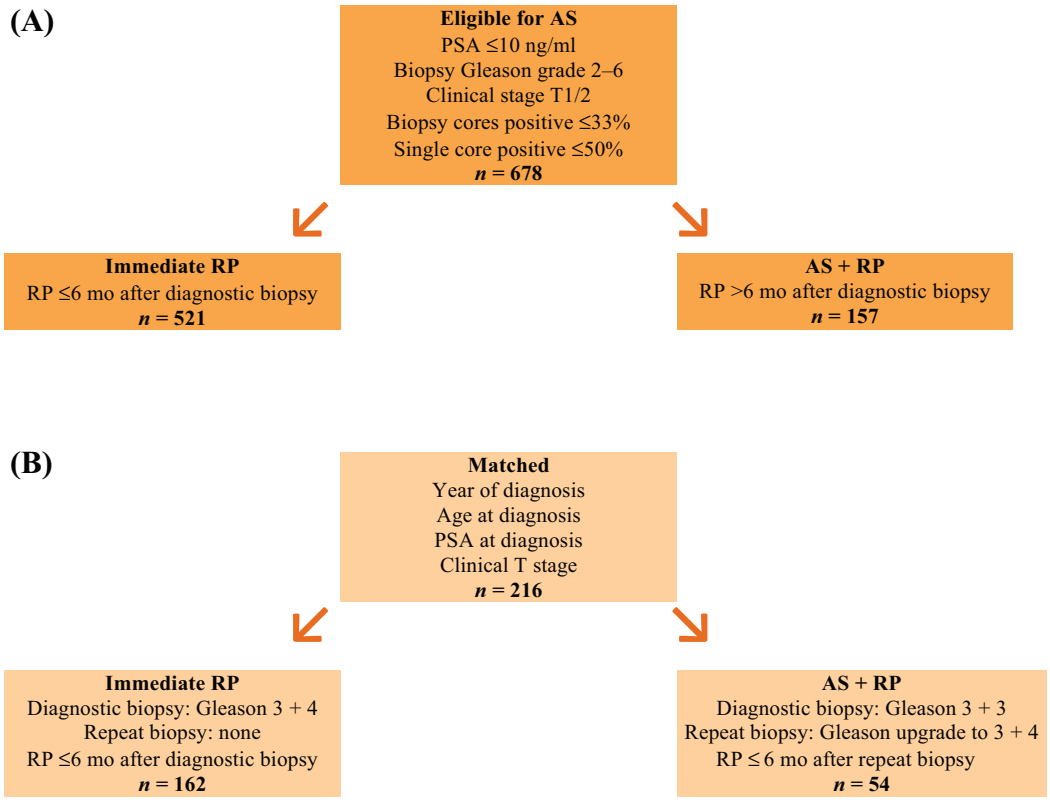

Fig. 1 – Description of cohorts for analysis of immediate versus delayed radical prostatectomy at University of California, San Francisco (UCSF). (A) Patient group for first analysis: men who met clinical criteria for very low risk for enrollment in active surveillance (AS) and underwent radical prostatectomy (RP) at UCSF. (B) Patient group for second analysis: men matched for presentation characteristics with Gleason grade 3 + 4 at last biopsy who underwent RP at UCSF. PSA = prostate-specific antigen.

median follow-up after surgery was 40 mo (range 7–166). Patient characteristics are shown in Table 1.

In the ASE cohort, adverse pathologic features were present in 118 (23%) men after IRP compared to 69 (44%) men after delayed RP ($p < 0.01$; Table 1). Of the 157 men in the delayed RP group, 121 (77%) experienced a GS upgrade or an increase in cancer volume on biopsy before treatment. Men in the delayed RP group had higher rates of upgrade to GS $\geq 4 + 3$ (12% vs 5%, $p < 0.01$), EPE (25% vs 11%, $p < 0.01$) and pSM (21% vs 11%, $p < 0.01$) than men in the IRP group. One patient in the delayed RP group had a positive lymph node. After RP, 65% of the delayed RP group had a low-risk CAPRA-S score compared to 86% of IRP patients ($p < 0.01$; Table 1). On multivariate analysis adjusted for demographic and clinical characteristics, the IRP group had lower odds for adverse pathology compared to the delayed RP group (odds ratio [OR] 0.34, 95% confidence interval [CI] 0.21–0.55; $p < 0.01$). Age (OR 1.06, 95% CI 1.02–1.09; $p < 0.01$) and log PSA density (OR 2.44, 95% CI 1.52–3.91; $p < 0.01$) also were significantly associated with the risk of adverse pathologic features. (Table 3). Survival free of biochemical recurrence or additional treatment at 3 yr was 93% in the AS + RP group and 96% in the IRP group ($p < 0.01$).

The matched analysis included 216 men matched for diagnostic year, age, PSA, and clinical stage; 162 (75%) underwent IRP and 54 (25%) underwent AS + RP. In contrast to the ASE analysis, unadjusted rates of adverse pathologic features did not differ between the immediate and delayed RP groups. Overall, 25 patients (46%) in the delayed RP group had at least one adverse pathologic feature, compared to 71 patients (44%) in the IRP group ($p = 0.75$; Table 2). Similarly, delayed RP was not associated with adverse pathology on multivariate conditional logistic regression in the matched analysis (OR 0.75, 95% CI 0.35–1.59, IRP vs delayed RP). The rate of recurrence-free survival at 3 yr was 88% after IRP and 100% after delayed RP ($p = 0.56$).

4. Discussion

This study describes the clinical and pathologic outcomes for men who underwent RP from a large prospective AS cohort. It expands on an earlier report on 33 men who underwent delayed RP that, possibly because of the small sample size, did not show an association between treatment timing and adverse surgical pathology among men eligible for AS [13]. The current study, with 241 patients who underwent AS + RP after a median of 22 mo from diagnosis, is one of the largest experiences of RP after AS reported to date.

One of the primary goals of AS is to spare treatment for men with indolent disease and delay treatment for men with clinically significant disease until it is necessary. An increase in adverse pathology at the time of delayed RP, as found in the ASE analysis, would be expected if the

Table 1 – Demographic, disease, and pathologic data for 678 men who met strict criteria for active surveillance at diagnosis and underwent immediate or delayed radical prostatectomy [a]

Patient characteristic	Immediate RP (n = 521)	Delayed RP (n = 157)	p value
At diagnosis			
Mean age, yr (SD)	58.8 (6.8)	60.6 (6.9)	<0.01
Median PSA, ng/ml (IQR)	5.4 (4.3–6.6)	4.9 (4.0–6.1)	0.02
Median PSA density (IQR)	0.15 (0.10–0.20)	0.14 (0.10–0.20)	0.92
Median biopsy cores sampled (IQR)	12 (9–16)	14 (12–16)	<0.01
Mean positive biopsy cores (SD)	2.1 (1.3)	2.0 (1.2)	0.22
Clinical T2 stage, n (%)	231 (44)	54 (34)	0.03
Low CAPRA clinical risk (0–2), n (%)	462 (100)	157 (100)	–
Median time to RP, mo (range)	3 (0–6)	20 (6–148)	<0.01
At radical prostatectomy			
CAPRA-S surgical risk, n (%)			
Low (0–2)	443 (86)	101 (65)	<0.01
Intermediate (3–5)	66 (13)	48 (31)	
High (≥6)	4 (0.8)	6 (4)	
Any adverse pathology, n (%)	118 (23)	69 (44)	<0.01
Major upgrade, n (%) [b]	28 (5)	19 (12)	<0.01
Extraprostatic extension, n (%)	58 (11)	39 (25)	<0.01
Seminal vesicle invasion, n (%)	8 (2)	7 (4)	0.03
Positive margins, n (%)	58 (11)	33 (21)	<0.01
Positive lymph nodes, n (%)	0 (0)	1 (0.6)	–
Median overall follow-up, mo (IQR)	33 (10–67)	40 (25–62)	0.66

RP = radical prostatectomy; PSA = prostate specific antigen; SD = standard deviation; IQR = interquartile range; CAPRA = Cancer of the Prostate Risk Assessment score.
[a] Numbers may not sum to the entire cohort because of missing data.
[b] Defined as an increase in Gleason score at surgery and the presence of primary pattern 4 or 5.

surveillance regimen and criteria used for intervention preferentially identified men with aggressive disease. For most of these men, a higher volume or higher GS cancer was probably present at the time of diagnosis. Multiple previous studies have documented a 20–30% risk of upgrading from GS 3 + 3 on biopsy to GS 3 + 4 or higher at the time of surgery among men with low-risk disease [15–17]. The question becomes not whether men with clinically indolent PCa progress while under surveillance, but whether a window for cure is missed for men with understaged or undergraded disease who are watched on AS.

This question was addressed by matching men who underwent delayed RP after biopsy reclassification to patients who underwent IRP on the basis of pretreatment

Table 2 – Clinical and surgical pathology for men reclassified to Gleason 3 + 4 disease while on active surveillance compared to matched controls diagnosed with 3 + 4 disease who underwent immediate radical prostatectomy (matched for age, PSA, cT stage, and year of diagnosis) [a]

Patient characteristic	Matched RP		p value
	Immediate (n = 162)	Delayed (n = 54)	
At diagnosis			
Median PSA density (IQR)	0.17 (0.13–0.21)	0.17 (0.11–0.27)	0.18
Biopsy Gleason grade, n (%)			
2–6	0 (0)	54 (100)	
7 (3 + 4)	162 (100)	0 (0)	
Median time to RP, mo (range)	3 (0–6)	19 (6–129)	<0.01
At radical prostatectomy			
CAPRA-S surgical risk, n (%)			
Low (0–2)	110 (68)	33 (61)	0.52
Intermediate (3–5)	44 (27)	19 (35)	
High (≥6)	8 (5)	2 (4)	
Any adverse pathology, n (%)	71 (44)	25 (46)	0.75
Major upgrade, n (%) [b]	24 (15)	7 (13)	0.74
Extraprostatic extension, n (%)	39 (24)	11 (20)	0.58
Seminal vesicle invasion, n (%)	7 (4)	4 (7)	0.37
Positive margins, n (%)	34 (21)	11 (20)	0.92
Positive lymph nodes, n (%)	1 (0.6)	0 (0)	–
Median overall follow-up, mo (IQR)	21.5 (11–43)	34 (24–52)	0.01

RP = radical prostatectomy; PSA = prostate specific antigen; IQR = interquartile range; CAPRA = Cancer of the Prostate Risk Assessment score.
[a] Numbers may not sum to the entire cohort because of missing data.
[b] Defined as an increase in Gleason score at surgery and the presence of primary pattern 4 or 5.

Table 3 – Logistic regression for odds of adverse pathology (stage ≥pT3, Gleason upgrade ≥ 4 + 3, seminal vesicle invasion, extracapsular extension) among men who met active surveillance eligibility criteria and men included in matched pair analysis who underwent radical prostatectomy after diagnosis of Gleason 3 + 4 disease

Effect	Univariate analysis		Multivariate analysis [a]	
	p value	OR (95% CI)	*p* value	OR (95% CI)
AS cohort (n = 678)				
Immediate versus delayed RP	<0.01	0.37 (0.26–0.54)	<0.01	0.34 (0.21–0.55)
Age at diagnosis (yr)			<0.01	1.06 (1.02–1.09)
Clinical CAPRA score (0–10)			0.50	1.14 (0.61–2.12)
PSA at diagnosis (ng/ml)			0.69	0.94 (0.78–1.15)
PSA density at diagnosis (log)			<0.01	2.44 (1.52–3.91)
Percentage pretreatment biopsy cores positive			0.48	1.00 (0.99–1.01)
Matched pair cohort (n = 216)				
Immediate vs. delayed RP	0.75	0.91 (0.49–1.68)	0.66	0.79 (0.27–2.28)
Age at diagnosis (yr)			0.48	1.02 (0.97–1.06)
Clinical CAPRA score (0–10)			0.87	1.06 (0.55–2.03)
PSA at diagnosis (ng/ml)			0.91	1.01 (0.83–1.24)
PSA density at diagnosis (log)			0.07	1.76 (0.96–3.23)

AS = active surveillance; OR = odds ratio; CI = confidence interval; RP = radical prostatectomy; PSA = prostate specific antigen; CAPRA = Cancer of the Prostate Risk Assessment.

[a] Adjusted for race and marital status in addition to the variables listed.

characteristics, the matched analysis in this study. The presence of adverse pathologic features after surgery did not differ between men initially diagnosed with GS 3 + 4 disease and treated and men reclassified to GS 3 + 4 on follow-up biopsy after a period of AS and then treated. This suggests that a window for cure was probably not missed during the time it took to identify the presence of higher-risk disease. In addition, with limited long-term follow-up after RP, there was no difference in the rate of biochemical recurrence.

One recently published, albeit smaller, study took a similar approach to assessing the potential risk associated with delayed treatment. Satkunasivam and colleagues [10] compared surgical outcomes between 41 men with low-risk disease who underwent AS + RP after a median of 35.2 mo and 112 men with low-risk disease who underwent IRP (group 1), and between 24 men who underwent RP after upgrading to GS 7 on AS and 70 men with GS 7 disease who underwent IRP (group 2). Similar to the present study, they found higher rates of adverse pathology for AS + RP in the group 1 comparison, but not in the group 2 comparison. The present study expands on these findings by including nearly four times the number of men in the ASE analysis and more than twice as many men in the matched analysis [10]. In addition, PSA density was associated with adverse pathologic outcome for patients in both groups. This association between PSA density and adverse pathology in this select group of ASE patients is consistent with prior analyses of our larger cohort [18,19].

Other studies have focused on matching patients by disease features at diagnosis, or included only historical controls. Warlick et al [20] found that the risk of noncurable cancer(<75% chance of remaining disease-free for 10 yr after RP according to GS, PSA, and disease organ confinement) was not higher in 38 patients who underwent RP after a period on AS compared to immediate surgery. In a retrospective cohort of 69 men with median AS of 29 mo, van den Bergh et al [16] similarly found no difference in the frequency of adverse pathologic characteristics between immediate and delayed RP groups. The lack of difference in adverse pathologic characteristics among patients matched on diagnostic features in these studies was probably a result of fewer patients in the AS + RP group and the control group selected. Abern et al [17] retrospectively analyzed a cohort of men from the SEARCH database who underwent delayed surgery, and found no difference in pathologic outcome among low-risk men with delayed treatment. However, many of these patients were probably not followed in a formal AS protocol and had other reasons for delaying surgery, limiting the relevance of this study to AS patients.

Identification of patients with higher risk disease while on AS often comes at the cost of more frequent surveillance biopsy, which may deter men from enrolling in AS. Several new modalities are available for better evaluation of the risk of occult higher-grade disease, including additional imaging techniques and genomic profiling [21]. These innovative solutions may be able to improve selection of patients for AS, reducing the risk of upgrading during AS. The evaluation of outcomes for patients who undergo treatment following AS, as was done in this study, continues to be of utmost importance.

As a retrospective analysis of an AS cohort from a single institution, there are limitations to this study. First, despite matching patients on the basis of available pretreatment biopsy data, potential for residual selection bias remains. Until a randomized trial with extended follow-up is available, results from prospective cohorts remain important. Second, many diagnostic biopsies were not performed at our institution. While the biopsy pathology was reviewed at our institution, different sampling patterns used by third-party providers may have affected the risk of undersampling. The median number of cores taken at diagnosis was 14, suggesting that sampling was adequate for most biopsies. The follow-up time between immediate and delayed RP groups varied, as evidenced by wide IQRs. Finally, additional

follow-up of this and other cohorts is needed to assess long-term clinical outcomes after delayed RP.

5. Conclusions

In our prospectively accrued, retrospectively analyzed AS cohort, men selected for surgery had a higher rate of adverse pathology than those who met strict AS criteria and underwent immediate prostatectomy. This is probably because of selection of men with higher-risk disease for treatment during follow-up. When compared to men with similar pretreatment biopsy features, those who underwent delayed RP were not at higher risk of adverse pathology, indicating a window for cure was probably not missed.

Author contributions: Peter R. Carroll had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Carroll, Welty, Cowan.
Acquisition of data: Filippou, Welty, Cowan, Perez, Shinohara, Carroll.
Analysis and interpretation of data: Filippou, Welty, Cowan.
Drafting of the manuscript: Filippou, Welty, Cowan.
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References

[1] Cooperberg MR, Broering JM, Kantoff PW, Carroll PR. Contemporary trends in low risk prostate cancer: risk assessment and treatment. J Urol 2007;178:S14–9.
[2] National Comprehensive Cancer Network. Prostate cancer (version 2.2014). www.nccn.org/professionals/physician_gls/pdf/prostate.pdf
[3] Heidenreich A, Bastian PJ, Bellmunt J, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis and local treatment with curative intent. Eur Urol 2014;65:124–37.
[4] Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. J Clin Oncol 2010;28:1117–23.
[5] Womble PR, Montie JE, Ye Z, Linsell SM, Lane BR, Miller DC. Contemporary use of initial active surveillance among men in Michigan with low-risk prostate cancer. Eur Urol 2015;67:44–50.
[6] Klotz L, Vesprini D, Sethukavalan P, et al. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol 2015;33:272–7.
[7] Tosoian JJ, Trock BJ, Landis P, et al. Active surveillance program for prostate cancer: an update of the Johns Hopkins experience. J Clin Oncol 2011;29:2185–90.
[8] Bul M, van den Bergh RC, Zhu X, et al. Outcomes of initially expectantly managed patients with low or intermediate risk screen-detected localized prostate cancer. BJU Int 2012;110:1672–7.
[9] Cary KC, Cowan JE, Sanford M, et al. Predictors of pathologic progression on biopsy among men on active surveillance for localized prostate cancer: the value of the patent of surveillance biopsies. Eur Urol 2014;66:337–42.
[10] Satkunasivam R, Kulkarni GS, Zlotta AR, et al. Pathological, oncologic and functional outcomes of men progressing from active surveillance to radical prostatectomy. J Urol 2013;190:91–5.
[11] Cooperberg MR, Pasta DJ, Elkin EP, et al. The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. J Urol 2005;173:1938–42.
[12] Cooperberg MR, Hilton JF, Carroll PR. The CAPRA-S score: a straightforward tool for improved prediction of outcomes after radical prostatectomy. Cancer 2011;117:5039–46.
[13] Dall'Era MA, Cowan JE, Simko J, et al. Surgical management after active surveillance for low risk prostate cancer: pathological outcomes compared with men undergoing immediate treatment. BJU Int 2010;10:1232–7.
[14] Bergstralh EJ, Kosanke JL. gmatch: computerized matching of cases to controls using the greedy matching algorithm with a fixed number of controls per case. Rochester, MN: Mayo Clinic; 2003. www.mayo.edu/research/departments-divisions/department-health-sciences-research/division-biomedical-statistics-informatics/software/locally-written-sas-macros
[15] Tosoian JJ, JohnBull E, Trock BJ, et al. Pathological outcomes in men with low risk and very low risk prostate cancer: implications on the practice of active surveillance. J Urol 2013;190:1218–22.
[16] van den Bergh RC, Steyerberg EW, Khatami A, et al. Is delayed radical prostatectomy in men with low-risk screen-detected prostate cancer associated with a higher risk of unfavorable outcomes? Cancer 2010;116:1281–90.
[17] Abern MR, Aronson WJ, Terris MK, et al. Delayed radical prostatectomy for intermediate-risk prostate cancer is associated with biochemical recurrence: possible implications for active surveillance from the SEARCH database. Prostate 2013;73:409–17.
[18] Cary KC, Cowan JE, Sanford M, et al. Predictors of pathologic progression on biopsy among men on active surveillance for localized prostate cancer: the value of the pattern of surveillance biopsies. Eur Urol 2014;66:337–42.
[19] Welty CJ, Cowan JE, Nguyen H, et al. Extended follow-up and risk factors for disease reclassification from a large active surveillance cohort for localized prostate cancer. J Urol 2015;193:807–11.
[20] Warlick C, Trock BJ, Landis P, Epstein JI, Carter HB. Delayed versus immediate surgical intervention and prostate cancer outcomes. J Natl Cancer Inst 2006;98:355–7.
[21] van den Bergh RC, Albertsen PC, Bangma CH, et al. Timing of curative treatment for prostate cancer: a systematic review. Eur Urol 2013;64:204–15.

Extended Followup and Risk Factors for Disease Reclassification in a Large Active Surveillance Cohort for Localized Prostate Cancer

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Purpose: Active surveillance to manage prostate cancer provides an alternative to immediate treatment in men with low risk prostate cancer. We report updated outcomes from a long-standing active surveillance cohort and factors associated with reclassification.

Materials and Methods: We retrospectively reviewed data on all men enrolled in the active surveillance cohort at our institution with at least 6 months of followup between 1990 and 2013. Surveillance consisted of quarterly prostate specific antigen testing, repeat imaging with transrectal ultrasound at provider discretion and periodic repeat prostate biopsies. Factors associated with repeat biopsy reclassification and local treatment were determined by multivariate Cox proportional hazards regression. We also analyzed the association of prostate specific antigen density and outcomes stratified by prostate size.

Results: A total of 810 men who consented to participate in the research cohort were followed on active surveillance for a median of 60 months. Of these men 556 (69%) met strict criteria for active surveillance. Five-year overall survival was 98%, treatment-free survival was 60% and biopsy reclassification-free survival was 40%. There were no prostate cancer related deaths. On multivariate analysis prostate specific antigen density was positively associated with the risk of biopsy reclassification and treatment while the number of biopsies and time between biopsies were inversely associated with the 2 outcomes (each $p < 0.01$). When stratified by prostate volume, prostate specific antigen density remained significantly associated with biopsy reclassification for all strata but prostate specific antigen density was only significantly associated with treatment in men with a smaller prostate.

Conclusions: Significant prostate cancer related morbidity and mortality remained rare at intermediate followup. Prostate specific antigen density was independently associated with biopsy reclassification and treatment while on active surveillance.

Key Words: prostatic neoplasms, prostate-specific antigen, disease progression, outcome assessment, biopsy

Abbreviations and Acronyms

5-ari = 5α-reductase inhibitor
ADT = androgen deprivation therapy
AS = active surveillance
BxD = biopsy density
BxR = biopsy reclassification
CAPRA = Cancer of the Prostate Risk Assessment
PCa = prostate cancer
PSA = prostate specific antigen
PSAD = PSA density
RP = radical prostatectomy

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Prostate cancer is the second leading cause of cancer death in men in the United States.[1] However, in the PSA era many newly diagnosed cases are low risk and potentially indolent.[2,3] AS is a management strategy

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involving PCa monitoring while delaying or avoiding definitive treatment.[4] Many published studies have demonstrated the short-term safety of AS but more data are needed to determine the intermediate and long-term safety of AS.[5–7]

More than 40% of men diagnosed with PCa in the United States are considered to have low risk disease.[8] However, about a third of the men with apparently low risk cancer are reclassified into a higher risk category upon followup biopsy.[9–12] The ability to identify men with low risk PCa who are likely to be reclassified would clearly be beneficial. Men with higher risk disease could be treated while the disease was still curable while those with truly indolent disease could be spared additional followup testing, risk and anxiety.

At several institutions, including ours, groups have reported risk factors for reclassification during AS, including initial biopsy characteristics, PSA velocity, PSAD, repeat biopsy results and other factors.[13–19] PSAD at diagnosis is one of the few metrics associated with the risk of disease reclassification and adverse pathological features in many of these studies. However, the relationship between PSAD and risk may vary across PCa risk levels and prostate volumes.[20,21] How to use PSAD when advising men on AS is still unclear.

The AS study at our institution has been accruing patients since 1990. We report outcomes from one of the longest running AS cohorts in North America. We assessed PSAD and a novel metric, BxD (defined as the number of total biopsy cores divided by prostate volume) as potential predictors of outcome during AS. We also evaluated the performance of PSAD as a predictor of outcome in men who did not meet our strict criteria for AS and across a wide range of prostate volumes.

METHODS

At the Department of Urology at our institution a study of AS for PCa began in 1990. Patients who consent to prospective data collection under internal review board supervision and who undergo no active treatment for at least 6 months after the first diagnostic biopsy are included in analysis. Eligibility criteria and monitoring protocol have evolved with time. Currently strict AS criteria at our institution are diagnostic PSA 10 ng/ml or less, clinical stage T1/2, biopsy Gleason grade 3 + 3 or less, 33% or less positive cores and 50% or less tumor in any single core. Carefully selected men who do not meet strict eligibility criteria may be enrolled. Recommended monitoring includes quarterly PSA testing, semiannual transrectal ultrasound and annual biopsy. The first surveillance (ie confirmatory) biopsy is recommended within 12 months of diagnostic biopsy. Subsequent surveillance biopsies are recommended every 12 to 24 months based on clinical risk. Surveillance biopsy sessions at our institution include at least 12 cores with sampling from each sextant (medial and lateral) and the anterior gland. The primary trigger for treatment has been biopsy reclassification. Additional indications for discussion of treatment were patient anxiety, CAPRA risk reclassification and change in clinical stage. PSA kinetics alone did not serve as an indication for treatment.

We retrospectively reviewed clinical data on men enrolled in the AS study from 1990 to 2013, evaluating the entire cohort as well as subgroups that met strict eligibility criteria or underwent multiple biopsies. We described independent demographics (age, race/ethnicity, relationship status and smoking status) and clinical characteristics (5-ari use, diagnostic T stage, biopsy Gleason grade and volume, PSA and prostate volume). Clinical risk at diagnosis was calculated using CAPRA on a scale of 0 to 10 and classified using validated CAPRA groups, including low—0 to 2, intermediate—3 to 5 and high risk—6 to 10.[22] PSAD at diagnosis was calculated as PSA at diagnosis divided by prostate volume in cc as measured on confirmatory transrectal ultrasound. BxD was calculated as the total number of biopsy cores taken divided by prostate volume. Outcomes were time to BxR and time to active treatment. BxR was defined as an increase in Gleason grade of 3 + 4 or greater, more than 33% positive cores or more than 50% of positive tissue in a single core. Time to Gleason grade reclassification in men with Gleason 3 + 3 cancer was included as a separate outcome. Men in whom disease at diagnosis exceeded these parameters were not included in BxR analysis. Active treatment included RP, radiotherapy or ADT that began more than 6 months after enrollment in AS.

Cohort demographic and clinical characteristics were described with frequency tables. The Pearson chi-square test was used for categorical variables, and the mean and ANOVA were used for continuous variables. Life tables, Kaplan-Meier curves and log rank test were applied for univariate time to event analysis of the outcomes. PSAD and other factors associated with outcomes served as independent variables and were assessed by multivariate Cox regression adjusted for demographic and clinical characteristics. Smoking was included as a predictor of interest due to prior research indicating an association of smoking history with poor PCa outcomes.[23] PSAD was analyzed in 3 ways (as a continuous variable, as a log-transformed variable to normalize the distribution of values and as a categorical variable for ease of interpretation). Models were used to assess the entire cohort and the subset that met strict low risk criteria. Model covariates were evaluated for interitem correlations. To assess the potential interaction between PSAD and prostate volume analysis was stratified by prostate size, including small—less than 30, medium—30 to 45 and large—greater than 45 cc based on the cohort distribution of values. Two-tailed $p < 0.05$ was considered statistically significant. All analysis was done with SAS® 9.2.

RESULTS

A total of 1,075 men were enrolled in the AS cohort at our institution from 1990 to 2013, of whom

810 with at least 6 months of followup consented to research. Of these men 556 (69%) met strict criteria for AS and 685 have undergone repeat biopsy. Those with repeat biopsy were similar to the cohort as a whole (supplementary table, http://jurology.com/). Mean ± SD age at diagnosis was 62.0 ± 7.9 years, 87% of patients were white, 76% were married/partnered and 80% had never smoked. Median PSA was 5.3 ng/ml (IQR 4.1–7.4) and median PSAD was 0.13 ng/ml/cc (IQR 0.09–0.19). At initial biopsy 738 men (92%) had a Gleason score of 6 or less, 716 (92%) had 33% or less of cores involved and 616 (90%) had 50% or less of any individual core involved.

At a median followup of 60 months (IQR 36–91, maximum 19 years) there were no deaths due to PCa. Metastatic disease developed in 1 patient (0.12%). Five-year overall survival was 98%, treatment-free survival was 60% and BxR-free survival was 40%. Median time to treatment was 25 months (IQR 15–45) and median time to reclassification was 17 months (IQR 10–33). The treatment rate was 60% in men who did and did not meet strict AS clinical criteria. Of the 348 treated men 240 (69%) underwent RP, 98 (28%) received some form of radiotherapy and 10 (3%) received ADT. PSA recurrence-free survival was 97% 1 year after RP.

In the multivariate model adjusted for clinical risk and sociodemographics a decreasing interval between biopsy and PSAD were positively associated with the risks of treatment and BxR. Age was associated with the risk of BxR but not with the risk of treatment. PSA at diagnosis and BxD were not associated with the risk of BxR or of treatment (see table).

Increasing logPSAD was associated with the risk of treatment (HR 1.59, 95% CI 1.24–2.03) and the risk of BxR (HR 1.90, 95% CI 1.55–2.33, see table). Patients with a PSAD of 0.1 to 0.15 ng/ml/cc were more likely to be treated (HR 1.75, 95% CI 1.20–2.56) and reclassified (HR 1.67, 95% CI 1.23–2.26) than those with PSAD less than 0.1 ng/ml/cc. Associations were stronger in men with PSAD greater than 0.15 ng/ml/cc (treatment and BxR HR 2.15, 95% CI 1.46–3.16 and 2.14, 95% CI 1.56–2.94, respectively, see table). Factors associated with Gleason grade reclassification alone did not meaningfully differ from those associated with BxR as a whole.

The interaction of prostate size and PSAD was explored by stratified analysis across small (less than 30 cc), intermediate (30 to 45 cc) and large (greater than 45 cc) prostates. Among men with a small prostate logPSAD was significantly associated with treatment and BxR (HR 1.52, 95% CI 1.03–2.24 and 1.92, 95% CI 1.41–2.62, respectively). In men with a medium or large prostate logPSAD remained associated with BxR but it was not significantly associated with treatment (see table).

Multivariate Cox proportional hazards regression of categorical and continuous PSAD, and outcomes of active treatment and BxR in men on AS at our institution

	Active Treatment*		BxR†	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Categorical PSAD				
Age at diagnosis	1.00 (0.98–1.01)	—	1.02 (1.00–1.03)	<0.05
Race (white)	1.15 (0.74–1.76)	—	1.06 (0.74–1.50)	—
Unmarried/widowed	0.96 (0.71–1.30)	—	0.96 (0.74–1.25)	—
Smoking history	0.68 (0.48–0.97)	<0.05	1.03 (0.78–1.36)	—
5-ari Use	0.50 (0.28–0.87)	<0.05	0.90 (0.60–1.33)	—
Met strict AS clinical risk criteria	0.95 (0.71–1.28)	—	0.93 (0.71–1.21)	—
Total No. biopsies	0.44 (0.39–0.50)	<0.01	0.47 (0.42–0.54)	<0.01
PSA at diagnosis (ng/ml)	0.99 (0.95–1.03)	—	0.99 (0.96–1.02)	—
BxD	0.97 (0.62–1.52)	—	1.05 (0.66–1.66)	—
Mos between biopsies	0.94 (0.92–0.95)	<0.01	0.93 (0.92–0.94)	<0.01
Biopsy reclassification	6.31 (4.30–9.25)	<0.01	—	—
PSAD (ng/ml/cc):				
0.1–0.15 vs less than 0.1	1.75 (1.20–2.56)	<0.01	1.67 (1.23–2.26)	<0.01
Greater than 0.15 vs less than 0.1	2.15 (1.46–3.16)	<0.01	2.14 (1.56–2.94)	<0.01
Continuous PSAD (logPSAD)				
All pts	1.59 (1.24–2.03)	<0.01	1.90 (1.55–2.33)	<0.01
Prostate vol only (cc):				<0.01
Less than 30	1.52 (1.03–2.24)	<0.05	1.92 (1.41–2.62)	
30–45	1.26 (0.70–2.29)	—	2.01 (1.32–3.05)	
Greater than 45	1.65 (0.86–3.19)	—	2.21 (1.29–3.77)	

* Also adjusted for diagnosis year, diagnosis age, race (white), married/partnered, prior smoking history, meeting strict AS clinical risk criteria, total number of biopsies, PSA at diagnosis, biopsy density and biopsy reclassification.
† Also adjusted for diagnosis year, diagnosis age, race (white), married/partnered, prior smoking history, meeting strict AS clinical risk criteria, total number of biopsies, PSA at diagnosis and biopsy density.

DISCUSSION

The short-term safety of AS has been demonstrated in multiple cohorts with only rare occurrences of PCa related death or metastasis reported.[10,11,19,24] Fewer cohorts reportedly have a median followup of beyond 5 years.[5–7] Our results extend the median followup previously reported in this cohort from 3.6 to 5 years and include more than 200 men with followup beyond 7.5 years. During this extended followup PCa metastasis and hormone therapy remained rare events. This is notable since this cohort included 125 men who did not meet strict criteria for AS inclusion and 67 with a diagnostic Gleason score of greater than 6.

The inclusion of men who did not meet very low risk entrance criteria is similar to inclusions in the University of Toronto cohort.[5] However, in contrast to the current cohort, there were 5 PCa related deaths in the University of Toronto cohort at a median followup of 6.8 years. More recently 15 PCa related deaths were reported at a median followup

of 8.3 years and an additional 12 patients survived with metastasis.[25] European studies of cohorts with intermediate followup describe death and metastasis in men who received AS or deferred treatment, including 2 deaths among 471 patients at the Royal Marsden Hospital[6] and 1 death among 439 in the Göteborg cohort.[7] Although the goal of AS is to identify men with less aggressive disease and treat them before PCa dissemination, it is possible that with additional followup some patients in the current cohort may have metastatic disease and die of PCa. Indeed, in a recent modeling study Xia et al estimated that men with very low risk PCa were at 2.8% risk for death compared to 1.6% in those treated immediately.[26]

As the acceptance and use of AS increase, an important question is whether the outcomes observed in current academic cohorts apply to the population at large. A key difference between our cohort and others is that men could be enrolled before repeat biopsy at our institution. This approach may better reflect the experience of men seen outside academic AS cohorts since men in the community are biopsied by many providers using various techniques. Lack of rebiopsy before inclusion may in part explain the higher observed rates of treatment and BxR compared to those of other cohorts. Notably even when including men before repeat biopsy and men who did not meet strict AS entry requirements, ADT, metastasis and PCa death remained extremely rare events.

Efforts are ongoing to improve risk assessment in men diagnosed with low and intermediate risk PCa. While tools such as magnetic resonance imaging and genetic tumor profiling hold promise, they require further validation before they can be widely incorporated into AS management protocols.[27] Even when these tools are available, they must be interpreted in the context of other well established predictors of risk. The current study expands the association of PSAD with disease reclassification and treatment by including men at higher risk and examining associations across multiple prostate sizes. The finding that PSAD remained a strong predictor of BxR in men who did not meet strict AS criteria could be helpful when counseling such patients who are still considering AS.

In addition, PSAD may have value even in men with PSAD less than 0.15 ng/ml/cc since that group was at higher risk for BxR than those with PSAD less than 0.1 ng/ml/cc. This is consistent with the findings of Tseng et al, who observed that of patients with PSAD less than 0.15 ng/ml/cc those with PSAD greater than 0.08 ng/ml/cc were at twofold increased risk of reclassification.[14] Lastly, PSAD was associated with the risk of active treatment independent of BxR and other clinical factors.

We postulated that PSAD may perform differently at the extremes of prostate size for several reasons. PSA production by benign prostate tissue varies. It is possible that the amount of incremental PSA produced by benign prostate glands in enlarged prostates is not linearly related to prostate size and PSAD becomes less sensitive as prostate size increases.[28] In addition, while absolute PSA tends to increase with increasing tumor volume, larger tumors may make less PSA per cc tumor volume than smaller tumors.[20] However, in our cohort PSAD was associated with BxR for all 3 strata of prostate size, indicating that PSAD is useful across a range of prostate sizes.

It is also possible that biopsy may not be as effective at detecting clinically significant disease in larger prostates.[29] In this study we used the metric BxD to assess the impact of the number of biopsy cores relative to prostate size on AS outcomes and we found no association. BxD may be associated with longer term outcomes that we cannot assess without further followup.

We included the use of 5-aris on multivariate analysis to control for the effect of these medications on prostate size and PSA. While 5-ari was associated with treatment in our cohort, this may have been for reasons other than clinical progression since 5-ari use was not associated with BxR. Not enough men in this cohort were receiving 5-ari to separately assess the performance of PSAD in these men.

Other caveats should be noted. While the current cohort is one of the longest standing AS cohorts reported, accrual has increased with time. The median recruitment year was 2006 and median followup has been 5 years. Treatment of patients on AS has evolved with time, which could have affected the results of this analysis. For example, men enrolled later in the cohort were biopsied more frequently than those enrolled earlier, increasing the chances of BxR and treatment. Notably BxR and subsequent active treatment are anticipated to occur during AS. Longer term clinical outcomes such as PCa metastasis and death would be preferable measures of the oncologic efficacy of AS.[30] However, we used the surrogate outcomes of BxR and treatment due to the rarity of metastasis and the absence of PCa related deaths. Also, since a PSA threshold of less than 10 ng/ml has been used to advise men on the safety of AS regardless of prostate size, the PSAD range in men with a larger prostate was smaller. This may limit the generalizability of our results to men with a large prostate. In addition, as with other AS cohorts, ours is an observational cohort and can only be compared to men who undergo immediate treatment using historical external comparison groups.

CONCLUSIONS

The incidence of significant PCa related events remained low in a cohort with followup beyond 5 years. Additional followup is needed to assess long-term outcomes. Independent of absolute PSA, increased PSAD is a strong marker of future BxR and active treatment. It should be considered along with Gleason score, tumor volume and other disease characteristics when counseling a man on AS.

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REFERENCES

1. Cancer Facts & Figures 2014. Atlanta: American Cancer Society 2014; pp 1—72.
2. Cooperberg MR, Lubeck DP, Meng MV et al: The changing face of low-risk prostate cancer: trends in clinical presentation and primary management. J Clin Oncol 2004; **22:** 2141.
3. Albertsen PC, Hanley JA and Fine J: 20-year outcomes following conservative management of clinically localized prostate cancer. JAMA 2005; **293:** 2095.
4. Venderbos LDF, Bokhorst LP, Bangma CH et al: Active surveillance: oncologic outcome. Curr Opin Urol 2013; **23:** 268.
5. Klotz L: Active surveillance: the Canadian experience. In: Active Surveillance for Localized Prostate Cancer. Totowa: Humana Press 2012; pp 95—105.
6. Selvadurai ED, Singhera M, Thomas K et al: Medium-term outcomes of active surveillance for localised prostate cancer. Eur Urol 2013; **64:** 981.
7. Godtman RA, Holmberg E, Khatami A et al: Outcome following active surveillance of men with screen-detected prostate cancer. Results from the Göteborg randomised population-based prostate cancer screening trial. Eur Urol 2013; **63:** 101.
8. Cooperberg MR, Cooperberg MR, Broering JM et al: Contemporary trends in low risk prostate cancer: risk assessment and treatment. J Urol, suppl., 2007; **178:** S14.
9. Klotz L: Active surveillance for prostate cancer: for whom? J Clin Oncol 2005; **23:** 8165.
10. Dall'Era MA, Cooperberg MR, Chan JM et al: Active surveillance for early-stage prostate cancer: review of the current literature. Cancer 2008; **112:** 1650.
11. Bul M, Zhu X, Valdagni R et al: Active surveillance for low-risk prostate cancer worldwide: the PRIAS study. Eur Urol 2013; **63:** 597.
12. Kryvenko ON, Carter HB, Trock BJ et al: Biopsy criteria for determining appropriateness for active surveillance in the modern era. Urology 2014; **83:** 869.
13. Lam A, Loblaw A, Nam R et al: Comparing prostate specific antigen triggers for intervention in men with stable prostate cancer on active surveillance. J Urol 2010; **184:** 1942.
14. Tseng KS, Landis P, Epstein JI et al: Risk stratification of men choosing surveillance for low risk prostate cancer. J Urol 2010; **183:** 1779.
15. Ross AE, Loeb S, Landis P et al: Prostate-specific antigen kinetics during follow-up are an unreliable trigger for intervention in a prostate cancer surveillance program. J Clin Oncol 2010; **28:** 2810.
16. Margel D, Nandy I, Wilson TH et al: Predictors of pathological progression among men with localized prostate cancer undergoing active surveillance: a sub-analysis of the REDEEM study. J Urol 2013; **190:** 2039.
17. Cary KC, Cowan JE, Sanford M et al: Predictors of pathologic progression on biopsy among men on active surveillance for localized prostate cancer: the value of the pattern of surveillance biopsies. Eur Urol 2013; **66:** 337.
18. Umbehr MH, Platz EA, Peskoe SB et al: Serum prostate-specific antigen (PSA) concentration is positively associated with rate of disease reclassification on subsequent active surveillance prostate biopsy in men with low PSA density. BJU Int 2014; **113:** 561.
19. Barayan GA, Brimo F, Bégin LR et al: Factors influencing disease progression of prostate cancer under active surveillance: a McGill University Health Center cohort. BJU Int 2014; **114:** E99.
20. Corcoran NM, Casey RG, Hong MKH et al: The ability of prostate-specific antigen (PSA) density to predict an upgrade in Gleason score between initial prostate biopsy and prostatectomy diminishes with increasing tumour grade due to reduced PSA secretion per unit tumour volume. BJU Int 2012; **110:** 36.
21. Vellekoop A, Loeb S, Folkvaljon Y et al: Population based study of predictors of adverse pathology among candidates for active surveillance with Gleason 6 prostate cancer. J Urol 2014; **191:** 350.
22. Cooperberg MR, Pasta DJ, Elkin EP et al: The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. J Urol 2005; **173:** 1938.
23. Gong Z, Agalliu I, Lin DW et al: Cigarette smoking and prostate cancer-specific mortality following diagnosis in middle-aged men. Cancer Causes Control 2008; **19:** 25.
24. Eggener SE, Mueller A, Berglund RK et al: A Multi-institutional evaluation of active surveillance for low risk prostate cancer. J Urol, suppl., 2013; **189:** S19.
25. Klotz L, Vesprini D and Loblaw A: Long term follow-up of a large active surveillance cohort. J Urol, suppl., 2014; **191:** e411, abstract PD14-03.
26. Xia J, Trock BJ, Cooperberg MR et al: Prostate cancer mortality following active surveillance versus immediate radical prostatectomy. Clin Cancer Res 2012; **18:** 5471.
27. van den Bergh RCN, Ahmed HU, Bangma CH et al: Novel tools to improve patient selection and monitoring on active surveillance for low-risk prostate cancer: a systematic review. Eur Urol 2014; **65:** 1023.
28. Partin AW, Carter HB, Chan DW et al: Prostate specific antigen in the staging of localized prostate cancer: influence of tumor differentiation, tumor volume and benign hyperplasia. J Urol 1990; **143:** 747.
29. Cohen YC, Liu KS, Heyden NL et al: Detection bias due to the effect of finasteride on prostate volume: a modeling approach for analysis of the Prostate Cancer Prevention Trial. J Natl Cancer Inst 2007; **99:** 1366.
30. Welty CJ, Cooperberg MR and Carroll PR: Meaningful end points and outcomes in men on active surveillance for early-stage prostate cancer. Curr Opin Urol 2014; **24:** 288.

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Application of a Clinical Whole-Transcriptome Assay for Staging and Prognosis of Prostate Cancer Diagnosed in Needle Core Biopsy Specimens

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Molecular and genomic analysis of microscopic quantities of tumor from formalin-fixed, paraffin-embedded biopsy specimens has many unique challenges. Herein, we evaluated the feasibility of obtaining transcriptome-wide RNA expression to measure prognostic classifiers in diagnostic prostate needle core biopsy specimens. One-hundred fifty-eight samples from diagnostic needle core biopsy specimens (BX) and radical prostatectomies (RPs) were collected from 33 patients at three hospitals; each patient provided up to six tumor and benign samples. Genome-wide transcriptomic profiles were generated using Affymetrix Human Exon arrays for comparison of gene expression alterations and prognostic signatures between the BX and RP samples. A sufficient amount of RNA (>100 ng) was obtained from all RP specimens ($n = 77$) and from 72 of 81 of BX specimens. Of transcriptomic features detected in RP, 95% were detectable in BX tissues and demonstrated a high correlation ($r = 0.96$). Likewise, an expression signature pattern validated on RPs (Decipher prognostic test) showed correlation between BX and RP ($r = 0.70$). Of matched BX and RP pairs, 25% showed discordant molecular subtypes. Genome-wide exon arrays yielded data of comparable quality from biopsy and RP tissues. The high concordance of tumor-associated gene expression changes between BX and RP samples provides evidence for the adequate performance of the assay platform with samples from prostate needle biopsy specimens with limited tumor volume. *(J Mol Diagn 2016, 18: 395—406; http://dx.doi.org/10.1016/j.jmoldx.2015.12.006)*

Prostate cancer is the second leading cause of cancer-related mortality in US men.[1] The American Cancer Society estimates that >220,000 new cases of prostate cancer will be recorded in 2015, accounting for >25% of all cancers in men.[2] Prostatic needle core biopsy is currently the most reliable standard for diagnosis of prostate cancer, and it is estimated that >800,000 patients undergo prostate biopsy annually in the United States.[3] However, in addition to the recognized concerns about tumor heterogeneity and sampling errors associated with biopsy, the pathological findings and tumor grade do not always accurately predict tumor behavior and patient outcome. In addition, tumor grading has poor interobserver reproducibility

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in some cases, which can lead to uncertainty in grade assignment and subsequent misclassification of disease severity.[4] Therefore, developing more sensitive and accurate biomarkers and prognostic tools is of critical clinical need to better risk-stratify patients when cancer is first diagnosed at biopsy, and will allow patients to make the most informed treatment management decisions possible.

Before molecular tests can be accepted into standard clinical practice, there is a need to demonstrate their analytical feasibility and clinical utility. When using formalin-fixed, paraffin-embedded (FFPE) tissue specimens as starting material, the FFPE processing causes degradation of RNA that generates challenges in using expression patterns as a clinical biomarker; the oxygen and hydroxyl radicals in formalin crosslink RNA, and the high temperatures of the wax involved in embedding the sample cause irreversible damage to RNA, with fragmentation into 150 to 200 bases long oligonucleotides.[5] Run-to-run variations in processing parameters, as well as processing and storage variations from one institution to the next, can also affect RNA levels and degradation rates in FFPE tissue specimens. Tumor heterogeneity and the limited amount of tumor in biopsy material further affect expression analyses, introducing multiple, sometimes discordant, expression signatures. In addition, the multifocal and heterogeneous nature of prostate cancers poses even more challenges.[6–8] More studies that use biopsy tumor samples and address the opportunities for biomarker and molecular signature evaluation studies are needed to improve patient management from the time of diagnosis.

Herein, we demonstrate the feasibility of using transcriptome-wide oligonucleotide microarray technology [Human Exon 1.0 ST GeneChips (Affymetrix Inc., Santa Clara, CA) with 1.4 million probe selection regions (PSRs)] optimized for use with RNA extracted from FFPE tissue specimens, and this protocol is performed in a Clinical Laboratories Improvement Amendment–certified reference laboratory, allowing it to be used to generate tumor expression data for clinical use in prognostic assays.[9] For example, the Decipher test score—performed on prostate tumor tissue—is designed to predict metastatic prostate cancer risk after radical prostatectomy (RP), and is based on the expression of 22 markers from the 1.4 million PSRs on the chip. These markers relate to cell proliferation, differentiation, androgen signaling, motility, and immune modulation[10,11] and have been validated to predict metastatic progression after RP in several independent cohorts from multiple institutions.[12–14] This genomic assay is currently covered by Medicare for helping to guide postoperative therapy decision making in patients with adverse pathological features.[15] Use of this expression array protocol also allows for evaluation of various combinations of PSRs, and thus permits one to simultaneously assess other expression marker panels and data sets,[10,16,17] as well as evaluate new ones.

To explore the transcriptomic differences between prostate biopsy and matched RP samples, and evaluate the effects of heterogeneity, we compared transcriptomic data generated using Human Exon arrays obtained from 158 different prostate tissue samples from 33 patients seen at three different institutions. This cohort provides, for the first time, an opportunity to compare the whole transcriptome array-based expression profiles obtained from matched biopsy and RP specimens from multiple institutional sources. It is particularly important as different procurement, processing, and sampling conditions are represented in this cohort for a more thorough evaluation of expression-based genomic classifiers such as Decipher test.[18–20] Finally, we use the data to explore tumor heterogeneity and the assignments of recently described molecular subtypes of prostate cancers by comparing and contrasting expression patterns in this specimen cohort.[20,21]

Materials and Methods

Patients and Samples

A total of 158 FFPE samples from 33 patients with matching biopsy and RP were collected from three institutions: University of California San Francisco (UCSF; $n = 13$), Cedars Sinai Medical Center (CSMC; Los Angeles, CA; $n = 11$), and the University Health Network (Toronto, ON, Canada; $n = 9$) (Supplemental Figure S1). Each institution's institutional review board committees gave approval of this study. These 158 samples comprised 64 tumor samples (33 from biopsy and 31 from RP), 47 benign adjacent to tumor tissue samples (24 from biopsy and 23 from RP), and 47 benign contralateral tissue samples (24 from biopsy and 23 from RP) (Table 1). For 23 patients from UCSF and CSMC, six prostate tissue samples were obtained from each patient: tumor biopsy, tumor RP, benign adjacent biopsy, benign adjacent RP, benign contralateral biopsy, and benign contralateral RP (Table 1). Tumor grade, tumor content, and stromal content were assigned by expert uropathologists (J.P.S., B.S.K., T.v.d.K.) on hematoxylin and eosin review for each biopsy core and RP tumor section used, with the area to be sampled for RNA extraction marked on each slide (Table 1). Except for cases analyzed before 2006 (two cases), all were categorized as robotic prostatectomies, which have similar warm and cold ischemia times. These cases were all fixed within 1 hour of resection by formalin injection technique. Biopsy specimens are all fixed immediately on removal of the specimen.

Tissue Selection and Sampling

At CSMC and UCSF, tumor tissues were sampled from FFPE biopsy cores by dissecting a portion of the tumor tissue directly from the blocks in the areas corresponding to marked areas on each hematoxylin and eosin–stained slide using either a 1-mm sterile biopsy punch tool (UCSF) or 0.6-mm cylindrical full-thickness cores using the Tissue microarrayer (Pathology Devices, Westminster, MD). Next, RNA was extracted as described below. Either one to two 0.6-mm punches from the center of the tumor (CSMC)

Table 1 Clinicopathologic Variables and QC Characteristics of Samples and Tissue Types

Variables/characteristics	Biopsy			Radical prostatectomy			Total
	Contralateral	Adjacent	Tumor	Contralateral	Adjacent	Tumor	
Sample storage age, mean (SD), years	3.29 (2.69)			3.06 (2.69)			
Available tissue	24	24	33	23	23	31	158
Clinicopathological							
Tumor content, %							
Median	0	0	70	0	0	80	
Range	0–0	0–0	10–90	0–0	0–0	50–90	
Stromal content, %							
Median	60	60	25	60	60	20	
Range	40–80	40–80	10–80	10–80	40–80	5–50	
Gleason score							
6	0	0	13	0	0	10	23
7	0	0	14	0	0	16	30
8	0	0	4	0	0	4	8
9	0	0	2	0	0	2	4
Quality control							
Successful RNA extraction	23	22	27	23	23	31	149
Failed RNA extraction	1	2	6	0	0	0	
Median RNA yield, ng	463.28	283.74	278.25	2814.3	2319.04	2846.7	
Successful cDNA amplification	23	22	27	22	23	31	148
Failed cDNA extraction	0	0	0	1	0	0	
Median cDNA yield, ng	7474.68	6984.275	7066.44	6704.88	7011.2	6461.91	
Array good QC	23	22	26	22	23	31	147
Median % present	54.34	51.16	48.65	46.91	51.03	49.44	

The array quality is equivalent across tissue types and samples.
QC, quality control.

or one to five superficial punches sampling half of the tumor in a single core (UCSF) were obtained. The histologically benign peripheral zone glandular tissue was sampled in an analogous manner. Benign tissues were defined as adjacent to tumor (benign adjacent) when they were within 1 to 5 mm of the tumor. Benign contralateral tissues were obtained from the side of the prostate opposite to the tumor, and as far away from any other tumor or high-grade prostatic intraepithelial neoplasia areas as possible. To minimize any effects because of tumor heterogeneity, the area matching the biopsy specimen was identified in the RP specimen and punched. Tissues were punched in the same manner at locations matching those where the biopsy punches were taken for each tissue type (tumor, benign adjacent to tumor, and benign contralateral to tumor). In the RP specimens, only a single punch was obtained for each tissue type. For example, if the biopsy specimen used for RNA extraction reportedly was from the right apex, then the tumor in that portion of the RP also was sampled for extraction. This matching was performed for all tissue types (tumor, benign adjacent, and benign contralateral). At University Health Network, both biopsy and RP specimens were divided into sections (4 μm thick) to generate unstained sections, and the areas of interest were macrodissected (scraped) from the slides for RNA extraction. Six unstained sections were used to isolate RNA from biopsy tissue, and four to isolate RNA from RP tissue. RP tissue was sampled in locations matching the location of tumor in the biopsy cores.

RNA Extraction, Quantification, and Quality Control

Total RNA was extracted and purified using the RNeasy FFPE kit (Qiagen, Valencia, CA). RNA was amplified and labeled using the Ovation WTA FFPE system (NuGen, San Carlos, CA) and hybridized to Human Exon 1.0 ST GeneChips (Affymetrix Inc.), according to the manufacturer's recommendations. Using this approach, the expression of >1.4 million PSRs was quantified. Quality and quantity of RNA extracted and cDNA amplified were measured with a NanoDrop 1000 (Thermo Scientific Inc., Wilmington, DE). RNA (50 to 100 ng) was required for cDNA amplification. The ratio of absorbance (260/280 nm) used to assess the purity of RNA and ratio values between 1.7 and 2.2 were considered of acceptable purity. Quality control for microarray data was performed with the Affymetrix Power Tools packages and with internally developed metrics, including percentage present—the percentage of probes detected above the background defined the detection level of background probes with similar GC content. The positive versus negative area under the curve (AUC) was used as an additional metric to assess microarray quality by measuring the signal between positive control probes, which measure the expression of housekeeping genes, and negative control probes, which measure antigenomic sequences and hence should exhibit background intensity levels.[10] This metric can represent the quality of the RNA sample, with an AUC of 1 reflecting perfect separation that

indicates no false positives are detected, whereas all true positives are measured.

Expression Data Processing Analysis

The expression of approximately 1.4 million PSRs was normalized and summarized using SCAN[22] to the Affymetrix core transcript cluster level (approximately 22,000 genes). Expression data were uploaded to Gene Expression Omnibus (*http://www.ncbi.nlm.nih.gov/geo*, accession number GSE72220). To reduce measurement error because of laboratory variability, matching biopsy and RP samples were processed in the same batch, and batch correction was performed using ComBat (*http://www.bu.edu/jlab/wp-assets/ComBat/Abstract.html*, last accessed January 7, 2015)[23] on the expression data before analysis. Differential expression analysis using paired median fold difference (MFD). (MFD, $\overline{x - x'}$) was used to identify discriminative features between tumor and benign tissues. Dimensionality reduction was performed using principal component analysis (PCA), and significant sources of experimental and biological signal associated with the genomic variance captured by each principal component were assessed using the Mann-Whitney *U* test. The Fisher's exact test was used to assess the significance of concordance between Decipher measured from biopsy and Decipher measured from RP samples.

Field Effect Assessment

Matching tumor, benign adjacent to tumor, and benign contralateral samples for each patient were used to assess a potential genomic prostate cancer field effect. The expression profiles for features on the array were evaluated between the tumor and the two benign samples using Pearson's correlation. One-tailed *P* values were computed and adjusted using the false discovery rate method. Features correlated between tumor and benign with a $P < 0.05$ were considered candidate field effect features. This assessment was performed on the RP and biopsy samples separately to minimize confounding.

Prostate Cancer Molecular Subtyping

Patients in this study were classified into four previously published molecular subtypes that are mutually exclusive of each other: ERG^+, ETS^+, $SPINK1^+$, and triple negative.[21] Outlier expression analysis of *ERG*, *ETS* (*ETV1*, *ETV4*, *ETV5*, and *FLI1*), and *SPINK1* was used to assign each tumor sample to one of the subtypes. Patients exhibiting an outlier profile in either of genes were annotated with +, and − otherwise. Patients with high *ERG* expression profile (ERG^+) and not exhibiting outlier profiles for the other genes were classified as ERG^+ subtype, patients who were $ETV1^+$, $ETV4^+$, $ETV5^+$, or $FLI1^+$ and ERG^- and $SPINK1^-$ were classified as ETS^+ subtype, patients who were $SPINK1^+$, ERG^-, and ETS^- were classified as $SPINK1^+$, patients not exhibiting outlier profiles of any of these six genes were classified as the triple-negative subtype.

Results

Clinical and Pathological Characteristics of Patient Samples

Matched biopsy and RP specimens from three institutions using several sampling techniques were used to investigate the feasibility of obtaining high-quality and comprehensive whole transcriptome profiling data from FFPE tissue samples using Human Exon arrays. Patients had a median time between RP and biopsy of 86 days. The tumors in the samples covered a wide spectrum of Gleason scores (GSs) (Table 1) and in the RPs included GS = 3 + 3 (13 cases), GS = 3 + 4 (six cases), GS = 4 + 3 (eight cases), GS = 4 + 4 (four cases), and GS = 4 + 5 (two cases). The individual GSs in the biopsy specimens that were sampled matched those in the corresponding RP specimen, with the exception of two cases with GS 3 + 3 in the biopsy that were upgraded in the RP specimen. None of the cases were downgraded at RP.

Tissue Characteristics of Biopsy versus RP Samples

Samples were stored for an average of 3.1 years ($\sigma = 2.6$ years) before processing (Supplemental Table S1). The percentage of tumor involvement in the biopsy cores ranged from 5% to 70%, and the percentage tumor in the cylindrical punches of these cores ranged from 10% to 90%. The percentage tumor involvement of punches from RP specimens was higher and ranged from 50% to 90%. The stromal content in the punches of the biopsy cores ranged from 10% to 80%, whereas the percentage of benign epithelial content was <5% (Table 1). The amount of tissue that was provided for RNA extraction also varied between the three sites because of differences in the diameter of the punches (ie, 0.6 versus 1.0 mm) and sampling method (ie, a single cylindrical core using a biopsy punch versus multiple cores versus scraping from unstained sections). When the tumor occupied only 5% (0.5 mm) of the length of the biopsy specimen, the sample did not yield enough RNA ($n = 2$, 100%). However, at 1-mm tumor length and at least 35% tumor content in the punch area, the amount of RNA was sufficient to pass the quality threshold and generated high-quality data from the assay.

Transcriptome Data Quality from Biopsy versus RP Samples

RNA was extracted from 0.6-mm (CSMC) and 1-mm (USCF) cylindrical punches and from macrodissected tumor from unstained sections (4 μm thick; University Health Network) from FFPE blocks. For each patient, the same procedure was used at each site for collecting samples from both biopsy and RP specimens. All 77 RP samples, but only 72 (89%) of 81 biopsy samples yielded sufficient RNA for cDNA amplification

(Table 1). The lower yield of RNA from biopsy punches is explained by the smaller depth of tissue in biopsy specimens (at most, 1-mm core diameters) compared with RP (at least 2-mm thick tissue slice). In addition, RNA yield from unstained sections was, in general, lower than from punches (data not shown). Although the RNA yield from RP samples was approximately 10-fold greater than the RNA yield from biopsy samples (Figure 1A), when using 100 ng of RNA as a starting material, comparable amounts of cDNA were amplified from both sample sources (Figure 1B). All samples passed cDNA amplification, except one biopsy sample. A positive correlation was observed between RNA yield from punches of the biopsy cores and amount of tumor tissue in the punches. Overall cDNA yield remained relatively consistent between sample sources (Figure 1C).

Quality assessment of expression data generated from the assay was performed by assessing the sensitivity and specificity of the microarray. Sensitivity is assessed using the percentage of probe sets that provide a signal higher than the level of detection (LOD), whereas specificity is measured by the discrimination of positive and negative control probes, as calculated by the AUC. Biopsy and RP samples had similar medians of probe sets higher than the LOD, 51.3% for biopsy and 48.6% for RP samples; however, biopsy samples possessed greater AUCs than RP samples, with median AUCs of 0.76 versus 0.70 ($P < 0.01$), respectively. This suggests that the RNA quality of biopsy samples is higher than that of RP samples. Significantly, the percentage of probes higher than the LOD did not correlate with the tumor content present in the biopsy cores or punches evaluated (Figure 1C).

Transcriptome-Wide Expression Analysis in FFPE Tissues Obtained Through Biopsy

Having demonstrated that RNA extracted from needle core biopsy FFPE tissue samples was sufficient to generate transcriptome data using the assay and of comparable quality to that obtained from RP-derived samples, we next investigated the correlations of expression between biopsy specimens and RPs. Approximately 50% of the 1.4 million probe sets on the array provided signal intensities higher than the LOD (Table 1). Of these probe sets, 70% (490K probe sets) were detected in all RP samples, and of those, 95% were also detected in all of the biopsy samples, supporting the analytical feasibility of applying genome-wide exon arrays to prostate biopsy specimens.

To examine if tumor-associated signals could be detected in the biopsy and RP samples, we first performed an unsupervised hierarchical clustering and PCA on the microarray expression data. The hierarchical clustering analysis revealed that biopsy and RP samples formed two large clusters (Figure 2A) and that the origin of the sample (ie, biopsy versus RP) was the main responsible determinant. Within each cluster, moderate sub-clustering of tumor and benign samples was also observed. The PCA confirmed the clustering results, demonstrating that the sample origin (biopsy versus RP) was the biggest source of variation at the global level. This was observed mainly in principle component 1 (PC1). Furthermore, with the exception of PC2, the first six PCs all associated with sample origin (biopsy versus RP) and tissue type (benign versus cancer). In addition to the origin of the sample, it became evident from the PCA that the tissue type (ie, tumor versus benign) was another main source of variance in the data set. As seen most clearly in PC6, samples from benign tissue in both biopsy and RP clustered together (Figure 2, B and C). Despite the limiting amount of tissue and sources of technical variability (eg, differences in time to fixation between biopsy and RP), tumor-associated signals were clearly identified in biopsy tissues using unsupervised and unbiased transcriptome-wide data analysis methods.

A comparison of overall gene expression levels between biopsy and RP samples demonstrated a highly positive

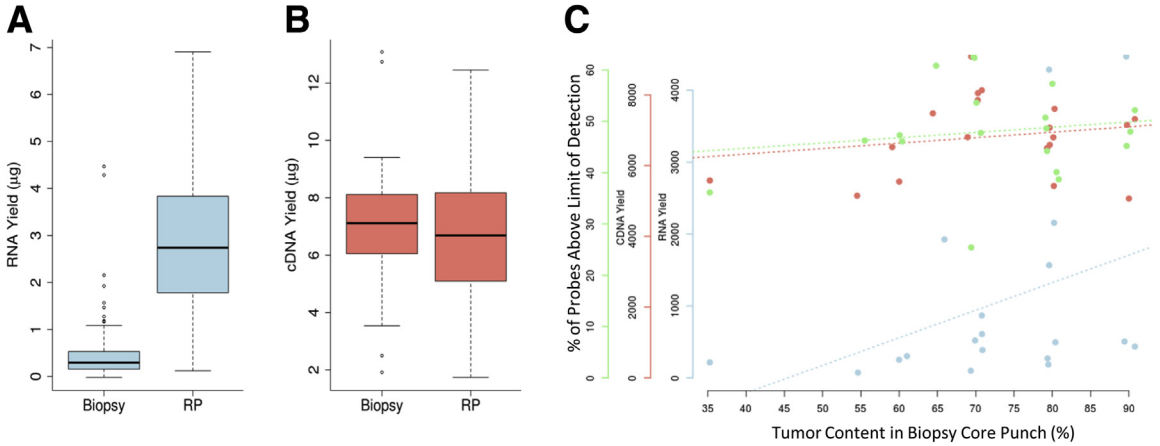

Figure 1 Quality control (QC) characteristics of biopsy and radical prostatectomy (RP) samples. **A:** Total RNA yield in RP samples is greater than in biopsy samples. **B:** An equal amount of extracted RNA was converted to cDNA, yielding similar amounts of cDNA in biopsy specimens and RPs. **C:** The three QC parameters (RNA yield, cDNA yield, and microarray probe signal intensity) are not affected by tumor content in the punch of the biopsy core samples. $n = 77$ (**A**, RP samples); $n = 81$ (**A**, biopsy samples).

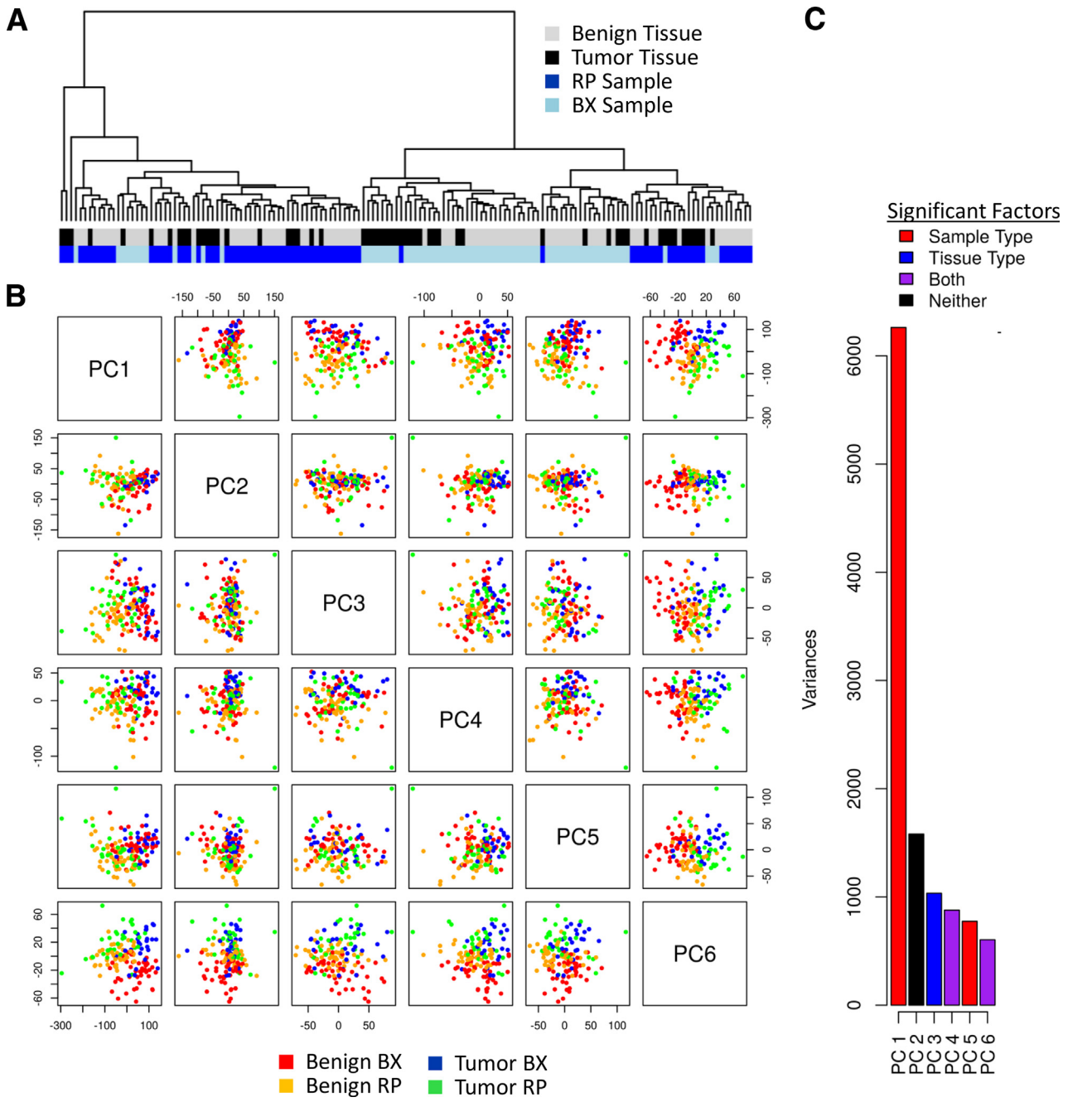

Figure 2 Sources of variance in radical prostatectomies (RPs) and biopsy (BX) samples. **A:** Hierarchical clustering of samples from tumor and benign tissues from BX and RP origins. The two main clusters are generated on the basis of the origin (BX versus RP), containing both benign and tumor samples. Additional clusters are obtained on the basis of the tissue type (tumor versus benign). **B** and **C:** Principal component analysis (PCA) shows that sample origin (BX versus RP) contributes the biggest variation and that tissue type (tumor versus benign) contributes less to the total variation.

correlation between expression values ($r = 0.96$) (Figure 3A and Supplemental Table S2). This suggested that, by comparison to expression levels generated from RP tissues, the assay could reliably quantify RNA expression levels in biopsy samples and, furthermore, that the expression profiles in biopsy and RP samples were highly analogous. A reduction in dynamic range in the biopsy samples was observed, but the effect was small. To further illustrate the within-patient RP-biopsy variability, correlation plots were generated for four randomly selected patients, which showed that there is strong consistency between RP and biopsy sample expression, even for individual patients (Supplemental Figure S2).

To compare changes in gene expression levels that are specific to cancer, we determined the differential expression for each gene between cancer and contralateral benign tissue for both the biopsy and RP specimens separately. Benign adjacent samples were removed because of possible tumor

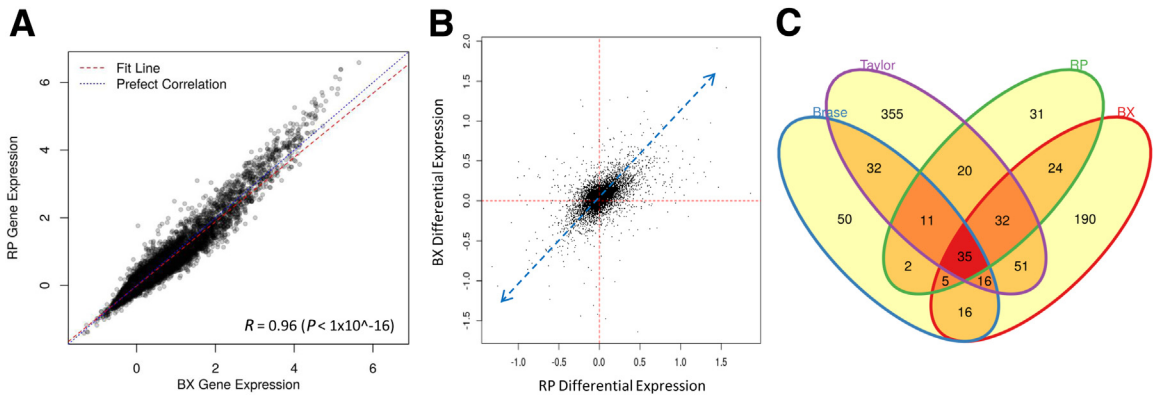

Figure 3 Expression analysis between radical prostatectomies (RPs) and biopsy (BX). **A:** High concordance of gene expression levels between BX and RP ($r = 0.96$). **B:** Differential expression analysis using median fold difference (MFD) shows that the MFD between RP tumor and RP benign contralateral, and BX tumor and BX benign contralateral, is consistent in terms of directionality. **C:** The genes differentially expressed between tumor and benign contralateral in BX and RP are overlapping with genes differentially expressed between tumor and benign contralateral in external public data sets. Statistical significance was assessed via bootstrapping ($P < 0.05$).

contamination and field effect, which may confound the analysis, and to allow for a pairwise analysis. Differential expression analysis using paired MFD demonstrated the same directionality of expression changes in biopsy and RP and, in addition, similar magnitudes of expression changes in both biopsy and RP samples (Figure 3B). Although the magnitude of the MFD is affected by the difference in tumor content and efficiency of RNA extraction between biopsy and RP, the results nevertheless demonstrate that the assay faithfully captured the biological signal in both specimen types. An assessment of individual patients revealed similar trends, where benign-tumor gene expression differences in the biopsy and RP samples had significant, positive correlations (Supplemental Figure S3). In addition, prostate cancer–related genes that were found to be differentially expressed in analysis of two public data sets (Taylor et al[24] and Brase et al[25]) were confirmed in our samples, independent of their origin from biopsy or RP (Figure 3C). Bootstrapping analysis revealed the observed overlap between these sets to be statistically significant ($P < 0.05$). Together, these data demonstrate that relevant and consistent biological prostate cancer–specific signals exist in data generated from both biopsy and RP specimen types.

Prostate Cancer Prognostic Signatures (Cuzick, Klein, Penney, and Decipher) in Prostate Needle Core Biopsy Specimens

Having demonstrated the feasibility of using the assay for evaluation of FFPE needle core biopsy specimens, we next assessed the robustness of four prostate cancer prognostic signatures. In addition to Decipher scores, we evaluated the expression levels of genes used in other published molecular signatures used for prostate cancer risk stratification, including expression signatures from Cuzick et al,[19] Klein et al,[18] and Penney et al,[20] as previously described.[5] For comparison, the RNA expression of the individual genes comprising these signatures was evaluated in both biopsy and RP samples. More than 94% of the features were higher than the LOD in all biopsy and RP samples, providing evidence of the ability of the assay to capture the biological signal of multiple prognostic signatures.

The Decipher scores showed a positive correlation ($r = 0.70$, $P < 0.001$) between biopsy and tumor RP samples (Figure 4A). Similarly, the Penney et al[20] signature also showed a positive correlation ($r = 0.65$, $P < 0.001$) (Figure 4B). To show that this result is robust, we progressively removed one and then two points driving the correlation from the analysis and reevaluated the model's correlations (Supplemental Figure S4). We did not observe major changes in the correlations, except for Penney et al,[20] which was found to have a borderline significant correlation after removing two of the driving points. Technical variables, such as percentage of tumor in punch, RNA yield, cDNA yield, and percentage of probes higher than the LOD, did not affect the Decipher scores in matched pairs of RP and biopsy tissues. Using validated cut points, Decipher patient risk categories between biopsy and RP were concordant in 75% of cases (Figure 4C). Most of the discordant cases were at the border between categories of low and intermediate risk of metastatic development. Using Fisher's exact test, the concordance in Decipher BX and RP scores trends toward significance ($P = 0.08$). This failure to reach significance is most likely because of the small sample size and the few patients classified as high risk. It is important to note that Decipher scores were independent of tumor content, demonstrating that the Decipher test is robust, despite limitations posed by formalin fixation and small amounts of cancer tissue in biopsy specimens.

Transcriptome Expression Assessment with Respect to Field Effect

The 22 Decipher features were measured across the three tissue sources in the study: tumor, benign adjacent to tumor,

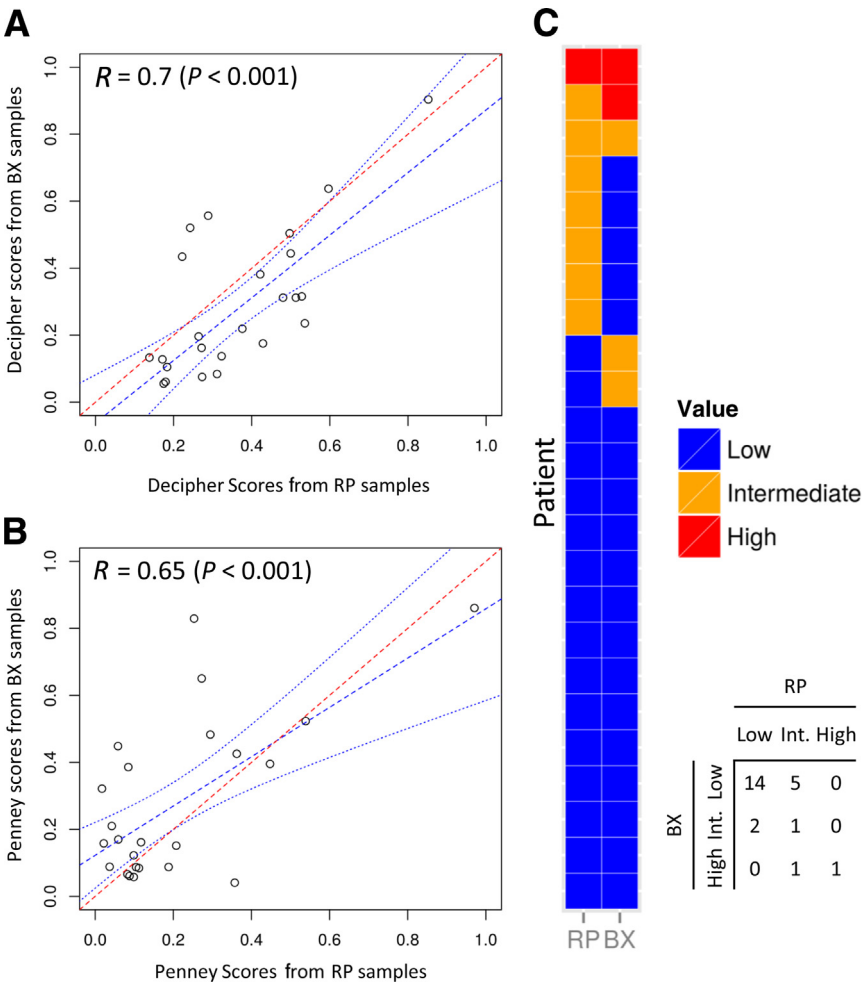

Figure 4 Robustness of the Decipher assay between biopsy (BX) and radical prostatectomies (RPs). **A:** Correlation of Decipher scores from BX and RP samples ($r = 0.7$). **B:** Correlation of scores for the Penney et al[20] signature from BX and RP samples ($r = 0.65$). The **blue dashed line** represents the line of best fit, whereas the **dotted blue lines** represent the 95% CI. **C:** Concordance of Decipher category between cancers in RP and BX (Fisher's exact test $P = 0.08$). Int., intermediate.

and benign contralateral to tumor (Figure 5A). We observed 15 (68%) of the 22 markers displaying the same pattern of gene expression in both biopsy and RP across the three tissue types. Overall, the 22 features showed highly concordant expression patterns between matched tumor samples from biopsy and RP, with generally higher expression of these genes in tumor compared with matched benign samples.

Next, we explored molecular heterogeneity by comparing expression of prostate cancer lineage and subtype markers (*ERG*, *ETV1*, *ETV4*, *ETV5*, and *SPINK1*) for each RP–biopsy pair for tumor and benign samples. Samples were grouped into four mutually exclusive molecular subtypes, ERG^+, ETS^+, $SPINK1^+$, and triple negative, as described in *Materials and Methods*.[26] As expected, these prostate cancer subtype markers were found mostly in tumor tissues; however, a few benign samples had outlier expression of these genes, suggesting contamination of some tumor cells in the histologically benign tissue (Supplemental Figure S5). In biopsy samples, 12, 3, 5, and 6 of 26 were assigned to ERG^+, ETS^+, $SPINK1^+$, and triple negative, respectively. In RP samples, 7, 4, 5, and 15 of 31 were assigned to ERG^+, ETS^+, $SPINK1^+$, and triple negative, respectively. In matched biopsy and RP sample pairs, overall 18 (75%) of 24 had concordant subtypes (Figure 5B). In RP samples, four cases of the 23 adjacent benign sample demonstrated outlier expression of *ERG*, *ETV5*, and *SPINK1* genes. Six of 24 matched biopsy and RP sample pairs showed different molecular subtypes: two were $SPINK1^+$ in biopsy and triple negative in RP, two were ERG^+ in biopsy and triple negative in RP, one was ERG^+ in biopsy and $SPINK1^+$ in RP, and, finally, one was ERG^+ in biopsy and ETS^+ in RP. The data herein indicate, for the first time, implementing molecular subtypes in prognostic assays to improve the currently available prognostic test for evaluation in prostate needle biopsy specimens and shed light on the, yet unmet, clinical need for integrating molecular subtypes and prognostic assays.

Finally, we assessed the extent of the prostate cancer field effect by examining the transcriptome-wide correlation between the tumor and matching benign samples within RP. We identified the genomic features on the microarray with significantly correlated expression ($P < 0.05$, after false discovery rate P value adjustment) between the tumor and the two types of benign samples. As expected, there were 7168 correlated features between RP tumor and benign adjacent samples compared with only 291 correlated features between RP tumor and benign contralateral samples

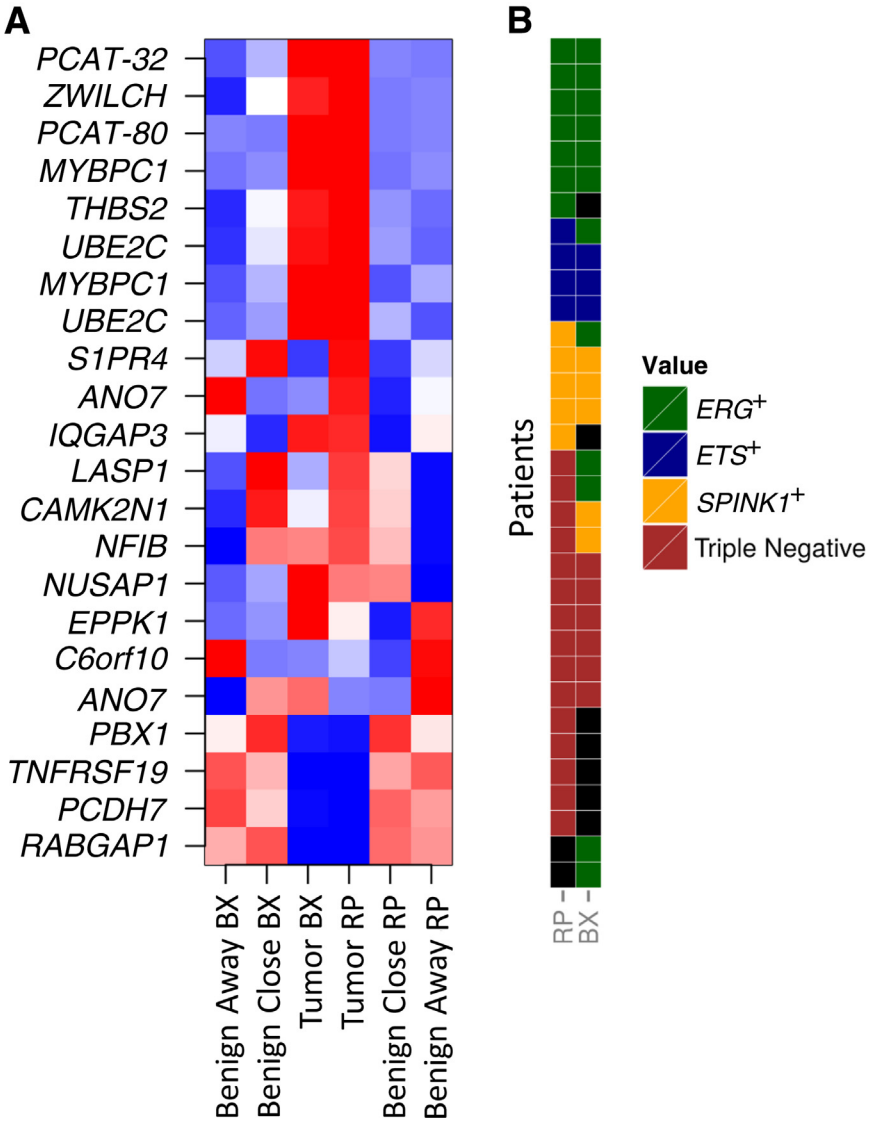

Figure 5 **A:** The expression of Decipher features is consistent across tissue types between radical prostatectomies (RPs) and biopsy (BX). Most features are overexpressed in tumor compared with benign in RP and BX. A group of features (*LASP1*, *CAMK2N1*, and *NFIB*) expressed at levels similar to the tumor specimens in benign adjacent to tumor (benign close to tumor) but not in benign contralateral to tumor (benign away from tumor). **B:** The concordance of prostate cancer molecular subtypes between BX and RP cancer samples.

(Supplemental Figure S6). Of 7168 features, 225 were identified in all three samples. As expected, the results show an overall higher correlation between tumor and benign adjacent samples compared with tumor and benign contralateral samples (Supplemental Figures S6 and S7 and Supplemental Tables S3 and S4). To shed further biological insights into the field effect genes, we used a subset (n = 2031) of the most correlated genes between paired tumor and adjacent benign samples (Pearson correlation >0.6 and $P < 0.001$) and compared it with a list of prostate cancer genes (n = 1114) that are differentially expressed between tumor and benign tissues in Memorial Sloan Kettering Cancer Center[24] and German Cancer Research Center[25] public data sets. Comparative analysis showed that field effect genes are distinct from prostate cancer genes, with only 95 genes overlapping (Figure 6A). Field effect genes are highly enriched with RNA splicing, chromosome organization, and intracellular transport biological processes (Figure 6B), and possess binding sites of key prostate cancer transcription factors, including *AR*, *TP53*, *ETS1*, *JUN*, *CREB1*, and *FOXO1*, on the basis of enrichment analysis of chromatin immunoprecipitation data sets using the ChEA tool[27] (Figure 6C). To assess if field effect genes are correlated with *ERG* and *ETV1* genomic rearrangements, a Pearson correlation coefficient between field effect genes and *ERG* and *ETV1* expression was determined and revealed only a poor correlation (Figure 6D).

Discussion

Accurate pretreatment risk assessment using prostatic needle biopsy specimens, although challenging, is essential to proper prostate cancer patient management. When prostate cancer is first diagnosed, it is necessary to determine the best individualized treatment plan for each patient. Accurate prognostication at the time of diagnosis is challenging because of several reasons. First, the standard 12 core

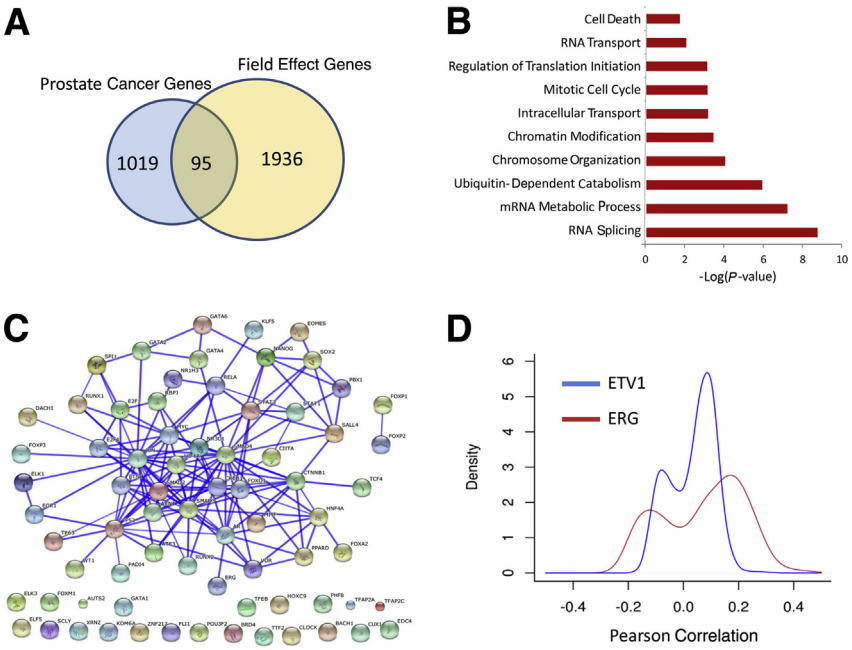

Figure 6 Functional analysis of the field effect signature. **A:** The field effect signature is distinct from prostate cancer genes differentially expressed between tumor and benign tissues (Memorial Sloan Kettering Cancer Center and German Cancer Research Center).[24,25] **B:** Functional enrichment analysis reveals that the field effect signature is highly enriched with gene categories of RNA splicing, ubiquitin-dependent catabolism, epigenetics, and cellular transport. **C:** Functional interaction networks generated by STRING of 75 transcription factors whose targets are enriched in the field effect signature on the basis of a chromatin immunoprecipitation enrichment analysis tool.[27] **D:** Density plots of Pearson's correlation between field effect signature and *ERG* and *ETV1* genes, suggesting that field effect is independent of *ERG* and *ETS*.

prostate needle biopsy only samples a small fraction of the prostate and, therefore, may not be a representative sampling of the most significant tumor, and may not provide sufficient material for deep expression profiling. Second, the accuracy of Gleason grading can be compromised by the sampling error of the biopsy process; the GS is increased at RP in approximately 20% to 50% of cases.[28] Thus, the severity of the cancer is often underestimated at biopsy. In addition, the subjective nature of tumor grading and assigning GSs complicates prognostication. The unpredictability of disease progression is further affected by the genetic heterogeneity and multiclonality of tumors that can appear histologically identical or, surprisingly, even lower grade.[6,7] All of these challenges highlight the need for more sensitive and robust genomic-based risk stratification methods that are applicable to prostate biopsy specimens so that men can more confidently choose a proper cancer management strategy. The small amount of tumor usually present in FFPE tissue specimens poses a barrier that is particularly difficult to overcome in the analysis of tissue biomarkers.

Characterizing the RNA expression of the tumor in biopsy tissues may provide informative clinical insights into the true aggressiveness of the tumor. Herein, we took advantage of Human Exon 1.0 ST arrays, a high-density oligonucleotide microarray that measures 1.4 million transcriptome-wide PSRs representing all known genes and many noncoding RNAs. Because of well-characterized assay characteristics and excellent performance in RP FFPE tissues, we decided to test the workflow with its quality control standards in biopsy specimens using the matched RP specimens as a reference.

Several studies have been performed demonstrating the generation of high-quality gene expression data from FFPE specimens. Bibikova et al[29] and Frank et al[30] assessed the reproducibility of FFPE samples profiled with oligonucleotide arrays and found high concordance between replicate samples. Likewise, high correlations ($r \geq 0.83$) were observed between array data from FFPE and snap-frozen tissues.[30] Pillai et al[31] profiled 462 FFPE metastatic tumor biopsy specimens with Pathchip arrays for a tumor of origin test and found high-quality data in 80% of cases. Our study builds on this foundation by focusing on the comparison between FFPE biopsy specimens and FFPE surgical samples from prostate cancer patients. Prostate biopsy specimens present a unique challenge because of issues of heterogeneity and significant stromal contamination. Likewise and unlike previously cited studies,[29–31] which were all preformed in research laboratories, all samples in this study were assayed in a Clinical Laboratories Improvement Amendment–certified laboratory. Finally, unlike the cited feasibility studies with FFPE tissues, which used gene expression arrays, this study uses a transcriptome-wide, high-density, exon array to profile samples.

To assess the technical feasibility of running transcriptome-wide arrays on biopsy samples, we took advantage of a multi-institutional cohort representing various tissue sampling methods. The way the samples were obtained (direct biopsy of the FFPE block versus scraped area of unstained sections) and the variability in institution processing did not hinder the ability to generate genome-wide transcriptome data. The generated data demonstrate that a sufficient amount of RNA of suitable quality for molecular genomic analysis can be consistently and successfully derived from the limited tumor tissue in biopsy cores, even with minuscule tumor content (1 mm length of tumor in a biopsy core with at least 40% concentration of tumor cells). Optimally, we found the direct biopsy method of the block using a punch tool to give the highest yields, and with a single 1-mm diameter punch, almost

all biopsy specimens could be investigated without depleting the block from cancer, as is often the case when biopsy specimens are divided into sections serially for hematoxylin and eosin diagnosis, immunohistochemistry, and genomic assays. This is important for many pathology laboratories, which do not want to deplete FFPE blocks of tumor cells for medicolegal reasons or want to preserve tumor for future clinical uses. Furthermore, not only have we demonstrated that biopsy-derived RNA is equivalent to that of RNA obtained from surgical specimens, it can be considered in some regards to be superior. This observation might not be surprising given that biopsy cores are typically fixed in formalin immediately after they are obtained from the patient, whereas surgical specimens experience a prolonged hypoxic period before they are immersed in the formalin fixative, and it takes more time for the fixative to penetrate and diffuse into the larger tissue volume if rapid fixation techniques, such as fixative injection, are not used. The delay could potentially lead to RNA degradation and perturb RNA expression profiles. However, we observed a high correlation overall between gene expression data generated from biopsy and RP samples ($r = 0.96$), suggesting that most genes do not change in abundance despite ischemic and/or slower fixation conditions.

We further examined the expression of prostate cancer genes from other prostate cancer prognostic tests (ie, Cuzick et al,[19] Klein et al,[18] and Penney et al[20]), which can be measured using Human Exon arrays. More than 94% of the genes from Cuzick et al,[19] Klein et al,[18] and Penney et al[20] had signal higher than the LOD. The expression of the 22 features in the Decipher test and the Decipher scores were in general concordant between RP and biopsy. A patient-per-patient pairwise agreement between biopsy and RP samples using the Decipher risk category between biopsy and RP tumor samples showed good overall concordance (75%). This result provides preliminary evidence that the Decipher score may be predictive of disease progression in diagnostic prostate needle biopsy specimens and warrants a larger, adequately powered study to validate this observation.

To provide additional molecular insights, we examined whether matched tissues harbor similar molecular subtypes of prostate cancer. Overall, there is strong agreement in molecular subtypes between cancers in RP and biopsy specimens. In addition, the molecular analysis revealed the coexistence of several clones within the region of cancer that was analyzed. These results demonstrate that, in addition to the Decipher score, the molecular subtypes in the biopsy are representative of the cancer in the RP. Taken together, these observations provide additional evidence as to the potential clinical utility of high-resolution expression arrays in the biopsy setting for use in molecular classification, risk stratification, and prognosis.

Further characterization of the transcriptome data from tissues adjacent or contralateral to the tumor revealed that there is higher concordance in gene expression profiles between tumors and adjacent benign compared with contralateral benign samples, and that the expression of many Decipher features is higher in the tumor compared with the benign tissues. Although, in the RP specimens, we could clearly detect a field effect, with a higher proportion of correlated expressed genes between tumor and adjacent compared with contralateral histologically nonneoplastic tissue, this was not detected in the biopsy specimens. Functional characterization of the field effect genes revealed that they are related to key biological processes involved in tumor progression and key prostate cancer transcription factors, including *AR*, *JUN*, *HIFA1*, and *TP53*. On the other hand, they are not correlated with genomic rearrangements, suggesting that adjacent benign tissues harbor a distinct biology. Furthermore, the detection of tumor-specific subtype markers, such as ERG^+, ETS^+, and $SPINK1^+$, in the benign specimens suggests the presence of tumor cells in histologically nonneoplastic tissue may be a confounder for the measurement of a field effect. Overall, the molecular subtyping results show that for 25% of patients analyzed, tumor heterogeneity could be detected between the biopsy and RP specimens.

Previous reports of investigating multifocality in prostate RP specimens have shown between 41% and 67% discordance rates between *ERG* status within the same patient.[26] Other studies have evaluated single prostates and detected numerous tumors with different clonal type and origin.[6] To our knowledge, no previous study has reported the rate of subtype discordance between matched biopsy-RP specimens using six molecular markers (*ERG*, *ETV1*, *ETV4*, *ETV5*, *FLI1*, and *SPINK1*).

Although the conclusions drawn from this study might be limited by the small sample size, the data provide, for the first time, evidence of concordance of a genomic classifier across matched tumor samples from biopsy and RP in a multi-institutional cohort. Altogether, the data demonstrate the feasibility of measuring RNA expression in FFPE prostate needle biopsy specimens with small amounts of tumor using high-density expression arrays. These data suggest that a high-density array run on prostate biopsy specimens also provides potentially useful information on tumor heterogeneity, which can be combined with currently validated RNA expression–based tests to improve prediction of cancer progression.

Supplemental Data

Supplemental material for this article can be found at http://dx.doi.org/10.1016/j.jmoldx.2015.12.006.

References

1. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2012. CA Cancer J Clin 2012, 62:10–29
2. Siegel R, Ma J, Zou Z, Jemal A: Cancer statistics, 2014. CA Cancer J Clin 2014, 64:9–29
3. Wojno K, Hornberger J, Schellhammer P, Dai M, Morgan T: The clinical and economic implications of specimen provenance

complications in diagnostic prostate biopsies. J Urol 2015, 193: 1170–1177
4. Chang AJ, Autio KA, Roach M, Scher HI: High-risk prostate cancer: classification and therapy. Nat Rev Clin Oncol 2014, 11:308–323
5. Xie R, Chung J-Y, Ylaya K, Williams RL, Guerrero N, Nakatsuka N, Badie C, Hewitt SM: Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. J Histochem Cytochem 2011, 59:356–365
6. Haffner MC, Mosbruger T, Esopi DM, Fedor H, Heaphy CM, Walker DA, Adejola N, Gürel M, Hicks J, Meeker AK, Halushka MK, Simons JW, Isaacs WB, De Marzo AM, Nelson WG, Yegnasubramanian S: Tracking the clonal origin of lethal prostate cancer. J Clin Invest 2013, 123:4918–4922
7. Lindberg J, Kristiansen A, Wiklund P, Grönberg H, Egevad L: Tracking the origin of metastatic prostate cancer. Eur Urol 2015, 67: 819–822
8. Boutros PC, Fraser M, Harding NJ, de Borja R, Trudel D, Lalonde E, et al: Spatial genomic heterogeneity within localized, multifocal prostate cancer. Nat Genet 2015, 47:736–745
9. Abdueva D, Wing M, Schaub B, Triche T, Davicioni E: Quantitative expression profiling in formalin-fixed paraffin-embedded samples by affymetrix microarrays. J Mol Diagn 2010, 12:409–417
10. Erho N, Crisan A, Vergara IA, Mitra AP, Ghadessi M, Buerki C, Bergstralh EJ, Kollmeyer T, Fink S, Haddad Z, Sierocinski T, Ballman KV, Triche TJ, Black PC, Karnes RJ, Klee G, Davicioni E, Jenkins RB: Discovery and validation of a prostate cancer genomic classifier that predicts early metastasis following radical prostatectomy. PLoS One 2013, 8:e66855
11. Alshalalfa M, Schliekelman M, Shin H, Erho N, Davicioni E: Evolving transcriptomic fingerprint based on genome-wide data as prognostic tools in prostate cancer. Biol Cell 2015, 107:232–244
12. Karnes R, Bergstralh E, Davicioni E, Ghadessi M, Buerki C, Mitra A, Crisan A, Erho N, Lam L, Carlson R, Haddad Z, Triche T, Kollmeyer T, Ballman K, Black P, Klee G, Jenkins R: Validation of a genomic classifier that predicts metastasis following radical prostatectomy in an at risk patient population. J Urol 2013, 190:2047–2053
13. Klein EA, Yousefi K, Haddad Z, Choeurng V, Buerki C, Stephenson AJ, Li J, Kattan MW, Magi-Galluzzi C, Davicioni E: A genomic classifier improves prediction of metastatic disease within 5 years after surgery in node-negative high-risk prostate cancer patients managed by radical prostatectomy without adjuvant therapy. Eur Urol 2014, 67:778–786
14. Ross AE, Feng FY, Ghadessi M, Erho N, Crisan A, Buerki C, Sundi D, Mitra AP, Vergara IA, Thompson DJ, Triche TJ, Davicioni E, Bergstralh EJ, Jenkins RB, Karnes RJ, Schaeffer EM: A genomic classifier predicting metastatic disease progression in men with biochemical recurrence after prostatectomy. Prostate Cancer Prostatic Dis 2014, 17:64–69
15. Den RB, Yousefi K, Trabulsi EJ, Firas A, Voleak C, Feng FY, Dicker AP, Lallas CD, Gomella LG, Davicioni E, Karnes RJ: Genomic classifier identifies men with adverse pathology after radical prostatectomy who benefit from adjuvant radiation therapy. J Clin Oncol 2015, 33:944–951
16. Prensner JR, Shuang Z, Erho N, Schipper M, Lyer MK, Dhanasekaran SM, Magi-Galluzzi C: RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. Lancet Oncol 2014, 15:1469–1480
17. Lalonde E, Ishkanian A, Sykes J, Fraser M, Ross-Adams H, Erho N, Dunning M: Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. Lancet Oncol 2014, 15:1521–1532
18. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, Chan JM, Li J, Pelham RJ, Tenggara-Hunter I, Baehner FL, Knezevic D, Febbo PG, Shak S, Kattan MW, Lee M, Carroll PR: A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol 2014, 66:550–560
19. Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM, Reid JE, Mesher D, Speights VO, Stankiewicz E, Scardino P, Younus A, Flake DD, Wagner S, Gutin A, Lanchbury JS, Stone S: Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. Lancet Oncol 2011, 12:245–255
20. Penney KL, Sinnott JA, Fall K, Pawitan Y, Hoshida Y, Kraft P, Stark JR, Fiorentino M, Setlur S, Johansson J-E, Adami H-O, Rubin MA, Loda M, Golub TR, Andrén O, Stampfer MJ, Mucci LA: mRNA expression signature of Gleason grade predicts lethal prostate cancer. J Clin Oncol 2011, 29:2391–2396
21. Tomlins SA, Alshalalfa M, Davicioni E, Erho N, Yousefi K, Zhao S, Haddad Z, Den RB, Dicker AP, Trock BJ, DeMarzo A, Ross A, Schaeffer EM, Klein EA, Magi-Galluzzi C, Karnes RJ, Jenkins RB, Feng FY: Characterization of 1,577 primary prostate cancers reveals novel biological and clinicopathological insights into molecular subtypes. Eur Urol 2015, 68:555–567
22. Piccolo SR, Sun Y, Campbell JD, Lenburg ME, Bild AH, Johnson WE: A single-sample microarray normalization method to facilitate personalized-medicine workflows. Genomics 2012, 100: 337–344
23. Johnson WE, Li C, Rabinovic A: Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 2007, 8: 118–127
24. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Wilson M, Heguy A, Eastham JA, Scardino PT, Sander C, Sawyers CL, Gerald WL: Integrative genomic profiling of human prostate cancer. Cancer Cell 2010, 18:11–22
25. Brase JC, Johannes M, Mannsperger H, Falth M, Metzger J, Kacprzyk LA, Andrasiuk T, Gade S, Meister M, Sirma H, Sauter G, Simon R, Schlomm T, Beissbarth T, Korf U, Kuner R, Sultmann H: TMPRSS2-ERG-specific transcriptional modulation is associated with prostate cancer biomarkers and TGF-beta signaling. BMC Cancer 2011, 11:507
26. Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA, Schalken JA: ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol 2009, 56:275–286
27. Lachmann A, Xu H, Krishnan J, Berger SI, Mazloom AR, Ma'ayan A: ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. Bioinformatics 2010, 26: 2438–2444
28. Corcoran NM, Hong MK, Casey RG, Hurtado-Coll A, Peters J, Harewood L, Goldenberg SL, Hovens CM, Costello AJ, Gleave ME: Upgrade in Gleason score between prostate biopsies and pathology following radical prostatectomy significantly impacts upon the risk of biochemical recurrence. BJU Int 2011, 108(8 Pt 2):E202–E210
29. Bibikova M, Yeakley JM, Chudin E, Chen J, Wickham E, Wang-Rodriguez J, Fan J-B: Gene expression profiles in formalin-fixed, paraffin-embedded tissues obtained with a novel assay for microarray analysis. Clin Chem 2004, 50:2384–2386
30. Frank M, Döring C, Metzler D, Eckerle S, Hansmann M-L: Global gene expression profiling of formalin-fixed paraffin-embedded tumor samples: a comparison to snap-frozen material using oligonucleotide microarrays. Virchows Arch 2007, 450:699–711
31. Pillai R, Deeter R, Rigl CT: Validation and reproducibility of a microarray-based gene expression test for tumor identification in formalin-fixed, paraffin-embedded specimens. J Mol Diagn 2011, 13: 48–56

Tissue Sources for Accurate Measurement of Germline DNA Genotypes in Prostate Cancer Patients Treated With Radical Prostatectomy

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BACKGROUND. Benign tissue from a tumor-containing organ is commonly the only available source for obtaining a patient's unmutated genome for use in cancer research. While it is critical to identify histologically normal tissue that is independent of the tumor lineage, few additional considerations are applied to the choice of this material for such measurements.
METHODS. Normal formalin-fixed, paraffin-embedded seminal vesicle, and urethral tissues, in addition to whole blood, were collected from 31 prostate cancer patients having undergone radical prostatectomy. Genotype concordance was evaluated for DNA from each tissue source in relation to whole blood.
RESULTS. Overall, there was a greater genotype call rate for DNA derived from urethral tissue (97.0%) in comparison with patient-matched seminal vesicle tissues (95.9%, $P = 0.0015$). Furthermore, with reference to patient-matched whole blood, urethral samples exhibited higher genotype concordance (94.1%) than that of seminal vesicle samples (92.5%, $P = 0.035$).
CONCLUSIONS. These findings highlight the heterogeneity between diverse sources of DNA in genotype measurement and motivate the consideration of normal tissue biases in tumor-normal analyses. *Prostate 77: 425–434, 2017.* © 2016 Wiley Periodicals, Inc.

KEY WORDS: **genotype concordance; archival FFPE tissue; urethra; seminal vesicle; tumor-normal**

INTRODUCTION

Disease screening and risk-modeling involve the integration of increasingly diverse sources of biological information. Innovations in high-throughput assay technologies have enabled the acquisition of biological data at an unprecedented scale. Subsequently, the development of a clinically actionable model of disease risk now involves traversing multiple dimensions of biological measurements, including protein levels, gene expression levels, and germline DNA polymorphisms, in addition to clinical and sociodemographic variables.

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Recent studies have demonstrated that the predictive power of risk models that integrate diverse biomarkers may be greatly improved in comparison to traditional screening approaches based on clinical data and limited biomarkers [1,2]. Hence, the methods by which biological data are acquired deserve special attention, as they may influence downstream predictive performance.

One consideration is the choice of appropriate biospecimen from which biomarkers will be measured. In genetic association studies of complex disease, the DNA used for measuring germline variants is often purified from blood, oral scrapings, or saliva [3,4]. However, retrospective tumor-normal research analyzing mutations, copy number, and gene expression in tissue from biopsy or surgery often relies on tumor-adjacent normal tissue as the only possible source for germline DNA genotypes [5]. While previous studies have examined the genotyping performance of select normal tissues in comparison with blood [6–8], the issue of how different sources of normal tissue influence the result of germline DNA genotyping, and accordingly the validity of disease risk predictions that model such genotypes, has been generally overlooked.

In the development of an integrated risk prediction model to discern aggressive versus indolent prostate cancer, we hypothesized that distinct sources of normal tissue may perform differently in the context of high-throughput genetic analyses. Here we analyze surgically resected specimens from patients with prostate cancer to compare genotyping results for DNA samples derived from archival normal tissues of the prostatic urethra and seminal vesicle.

MATERIALS AND METHODS

Tissue Preparation

We obtained 93 normal samples (patient-matched blood, urethral tissue, and seminal vesicle tissue) from 31 patients who had undergone radical prostatectomy. All tissue was obtained using a 2 mm dermal punch to biopsy archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks. A new punch was used to collect each biopsy from each block, and a single punch was made each time and placed into an Eppendorf tube for DNA extraction. The region of interest from each block to be biopsied was marked for punching. For each prostatectomy, the slides and pathology report of each case were reviewed. Seminal vesicle tissue from the side opposite to that most involved by prostate cancer was used; the area marked included both the seminal vesicle epithelium and the muscle wall, and the punch was taken to include both. The area of the urethra to be punched was marked in an area at least 5 mm from any tumor foci and included both urothelium and underlying stromal tissue, in a manner to exclude prostate glandular tissue. Note that while all punches of seminal vesicle contained 100% tissue throughout, many punches of urethra were taken from the border of the tissue with surrounding FFPE such that the punch may not have been completely composed of tissue. Normal prostate tissue was excluded from consideration due to several known obstacles to the identification of histologically pure samples of normal prostate, including the presence of multiple, scattered heterogeneous tumor foci [9], prostatic intraepithelial neoplasia [10–12], and field effects due to the presence of nearby neoplasia(s) [13], all of which are known to induce genetic abnormalities.

DNA Purification and Genotyping

After the paraffin layer was removed, 1 mm diameter cores punched from FFPE tissue blocks were sectioned into 20–30 pieces using a sterile razor blade. Samples were then vortexed with 1 ml xylene, followed by 2 min of centrifugation at room temperature. Next, samples were again vortexed with 1 ml of 100% ethanol and pelleted by centrifugation. The supernatant was discarded and residual solvent was evaporated at room temperature. Next, DNA was purified from blood samples (Promega Wizard Genomic DNA Purification Kit) and FFPE tissues (QIAamp DNA FFPE Tissue Kit). To boost DNA yields prior to genotyping, 200 ng of input DNA from each sample was amplified (Affymetrix Axiom 2.0 Reagent Kit) via isothermal incubation at 37 °C for 48 hr. The sample DNA was next fragmented into pieces ranging from 25 to 125 base pairs, followed by isopropanol precipitation. The Affymetrix GeneTitan Multi-Channel Instrument was used for sample genotyping.

Custom Microarray Design

In collaboration with Affymetrix Inc., we designed a custom DNA microarray to assay functional and putative prostate cancer specific variation. While the array features many rare (<1% minor allele frequency) and coding variants, its design was not limited to rare or exonic variation and broadly targeted genetic markers of interest genome-wide in a number of different functional categories.

The variant selection procedure was conducted as follows. First, a set of target markers, including both single nucleotide polymorphisms (SNPs) and insertion-deletion (indel) mutations, was constructed. The targets included previous GWAS findings

(genome-wide significant and suggestive) in prostate cancer, associated traits (PSA level and prostate cancer gene-by-gene interactions), other correlated traits (breast cancer, height, BMI, obesity, diabetes), and uncorrelated traits (NHGRI GWAS catalog polymorphisms). Additionally, a list of pan-cancer candidate genes was compiled and rare variants in windows centered around these genes were included in the target set. Rare variants in frequently mutated genes from the somatic cancer database COSMIC were also included. Furthermore, rare variants from a series of in-house whole genome and whole exome sequence analyses (of African American prostate cancer patient normal genomes [14], normal prostate exomes from the TCGA and dbGaP [15,16], and prostate cell line DNAse I hypersensitive sites [17]) were added to the target set. Finally, variants from previous Affymetrix microarrays were also targeted. These included the Exome 319 chip and the UK Biobank [18] array (excluding the GWAS backbone), which covered a broad range of functional categories including missense mutations and putative deleterious variants from the Human Gene Mutation Database.

The next step was to select which probesets would be directly genotyped on the microarray. Probesets were selected from a pool of candidate markers by an iterative, greedy algorithm which prioritized candidates based on their coverage of the target set. In order to reduce redundancy with previous GWAS arrays, candidates were chosen with complementarity to GWAS arrays previously assayed in the Kaiser Permanente GERA cohort [3,19,20] by drawing from a candidate set disjoint from the GWAS array markers. This produced a set of markers optimized for coverage of the target set.

Sample and Variant Quality Control

We excluded samples from our analyses if there was insufficient resolution between marker probeset intensities (axiom_dishqc_DQC <0.75) in any of the three tissue sources. This resulted in the exclusion of two samples and decreased the sample size from 31 initial subjects to 29 total. Out of the 29 subjects, 25 self-identified ethnically as Caucasian, one as African American, and three as "Other." All subjects were designated as clinical T stage one or two and Gleason 6 $(3+3)$ at diagnosis, although certain patients were upgraded and upstaged after surgery. These subject demographics and others are described in detail in Table I. Genotyping and sample quality control was performed using the Affymetrix Power Tools software suite.

To exclude variants susceptible to low-confidence genotype calls due to misclustering, variants with a minor allele frequency less than 5% are omitted from the reported concordance estimates. This minor allele frequency filter reduced the number of markers from 416,047 total variants to 127,847 common polymorphisms, from which call rates and concordance estimates were computed and summarized in Table I. However, for completeness, analyses where markers were stratified by minor allele frequency (in the main text and supplementary figures) include all 416,047 markers segregated into their respective minor allele frequency bins. These minor allele frequencies were based on the European (EUR) super population of the 1000 Genomes Project Phase 3 release [21].

Statistical Analyses

Tissue sources were compared using several sample statistics (DNA quantity, genotype call rate, and genotype concordance; Table I), as well as clinicopathologic factors ("subject-level" factors). For a given genetic variant, genotype concordance was defined as the agreement of both called alleles at a given marker (in samples from the same subject). Genotype pairs containing any no-calls were excluded from concordance calculations. Hypothesis testing for detecting statistically significant differences between tissue sources was conducted via paired-sample, two-tailed *t*-tests. Comparisons of genotype statistics ("variant-level" factors) between tissue sources (Figs. 1, 2, and S2) were likewise conducted using weighted, paired-sample, two-tailed *t*-tests, with the weight values equal to the number of markers in a given minor allele frequency bin. Linear regression model selection was conducted via stepwise bidirectional elimination using the Akaike Information Criterion. Concordance calculations and variant QC was conducted using PLINK [22], while all statistical analyses and figure generation were performed using the R statistical computing language [23,24].

RESULTS

Sample Quality of Source DNA

We evaluated the concordance between genotypes calls in DNA samples isolated from patient-matched blood, prostatic urethra (UR), and seminal vesicle (SV) normal tissues for 31 men with prostate cancer. Quality control procedures are described in the Materials and Methods section, and yielded a dataset comprised of 127,847 common polymorphisms measured in 29 men across each of three DNA sources (blood, UR, SV).

As expected, we observed the superior performance of blood to both normal FFPE tissue sources

TABLE I. Genotype Summary Statistics and Concordance Stratified by Tissue Source and Clinicopathologic Factors

Summary statistics and variables for 29 research subjects and 127,847 common SNPs (MAF >5%)		Whole blood	Urethra (UR)	Seminal vesicle (SV)	Percent difference (UR - SV)	*P*-value
Average DNA quantity (± std. dev.) post-amplification		1543 ng (±163)	1472 ng (±129)	1425 ng (±192)	–	0.12
Genotype call rate (post-Hardy–Weinberg equilibrium QC)		98.3% (97.9%)	97.0% (96.8%)	95.9% (95.8%)	+1.1% (+1.0%)	0.0015 (0.0010)
Concordance of genotype calls with whole blood genotypes (post-Hardy–Weinberg equilibrium QC)		100% (100%)	94.1% (95.4%)	92.5% (94.0%)	+1.6% (+1.4%)	0.035 (0.037)
Concordance of of genotype calls with whole blood genotypes, stratified by clinicopathologic factors						
Age at diagnosis (years)						
<55	n = 6	–	95.8%	94.2%	+1.6%	0.045
55–60	n = 12	–	92.0%	91.1%	+0.9%	0.21
>60	n = 11	–	95.6%	93.2%	+2.4%	0.25
DNA quantity difference post-amplification, by quartile (UR - SV, ng)						
Q1 [−178.4, −47.7]	n = 7	–	95.6%	95.4%	+0.2%	0.49
Q2 [−47.6, 14.2]	n = 7	–	89.2%	88.0%	+1.2%	0.70
Q3 [14.3, 90.4]	n = 8	–	95.8%	94.7%	+1.1%	0.33
Q4 [90.5, 663.8]	n = 7	–	96.0%	91.6%	+4.4%	0.063
Pathologic gleason score						
6	n = 20	–	93.5%	92.2%	+1.3%	0.042
7 (3 + 4)	n = 7	–	95.7%	92.8%	+2.9%	0.26
7 (4 + 3)	n = 2	–	93.8%	94.5%	−0.7%	0.79
Pathologic T-stage						
T2a	n = 1	–	92.0%	91.6%	+0.4%	–
T2c	n = 25	–	94.0%	92.3%	+1.7%	0.048
T3a	n = 3	–	95.6%	94.8%	+0.8%	0.32
PSA at diagnosis (ng/ml)						
<4.5	n = 10	–	95.7%	92.9%	+2.8%	0.08
4.5–6.5	n = 11	–	95.8%	94.9%	+0.9%	0.14
>6.5	n = 8	–	89.3%	88.5%	+0.8%	0.95

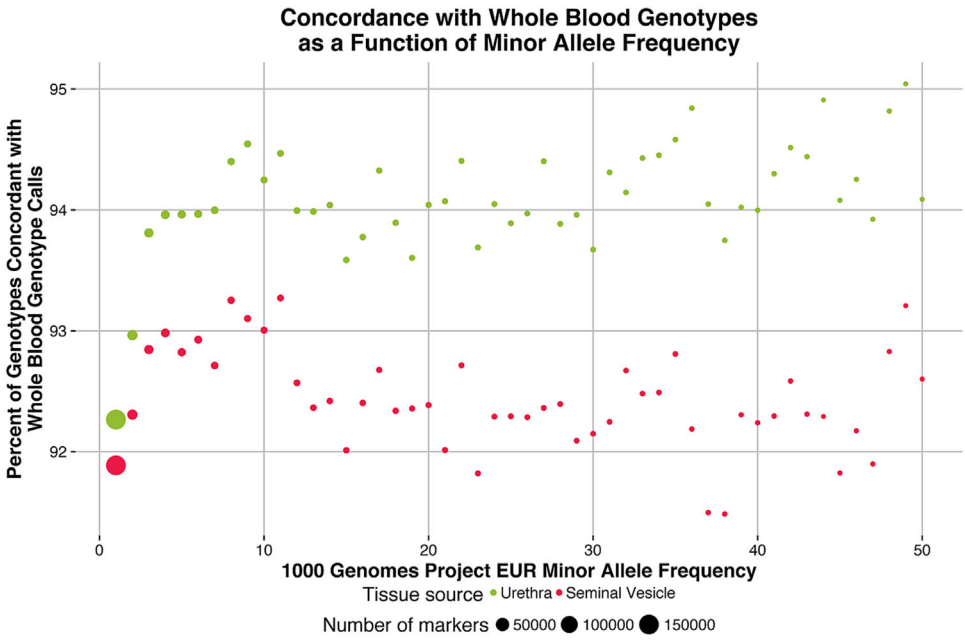

Fig. 1. Urethra-blood genotype concordance compared with seminal vesicle-blood over a range of variant minor allele frequency bins.

with respect to several measures. Across all samples, post-amplification DNA yields (Table I) were significantly greater for blood than for UR ($P = 0.0091$) and for SV ($P = 0.0012$). In turn, genotype call rate was significantly greater in blood (98.3%) than in UR (97.0%; $P = 3.8 \times 10^{-6}$) and SV (95.9%; $P = 4.1 \times 10^{-10}$). This observation supported using blood genotypes as a gold-standard reference. Hence, in all subsequent comparisons, concordance estimates were computed with reference to blood genotypes.

Although the genotype call rate was higher overall for UR samples in comparison with SV (97.0% vs. 95.9%, $P = 0.0015$), DNA quantities did not differ significantly between UR and SV

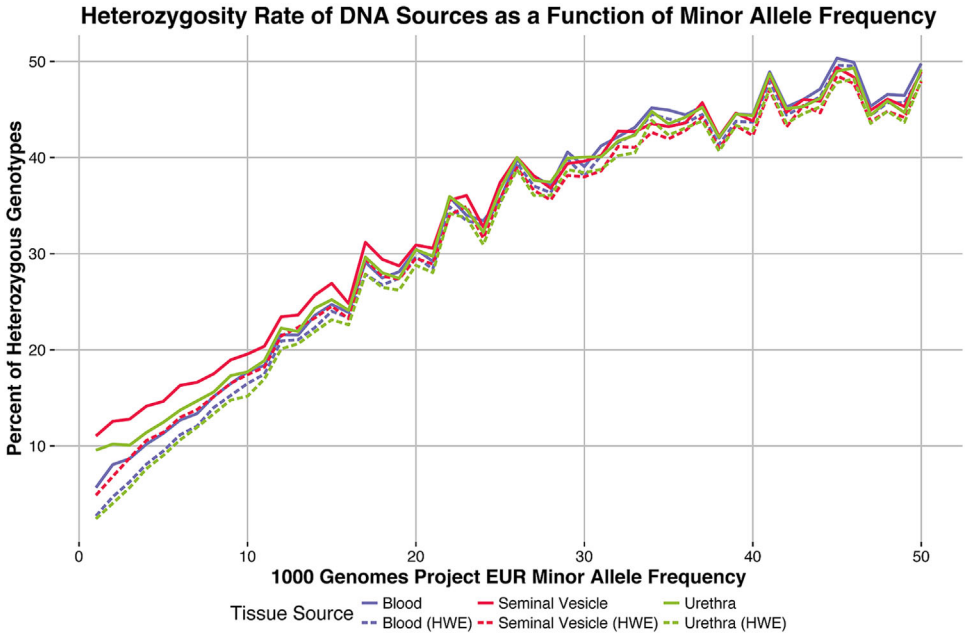

Fig. 2. Heterozygosity rate for blood, urethra, and seminal vesicle genotypes over a range of variant minor allele frequency bins, before (solid) and after (dashed) Hardy–Weinberg equilibrium filtering.

($P = 0.12$), suggesting that the observed difference in call rate was not merely a consequence of DNA quality and may reflect physiological differences between normal tissue sources.

Genotype Concordance Across Subject-Level Factors

Furthermore, UR genotypes were more concordant with blood than SV genotypes (94.1% vs. 92.5%, $P = 0.035$). To determine whether certain subject-level factors may explain this 1.6% concordance difference between UR and SV, we considered the potential confounding effect of specific variables on concordance. First, we stratified concordance estimates by subject age at diagnosis and found that the superiority of UR genotype concordance was consistent across age groups (Table I). Next, we stratified concordance with respect to two variables significantly associated ($P < 0.05$) with UR and SV concordance differences in a linear regression model: prostate specific antigen (PSA) level at diagnosis and the source DNA quantity difference (post-amplification) between UR and SV. Again, we found that concordance for UR was slightly better than for SV across all strata. This included the first and second quartiles of DNA quantity differences, where SV DNA was more abundant than UR DNA in all samples (Q1) or in the majority (Q2), although these subsets contained rather few counts and the differences therein were thus not individually significant. Finally, we stratified concordance with respect to two clinical variables of interest: pathologic Gleason score and pathologic T stage. These variables generally reflected the trend of higher concordance of UR with blood, with the exception of Gleason 7 ($4 + 3$), which was comprised of a small sample size of only two subjects. These observations support the notion that true differences between UR and SV tissue, rather than confounding by other factors, underlie the observed differences in genotype concordance with blood.

We also examined whether cigarette smoking status at diagnosis may have impacted our results. Smoking was categorized into three levels: never (18 subjects), past (8), and current (3). One current smoker at diagnosis had 17.1% higher genotype concordance between UR and blood than between SV and blood, by far the greatest concordance difference among all studied subjects. When this subject was removed from our analysis, the pairwise difference in concordance among the remaining 28 subjects weakened but remained statistically significant (94.0% vs. 92.9%; $P = 0.04$), and the concordance of UR with blood still exceeded that of SV concordance across all rows in Table I from which the subject was omitted.

We additionally identified another potential outlier subject for whom concordance between UR and blood was 57.4%, concordance between SV and blood was 56.9%, and concordance between UR and SV genotypes was 97.7%. Removal of this subject did not impact the statistical significance of UR and SV concordance differences ($P = 0.037$). However, it did increase the average concordance levels for UR and SV to 95.4% and 93.7%, respectively. Core punch slides for these two subjects were reviewed and revealed no tumor contamination, dysplasia, or general explanation for why these samples would have such poor concordance with blood.

Genotype Concordance Across Variant-Level Factors

We examined the concordance levels in different minor allele frequency (MAF) bins across all genotyped markers (total of 416,047 probesets, including previously filtered rare variants with MAF <5%). We found that genotype concordance for UR samples exceeded that of SV samples across the MAF spectrum ($P = 8.2 \times 10^{-14}$; Fig. 1). In most cases, the margin of concordance differences within a given bin approached or exceeded one percent, reflecting the 1.6% difference observed over all common polymorphisms. However, while the trend of superior concordance of UR was maintained over all MAF bins, the margin narrowed substantially in two bins: MAF <1% (+0.38%) and 1% ≤MAF <2% (+0.66%). One explanation for the observation of decreased concordance with blood and smaller differences in concordance between UR and SV in rare variants is simply a lack of variation, and hence potential differences, at such low MAFs. Another possible explanation is genotype misclustering: as the minor allele count at a given marker approaches zero, genotype clustering algorithms face the substantial difficulty of distinguishing heterozygotes from major allele homozygotes. This in turn contributes to errant clustering, whereby major allele homozygotes are incorrectly classified as heterozygotes and minor allele homozygotes, increasing the rate of heterozygosity. Accordingly, we observed a significant excess of heterozygosity in UR ($P = 8.8 \times 10^{-13}$) and SV ($P = 6.3 \times 10^{-18}$) in comparison with blood (Fig. 2) as well as an increasing proportion of samples with discordant genotypes in markers of decreased MAF (Figs. S1A and B). Moreover, SV heterozygosity significantly exceeded that of UR ($P = 4.7 \times 10^{-19}$) across the MAF spectrum and, as the difference between UR and SV heterozygosity narrowed in bins of increasing MAF, the difference in their concordance with blood simultaneously increased (Pearson's

r = −0.67, 95% CI [−0.80, −0.49], $P = 8.5 \times 10^{-8}$), suggesting that genotype misclustering may explain the narrower margins of concordance between UR and SV in rare variants.

To control for the effect of poor genotype clustering in rare variants, variant quality control was performed by Hardy–Weinberg equilibrium (HWE) filtering. When variants violating HWE were removed ($\alpha = 5 \times 10^{-5}$), heterozygosity for all tissue sources decreased significantly ($P < 5 \times 10^{-19}$) towards expected levels. However, heterozygosity of SV genotypes remained elevated in comparison with UR ($P = 7.2 \times 10^{-21}$) and blood ($P = 1.4 \times 10^{-13}$), suggesting that the superior concordance of UR and blood is not simply an artifact of poor genotype clustering (Fig. 2). This conclusion was further supported upon reexamination of concordance after HWE filtering, with UR concordance more clearly separated from SV concordance across all MAF categories ($P = 1.2 \times 10^{-36}$; Fig. S2A). Finally, while the differences between UR and SV call rate ($P = 0.0010$) and genotype concordance ($P = 0.037$) did not change substantially after HWE filtering, the overall genotype concordance with blood across the set of HWE filtered markers increased to 95.4% for UR and 94.0% for SV (Table I). These concordance figures for UR and SV increased to 96.6% and 95.4%, respectively, when increased stringency was applied to HWE filtering ($\alpha = 0.05$; Fig. S2B). However, while more stringent variant quality control can increase the accuracy of FFPE tissue genotype calls in comparison with blood, there exists a tradeoff between increasing concordance and potentially eliminating large numbers of accurate genotype calls from the final dataset.

Finally, further examination of the classes of discordant genotype calls confirmed the trend of excess heterozygosity in the genotypes from UR and SV tissue. Among all genotyped variants (common and rare), the percentage of discordant genotypes switching from a homozygous call in blood to a heterozygous call was 69.8% for UR and 71.5% for SV. For both tissues, the next most frequent change among discordant genotypes was in the opposite direction, from heterozygous to homozygous (27.5% for UR, 26.1% for SV). To assess whether changes in copy number or loss of heterozygosity may contribute to the observed genotype discordances, we used the Affymetrix CNV Summary Tools Software package to examine copy number in each sample set (blood, SV, and UR) and calculate B allele frequencies for each sample. When considering the deviation of the B allele frequencies from expected diploid allelic ratios (1.0, 0.5, 0.0), we found that the genome-wide variance of this deviation was significantly greater for UR ($P = 7.1 \times 10^{-4}$) and SV ($P = 7.9 \times 10^{-8}$) in comparison with blood, and was also greater for SV than for UR ($P = 0.01$). Thus, significant differences were observed between DNA from FFPE tissue and blood DNA, and, more remarkably, between DNA from FFPE seminal vesicle and urethral tissue. Increased noise in the raw fluorescent intensities used to derive B allele frequencies (and genotype calls) may account for these increases in allelic fraction variance. However, it is also possible that there are true differences in copy number between these different DNA sources.

DISCUSSION

In this study, we evaluated the differences between sources of FFPE normal tissue from prostate cancer patients in assaying germline genetics and found that urethral tissue performs more favorably than seminal vesicle tissue in relation to patient-matched whole blood. While germline DNA from normal seminal vesicle tissue may serve as an adequately concordant proxy for blood DNA in the absence of alternatives, genotype measurements derived from urethral tissue DNA exhibited significantly higher call rate, lower heterozygosity, and greater concordance with blood in comparison with seminal vesicle derived genotypes. Although blood remains the ideal biospecimen for genomic analysis, normal tissue may serve as a suitable replacement, in particular for retrospective and tumor-normal studies when a blood specimen can no longer be obtained.

Although studies have revealed substantial technical reproducibility (generally exceeding 99%) among DNA biospecimens (including blood, FFPE tissue, saliva, and fresh frozen vs. FFPE tissue) genotyped in replicate [25–28], our findings suggest that significant heterogeneity may exist between genotype calls derived from different tissues. In general, special attention should be placed on the choice of normal tissue for germline genotyping, as distinct normal tissues may yield substantially different results. This insight may have particular relevance to tumor-normal analyses such as whole genome and exome sequencing, array comparative genomic hybridization (aCGH), and RNA-seq, where the discovery of somatic aberrations in tumors is often predicated on the comparison to FFPE normal tissue as a reference [15,29–31]. Consequently, inaccuracies in germline measurements may lead to miscalled somatic mutations. While our results are based on data from a microarray genotyping platform, further study may determine that systematic differences among normal tissue sources potentially influence the results of next generation sequencing analyses.

There are several explanations for why genotype calls may vary significantly between normal tissue

sources. One potential source of heterogeneity is somatic mosaicism, whereby mutations arising during development and aging propagate into specific tissues. Although the variability of somatic mutation rates among normal tissues is supported by observed differences in somatic mutation frequencies across tumor types [32], the expected number of somatic mutations is relatively modest when considering the generational human germline mutation rate [33]. Additionally, while studies of genome-wide somatic copy number mosaicism have discovered heterogeneity in several tissues, the size and number of validated somatic copy number variants suggests that structural variation may play only a minor role in germline genotype discordance across tissues [34]. Another potential source of heterogeneity is the differential invasion of the glands and ducts peripheral to the prostate: if one tissue is particularly susceptible to prostate tumor cell invasion, the purity of the DNA extracted from that tissue may be compromised and impact genotype call rate and concordance. While prostate cancer can metastasize to the urethra in rare cases, roughly 10–18% of patients having undergone radical prostatectomy are estimated to have pathological seminal vesicle invasion [35]. In our study, however, the majority of subjects were designated as pathologic T-stage 2 (Table I), and thus tumor cell invasion would not be expected to influence peripheral tissues. Furthermore, while field effects are known to influence many different classes of aberrations in tumor-adjacent, normal tissue, including epigenetic, genotypic, cytogenetic, and morphological changes [13,36,37], the extent to which field effects differ between different tumor-adjacent tissues has not been well characterized. The contributions of each of these determinants of heterogeneity and mosaicism to genotype discordance among normal radical prostatectomy tissues are subject to future research.

Finally, this work represents a novel application of the Affymetrix Axiom microarray technology to FFPE urethra and seminal vesicle tissue genotyping. Despite documented issues with purification of DNA fragments longer than 100–200 base pairs from formalin cross-linked tissue, researchers have been able to successfully profile FFPE samples that are up to 30 years old [38]. Furthermore, a recent study found expression profiles from paired fresh frozen and FFPE samples to be highly correlated, both between those newly collected and others archived 14 years earlier [39]. Although there is a tendency for sample degradation to increase with storage time, DNA isolated from FFPE tissue remains relatively intact, further demonstrating the potential to study the large numbers of samples stored in hospitals and tissue banks worldwide. Still, not all samples are equal, and for the purposes of obtaining the best quality DNA for germline genotyping from radical prostatectomy tissues, our findings suggest that urethral tissue DNA is preferential to that of the seminal vesicle.

CONCLUSIONS

By comparing germline genotype concordance between different sources of tissue from radical prostatectomy specimens, we found that various normal tissue sources may in fact have different levels of concordance with blood. Urethral tissue genotypes exhibited not only increased genotype call rate, but also increased genotype concordance with blood in comparison with seminal vesicle derived genotypes when controlling for subject-level and variant-level factors. These findings motivate characterization of different sources of genetic heterogeneity and mosaicism in radical prostatectomy normal tissues and highlight the importance of identifying the source of normal tissue that produces the greatest validity for any given biomarker assay, including microarray, genotyping, and tumor-normal sequencing.

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REFERENCES

1. Grönberg H, Adolfsson J, Aly M, Nordström T, Wiklund P, Brandberg Y, Thompson J, Wiklund F, Lindberg J, Clements M, Egevad L, Eklund M. Prostate cancer screening in men aged 50–69 years (STHLM3): A prospective population-based diagnostic study. Lancet Oncol 2015;16(16):1667–1676.
2. Vachon CM, Pankratz VS, Scott CG, Haeberle L, Ziv E, Jensen MR, Brandt KR, Whaley DH, Olson JE, Heusinger K, Hack CC, Jud SM, Beckmann MW, Schulz-Wendtland R, Tice JA, Norman AD, Cunningham JM, Purrington KS, Easton DF, Sellers TA, Kerlikowske K, Fasching PA, Couch FJ. The contributions of breast density and common genetic variation to breast cancer risk. J Natl Cancer Inst 2015;107(5):dju397.
3. Kvale MN, Hesselson S, Hoffmann TJ, Cao Y, Chan D, Connell S, Croen LA, Dispensa BP, Eshragh J, Finn A, Gollub J, Iribarren C, Jorgenson E, Kushi LH, Lao R, Lu Y, Ludwig D, Mathauda GK, McGuire WB, Mei G, Miles S, Mittman M, Patil M, Quesenberry CP, Ranatunga D, Rowell S, Sadler M, Sakoda LC, Shapero M, Shen L, Shenoy T, Smethurst D, Somkin CP, Van Den Eeden SK, Walter L, Wan E, Webster T, Whitmer RA, Wong S, Zau C, Zhan Y, Schaefer C, Kwok PY, Risch N. Genotyping

informatics and quality control for 100, 000 subjects in the genetic epidemiology research on adult health and aging (GERA) cohort. Genetics 2015;200(4):1051–1060.

4. Roden DM, Pulley JM, Basford MA, Bernard GR, Clayton EW, Balser JR, Masys DR. Development of a large-scale de-identified DNA biobank to enable personalized medicine. Clin Pharmacol Ther 2008;84(3):362–369.
5. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455(7216):1061–1068.
6. Vos HI, van der Straaten T, Coenen MJ, Flucke U, te Loo DM, Guchelaar HJ. High-quality genotyping data from formalin-fixed, paraffin-embedded tissue on the drug metabolizing enzymes and transporters plus array. J Mol Diagn 2015;17(1):4–9.
7. Yu YP, Michalopoulos A, Ding Y, Tseng G, Luo JH. High fidelity copy number analysis of formalin-fixed and paraffin-embedded tissues using Affymetrix Cytoscan HD chip. PLoS ONE 2014;9(4):e92820.
8. Cannon-Albright LA, Cooper KG, Georgelas A, Bernard PS. High quality and quantity genome-wide germline genotypes from FFPE normal tissue. BMC Res Notes 2011;4:159.
9. Cheng L, Song SY, Pretlow TG, Abdul-Karim FW, Kung HJ, Dawson DV, Park WS, Moon YW, Tsai ML, Linehan WM, Emmert-Buck MR, Liotta LA, Zhuang Z. Evidence of independent origin of multiple tumors from patients with prostate cancer. J Natl Cancer Inst 1998;90(3):233–237.
10. Bostwick DG, Qian J, Frankel K. The incidence of high grade prostatic intraepithelial neoplasia in needle biopsies. J Urol 1995;154(5):1791–1794.
11. Jung SH, Shin S, Kim MS, Baek IP, Lee JY, Lee SH, Kim TM, Lee SH, Chung YJ. Genetic progression of high grade prostatic intraepithelial neoplasia to prostate cancer. Eur Urol 2016; 69(5):823–830.
12. Epstein JI, Grignon DJ, Humphrey PA, McNeal JE, Sesterhenn IA, Troncoso P, Wheeler TM. Interobserver reproducibility in the diagnosis of prostatic intraepithelial neoplasia. Am J Surg Pathol 1995;19(8):873–886.
13. Chai H, Brown RE. Field effect in cancer-an update. Ann Clin Lab Sci 2009;39(4):331–337.
14. Lindquist KJ, Paris PL, Hoffmann TJ, Cardin NJ, Kazma R, Mefford JA, Simko JP, Ngo V, Chen Y, Levin AM, Chitale D, Helfand BT, Catalona WJ, Rybicki BA, Witte JS. Mutational landscape of aggressive prostate tumors in african american men. Cancer Res 2016;76(7):1860–1868.
15. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163(4):1011–1025.
16. Kumar A, White TA, MacKenzie AP, Clegg N, Lee C, Dumpit RF, Coleman I, Ng SB, Salipante SJ, Rieder MJ, Nickerson DA, Corey E, Lange PH, Morrissey C, Vessella RL, Nelson PS, Shendure J. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci USA 2011;108(41):17087–17092.
17. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature 2012; 489(7414):57–74.
18. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 2015;12(3):e1001779.
19. Hoffmann TJ, Kvale MN, Hesselson SE, Zhan Y, Aquino C, Cao Y, Cawley S, Chung E, Connell S, Eshragh J, Ewing M, Gollub J, Henderson M, Hubbell E, Iribarren C, Kaufman J, Lao RZ, Lu Y, Ludwig D, Mathauda GK, McGuire W, Mei G, Miles S, Purdy MM, Quesenberry C, Ranatunga D, Rowell S, Sadler M, Shapero MH, Shen L, Shenoy TR, Smethurst D, Van den Eeden SK, Walter L, Wan E, Wearley R, Webster T, Wen CC, Weng L, Whitmer RA, Williams A, Wong SC, Zau C, Finn A, Schaefer C, Kwok PY, Risch N. Next generation genome-wide association tool: design and coverage of a high-throughput European-optimized SNP array. Genomics 2011;98(2):79–89.
20. Hoffmann TJ, Zhan Y, Kvale MN, Hesselson SE, Gollub J, Iribarren C, Lu Y, Mei G, Purdy MM, Quesenberry C, Rowell S, Shapero MH, Smethurst D, Somkin CP, Van den Eeden SK, Walter L, Webster T, Whitmer RA, Finn A, Schaefer C, Kwok PY, Risch N. Design and coverage of high throughput genotyping arrays optimized for individuals of East Asian, African American, and Latino race/ethnicity using imputation and a novel hybrid SNP selection algorithm. Genomics 2011;98(6):422–430.
21. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature 2015;526(7571):68–74.
22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(3):559–575.
23. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015.
24. Wickham H. ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag; 2009.
25. Abraham JE, Maranian MJ, Spiteri I, Russell R, Ingle S, Luccarini C, Earl HM, Pharoah PP, Dunning AM, Caldas C. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BMC Med Genomics 2012;5:19.
26. Hong H, Xu L, Liu J, Jones WD, Su Z, Ning B, Perkins R, Ge W, Miclaus K, Zhang L, Park K, Green B, Han T, Fang H, Lambert CG, Vega SC, Lin SM, Jafari N, Czika W, Wolfinger RD, Goodsaid F, Tong W, Shi L. Technical reproducibility of genotyping SNP arrays used in genome-wide association studies. PLoS ONE 2012;7(9):e44483.
27. Wang Y, Carlton VE, Karlin-Neumann G, Sapolsky R, Zhang L, Moorhead M, Wang ZC, Richardson AL, Warren R, Walther A, Bondy M, Sahin A, Krahe R, Tuna M, Thompson PA, Spellman PT, Gray JW, Mills GB, Faham M. High quality copy number and genotype data from FFPE samples using Molecular Inversion Probe (MIP) microarrays. BMC Med Genomics 2009;2:8.
28. Zhang S, Tan IB, Sapari NS, Grabsch HI, Okines A, Smyth EC, Aoyama T, Hewitt LC, Inam I, Bottomley D, Nankivell M, Stenning SP, Cunningham D, Wotherspoon A, Tsuburaya A, Yoshikawa T, Soong R, Tan P. Technical reproducibility of single-nucleotide and size-based DNA biomarker assessment using DNA extracted from formalin-fixed, paraffin-embedded tissues. J Mol Diagn 2015;17(3):242–250.
29. Wang M, Escudero-Ibarz L, Moody S, Zeng N, Clipson A, Huang Y, Xue X, Grigoropoulos NF, Barrans S, Worrillow L, Forshew T, Su J, Firth A, Martin H, Jack A, Brugger K, Du MQ. Somatic mutation screening using archival formalin-Fixed, paraffin-Embedded tissues by fluidigm multiplex PCR and illumina sequencing. J Mol Diagn 2015;17(5):521–532.

30. Fisher KE, Zhang L, Wang J, Smith GH, Newman S, Schneider TM, Pillai RN, Kudchadkar RR, Owonikoko TK, Ramalingam SS, Lawson DH, Delman KA, El-Rayes BF, Wilson MM, Sullivan HC, Morrison AS, Balci S, Adsay NV, Gal AA, Sica GL, Saxe DF, Mann KP, Hill CE, Khuri FR, Rossi MR. Clinical validation and implementation of a targeted next-Generation sequencing assay to detect somatic variants in non-Small cell lung, melanoma, and gastrointestinal malignancies. J Mol Diagn 2016;18(2):299–315.
31. Clynick B, Tabone T, Fuller K, Erber W, Meehan K, Millward M, Wood BA, Harvey NT. Mutational analysis of BRAF inhibitor-associated squamoproliferative lesions. J Mol Diagn 2015; 17(6):644–651.
32. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjörd JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jäger N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, López-Otín C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdés-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR. Australian Pancreatic Cancer Genome Initiative, ICGC Breast Cancer Consortium, ICGC MMML-Seq Consortium, ICGC PedBrain, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR. Signatures of mutational processes in human cancer. Nature 2013;500(7463):415–421.
33. Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, Al Turki S, Dominiczak A, Morris A, Porteous D, Smith B, Stratton MR, UK10K Consortium, Hurles ME. Timing, rates and spectra of human germline mutation. Nat Genet 2016; 48(2):126–133.
34. O'Huallachain M, Karczewski KJ, Weissman SM, Urban AE, Snyder MP. Extensive genetic variation in somatic human tissues. Proc Natl Acad Sci USA 2012;109(44):18018–18023.
35. Lee HM, Solan MJ, Lupinacci P, Gomella LG, Valicenti RK. Long-term outcome of patients with prostate cancer and pathologic seminal vesicle invasion (pT3b): Effect of adjuvant radiotherapy. Urology 2004;64(1):84–89.
36. Teschendorff AE, Gao Y, Jones A, Ruebner M, Beckmann MW, Wachter DL, Fasching PA, Widschwendter M. DNA methylation outliers in normal breast tissue identify field defects that are enriched in cancer. Nat Commun 2016;7:10478.
37. Troester MA, Hoadley KA, D'Arcy M, Cherniack AD, Stewart C, Koboldt DC, Robertson AG, Mahurkar S, Shen H, Wilkerson MD, Sandhu R, Johnson NB, Allison KH, Beck AH, Yau C, Bowen J, Sheth M, Hwang ES, Perou CM, Laird PW, Ding L, Benz CC. DNA defects, epigenetics, and gene expression in cancer-adjacent breast: a study from The Cancer Genome Atlas. Npj Breast Cancer 2016;2:16007.
38. Blow N. Tissue preparation: tissue issues. Nature 2007; 448(7156):959–963.
39. Hedegaard J, Thorsen K, Lund MK, Hein AM, Hamilton-Dutoit SJ, Vang S, Nordentoft I, Birkenkamp-Demtröder K, Kruhøffer M, Hager H, Knudsen B, Andersen CL, Sørensen KD, Pedersen JS, Ørntoft TF, Dyrskjøt L. Next-generation sequencing of RNA and DNA isolated from paired fresh-frozen and formalin-fixed paraffin-embedded samples of human cancer and normal tissue. PLoS ONE 2014;9(5):e98187.

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Association between a 17-gene genomic prostate score and multi-parametric prostate MRI in men with low and intermediate risk prostate cancer (PCa)

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Abstract

Background

We aimed to directly compare results from multi-parametric prostate MRI (mpMRI) and a biopsy-based 17-gene RT-PCR assay providing a Genomic Prostate Score (GPS) among individuals who were candidates for active surveillance with low and intermediate risk prostate cancer (PCa).

Patients and methods

We evaluated the association between GPS results (scale 0–100) and endorectal mpMRI findings in men with clinically localized PCa. MR studies were reviewed to a five-tier scale of increasing suspicion of malignancy. Mean apparent diffusion coefficient (ADC) was calculated from a single dominant lesion. Mean rank of the GPS (0–100) among MRI strata was compared with the Kruskal-Wallis test and Dunn's multiple comparison test. Spearman's correlation was performed to examine the association between mean ADC and scaled GPS.

Results

Of 186 patients who received GPS testing, 100 were identified who received mpMRI. Mean GPS results differed between mpMRI categories ($p = 0.001$); however a broad range was observed in all mpMRI categories. Among men with biopsy Gleason pattern 3+3, mean GPS results were not significantly different among MRI groups ($p = 0.179$), but GPS differences were seen among MRI categories for patients with pattern 3+4 ($p = 0.010$). Mean ADC was weakly associated with GPS ($\sigma = -0.151$). Stromal response ($p = 0.015$) and cellular organization ($p = 0.045$) gene group scores differed significantly by MRI findings, but no differences were seen among androgen signaling or proliferation genes.

Conclusions

Although a statistically significant association was observed between GPS results and MRI scores, a wide range of GPS values were observed across imaging categories suggesting that mpMRI and genomic profiling may offer non- overlapping clinical insights.

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Introduction

A majority of men diagnosed with prostate cancer (PCa) have what appears to be low and intermediate-risk disease at presentation on the basis of clinical and pathological factors[1]. Yet the performance of even best clinical prediction instruments will mischaracterize a proportion of men who harbor occult, higher grade or stage disease[2]. The ability to offer improved risk stratification among such men is therefore important as such efforts may better shape the trajectory of initial management, a decision choice increasingly defined by active surveillance (AS) versus immediate treatment[3]. To this end, both tissue-based gene expression assays and multi-parametric prostate magnetic resonance imaging (mpMRI) have received considerable attention for the potential to add predictive value beyond conventional clinical models to determine the presence of high grade or non-organ confined disease and are gaining utilization in early disease management[4–7].

Biopsy based assays reflecting the quantitative expression of genes associated with tumor aggressiveness have demonstrated predictive value for adverse pathology at radical prostatectomy (RP), as well as downstream oncologic endpoints including biochemical recurrence (BCR) and metastatic progression[8]. In validation studies, these tools have added predictive performance that exceeds conventional clinical and pathological variables[5]. Similarly, high resolution MRI examining multiple imaging parameters appears to offer anatomic and biological insights into tumor stage and grade that offer higher degrees of accuracy with regard to clinical staging, tumor localization, and the likelihood of adverse downstream events[9, 10].

It is unclear, however, whether these modalities offer congruent or independent biologic information. To date, no published studies exist that compare the directionality of these tests in the same subjects. Specifically, it is unknown whether men with adverse findings on MRI will derive further benefit from tissue-based assays; or conversely, whether MRI will add meaningful information to those who have already had tissue based testing. In this context, we sought to evaluate the association between mpMRI findings and a 17- gene GPS among men with clinically favorable PCa following initial diagnosis. We hypothesized that a strong correlation would exist between MRI findings and GPS results.

Patients and methods

Patient selection

Under the University of California San Francisco (UCSF) institutional review board approval, we retrospectively identified all consenting patients with low or intermediate-risk PCa who underwent a 3T endorectal coil mpMRI and a biopsy-based RT-PCR assay (Onco*type* DX® Prostate Assay) providing a Genomic Prostate Score (GPS) as a measure of tumor aggressiveness within a maximum of two years between studies. Patient records were de-identified and analyzed anonymously. Among patients with initial biopsy at our institution diagnostic biopsies were performed using extended sextant systematic sampling techniques including a minimum of 12 cores; those performed at referring centers were reviewed by genitourinary pathologists to establish the Gleason score (GS) and volume of disease. Disease risk was defined using the validated Cancer of the Prostate Risk Assessment score (UCSF-CAPRA)[11].

mpMRI tests were obtained at the discretion of the providers as a local staging tool for men with early stage disease who were considering or enrolled in AS to establish disease stability. MR sequences included T2, high B-value diffusion-weighted imaging (DWI), MR spectroscopic imaging (MRSI) and dynamic contrast enhancement (DCE)[12]. Scans were acquired on a 3-Tesla scanner (GE Healthcare, Waukesha, WI) using the body coil for excitation and an endorectal coil (E-Coil, Medrad, Pittsburgh, PA) filled with perfluorocarbon and a phased-array coil for reception. Images were post-processed to compensate for the reception profile of the endorectal coil. All MRI studies were re-reviewed by a genitourinary radiologist with 10 years of experience blinded to the biopsy and GPS results and graded on a 1–5 scale of increasing suspicion of malignancy, a modification of the PI-RADS version 1 system (Table 1). The mean apparent diffusion coefficient (ADC) was calculated from a single dominant lesion[13]. The combination of MR imaging and genomic profiling was routinely recommended for men with low and intermediate clinical risk disease prior to enrollment in AS as a means of refined risk assessment.

T2 –Peripheral Zone			
1	Homogeneous high SI		
2	Streaky, triangular, geographic areas of low SI		
3	Not 1/2 not 3/4		
4	Discrete nodule of low SI		
5	Same as 4 + evidence of ECE and/or > 1.5 cm of capsular contact		
T2 –Transitional Zone			
1	Heterogeneous SI, "organized chaos"		
2	Foci of low SI, well marginated /encapsulated		
3	Not 1/2 not 3/4		
4	Foci of homogeneous low SI, ill defined		
5	Same as 4 + evidence of ECE and/or > 1.5 cm of capsular contact		
Diffusion Weighted Imaging (DWI)			
	High B value DWI	*ADC*	*ADC Value*
1	Iso SI	Iso SI	N/A
2	High SI	Iso SI	N/A
3	Iso SI	Low SI	N/A
4	High SI	Low SI	> 850
5	High SI	Low SI	< 850
Magnetic Resonance Spectroscopy Imaging (MRSI)			
1	Citrate peak 2 x > choline peak		
2	Citrate peak 1–2 x > choline peak		
3	Citrate peak = choline peak		
4	Choline peak 1–2 x > citrate peak		
5	Choline peak 2 x > citrate peak		
Dynamic Contrast Enhancement (DCE)			
+	Asymmetric early enhancement with quick washout or plateau		
-	Other patterns of enhancement		

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Table 1. Multi-parametric prostate MRI scoring rubric.
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The OncotypeDX Prostate Cancer assay (Genomic Health, Inc., Redwood City, CA) was performed using RNA extracted from fixed paraffin-embedded diagnostic prostate needle biopsies. This biopsy-based RT-PCR assay generates a Genomic Prostate Score (GPS–scaled 0–100) as a biologic measure of tumor aggressiveness, and has been clinical validated as an independent predictor of favorable surgical pathology (surgical GS <4+3 and organ-confined disease) [5] [14]. The GPS represents a weighted calculation of a 17-gene expression signature including 12 genes highly associated with prostate cancer recurrence and metastases and five reference genes to control for RNA quality and quantity[5] [15]. The four constituent gene groups represented in the GPS include androgen signaling (AZGP1, KLK2, SRD5A2, RAM13C), stromal response (BGN, COL1A1, SFRP4), cellular organization (FLNC, GSN, TPM2, GSTM2) and proliferation (TPX2).

Statistical analysis

Our primary objective was to evaluate the association between mpMRI findings and GPS results among an observational cohort of men with clinically localized prostate cancer; we compared the mean rank of the scaled GPS (0–100) across MRI results characterized as negative (score 1–2); indeterminate (3); or positive/suspicious (4–5) using the Kruskal-Wallis test. To examine differences among groups, we further performed a post hoc pairwise analysis using Dunn's multiple comparison test, a method that retains the dependent ranking produced by the Kruskal-Wallis test statistic and incorporates the pooled variance estimate and also preserves the family-wise error rate by using adjusted significance level defined as $\alpha/(k(k-1))$, where k is the number of comparisons[16]. To examine particular associations among individual gene groups with MR findings we repeated the Kruskal-Wallis analyses within expression scores for all gene groups. As diffusion weighted imaging (DWI) been proposed as a quantitative measurement associated with tumor aggressiveness, we further sought to examine the association between mean apparent diffusion coefficient (ADC) and GPS using Spearman's correlation[17]. Analysis was performed among all pooled 100 patients, as well as stratified by CAPRA risk category and biopsy GS 3+3 or 3+4. Statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, North Carolina, USA).

Results

Among 186 consented men who have undergone GPS testing at our institution, we identified 100 with mpMRI within a two year window of genomic testing. Compared with men undergoing combined MRI and GPS testing those undergoing GPS testing alone were similarly matched with regard to baseline demographic and disease characteristics. Among patients receiving both MRI and GPS median age was 62.5 years and median PSA was 5.6 ng/mL (IQR 4.3–8.3). Biopsies included a median of 16 cores (interquartile range, IQR 13–19), and a median of three cores were positive for cancer (IQR 1–4). 53 men had biopsy GS3+3 and 47 GS 3+4. The majority of patients (n = 63) sought initial management with AS while 41 ultimately underwent treatment with radical prostatectomy. The complete clinical and pathologic features are presented in Table 2.

Patient Characteristics	Value	Statistic
Age (Years), Median (IQR)		62.5 (55.0–68.0)
PSA (ng/ml), Median (IQR)		5.6 (4.3–8.3)
Prostate volume (cm^3), Median (IQR)		38.0 (28.0–47.0)
PSA density, Median (IQR)		0.138 (0.098–0.189)
Race/ethnicity, N (%)*	Asian/Pacific Islander	2 (2.2)
	White	80 (86.0)
	Other	11 (11.8)
Clinical T-stage, N (%)	T1c	75 (75.0)
	T2	25 (25.0)
# Cores taken, Median (IQR)		16.0 (13.0–19.0)
# Cores positive, Median (IQR)		3.0 (1.0–4.0)
% Cores positive, Median (IQR)		17.0 (8.0–25.0)
% Single core positive, Median (IQR)		28.0 (15.0–44.0)
CAPRA, N (%)*	Low (0–2)	66 (79.5)
	Intermediate (3–5)	16 (19.3)
	High (6–10)	1 (1.2)

Abbreviations: IQR = interquartile range; PSA = prostate-specific antigen; CAPRA = Cancer of the Prostate Risk Assessment

*Due to missing data, totals do not sum to 100 patients

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Table 2. Baseline characteristics of 100 patients receiving multi-parametric prostate MRI and GPS testing.
https://doi.org/10.1371/journal.pone.0185535.t002

MRI findings were negative in 13 patients, indeterminate in 26, and positive in 61. The median GPS was 16 (IQR 13–21) for men with negative, 23 (IQR 14–27) for indeterminate and 28 for positive (IQR 21–34) MRI studies, Fig 1. There was a significant difference in the mean rank of the GPS results among the 3 MRI categories ($p = 0.001$) for the entire group of 100 patients, with mean GPS increasing with increasing MRI category. Nonetheless there was a wide distribution of GPS values within each MRI category. When patients were stratified by biopsy GS, no significant association ($p = 0.18$) was observed between GPS and MRI in patients with GS 3+3 (Fig 2A), however there was a significant association among men with GS 3+4 tumors ($p = 0.010$ –Fig 2B). Post-hoc pairwise comparisons showed significant difference in mean rank of GPS between negative and positive MRI categories ($p<0.001$), however no statistically significant difference was observed between negative and indeterminate or positive and indeterminate results. Among men with high suspicion MRI lesions we evaluated the association between scaled GPS results and meanADC values. A weak trend towards higher GPS results was observed with lower meanADC values, (Rho = -0.151), (Fig 3).

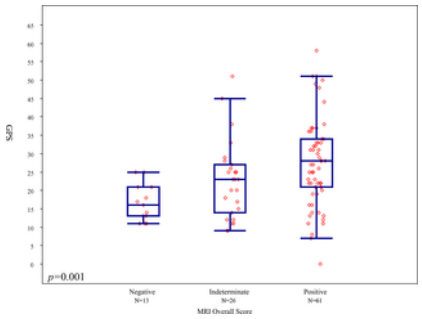

Fig 1. Distribution of GPS scores by MRI findings among all 100 patients.
https://doi.org/10.1371/journal.pone.0185535.g001

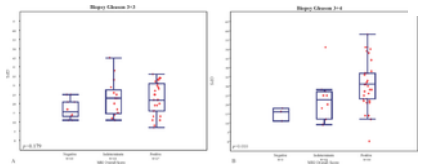

Fig 2.
A. Distribution of GPS results by MRI findings among men with biopsy Gleason grade 3+3 and B. Among patients with Gleason grade 3+4.
https://doi.org/10.1371/journal.pone.0185535.g002

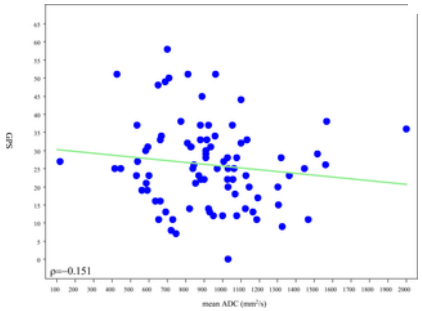

Fig 3. Relationship between meanADC value (mm^2/s) of the dominant MRI lesion and GPS result.
https://doi.org/10.1371/journal.pone.0185535.g003

To determine whether further associations may exist within individual gene pathways measured by GPS and MRI findings, we examined the association of gene group expression levels with MRI findings. Stromal response and cellular organization gene expression scores showed a modest but statistically significant association with MRI category ($p = 0.015$ and $p = 0.045$, respectively), however no significant trends were observed in androgen response or proliferation ($p = 0.101$ and 0.074, respectively). meanADC also exhibited weak association with individual gene groupings (stromal response: Rho = -0.221; cellular organization Rho = -0.01; androgen signaling Rho = 0.106; and proliferation Rho = 0.01). Within each gene pathway, considerable variation in expression levels was observed among all MRI groups, particularly among the 61 patients with category 4 and 5 lesions (Fig 4A–4D).

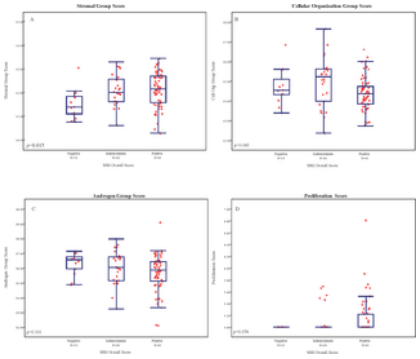

Fig 4.
A-D. Distribution of individual gene group expression levels among mpMRI categories.
https://doi.org/10.1371/journal.pone.0185535.g004

Discussion

The limitations of conventional clinical variables to reliably characterize the extent of disease among men with newly diagnosed PCa have been met with a growing arsenal of tools aimed to improve risk estimation in early stage disease. Genomic signatures have been validated to predict the occurrence of higher grade or stage elements among clinically favorable risk patients, while high resolution mpMRI as both an anatomic and biologically informative modality has received extensive study for the ability to detect significant cancer[4, 5, 18]. Comparative analyses of such advanced risk stratification tools, however, are lacking and it remains unclear whether individuals who have received MR imaging will derive further benefit from genomic testing, or conversely, if imaging may be of benefit among men following tissue-based assays.

In this study, we examined the association between MRI findings and the GPS signature in a cohort of 100 men with clinically favorable risk PCa undergoing evaluation with both modalities. Although a trend towards higher GPS results existed among higher MR-suspicion lesions, we observed considerable variation across all mpMRI findings, suggesting biologic heterogeneity within each of the MRI categories. Among men without MR-evident tumors, GPS results tended to be lower and more narrowly distributed, however this represented a minority of patients in the study (13%). For men with indeterminate or positive MR studies, GPS results ranged significantly, a relationship that persisted after stratification by GS or clinical risk group. Though not directly correlated with patient outcome, these findings suggest that further clinical refinement may be possible among patients beyond MRI findings alone.

The GPS assay is derived from a set of highly predictive genes in a fashion independent of Gleason pattern, and has been validated by independent studies as a predictor of pathological upgrading and/or upstaging among men with favorable risk disease [5] [14]. It has also been shown to predict distant oncological outcomes, including biochemical recurrence and metastatic progression[5, 14]. We speculated that imaging findings would recapitulate tumor gene expression levels: higher suspicion MRI lesions would be associated with higher GPS scores, higher expression levels of stromal response and proliferation genes would be associated with MR-evident disease, and that areas of restricted diffusion would be associated with higher GPS findings. We observed that GPS results were significantly different among patients with negative, indeterminate, or positive MRI scans, and among men with biopsy pattern 3+4, though no significant differences were seen among MRI groups for men with biopsy pattern 3+3. Moreover, stromal response and cellular organization gene expression scores were associated with MR findings, though no associations were seen among androgen signaling or proliferation groups. As prior clinical validation studies have demonstrated significant associations of the four primary gene groupings with clinical outcome, including recurrence, these finding may indicate a novel radiomic basis for stromal response and cellular organization genes and MR pattern. Taken together these findings suggest that a degree of agreement in signal direction exists between MRI and the GPS assay, however the information provided by these two modalities appears to be largely non-overlapping.

The prognostic significance of a visible lesion on mpMRI has been evaluated on several PCa endpoints including the detection of higher grade (Gleason ≥3+4) disease on biopsy[19], progression during AS, high grade/stage pathology at prostatectomy[20], and biochemical recurrence[21]. For men with newly diagnosed PCa, the presence of MRI-discernable lesions has been suggested as an adverse prognostic characteristic particularly for men initiating management with AS, where retrospective studies have suggested an increased risk for disease reclassification due to changes in tumor volume estimates[22]. As MRI studies incorporate multiple sequences believed to reflect anatomic, tumor vascularity, and cellularity it is not known whether higher rates of upgrading reflect improved sampling associated with larger tumors in some men or the occurrence of genuine biological progression.

Though MRI has exhibited the capacity to identify higher Gleason pattern PCa, the genetic mechanisms underlying characteristic MRI findings remain to be fully elucidated. In this study mean, ADC values exhibited weak, negative correlation with overall GPS or constituent gene groups. These findings are notable given prior studies addressing the association of apparent diffusion coefficient (ADC) analysis and prostate cancer aggressiveness[17]. The directionality of the relationship is consistent with the implication of restricted diffusion with tumor aggressiveness, however the strength of the association appears to imply that meanADC analysis does not closely parallel a panel of genes highly associated with PCa aggressiveness.

We included biopsy samples obtained using systematic extended sextant sampling and not direct MR-ultrasound fusion acquisition, reflecting the era of collection at our institution. As a result, we were not able to compare gene expression profiles on a per-lesion basis. In addition, because of a growing appreciation for improved diagnostic yield associated with MR-ultrasound fusion, it is conceivable that some degree of undersampling may be present relative to a targeted method[18] [23]. As the GPS assay has been previously shown to predict prostate cancer aggressiveness in the face of biopsy under-sampling and heterogeneity in Gleason

grade, our approach may hold validity in assessing the broader tumor profile of the gland, though further study is warranted using image-guidance or whole-mount correlation[5]. In this regard, important insights appear to be offered by intriguiging preliminary directed radiomic study matching gene expression to gene expression features. For example, in an analysis of 17 mpMRI-directed diagnostic biopsies, associations were observed between radiographic abnormalities and aberrant gene expression, highlighting a measurable molecular basis for characteristic MR findings [24].

An acknowledged limitation of this study is that our comparison is restricted to the two modalities themselves without direct evaluation of clinical outcomes. This finding reflects the favorable risk nature of the study cohort and the routine use of AS for initial management in appropriate candidates. As a result, only a small proportion received immediate definitive treatment with prostatectomy, which limited our ability to offer a pathological comparison. Additionally, although patients receiving genomic testing with or without MRI were evenly matched, we cannot conclude with certainty that the decision to pursue both MRI and GPS testing in these patients occurred at random. While individuals may opt for multiple modalities of advanced testing due to preference, it is possible that such patients possessed unrecognized clinical complexity which warranted additional evaluation. In addition, all MR images were reviewed by a single radiologist, which may limit reproducibility. We utilized a two-year interval between MRI and GPS testing as inclusion criteria under the assumption of disease stability during this time based on modeling and observational studies, however we cannot exclude that changes in tumor biology may have occurred during this time period[25, 26]. Lastly, the study design did not examine GPS results from biopsies of selected MRI abnormalitites. Given the growing utilization of MRI-ultrasound fusion platforms affording directed sampling of MR-apparent lesions, this limitations prevented us from comparing in genomic profiles based on direct biopsy.

Both tissue based genomic profiling and MRI seek to offer refined clinical staging and risk stratification at the time of diagnosis, including the risk of adverse pathology at the time of surgery. A host of publications support the use of MRI to predict clinical stage, and presence for extraprostatic extension or seminal vesicle invasion [27]. Similarly, the GPS assay has been clinically validated to predict pathologic outcomes among two studies including 732 patients receiving radical prostatectomy[28]. As patients in this study received imaging and genomic profiling in the clinical context of establishing eligibility for AS, a minority (n = 41) underwent treatment with prostatectomy at last follow. As a resut, direct comparisons are limited due to sample size, and selection bias as patients often receive treatment in the context of biopsy upgrade or concern for progression. To optimally detect significant differences in gene expression across five strata of MRI findings corresponding to all PI-RADS classifications, we estimate that over 300 patients would be required. Further, appropriately powered studies are required to assess the relative clinical utility of imaging and genomic profiling in initial risk stratification.

Few other studies have directly compared associations among a new generation of PCa risk prediction tools. Recently, Renard-Penna et al. examined 106 patients treated with RP in whom pre-operative mpMRI findings were compared with genomic testing of tumor tissue with a 31-gene cell progression assay as a measure of aggressiveness, noting associations among tumor size and diffusion abnormalities with adverse cell-cycle progression (CCP) scores[29]. Interestingly, the authors utilized CCP as a benchmark for assessing the performance of preoperative imaging, however, a valuable opportunity remains to compare these modalities, particularly in the pre-treatment setting. As the inventory for such risk refinement aids expands in size and complexity, it is likely that such direct comparisons will be of increasing clinical impact and potentially serve to more efficiently direct resources. Our findings suggest that mpMRI and the 17-gene GPS assay offer predictive information that may be distinctive in many circumstances reflecting intrinsic differences between these tools. Prostate MR scoring systems, including the PI-RADS framework, have been developed and calibrated to detect clinically significant cancer apprised largely on the basis of Gleason score, a powerful though altogether incomplete predictor of disease outcome[30]. In contrast, the genes selected for the GPS assay and their relative weights within the generated score were validated to predict adverse pathologic and oncological events independently of pathological assessment. As a result, we anticipated that heterogeneity in gene expression levels would exist within MRI groups, a finding which underscores the clinical variability within MRI groups.

The intersection of multiple PCa risk refinement tools in early stage PCa is a presently unexplored avenue of investigation with implications for disease management. Our findings, reflecting one commercial assay, demonstrate stratification among MRI findings, yet are, alone, inadequate to dictate definitive clinical sequencing of these tests. Additional comparative studies of imaging and serum detection-oriented biomarkers may also prove fruitful in clarifying optimal diagnostic pathways. Additionally, larger study populations assessing downstream PCa outcomes will be required to assess the optimal sequencing of such emerging modalities.

Conclusion

We compared mpMRI and tissue based gene expression testing among low and intermediate clinical risk patients. Prostate MRI and genomic testing with the GPS assay exhibited weak correlation, suggesting that the two modalities represent independent predictors and may be complementary in guiding patient management.

References

1. Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. J Clin Oncol. 2010;28(7):1117–23. Epub 2010/02/04. pmid:20124165; PubMed Central PMCID: PMCPMC2834465.
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2. Shariat SF, Karakiewicz PI, Suardi N, Kattan MW. Comparison of nomograms with other methods for predicting outcomes in prostate cancer: a critical analysis of the literature. Clin Cancer Res. 2008;14(14):4400–7. Epub 2008/07/17. pmid:18628454.
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3. Thompson I, Thrasher JB, Aus G, Burnett AL, Canby-Hagino ED, Cookson MS, et al. Guideline for the management of clinically localized prostate cancer: 2007 update. The Journal of urology. 2007;177(6):2106–31. Epub 2007/05/19. pmid:17509297.
View Article • PubMed/NCBI • Google Scholar

4. Bishoff JT, Freedland SJ, Gerber L, Tennstedt P, Reid J, Welbourn W, et al. Prognostic utility of the cell cycle progression score generated from biopsy in men treated with prostatectomy. J Urol. 2014;192(2):409–14. Epub 2014/02/11. pmid:24508632.
View Article • PubMed/NCBI • Google Scholar

5. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. European urology. 2014;66(3):550–60. Epub 2014/05/20. pmid:24836057.
View Article • PubMed/NCBI • Google Scholar

6. Somford DM, Hamoen EH, Futterer JJ, van Basten JP, Hulsbergen-van de Kaa CA, Vreuls W, et al. The predictive value of endorectal 3 Tesla multiparametric magnetic resonance imaging for extraprostatic extension in patients with low, intermediate and high risk prostate cancer. J Urol. 2013;190(5):1728–34. Epub 2013/05/18. pmid:23680307.
View Article • PubMed/NCBI • Google Scholar

7. Gondo T, Hricak H, Sala E, Zheng J, Moskowitz CS, Bernstein M, et al. Multiparametric 3T MRI for the prediction of pathological downgrading after radical prostatectomy in patients with biopsy-proven Gleason score 3 + 4 prostate cancer. Eur Radiol. 2014;24(12):3161–70. pmid:25100337.
View Article • PubMed/NCBI • Google Scholar

8. Cullen J, Rosner IL, Brand TC, Zhang N, Tsiatis AC, Moncur J, et al. A Biopsy-based 17-gene Genomic Prostate Score Predicts Recurrence After Radical Prostatectomy and Adverse Surgical Pathology in a Racially Diverse Population of Men with Clinically Low- and Intermediate-risk Prostate Cancer. European urology. 2015;68(1):123–31. Epub 2014/12/04. pmid:25465337.
View Article • PubMed/NCBI • Google Scholar

9. Salami SS, Vira MA, Turkbey B, Fakhoury M, Yaskiv O, Villani R, et al. Multiparametric magnetic resonance imaging outperforms the Prostate Cancer Prevention Trial risk calculator in predicting clinically significant prostate cancer. Cancer. 2014;120(18):2876–82. Epub 2014/06/12. pmid:24917122.
View Article • PubMed/NCBI • Google Scholar

10. Gupta RT, Faridi KF, Singh AA, Passoni NM, Garcia-Reyes K, Madden JF, et al. Comparing 3-T multiparametric MRI and the Partin tables to predict organ-confined prostate cancer after radical prostatectomy. Urol Oncol. 2014;32(8):1292–9. pmid:24863013.
View Article • PubMed/NCBI • Google Scholar

11. Cooperberg MR, Pasta DJ, Elkin EP, Litwin MS, Latini DM, Du Chane J, et al. The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. J Urol. 2005;173(6):1938–42. pmid:15879786; PubMed Central PMCID: PMC2948569.
View Article • PubMed/NCBI • Google Scholar

12. Flavell RR, Westphalen AC, Liang C, Sotto CC, Noworolski SM, Vigneron DB, et al. Abnormal findings on multiparametric prostate magnetic resonance imaging predict subsequent biopsy upgrade in patients with low risk prostate cancer managed with active surveillance. Abdom Imaging. 2014;39(5):1027–35. Epub 2014/04/18. pmid:24740760; PubMed Central PMCID: PMCPMC4169752.
View Article • PubMed/NCBI • Google Scholar

13. Pickles MD, Gibbs P, Sreenivas M, Turnbull LW. Diffusion-weighted imaging of normal and malignant prostate tissue at 3.0T. J Magn Reson Imaging. 2006;23(2):130–4. Epub 2005/12/24. pmid:16374882.
View Article • PubMed/NCBI • Google Scholar

14. Cullen J, Rosner IL, Brand TC, Zhang N, Tsiatis AC, Moncur J, et al. A Biopsy-based 17-gene Genomic Prostate Score Predicts Recurrence After Radical Prostatectomy and Adverse Surgical Pathology in a Racially Diverse Population of Men with Clinically Low- and Intermediate-risk Prostate Cancer. Eur Urol. 2014. pmid:25465337.
View Article • PubMed/NCBI • Google Scholar

15. Knezevic D, Goddard AD, Natraj N, Cherbavaz DB, Clark-Langone KM, Snable J, et al. Analytical validation of the Oncotype DX prostate cancer assay—a clinical RT-PCR assay optimized for prostate needle biopsies. BMC Genomics. 2013;14:690. Epub 2013/10/10. pmid:24103217; PubMed Central PMCID: PMCPMC4007703.
View Article • PubMed/NCBI • Google Scholar

16. Dunn OJ. “Multiple comparisons among means”. Journal of the American Statistical Association. 1961;56(293):52–64.
View Article • Google Scholar

17. Donati OF, Mazaheri Y, Afaq A, Vargas HA, Zheng J, Moskowitz CS, et al. Prostate cancer aggressiveness: assessment with whole-lesion histogram analysis of the apparent diffusion coefficient. Radiology. 2014;271(1):143–52. Epub 2014/01/31. pmid:24475824.
View Article • PubMed/NCBI • Google Scholar

18. Siddiqui MM, Rais-Bahrami S, Turkbey B, George AK, Rothwax J, Shakir N, et al. Comparison of MR/ultrasound fusion-guided biopsy with ultrasound-guided biopsy for the diagnosis of prostate cancer. JAMA. 2015;313(4):390–7. pmid:25626035.
View Article • PubMed/NCBI • Google Scholar

19. Pokorny MR, de Rooij M, Duncan E, Schroder FH, Parkinson R, Barentsz JO, et al. Prospective study of diagnostic accuracy comparing prostate cancer detection by transrectal ultrasound-guided biopsy versus magnetic resonance (MR) imaging with subsequent MR-guided biopsy in men without previous prostate biopsies. Eur Urol. 2014;66(1):22–9. pmid:24666839.
View Article • PubMed/NCBI • Google Scholar

20. Abd-Alazeez M, Ahmed HU, Arya M, Allen C, Dikaios N, Freeman A, et al. Can multiparametric magnetic resonance imaging predict upgrading of transrectal ultrasound biopsy results at more definitive histology? Urol Oncol. 2014;32(6):741–7. Epub 2014/07/02. pmid:24981993.
View Article • PubMed/NCBI • Google Scholar

21. Park JJ, Kim CK, Park SY, Park BK, Lee HM, Cho SW. Prostate cancer: role of pretreatment multiparametric 3-T MRI in predicting biochemical recurrence after radical prostatectomy. AJR Am J Roentgenol. 2014;202(5):W459–65. pmid:24758681.
View Article • PubMed/NCBI • Google Scholar

22. Dianat SS, Carter HB, Pienta KJ, Schaeffer EM, Landis PK, Epstein JI, et al. Magnetic resonance-invisible versus magnetic resonance-visible prostate cancer in active surveillance: a preliminary report on disease outcomes. Urology. 2015;85(1):147–53. pmid:25440986.
View Article • PubMed/NCBI • Google Scholar

23. Filson CP, Natarajan S, Margolis DJ, Huang J, Lieu P, Dorey FJ, et al. Prostate cancer detection with magnetic resonance-ultrasound fusion biopsy: The role of systematic and targeted biopsies. Cancer. 2016;122(6):884–92. Epub 2016/01/11. pmid:26749141; PubMed Central PMCID: PMCPMC4777653.
View Article • PubMed/NCBI • Google Scholar

24. Stoyanova R, Pollack A, Takhar M, Lynne C, Parra N, Lam LL, et al. Association of multiparametric MRI quantitative imaging features with prostate cancer gene expression in MRI-targeted prostate biopsies. Oncotarget. 2016. Epub 2016/07/21. pmid:27438142.
View Article • PubMed/NCBI • Google Scholar

25. Klotz L, Vesprini D, Sethukavalan P, Jethava V, Zhang L, Jain S, et al. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol. 2015;33(3):272–7. pmid:25512465.
View Article • PubMed/NCBI • Google Scholar

26. Inoue LY, Trock BJ, Partin AW, Carter HB, Etzioni R. Modeling grade progression in an active surveillance study. Stat Med. 2014;33(6):930–9. pmid:24123208; PubMed Central PMCID: PMC3955104.
View Article • PubMed/NCBI • Google Scholar

27. Morlacco A, Sharma V, Viers BR, Rangel LJ, Carlson RE, Froemming AT, et al. The Incremental Role of Magnetic Resonance Imaging for Prostate Cancer Staging before Radical Prostatectomy. European urology. 2017;71(5):701–4. Epub 2016/09/01. pmid:27576750.
View Article • PubMed/NCBI • Google Scholar

28. Brand TC, Zhang N, Crager MR, Maddala T, Dee A, Sesterhenn IA, et al. Patient-specific Meta-analysis of 2 Clinical Validation Studies to Predict Pathologic Outcomes in Prostate Cancer Using the 17-Gene Genomic Prostate Score. Urology. 2016;89:69–75. Epub 2016/01/03. pmid:26723180.
View Article • PubMed/NCBI • Google Scholar

29. Renard-Penna R, Cancel-Tassin G, Comperat E, Varinot J, Leon P, Roupret M, et al. Multiparametric Magnetic Resonance Imaging Strongly Predicts Postoperative Pathology but Misses Aggressive Prostate Cancers as Assessed by Cell Cycle Progression Score. J Urol. 2015. Epub 2015/08/15. pmid:26272031.
View Article • PubMed/NCBI • Google Scholar

30. Barentsz JO, Richenberg J, Clements R, Choyke P, Verma S, Villeirs G, et al. ESUR prostate MR guidelines 2012. Eur Radiol. 2012;22(4):746–57. Epub 2012/02/11. pmid:22322308; PubMed Central PMCID: PMCPMC3297750.
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Platinum Priority – Prostate Cancer – Editor's Choice

Editorial by Daniel E. Spratt and Edward M. Schaeffer on pp. 453–454 of this issue

The Diverse Genomic Landscape of Clinically Low-risk Prostate Cancer

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Abstract

Background: Among men with clinically low-risk prostate cancer, we have previously documented heterogeneity in terms of clinical characteristics and genomic risk scores.
Objective: To further study the underlying tumor biology of this patient population, by interrogating broader patterns of gene expression among men with clinically low-risk tumors.
Design, setting, and participants: Prostate biopsies from 427 patients considered potentially suitable for active surveillance underwent central pathology review and genome-wide expression profiling. These cases were compared with 1290 higher-risk biopsy cases with diverse clinical features from a prospective genomic registry.
Outcome measurements and statistical analysis: Average genomic risk (AGR) was determined from 18 published prognostic signatures, and MSigDB hallmark gene sets were analyzed using bootstrapped clustering methods. These sets were examined in relation to clinical variables and pathological and biochemical outcomes using multivariable regression analysis.
Results and limitations: A total of 408 (96%) biopsies passed RNA quality control. Based on AGR quartiles defined by the high-risk multicenter cases, the University of California, San Francisco (UCSF) low-risk patients were distributed across the quartiles as 219 (54%), 107 (26%), 61 (15%), and 21 (5%). Unsupervised clustering analysis of the hallmark gene set scores revealed three clusters, which were enriched for the previously described PAM50 luminal A, luminal B, and basal subtypes. AGR, but not the clusters, was associated with both pathological (odds ratio 1.34, 95% confidence interval [CI] 1.14–1.58) and biochemical outcomes (hazard ratio 1.53, 95% CI 1.19–1.93). These results may underestimate within-prostate genomic heterogeneity.
Conclusions: Prostate cancers that are homogeneously low risk by traditional characteristics demonstrate substantial diversity at the level of genomic expression. Molecular substratification of low-risk prostate cancer will yield a better understanding of its divergent biology and, in the future may help personalize treatment recommendations.
Patient summary: We studied the genomic characteristics of tumors from men diagnosed with low-risk prostate cancer. We found three main subtypes of prostate cancer with divergent tumor biology, similar to what has previously been found in women with breast cancer. In addition, we found that genomic risk scores were associated with worse pathology findings and prostate-specific antigen recurrence after surgery. These results suggest even greater genomic diversity among low-risk patients than has previously been documented with more limited signatures.

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1. Introduction

Among human malignancies, prostate cancer is remarkable both for its pervasiveness and for its exceptionally variable natural history. Roughly one man in six is diagnosed in his lifetime, a high outlier incidence that belies an even higher histological prevalence as indicated by autopsy studies [1]. A large majority of prostate cancers are entirely quiescent, and would never cause any symptoms or loss of life years if undiagnosed; yet the fraction that are more aggressive still amount to the second leading cause of cancer death among men [2]. Recent molecular studies among higher-risk tumors have documented genomic heterogeneity to match prostate cancer's clinical variability [3,4].

Clinical risk stratification of prostate cancer at diagnosis is relatively accurate in identifying cancers unlikely to progress to clinically relevant stages [5], and can be further enhanced through imaging and/or use of prognostic biomarker signatures [6]. Such approaches, however, assess aggressiveness along only one or a few biological axes, and do not allow the sort of molecular subtyping that is now standard for other tumors such as breast cancer. In fact, recent studies of high-risk prostate cancers have identified luminal and basal subtypes that echo those found in breast cancer and other cancers to a remarkable degree [7].

Active surveillance (AS) rather than immediate treatment is now widely endorsed as a preferred management strategy for low-risk prostate cancer [8,9] and is offered to a growing proportion of men both in the USA [10,11] and internationally [12]. An important goal of contemporary investigation into the biology of low-risk prostate cancer is to help determine which of the low-grade prostate cancers are highly aggressive and merit immediate treatment, need close AS, and could be safely followed with a less active monitoring strategy. We aimed to characterize the genomic expression patterns of tumors with relatively homogeneous, low-risk clinical characteristics, to determine whether such tumors are characterized by similarly homogeneous expression patterns, both in terms of genomically determined clinical risk and in terms of tumor subtyping based on broader expression analysis.

2. Patients and methods

Paraffin-embedded prostate biopsy specimens were collected from 427 men who underwent radical prostatectomy at the University of California, San Francisco (UCSF), based on patient treatment preference, for tumors that would have been eligible for AS based on low-risk (clinical stage ≤T2N0M0, prostate-specific antigen [PSA] ≤20 ng/ml, and biopsy grade group 1) or low-volume (≤3 cores positive overall) grade group 2 tumor characteristics. Additional inclusion criteria were at least 1 mm of cancer on biopsy, prostatectomy slides available for review, and provision of informed consent under institutional review board supervision. Clinical risk was summarized using the extensively validated Cancer of the Prostate Risk Assessment (CAPRA) score [13].

Biopsies and prostatectomy slides were centrally reviewed for grade and stage (by J.P.S.), and RNA was extracted as previously described [14]. In cases with multiple positive biopsy cores, the core with the longest length of the highest-grade cancer was selected. RNA from each case was amplified and labeled using the Ovation FFPE WTA system (NuGen, San Carlos, CA, USA) and hybridized to a Human Exon 1.0 ST GeneChip (Affymetrix, Santa Clara, CA, USA).

The UCSF cases in this study, as with similar prior studies of biopsy-based genomic risk assessment, were restricted by design to low/low-intermediate risk cases. For comparison, and to provide a wider dynamic range of genomic risk, we analyzed prostate cancer cases in the Decipher Genomic Resource Information Database (GRID), a prospective, genome-wide expression registry for urological oncology (NCT02609269), which includes basic demographic and baseline clinical information. These cases were profiled as part of clinical care to facilitate a variety of treatment decisions per clinician discretion. All 1290, mostly higher-risk prostate biopsy, cases currently represented in the GRID were included. For GRID cases, the needle biopsy core with the highest grade and percentage of tumor was selected for RNA extraction and purified using the RNeasy FFPE kit (Qiagen, Valencia, CA, USA), and amplified, labeled, and hybridized as described above for the UCSF cases.

Both GRID and UCSF samples were processed in a Clinical Laboratory Improvement Amendments (CLIA/CAP)-certified laboratory (GenomeDx Biosciences, San Diego, CA, USA). Quality control, normalization and gene level summarization, and batch effect correction were performed using Affymetrix Power Tools (v 1.19.0), Single Channel Array Normalization [15], and ComBat [16], respectively.

2.1. Pathway summarization and average genomic risk

The Molecular Signatures Database (MSigDB) was queried for 50 hallmark gene sets [17]. Non–prostate or non–cancer-related gene sets were filtered, leaving 37 gene sets for the analysis. Each hallmark set includes a variable number of genes (ranging from 32 to 200) and summarizes expression related to the given biological process. Hallmark gene sets are further grouped into seven biological process categories based on highly correlated expression profiles [17]. Hallmark gene set scores were computed by averaging the expression of each gene in the set, excluding genes not captured by the array.

A substantial number of genomic expression risk scores have previously been published, and we adapted them to the array, as previously described [18]. Eighteen prognostic signatures that achieved univariate significance for the metastasis endpoint in the study were combined into an average genomic risk (AGR) score [18] for each patient by computing the mean of their normalized scores. This AGR score, which serves as a genomic metascore, was analyzed with respect to clinical outcomes.

2.2. Clustering analysis

Patient pathway expression profiles were partitioning around medoids (PAM) clustered based on Spearman's correlation distances. Consensus clustering [19] bootstrapped over 1000 iterations with 80% sampling of both patients and pathways was used to arrive at a robust clustering solution. Pathway cluster expression patterns were specifically examined in reference to the PAM50 genomic classifier originally developed for breast cancer, and recently found to identify patterns in prostate cancer highly analogous to tumor subtypes described as “luminal A,” “luminal B,” and “basal,” which are prognostic in prostate cancer and strongly predict response to androgen deprivation therapy in particular [7]. Subset analyses focused on men with Gleason grade group 1 tumors on biopsy and on those confirmed at prostatectomy to have a “pure” Gleason grade group 1 tumor on final pathology.

2.3. Statistical analysis

Univariate association between genomic consensus clusters and clinical variables were tested using Kruskal-Wallis and Fisher exact tests with

Bonferroni corrections for multiple comparisons. AGR quartiles were calculated with respect to all GRID samples. These risk groups were verified in an independent retrospective biopsy dataset for stratification of metastasis risk using Kaplan-Meier estimates (data not shown). Adverse pathology at prostatectomy was defined by pathological grade group ≥3 or stage ≥T3a, and was analyzed using multivariable logistic regression controlling for CAPRA score and PSA density. Recurrence after surgery was defined by PSA >0.2 with verification or any secondary treatment, analyzed using multivariable Cox proportional hazards regression adjusting for CAPRA score and PSA density. Analyses were performed in R v3.3.3, and all p values were two tailed.

3. Results

Of the 427 UCSF cases, 408 (96%) had sufficient RNA. Table 1 summarizes the demographic and clinical characteristics of the UCSF and GRID patients. Compared with the UCSF AS-eligible patients, GRID patients were older and had higher PSAs, much less grade group 1 disease (37% of GRID vs 74% of UCSF patients), and higher-volume, higher-risk prostate cancer at diagnosis.

Figure 1 presents a heat map summarizing average expression in each patient for each of 37 hallmark gene sets. Patients (UCSF and GRID) were sorted by increasing AGR. Most of the prognostic signature scores summarized into the AGR correlated closely with each other. Gene sets with a significant positive correlation ($p < 0.05$) with high AGR included cell cycle/proliferation gene sets (MYC targets, G2 M checkpoint, E2F targets), immune response (tumor necrosis factor [TNF] signaling via nuclear factor kappa B [NFKB], IL2 STAT5 signaling), angiogenesis, upregulation of Kras signaling, cellular stress, and metabolism (unfolded protein response, oxidative phosphorylation). Gene sets negatively associated with AGR included those involved in steroid signaling (androgen and estrogen response, cholesterol homeostasis), cell-cell interactions (apical surface, apical junction), downregulation of Kras signaling, apoptosis, and metabolism (xenobiotic and fatty acid metabolism).

Supplementary Figure 1 presents a similar heat map including only the UCSF cases, with very similar overall findings. Of the UCSF cases, 54%, 26%, 15%, and 5%were sorted into each quartile of AGR with thresholds defined based on the GRID cases. Among grade group 1 and 2 cancers, 2.0% and 14%, respectively, were in the top quartile of AGR, and 13% and 20%, respectively, were in the third quartile. Likewise, 2.1% and 9.2% of tumors with the lowest clinical risk, as defined by CAPRA 0-1 tumors, were in the top and third quartiles, respectively.

A second heat map presented in Figure 2 illustrates the results of an unsupervised clustering analysis of the hallmark gene sets for the UCSF cases. Three clusters of cases clearly emerge from this analysis. Cluster 1 (from left) is driven by gene sets associated with migration and invasion, and immune response (IL2 STAT5 signaling, IL6 JAK STAT3 signaling, TNFA signaling via NFKB, interferon alpha, gamma and inflammatory response, complement). The second cluster is enriched by gene sets for cell cycle/proliferation (G2 M checkpoint, MYC, and E2F targets), DNA repair, canonical beta-catenin signaling, and metabolism (oxidative phosphorylation, glycolysis, fatty acid metabolism, mTORC1 signaling, PI3K signaling via AKT to mTORC1). The third cluster is driven by androgen and estrogen signaling (androgen and estrogen response, cholesterol homeostasis), mitotic spindle, p53, Hedgehog, Notch signaling and Kras downregulation, xenobiotic metabolism, reactive oxygen species, and apical surface gene sets. Supplementary Figure 2 summarizes the extent of overlap across the various gene sets, indicating their relative independence in contributing to the cluster definitions.

Next, we evaluated the three clusters for enrichment of previously described subtypes [20]. Cluster 1 was enriched

Table 1 – Clinical characteristics of the UCSF and GRID cohorts

Variables	Values	UCSF (*N*, %)	GRID (*N*, %)
		408 (24)	1290 (76)
Patient age at biopsy	Median (Q1, Q3)	59 (54, 64)	68 (62, 73)
% Positive biopsy cores	Median (Q1, Q3)	20 (13, 33)	33 (17, 50)
PSA	Median (Q1, Q3)	5.5 (4.3, 7.5)	6.4 (4.7, 9.3)
Clinical stage	T1	232 (57)	719 (56)
	T2	176 (43)	196 (15)
	T3		15 (1.2)
	T4		3 (0.23)
	Unknown		357 (28)
Gleason grade group	1	301 (74)	482 (37)
	2	107 (26)	445 (35)
	3		175 (14)
	4		109 (8.4)
	5		79 (6.1)
CAPRA	0–2	308 (76)	334 (26)
	3–5	92 (23)	389 (30)
	6+		150 (12)
	Unknown	8 (2.0)	417 (32)
PSA density	Median (Q1, Q3)	0.17 (0.11, 0.24)	–

CAPRA = Cancer of the Prostate Risk Assessment; GRID = Decipher Genomic Resource Information Database; PSA = prostate-specific antigen; UCSF = University of California, San Francisco.

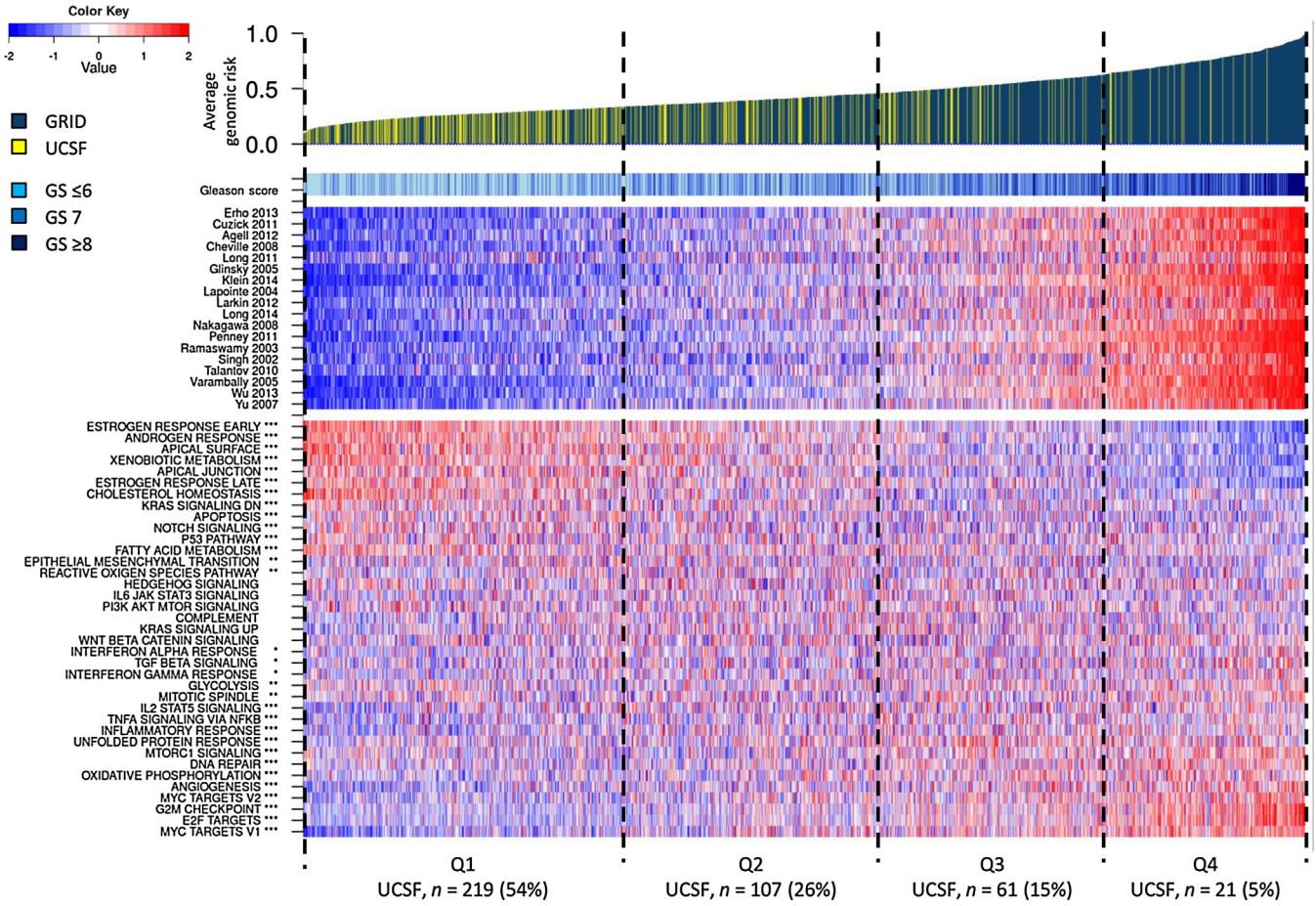

Fig. 1 – Heatmap of UCSF and GRID patients ($n = 1698$) ordered by increasing AGR. The map indicates the following (from top to bottom): (1) the average genomic risk colored by the study the patient is part of, (2) Gleason score, (3) normalized scores for 18 prognostic signatures, and (4) hallmark gene set scores and their correlations to AGR indicated. The patients are broken up into quartiles based on the GRID reference set, and the number (%) of UCSF patients in each quartile is annotated. UCSF patients are associated with lower AGR; however, some UCSF patients are also found in the highest-risk quartile. AGR = average genomic risk; GRID = Decipher Genomic Resource Information Database; GS = Gleason score; UCSF = University of California, San Francisco. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

with triple negative tumors (ERG–, ETS–, and SPINK1) [20] and cluster 2 with ERG+ tumors. Likewise, cluster 1 was enriched with basal-like tumors, whereas the other clusters were almost exclusively luminal like [21]. A related analysis based on PAM50 subtypes found cluster 1 to be enriched with basal, cluster 2 with luminal B, and cluster 3 with luminal A subtypes (Supplementary Table 1) [7]. Supplementary Figures 3 and 4 present similar clustering analyses including both UCSF and GRID cases, and restricted to grade group 1 cases, again identifying the same three clusters similar to basal, luminal B, and luminal A classifications. Supplementary Figure 4C indicates that substantial heterogeneity exists in cancer-related gene pathway expression even among men with pathologically confirmed Gleason grade group 1 tumors.

Among the UCSF cases, the clusters analogous to luminal A (cluster 3), basal (cluster 1), and luminal B (cluster 2) cancers had median (interquartile range) AGR scores of 0.27 (0.22–0.32), 0.34 (0.28–0.42), and 0.41 (0.31–0.49), respectively ($p < 0.001$; Fig. 3). On the contrary, aside from a statistically significant but clinically small difference in percent of biopsy cores involved and stage, there were no differences in standard clinical parameters or clinical risk stratification by CAPRA score across the three subtypes (Table 2).

Adverse pathology at prostatectomy was identified in 105 (26%) of cases. On logistic regression for this outcome of adverse pathology, both CAPRA and AGR were independently prognostic, but the three genomic clusters were not (Table 3). AGR was more predictive of adverse pathology than any of the individual signatures reflected in the AGR. Biochemical recurrence was identified in 30 of 357 of men with median follow-up time of 39 mo among censored patients. For this outcome, on proportional hazards regression, only AGR (not CAPRA or genomic clusters) was prognostic (Table 3).

4. Discussion

In the current analysis, we conducted the most comprehensive expression profiling study to date focused on newly diagnosed, relatively low-risk prostate cancer. These cases,

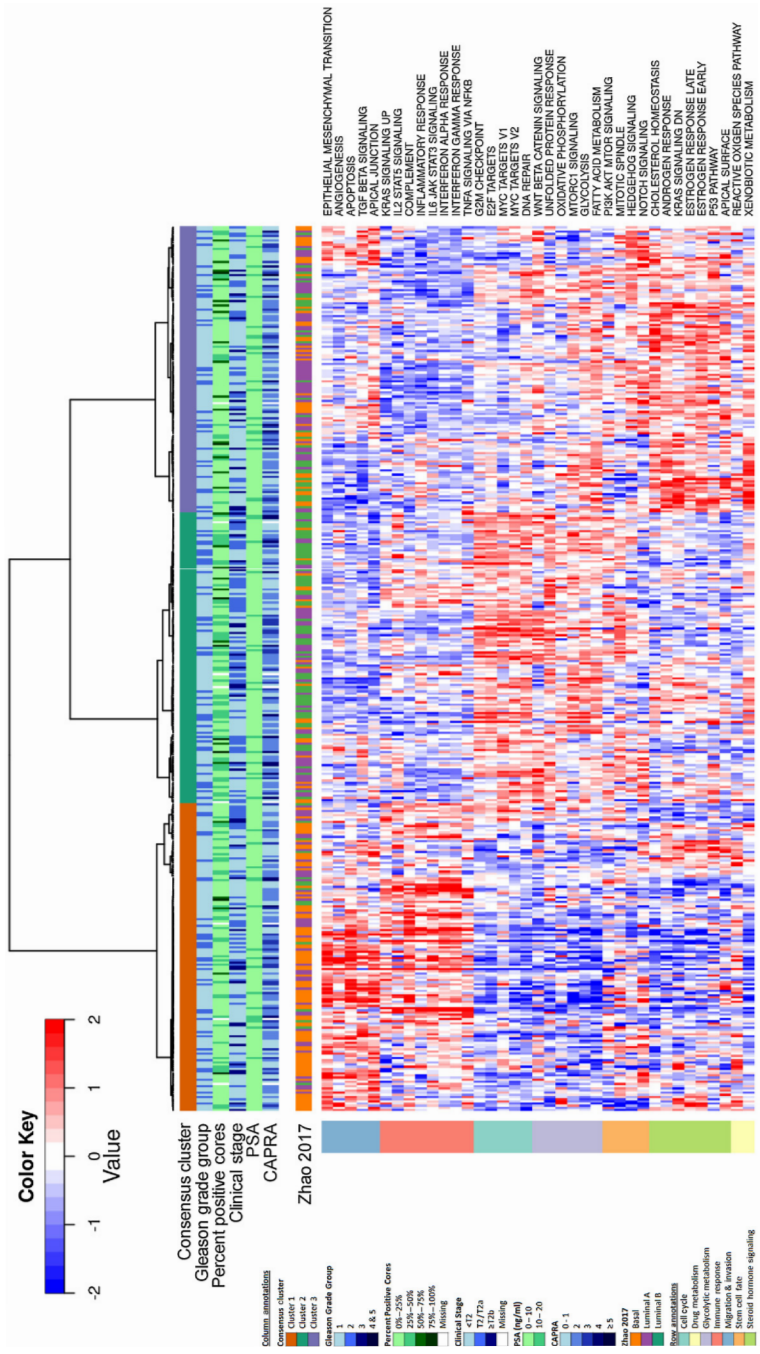

Fig. 2 – Heatmap of the UCSF patients ($n = 408$) consensus clustered based on the expression score of 37 hallmark gene sets. The patients tend to cluster into three distinct groups, which are loosely associated with PAM50's basal, luminal A, and luminal B subtypes. CAPRA = Cancer of the Prostate Risk Assessment; PSA = prostate-specific antigen; UCSF = University of California, San Francisco.

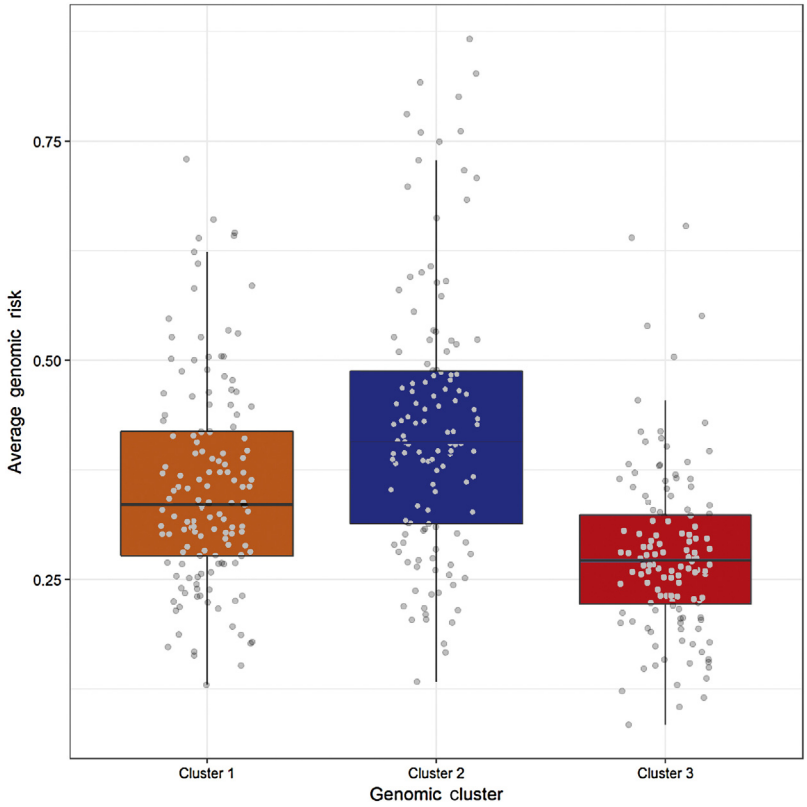

Fig. 3 – Boxplot showing the AGR for each of the clusters from Figure 2. Patients in cluster 3 are found to be at the lowest AGR, while patients in cluster 2 are at the highest AGR ($p < 0.001$). AGR = average genomic risk.

Table 2 – Clinical characteristics of genomic cluster groups

Variables	Values	Cluster 1	Cluster 2	Cluster 3	*p* value
		142 (35)	134 (33)	132 (32)	
Patient age at biopsy	Median (Q1, Q3)	60 (55, 65)	59 (53, 64)	58 (53, 62)	0.5
% Positive biopsy cores	Median (Q1, Q3)	18.8 (12.1, 30)	25 (16.7, 34.8)	16.7 (12.5, 28.6)	0.008
Preop PSA	Median (Q1, Q3)	5.48 (4.15, 7.73)	5.50 (4.18, 7.10)	5.78 (4.59, 7.82)	1
Clinical stage	T1	85 (60)	61 (46)	86 (65)	0.03
	T2	57 (40)	73 (54)	46 (35)	
Gleason grade group	1	105 (74)	94 (70)	102 (77)	
	2	37 (26)	40 (30)	30 (23)	1
CAPRA	0–1	52 (37)	44 (33)	46 (35)	
	2	58 (41)	57 (43)	51 (39)	1
	3	24 (17)	17 (13)	25 (19)	
	4–5	5 (3.5)	12 (9.0)	9 (6.8)	
	Unknown	3 (2.1)	4 (3.0)	1 (0.8)	
PSA density	Median (Q1, Q3)	0.17 (0.12, 0.25)	0.16 (0.12, 0.24)	0.17 (0.11, 0.24)	1
Average genomic risk	Median (Q1, Q3)	0.34 (0.28, 0.42)	0.41 (0.31, 0.49)	0.27 (0.22, 0.32)	<0.001

CAPRA = Cancer of the Prostate Risk Assessment; PSA = prostate-specific antigen.

characterized by low PSA, low stage, and either grade group 1 or low-volume grade group 2, would be considered at least potentially eligible for AS at our institution (with the men understanding clearly that AS for them is more likely to imply deferred rather than avoided treatment) [9,22]. We identified very substantial genomic heterogeneity within this cohort. About 15% of low-risk cases—even those defined as lowest risk based on either Gleason group or multivariable CAPRA score—are characterized by higher-risk genomic features, with approximately 2% of grade group 1 cases found at the top of the genomic risk range.

More importantly, we identified three distinct cancer subtypes at the genomic expression level, which correspond with similar subtypes previously described for breast, lung, and bladder cancers. These subtypes had minimal differences in clinical risk profiles and did not predict outcome, suggesting that the subclassification reflects biological distinctions not reflected in clinical parameters, or in the

Table 3 – Genomic risk and clusters as predictors of outcomes

	Adverse pathology		Biochemical recurrence	
	OR (95% CI)	*p* value	HR (95% CI)	*p* value
CAPRA	1.54 (1.14–2.07)	0.004	1.14 (0.72–1.80)	0.6
PSA density	1.16 (0.92–1.45)	0.2	1.32 (0.99–1.63)	0.06
AGR	1.23 (1.03–1.47)	0.02	1.58 (1.21–2.03)	0.001
CAPRA	1.65 (1.23–2.22)	<0.001	1.20 (0.75–1.90)	0.4
PSA density	1.14 (0.9–1.43)	0.3	1.28 (0.95–1.60)	0.1
Genomic cluster 1	REF		REF	
Genomic cluster 2	1.19 (0.61–2.28)	0.6	1.17 (0.45–2.96)	0.7
Genomic cluster 3	1.78 (0.98–3.23)	0.06	0.95 (0.35–2.46)	0.9

AGR = average genomic risk; CI = confidence interval; CAPRA = Cancer of the Prostate Risk Assessment; HR = hazard ratio; OR = odds ratio; REF = reference. Adverse pathology outcomes are based on multivariable logistic regression and biochemical recurrence outcomes are based on Cox proportional hazards regression.

genomic risk signatures summarized in the AGR score. AGR, by contrast, was the variable most strongly associated with both pathological and biochemical outcomes. We stress, however, that AGR is not intended to serve as yet another genomic predictor of oncological outcomes for clinical practice, but rather as a metascore that reflects an overall summary of genomic risk and aggressiveness, as reflected in multiple previously validated scores.

We found strong positive correlations for cell cycle/proliferation gene sets and high AGR, and an inverse correlation for this surrogate for androgen signaling. Using hallmarks of oncology gene set analysis, we identified clusters quite similar to those described by the PAM50 classification. PAM50 was originally developed for women with breast cancer, and recently, Zhao et al [7] analyzed the PAM50 breast cancer subtypes in several large cohorts of high-risk radical prostatectomy tumors. The authors found that luminal B tumors had the worst oncological outcomes, similar to our findings, in which cluster 2 patients had the highest AGR for metastasis. We found in cluster 1 higher levels of invasion/migration as well as immune response gene sets, suggestive of higher levels of immune infiltration or inflammatory response in these basal-like tumors. Finally, we observed that cluster 3 (enriched with luminal A) tumors had higher levels of androgen response but lower levels of immune response gene sets. These subtypes may ultimately predict responses to emerging targeted and immunological therapies for progressive prostate cancer.

Low-risk prostate cancer rarely progresses to a clinically meaningful stage over at least the 1st decade of follow-up [23,24], but at least a quarter of biopsies indicating low-risk features in fact undersample the tumor in terms of stage or grade [25]. More importantly, with extended follow-up, the risk of progression to metastasis or cancer-specific mortality is not insubstantial for men on AS [24,26]. While many of these adverse outcomes likely could have been avoided with closer monitoring and/or earlier treatment, it is not always clear what the triggers for intervention should have been, even in retrospect [27]. Moreover, the burden of surveillance is significant, and men whose tumors have negligible biological potential for metastasis are still subjected to repeated PSA checks, biopsies, and other interventions, with ongoing anxiety of the attendant, risks of infection, and opportunities for overtreatment [9].

Emerging tests based on assessment of RNA expression in paraffin-embedded prostate biopsy tissue have shown promise for improving prognostic assessments over clinical parameters alone, and have clearly shown genomic heterogeneity among tumors that are relatively homogeneous clinically [14,28]. These tests are now endorsed to aid in treatment decision making by the National Comprehensive Cancer Network prostate cancer guideline. Nearly all the other published signatures reflected in the AGR, likewise, are based on genes originally selected to predict PSA recurrence, metastasis, and other relatively distal clinical events. However, these existing assays are based on measurement of relatively small numbers of genes in one or a few pathways, and therefore provide relatively limited insight into the extent of biological heterogeneity. For this reason, among others, existing tests are independently prognostic but cannot clearly guide treatment selection.

Moreover, most genomic studies on the molecular variability of prostate cancer have focused on advanced disease and, not surprisingly, have found great heterogeneity among heavily treated progressive cancers. Much less prior work has focused on untreated, early-stage clinically localized tumors. The Cancer Genome Atlas is a notable exception, but even in this cohort of 333 cases, in which the full spectrum of clinical risk was represented, only 65 (20%) of the cases had grade group 1 disease [3]. Moreover, this study focused primarily on mutations, chromosomal rearrangements, and methylation events [4]. Both studies also performed limited RNA analysis, focusing on expression directly related to identified DNA changes.

There are important limitations to this analysis which must be acknowledged. All the UCSF cases underwent a central pathology review, whereas the GRID cases were read and submitted by many different pathologists. RNA processing was slightly different for the UCSF and GRID cases, but quality control results were substantively similar between the two. Cases were all microdissected (without laser capture) to enrich for malignant glands. The sampled tumor specimen and resultant transcriptomic profile may therefore represent a complex mixture including other nonmalignant cells from the immediate

tumor microenvironment, such as stromal, benign, and immune infiltrate (if present). Therefore, these results could also reflect heterogeneity in overall cellular composition of tumor and not only of the malignant cells in lower-risk prostate cancer. However, all three commercial RNA-based assays are based on similar microdissection without laser capture, suggesting that these techniques are at least partially robust to variation in local cell type populations.

The AGR score is only a surrogate for clinical outcomes, but it is based on multiple scores that have been validated for such outcomes in many prior studies, and we believe that averaging the existing scores reduces the potential for bias in reflecting overall genomic risk. The analysis of biochemical outcomes is limited by small event numbers and a relatively narrow range of risk. Only one biopsy per case was processed. We acknowledge that there may be within-patient heterogeneity in terms of AGS and/or tumor subtype classifications. Indeed, the consistency or variability of gene expression even within normal prostate tissue is not well defined. However, the success of all existing tissue-based biopsy tests is based on the presumption that the selected biopsy can accurately reflect the biopsy of the cancer overall. Adverse pathology is an imperfect surrogate for distal clinical outcomes, but has been used in multiple prior genomic studies. The biochemical recurrence analyses in the present manuscript are further limited by a relatively low event rate, reflecting the low-risk clinical characteristics.

5. Conclusions

The diversity that we observed, in terms of both the AGR spectrum and subtyping across the luminal A/luminal B/basal spectrum, was present both in the clinically diverse GRID cohort and in the much more clinically homogeneous UCSF AS-eligible cohort. While we cannot yet confirm the differential response to androgen deprivation therapy observed for luminal B versus basal and luminal A tumors, we believe that these data underscore the potential utility of a taxonomy and nomenclature that considers molecular characteristics and biological potential for progression at least as strongly as the organ of origin and traditional histology. With future studies planned in our prospective AS cohort, we hope to demonstrate that such molecular substratification can help stratify men not only to surveillance versus immediate treatment, but also to more or less intensive surveillance strategies.

Author contributions: Matthew R. Cooperberg had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Cooperberg, Chan, Davicioni, Carroll.
Acquisition of data: Cooperberg, Chan, Cowan, Kolisnik, Chelliserry, Margrave, du Plessis, Davicioni.
Analysis and interpretation of data: Cooperberg, Erho, Fishbane, Zhao, Lehrer, Alshalalfa, Davicioni.
Drafting of the manuscript: Cooperberg, Erho, Chan, Feng, Fishbane, Simko, Lehrer, Alshalalfa, Davicioni.
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Statistical analysis: Fishbane, Aranes.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.05.014.

References

[1] Jahn JL, Giovannucci EL, Stampfer MJ. The high prevalence of undiagnosed prostate cancer at autopsy: implications for epidemiology and treatment of prostate cancer in the prostate-specific antigen-era. Int J Cancer 2015;137:2795–802.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7–30.
[3] Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25.
[4] Fraser M, Sabelnykova VY, Yamaguchi TN, et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature 2017;541:359–64.
[5] Cooperberg MR, Hinotsu S, Namiki M, et al. Risk assessment among prostate cancer patients receiving primary androgen deprivation therapy. J Clin Oncol 2009;27:4306–13.
[6] Leapman MS, Nguyen HG, Cooperberg MR. Clinical utility of biomarkers in localized prostate cancer. Curr Oncol Rep 2016;18:30.
[7] Zhao SG, Chang L, Erho N, et al. Luminal and basal subtyping of prostate cancer is prognostic and predicts response to androgen deprivation therapy. JAMA Oncol 2017;3:1663–72.
[8] Ganz PA, Barry JM, Burke W, et al. National Institutes of Health State-of-the-Science Conference: role of active surveillance in the management of men with localized prostate cancer. Ann Intern Med 2012;156:591–5.
[9] Chen RC, Rumble RB, Loblaw DA, et al. Active surveillance for the management of localized prostate cancer (Cancer Care Ontario guideline): American Society of Clinical Oncology clinical practice guideline endorsement. J Clin Oncol 2016;34:2182–90.
[10] Womble PR, Montie JE, Ye Z, et al. Contemporary use of initial active surveillance among men in Michigan with low-risk prostate cancer. Eur Urol 2015;67:44–50.

[11] Cooperberg MR, Carroll PR. Trends in management for patients with localized prostate cancer, 1990-2013. JAMA 2015;314:80–2.
[12] Loeb S, Folkvaljon Y, Curnyn C, Robinson D, Bratt O, Stattin P. Uptake of active surveillance for very-low-risk prostate cancer in Sweden. JAMA Oncol 2017;3:1393–8.
[13] Brajtbord JS, Leapman MS, Cooperberg MR. The CAPRA score at 10 years: contemporary perspectives and analysis of supporting studies. Eur Urol 2017;71:705–9.
[14] Klein EA, Cooperberg MR, Magi-Galluzzi C, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol 2014;66:550–60.
[15] Piccolo SR, Sun Y, Campbell JD, Lenburg ME, Bild AH, Johnson WE. A single-sample microarray normalization method to facilitate personalized-medicine workflows. Genomics 2012;100:337–44.
[16] Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 2007;8:118–27.
[17] Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 2015;1:417–25.
[18] Ross AE, Johnson MH, Yousefi K, et al. Tissue-based genomics augments post-prostatectomy risk stratification in a natural history cohort of intermediate- and high-risk men. Eur Urol 2015;69:157–65.
[19] Wilkerson MD, Hayes DN. ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics 2010;26:1572–3.
[20] Tomlins SA, Alshalalfa M, Davicioni E, et al. Characterization of 1577 primary prostate cancers reveals novel biological and clinicopathologic insights into molecular subtypes. Eur Urol 2015;68:555–67.
[21] Zhang D, Park D, Zhong Y, et al. Stem cell and neurogenic gene-expression profiles link prostate basal cells to aggressive prostate cancer. Nat Commun 2016;7, 10798.s.
[22] Welty CJ, Cowan JE, Nguyen H, et al. Extended followup and risk factors for disease reclassification in a large active surveillance cohort for localized prostate cancer. J Urol 2015;193:807–11.
[23] Wilt TJ, Brawer MK, Jones KM, et al. Radical prostatectomy versus observation for localized prostate cancer. N Engl J Med 2012;367:203–13.
[24] Hamdy FC, Donovan JL, Lane JA, et al. 10-Year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. N Engl J Med 2016;375:1415–24.
[25] Conti SL, Dall'era M, Fradet V, Cowan JE, Simko J, Carroll PR. Pathological outcomes of candidates for active surveillance of prostate cancer. J Urol 2009;181:1628–33, discussion 1633-4.
[26] Klotz L, Vesprini D, Sethukavalan P, et al. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol 2015;33:272–6.
[27] Tosoian JJ, Mamawala M, Epstein JI, et al. Intermediate and longer-term outcomes from a prospective active-surveillance program for favorable-risk prostate cancer. J Clin Oncol 2015;33:3379–85.
[28] Cooperberg MR, Simko JP, Cowan JE, et al. Validation of a cell-cycle progression gene panel to improve risk stratification in a contemporary prostatectomy cohort. J Clin Oncol 2013;31:1428–34.

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ORIGINAL RESEARCH

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Development and pilot evaluation of a personalized decision support intervention for low risk prostate cancer patients

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Abstract

Objectives: Development and pilot evaluation of a personalized decision support intervention to help men with early-stage prostate cancer choose among active surveillance, surgery, and radiation.

Methods: We developed a decision aid featuring long-term survival and side effects data, based on focus group input and stakeholder endorsement. We trained premedical students to administer the intervention to newly diagnosed men with low-risk prostate cancer seen at the University of California, San Francisco. Before the intervention, and after the consultation with a urologist, we administered the Decision Quality Instrument for Prostate Cancer (DQI-PC). We hypothesized increases in two knowledge items from the DQI-PC: How many men diagnosed with early-stage prostate cancer will eventually die of prostate cancer? How much would waiting 3 months to make a treatment decision affect chances of survival? Correct answers were: "Most will die of something else" and "A little or not at all."

Results: The development phase involved 6 patients, 1 family member, 2 physicians, and 5 other health care providers. In our pilot test, 57 men consented, and 44 received the decision support intervention and completed knowledge surveys at both timepoints. Regarding the two knowledge items of interest, before the intervention, 35/56 (63%) answered both correctly, compared to 36/44 (82%) after the medical consultation ($P = .04$ by chi-square test).

Conclusions: The intervention was associated with increased patient knowledge. Data from this pilot have guided the development of a larger scale randomized clinical trial to improve decision quality in men with prostate cancer being treated in community settings.

KEYWORDS

behavioral science, cancer education, ethical considerations, prostate cancer

1 | BACKGROUND

Patients with low-risk prostate cancer are vulnerable to making decisions based on incomplete information.[1] Patients have misconceptions about the risks and benefits of surgery, radiation, and active surveillance.[2-4] This can result in prostate cancer survivors feeling that they had more or less treatment than they would have chosen if they had been fully informed and more involved in their decisions.[5-8]

A systematic review with meta-analysis of randomized controlled trials concluded that decision aids are associated with increases in patient knowledge,[9] among other benefits. Communication aids include question-listing interventions.[10] A systematic review with meta-analysis found that these are associated with increased involvement in the form of question-asking.[11] Members of our team have developed communication aiding interventions showing psycho-social benefits for men with prostate cancer, including increases in decision self-efficacy (DSE) and reductions in decisional conflict and regret.[12]

It appears decision and communication aids can address deficits in patients being informed and involved. However, we identified two gaps in the literature.

First, decision aids in prostate cancer have not yet provided personalized estimates of risk and benefit. Two randomized controlled studies of decision aids in prostate cancer found increased knowledge.[13,14] However, these decision aids were not targeted specifically at low-risk prostate cancer using personalized estimates of risk.[15] Greater personalization of decision aids for low-risk patients is now possible because researchers are beginning to report long-term outcomes data about mortality and side effects while stratifying results according to risk level.[16,17]

The second gap in the literature is that researchers have not yet studied the delivery of decision and communication aids by students as part of their pre-medical training. A recent review of evidence[18] found three studies, in domains other than prostate cancer, where professional health coaches delivered decision aids that were associated with increased patient knowledge.[19-21] Subsequent to the publication of this review, one study by Mishel et al in prostate cancer found a strong effect on knowledge when nurses coached patients in the use of decision and communication aids.[14]

Addressing these gaps would contribute important knowledge about the impact of more specific patient education; and whether delivery of patient education interventions can be task-shifted to students.

To address these gaps, our team developed a multi-component decision support intervention. First, we asked whether an intervention delivered by pre-medical student interns would be acceptable to a focus group of stakeholders. Second, we asked if a personalized decision support intervention was associated with improved patient knowledge about early-stage prostate cancer.

2 | METHODS

2.1 | Approach and study design

We approached this research as formative work to assess the acceptability and efficacy of a novel decision support intervention, while generating pilot data to estimate effect sizes for a future randomized controlled trial. We developed our intervention using qualitative methods and conducted a pre/post test with patients at our academic medical center. We obtained ethics approval (14-13332) from the Committee on Human Research at the University of California, San Francisco (UCSF). We registered the study on ClinicalTrials.gov as number NCT02451345.

2.2 | Target population and study samples

Our target population was men with low-risk prostate cancer being treated in academic and community settings in the United States. For the intervention design phase, we convened a sample of 8 patients, 1 family member/caregiver, and 7 healthcare professionals. For the intervention testing phase, we approached patients diagnosed with low-risk disease at the University of California, San Francisco to discuss treatment options with a urologist. The inclusion criteria included men age ≥18, who could speak and read English, and with newly diagnosed (within 6 months), low-risk prostate cancer, who have not yet received therapy. Low-risk was defined as: Gleason score ≤3+4, stage ≤T2N0M0, PSA ≤10 ng/mL.

2.3 | Outcomes, measures, and instruments

2.3.1 | Intervention design phase

We used a survey instrument from the International Patient Decision Aids Standards (IPDAS) to measure the stakeholder endorsement of our decision aid.[22] We limited our questionnaire to 12 questions in the qualifiying and certifying criteria. See online supplemental materials Data S1.

We also asked stakeholders to rate the acceptability of our coaching intervention using the Decision Support Assessment Tool,[23] a written survey instrument designed to evaluated the provision of decision coaching. See online supplemental materials Data S1.

2.3.2 | Intervention testing phase

For the intervention testing phase, we collected patient demographics at baseline, and measured decision self-efficacy immediately before and immediately after the intervention. Our intention was to orient patients to their treatment options and outcomes using the decision aid before the urologist consultation. We also wanted to help them list questions. We

hypothesized that the decision aid and question listing would increase patient decision self-efficacy. We measured patient knowledge, as described below, before the intervention and after the medical consultation. We wanted the patient to ask questions and emerge from the consultation with increased knowledge.

Decision quality instrument with knowledge subscale

The Decision Quality Instrument-Early Prostate Cancer Treatment has a knowledge subscale with 11 questions which can be provided to patients in the form of a multiple-choice quiz.[24] We chose five questions about survival outcomes and side effects that were addressed by our decision aid. (See Table 1). We hypothesized that we would see a pre/post increase in the proportion of patients who answered the first two knowledge items correctly: How many men diagnosed with early-stage prostate cancer will eventually die of prostate cancer? How much would waiting 3 months to make a treatment decision affect chances of survival? Correct answers were: "Most will die of something else" and "A little or not at all." These items are most relevant to patient understanding that their condition is not urgently life-threatening, and there is time to weigh all options thoroughly.

Decision self-efficacy item

To assess decision self-efficacy, we used an item from the decision self-efficacy scale.[25] This item was sensitive to our question-listing intervention in a prior randomized controlled trial.[12] The item requested a 0-4 confidence rating that I can "Figure out the treatment choices that best suit me."

TABLE 1 Survey to assess patient knowledge

Five items from decision quality instrument-early prostate cancer treatment[24]
1. Without treatment, about how many men diagnosed with early-stage prostate cancer will eventually die of prostate cancer? Responses: Most will die of prostate cancer; About half will die of prostate cancer; Most will die of something else*.
2. For most men with early-stage prostate cancer, how much would waiting a few months to make a treatment decision hurt their chances of survival? Responses: A lot; Somewhat; A little or not at all*.
3. In the first few years after treatment for prostate cancer, which is more likely to cause bowel problems? Responses: Surgery; Radiation*; Both surgery and radiation are equally likely to cause bowel problems.
4. In the first few years after treatment for prostate cancer, which is more likely to cause sexual problems with erections? Surgery*; Radiation; Both surgery and radiation are equally likely to cause sexual problems.
5. In the first few years after treatment for prostate cancer, which is more likely to cause dripping or leaking urine? Responses: Surgery*; Radiation; Both surgery and radiation are equally likely to cause dripping or leaking urine.

*denotes the correct answer.

2.4 | Data collection procedures

2.4.1 | Intervention design phase

Decision scientists on our team designed an initial prototype of our decision aid, using the SCOPED model as a conceptual framework.[26] SCOPED is an acronym whose letters represent steps in reflecting critically on a decision: Situation, Choices, Objectives, People, Evaluation, and Decisions.

To refine this prototype, we identified a representative group of stakeholders. We conducted rounds of feedback until all the stakeholders endorsed the decision aid according to the IPDASi standards described above.

In order to systematically incorporate stakeholder feedback, we used the Nominal Group Technique.[27] The Nominal Group Technique is a focus group technique that captures stakeholder input in writing first, to prevent dominance of any especially verbal members of the group. In each round of feedback, we surveyed the stakeholders about the current version of the decision aid; discussed their survey responses; and then voted on the acceptability of the decision aid. We continued making changes to the decision aid and getting feedback until all stakeholders rated the decision aid as acceptable.

Our coaching intervention to deliver the decision aid was based on an established approach to question listing, described in the literature as Consultation Planning.[26] To adapt Consultation Planning for this intervention, we recorded all coaching sessions, and reviewed recordings with the stakeholder team during our biweekly calls. We asked stakeholders to rate the coaching process using the Decision Support Analysis Tool (DSAT) survey, capturing suggestions for improvement and repeating until we arrived at consensus endorsement of the intervention design.

2.4.2 | Intervention testing phase

Study sample

We enrolled a convenience sample of 51 patients seeing 7 urologists at UCSF, between 4/1/2015 and 2/7/17. Part-time study coordinators approached these patients based on the coordinator's availability and overlap with their urologist's schedule. After enrollment, student coaches contacted the patients to administer the intervention by telephone and survey instruments by email.

2.5 | Analysis plan

2.5.1 | Intervention design phase

We documented the ongoing suggestions for improvement from stakeholders. All suggested modifications were

considered by study personnel (including investigators, software developers, and patient representatives). The study's co-investigators weighed additional factors such as cost and technical feasibility in incorporating panelist feedback.

2.5.2 | Intervention testing phase

Decision self-efficacy: We graphed the distribution of DSE scores before compared to after the intervention, and counted the number and proportion of patients whose DSE score rose vs fell. We also compared the mean DSE before and after the intervention using a paired *t* test.

Decision Quality Instrument—Knowledge: We graphed the distribution of knowledge scores before compared to after the intervention, and counted the number and proportion of patients whose knowledge scores rose vs fell. We compared the mean knowledge score before and after the intervention using a paired *t* test. We computed the proportion of patients who answered the first two knowledge items correctly. Then, we used McNemar's test for the hypothesis of no difference in number of patients who answered both correctly before compared to after the intervention. We used Release 10 of Stata Statistical Software for all statistical analyses.[28]

3 | RESULTS

3.1 | Intervention design phase

The stakeholder team arrived at a consensus endorsement of our initial decision aid after three rounds of feedback, and a consensus endorsement of our coaching intervention after one round. Based on this feedback, we concluded the decision aid and coaching intervention were feasible and acceptable for inclusion in the intervention testing phase of the study. We trained our existing premedical student interns to deliver the intervention.[29]

The training for this intervention was based on a question-listing curriculum we have implemented since 2012 with the premedical student intern workforce at UCSF. These student interns participate in a service learning program known as the Patient Support Corps. Through the Patient Support Corps, the students earn academic credit while gaining experience working as health coaches in our medical center. Each year, we recruit our interns from the undergraduate student population at the University of California, Berkeley. We select students after a screening process that includes a written application and interview. The application and interview focus on the student's competence in neutral, non-directive coaching.

Through this screening process, we identify students who are skilled at gathering information from others; who can summarize and paraphrase information in a neutral fashion; and who will escalate problems to supervisors when situations arise outside of their scope. Then the Director of the Patient Support Corps (first author JB), along with the program coordinator (author TW), train the students in their specific question-listing tasks.

We administer 16 hours of classroom training in which the students learn a process for eliciting and documenting patient questions, known as the SLCT process.[29] After reviewing videos of the process in action, trainees role-play in pairs and the instructors review recordings of their role-plays and provide feedback. Then the trainees are paired up with experienced student interns, who shadow them during patient interactions until the trainees are ready to interact with patients alone.

After the trainees begin interacting with patients alone, they submit recordings of their interactions to the program director and coordinator, who review recordings in group meetings every week, and provide ongoing training and quality improvement.

3.1.1 | Coaching and question-listing process

We were able to leverage our existing training for coaches because the final study coaching process closely resembled our existing question-listing intervention, described in the literature.[26] The only material difference in this project was that, in addition to open-ended question prompts, the coach also used the decision aid content as additional question prompts. The coach did this by reviewing the decision aid with the patient one screen at a time, checking for questions, then writing the questions down and asking for elaboration. Also, based on focus group feedback, we asked the coaches to check for patient understanding and direct the patient to additional help text in the decision aid when something was unclear.

3.1.2 | Decision aid content and interface

Our software team coded four versions of our decision aid as a result of the iterative feedback we collected. Readers may request a copy of the decision aid in portable document format from the corresponding author. Our software development team deployed this decision aid as a web application using a JavaEE web profile and ran it on Amazon Elastic Beanstalk to provide automatic updates and resiliency. The application stored and retrieved data from a database configured using the Research Electronic Data Capture platform (REDCap).[30]

3.2 | Intervention testing phase

3.2.1 | Sample description

Between April 2015 and February 2017 (4/1/15-2/7/17), our clinic research coordinators enrolled a convenience sample

of 51 men seeing 7 urologists in our clinic. The men had a median age of 63 (mean 62). Racial and ethnic representation was 43 Caucasian/White, 2 Asian/Pacific, 6 Other/Mixed/Unknown including 4 Hispanic/Latino. For employment status, 21 reported an employment status of Working, 14 others Self-employed, 11 Retired, and 5 Other/Unknown. Cancer of the Prostate Risk Assessment (UCSF-CAPRA) scores ranged from 1-6 (median: 2). T1c was the most common stage (63%).

3.2.2 | Decision self-efficacy

For decision self-efficacy (0-4 confidence rating that I can "Figure out the treatment choices that best suit me"), the distribution of scores was similar before compared to after the intervention. The scale showed a ceiling effect, as the most frequent score before and after was the highest score, 4. The pre and post-intervention means were not statistically significantly different (3.43 to 3.47, $P = .62$).

We graphed the joint distribution of ratings, pre and post (see Figure 1). Figure 1 shows parallel 45-degree lines corresponding to changes in score of −2, −1, 0, and +1, while the responses are superimposed on the lines in bubbles whose size reflects the frequency of each pre/post combination. For example, a bubble on the +1 line shows 2 respondents rated their self-efficacy at 2 before and 3 after the intervention.

Overall, Of 51 respondents to the DSE pre and post-intervention, 38 scores (72%) stayed the same (shown on the 0-change line), with 27 (51%) holding perfect at 4/4. Six scores (11%) went up one point, while six (11%) went down 1 point and one (2%) went down 2 points.

3.2.3 | Decision quality instrument—knowledge

For knowledge, the distribution of total knowledge score after was shifted upwards compared to before. The raw improvement in means (2.84-3.16) was not statistically significant ($P = .16$).

We graphed the joint distribution of knowledge scores (see Figure 2). Figure 2 shows parallel 45 degree lines corresponding to changes in score of −2, −1, 0, +1, +2, and +3, while the responses are superimposed on the lines in bubbles whose size reflects the frequency of each pre/post combination. For example, a bubble on the +1 line shows 3 respondents answered 1 item correctly before and 2 items correctly after the intervention.

Overall, Figure 2 reveals 15 scores (29%) staying flat, 12 (27%) going up one point, 5 (11%) going up two points, and one (2%) going up 3 points; while 8 (18%) went down 1 point, and 3 (7%) went down 2 points. There were 18 (41%) scores that went up, and 11 (25%) that went down. This raw difference was not a statistically significant difference (binomial sign test $P = .13$).

We had previously identified the first two items of the DQI knowledge survey as most relevant to our decision support intervention. Before the intervention, 35/56 (63%) got both these questions right. After the consultation, 36/44 (82%) got both these questions right ($P = .04$ by Chi-Square test).

Among respondents who answered the questions at both timepoints, the number moving from at least one incorrect to both correct (7, or 16%) was higher than the number

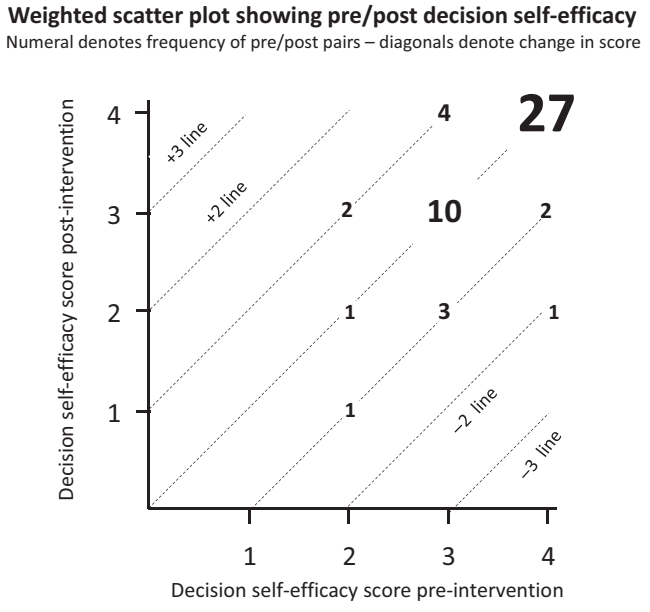

FIGURE 1 Scatterplot of paired decision self-efficacy scores (before and after)

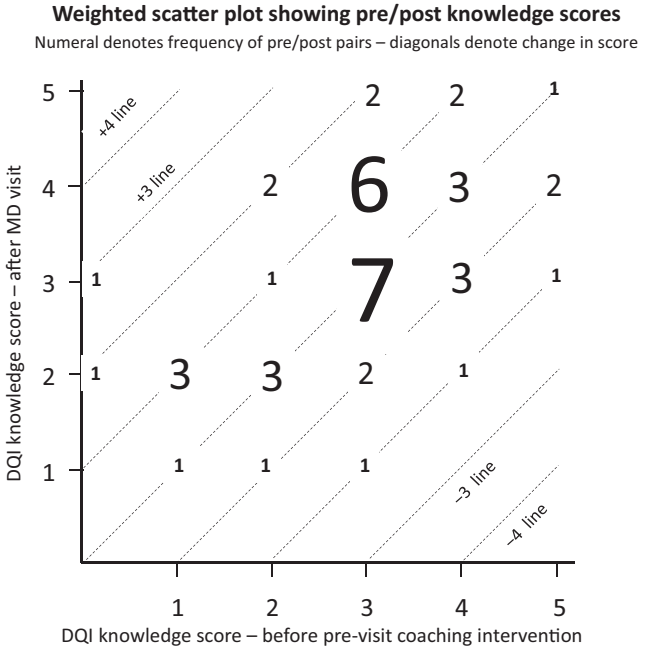

FIGURE 2 Scatterplot of paired total knowledge scores (before intervention and after consultation)

moving from both correct to at least one incorrect (1, or 2%; McNemar $P = .08$). See Table 2.

4 | DISCUSSION

We designed and tested a multi-component intervention with personalized decision and communication aids that premedical students could deliver by telephone. Our decision aid broke new ground by incorporating long-term, patient-specific, and personalized data on both survival and side effects. We adapted a prior communication aid (Consultation Planning) and prompted patient questions in categories corresponding to the six decision aid topics. The close integration of a personalized decision aid with coached question-listing is also novel.

We were surprised that the intervention was not associated with pre/post changes in decision self-efficacy. A recent study of prostate cancer patients in the UK found that Consultation Planning alone was associated with a significant pre/post change in DSE.[12] As opposed to that study, our population demonstrated a ceiling effect that left little room for improvement. We believe that patients in community settings may demonstrate lower self-efficacy levels and benefit more from the intervention.

The direction and magnitude of improvement in knowledge was encouraging. A subset of two key knowledge questions were especially sensitive to our intervention. We will use these two items as the primary outcome in a randomized controlled trial of our intervention vs usual care in community settings, with decision self-efficacy as a secondary outcome.

4.1 | Study strengths

The strengths of our study included the participation of diverse stakeholders, notably patient representatives, during the design phase. We designed an innovative multi-component intervention that was delivered by members of an untapped workforce—premedical students who earned academic credit while serving as health coaches. The intervention broke new ground in prostate cancer education by personalizing our decision aid with risk information based on each patient's clinical characteristics. In addition, we integrated the decision aid with our coach-led question-listing intervention, to assure that each component of our intervention flowed smoothly into the next.

TABLE 2 Patient performance on first two knowledge items before and after intervention

	After Intervention	
Before Intervention	**At least 1 incorrect**	**Both correct**
At least one incorrect	7	7
Both correct	1	29

4.2 | Study limitations

Our formative study recruited a convenience sample of patients in an academic medical center, had no control group, and relied on self-reported measures collected before and after the intervention (self-efficacy) and consultation with a urologist (knowledge). We observed knowledge gains but in the absence of a control group, we do not know if they would have occurred even without the intervention. Other limitations include sampling bias (we invited patients at convenient times for the study coordinator), motivational bias (patients who consented may be different than those who did not), agreement bias (patients may have wanted to please study personnel or the clinical care team with their answers), and maturation bias (there could have been changes in the environment over time relevant to our study outcomes). One of our measures exhibited a strong ceiling effect, which may not be as evident in other settings. This study was conducted in an academic center with urologists who are highly specialized in the care of patients with low-risk prostate cancer, which may not be representative of all prostate cancer care settings.

4.3 | Clinical implications

The ceiling effect in decision self-efficacy is surprising when juxtaposed with relatively low knowledge scores. The mode of the DSE distribution was the maximum score (4/4), while the mode of the total knowledge score was 60% (3/5). This suggests that patient confidence about making decisions exceeded patient knowledge. Our finding suggests that patients may need more education than they report, if they are to make decisions based on valid information. Therefore, in order to assure truly informed consent, clinicians should check explicitly for understanding on key facts, whether or not the patient asserts self-efficacy for decision making. In the case of early-stage prostate cancer, two key misconceptions include how many men diagnosed with early-stage prostate cancer will eventually die of prostate cancer (most will die of something else); and how much would waiting 3 months to make a treatment decision affect chances of survival (a little or not at all). These items are most relevant to patient understanding that their condition is not urgently life-threatening, and there is time to weigh all options thoroughly.

5 | CONCLUSIONS

Our multi-component decision aid intervention has potential for reducing knowledge deficits about early-stage

prostate cancer. We would like to further examine whether the intervention will improve knowledge and decision self-efficacy, in a population with lower decision self-efficacy than seen in our sample. To more definitively address these questions we have designed and are implementing a cluster-randomized controlled trial with sites in community settings.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Daum LM, Reamer EN, Ruterbusch JJ, et al. Patient knowledge and qualities of treatment decisions for localized prostate cancer. *J Am Board Fam Med*. 2017;30:288.
2. Beydoun HA, Mohan R, Beydoun MA, et al. Development of a scale to assess patient misperceptions about treatment choices for localized prostate cancer. *BJU Int*. 2010;106:334.
3. McGregor S. What information patients with localised prostate cancer hear and understand. *Patient Educ Couns*. 2003;49:273.
4. Denberg TD, Melhado TV, Steiner JF. Patient treatment preferences in localized prostate carcinoma: the influence of emotion, misconception, and anecdote. *Cancer*. 2006;107:620.
5. Hoffman RM, Lo M, Clark JA, et al. Treatment decision regret among long-term survivors of localized prostate cancer: results from the prostate cancer outcomes study. *J Clin Oncol*. 2017;35:2306.
6. Schroeck FR, Krupski TL, Sun L, et al. Satisfaction and regret after open retropubic or robot-assisted laparoscopic radical prostatectomy. *Eur Urol*. 2008;54:785.
7. Holmes JA, Bensen JT, Mohler JL, et al. Quality of care received and patient-reported regret in prostate cancer: analysis of a population-based prospective cohort. *Cancer*. 2017;123:138.
8. Morris BB, Farnan L, Song L, et al. Treatment decisional regret among men with prostate cancer: racial differences and influential factors in the North Carolina Health Access and Prostate Cancer Treatment Project (HCaP-NC). *Cancer*. 2015;121:2029.
9. Stacey D, Legare F, Col NF, et al. Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev*. 2014;1:CD001431.
10. Roter DL. Patient participation in the patient-provider interaction: the effects of patient question asking on the quality of interaction, satisfaction and compliance. *Health Educ Monogr*. 1977;5:281.
11. Kinnersley P, Edwards A, Hood K, et al. Interventions before consultations to help patients address their information needs by encouraging question asking: systematic review. *BMJ*. 2008;337:a485.
12. Hacking B, Wallace L, Scott S, et al. Testing the feasibility, acceptability and effectiveness of a 'decision navigation' intervention for early stage prostate cancer patients in Scotland–a randomised controlled trial. *Psychooncology*. 2013;22:1017.
13. Chabrera C, Zabalegui A, Bonet M, et al. A decision aid to support informed choices for patients recently diagnosed with prostate cancer: a randomized controlled trial. *Cancer Nurs*. 2015;38:E42.
14. Mishel MH, Germino BB, Lin L, et al. Managing uncertainty about treatment decision making in early stage prostate cancer: a randomized clinical trial. *Patient Educ Couns*. 2009;77:349.
15. Hamdy FC, Donovan JL, Lane JA, et al. 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. *N Engl J Med*. 2016;375:1415.
16. Wilt TJ, Brawer MK, Jones KM, et al. Radical prostatectomy versus observation for localized prostate cancer. *N Engl J Med*. 2012;367:203.
17. Xia J, Trock BJ, Cooperberg MR, et al. Prostate cancer mortality following active surveillance versus immediate radical prostatectomy. *Clin Cancer Res*. 2012;18:5471.
18. Stacey D, Kryworuchko J, Belkora J, et al. Coaching and guidance with patient decision aids: a review of theoretical and empirical evidence. *BMC Med Inform Decis Mak*, 2013;13 (Suppl 2):S11.
19. Hamann J, Langer B, Winkler V, et al. Shared decision making for in-patients with schizophrenia. *Acta Psychiatr Scand*. 2006;114:265.
20. Lerman C, Biesecker B, Benkendorf JL, et al. Controlled trial of pretest education approaches to enhance informed decision-making for BRCA1 gene testing. *J Natl Cancer Inst*. 1997;89:148.
21. van Peperstraten A, Nelen W, Grol R, et al. The effect of a multifaceted empowerment strategy on decision making about the number of embryos transferred in in vitro fertilisation: randomised controlled trial. *BMJ*. 2010;341:c2501.
22. Elwyn G, O'Connor AM, Bennett C, et al. Assessing the quality of decision support technologies using the International Patient Decision Aid Standards instrument (IPDASi). *PLoS ONE*. 2009;4:e4705.
23. Stacey D, Taljaard M, Drake ER, et al. Audit and feedback using the brief decision support analysis tool (DSAT-10) to evaluate nurse-standardized patient encounters. *Patient Educ Couns*. 2008;73:519.
24. Sepucha K. Prostate cancer decision quality instrument v. 1.0. Massachusetts Gen Hosp: 1, 2013. https://mghhealthdecisions.files.wordpress.com/2018/06/pcadqi_usermanual.pdf. Accessed November 7, 2019.
25. Bunn H, O'Connor A. Validation of client decision-making instruments in the context of psychiatry. *Can J Nurs Res*. 1996;28:13.

26. Belkora JK, Loth MK, Volz S, et al. Implementing decision and communication aids to facilitate patient-centered care in breast cancer: a case study. *Patient Educ Couns*. 2009;77:360.
27. van Teijlingen E, Pitchforth E, Bishop C, et al. Delphi method and nominal group technique in family planning and reproductive health research. *J Fam Plann Reprod Health Care*. 2006;32:249.
28. StataCorp. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP; 2007.
29. Belkora J, Volz S, Loth M, et al. Coaching patients in the use of decision and communication aids: RE-AIM evaluation of a patient support program. *BMC Health Serv Res*. 2015;15:209.
30. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Stability of a 17-Gene Genomic Prostate Score in Serial Testing of Men on Active Surveillance for Early Stage Prostate Cancer

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Abbreviations and Acronyms

AS = active surveillance

GPS = Genomic Prostate Score

LR = likelihood ratio

PSA = prostate specific antigen

RP = radical prostatectomy

UCSF = University of California San Francisco

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Editor's Note: This article is the second of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 828 and 829.

Purpose: Genomic testing may improve risk stratification in men with prostate cancer managed by active surveillance. We aimed to characterize the stability and usefulness of serial genomic test scores in men undergoing serial biopsies during active surveillance.

Materials and Methods: We compiled clinical and disease characteristics of men on active surveillance using an institutional Urologic Outcomes Database. We included patients initially diagnosed with Gleason 3 + 3 prostate cancer who elected active surveillance and received 2, 17-gene GPS (Genomic Prostate Score) results. We examined the association of GPS results and Gleason grade reclassification (Gleason 3 + 4 or greater) with definitive treatment using multivariable Cox proportional hazards regression models.

Results: We identified 111 men who underwent serial genomic testing. There were 49 grade reclassification events (44%) at a median followup of 64 months. The mean ± SD GPS change between the first and second biopsies was 2.1 ± 10.3. The GPS at first biopsy (per 5 units HR 1.04, 95% CI 1.00−1.07, p=0.03) was associated with an upgrade at second biopsy, although the second GPS was not (HR 1.02, 95% CI 0.99−1.05, p=0.13). The first and second GPSs (HR 1.09, 95% CI 1.04−1.14 and HR 1.09, 95% CI 1.04−1.14, each p <0.01) were associated with active treatment.

Conclusions: The GPS undergoes small changes with time. Absolute GPS results at the first and second biopsies were associated with Gleason upgrading and transition from active surveillance to active treatment.

Key Words: prostatic neoplasms, biopsy, watchful waiting, molecular diagnostic techniques, gene expression

The adoption of AS as the initial treatment approach in men with early stage and low grade prostate cancer has increased substantially in recent years.[1−3] Close monitoring of well selected patients has been shown to safely avoid definitive treatment and associated side effects in many men.[4] AS provides the opportunity to offer timely treatment to patients with curative intent if disease progression is later detected. Disease progression has typically been defined as an upgrade in the Gleason score on biopsy, which is the most common reason for transition to active treatment.[5−7]

Despite the good performance of clinical risk prediction models to identify individuals at greater risk

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for progression, the detection of progression and the subsequent timing of active treatment remain imperfect. Patients on AS are at 25% to 65% 10-year cumulative risk for upgrading.[8] Furthermore, the treatment rate is approximately 50% by 15 years.[5,9] In light of the potential for overtreatment and underestimation of disease severity, efforts to improve risk stratification are needed.

One approach to improving risk assessment involves genomic characterization of prostate cancer tissue.[10] Several tests have been developed which assess the expression status of multiple genes and they have been clinically validated to predict outcomes. The GPS is a biopsy based 17-gene assay which uses real-time quantitative polymerase chain reaction to measure expression levels of genes related to 4 tumor aggressiveness pathways, including androgen signaling, cellular organization, stromal response and cellular proliferation.[11,12] The GPS is a weighted average of gene expression levels on a scale of 0 to 100, representing increasing aggressiveness. GPS clinical reporting is embedded in the risk category of the patient as defined by PSA, the Gleason score and stage (ie very low, low and intermediate with the latter subcategorized as favorable or unfavorable).[13]

In this study we focused on the absolute score and the control of other disease characteristics to study the independent impact of the score (ie tumor biology). The GPS has been studied as an independent predictor of multiple end points relevant to selecting patients for AS, including the risk of adverse pathology at RP[11] and the risk of recurrence after treatment.[12] An important knowledge gap persists in assessing how scores may or may not change with time and whether any changes are associated with progression and/or active treatment.

Serial genomic testing of biopsy specimens during active surveillance may offer a means to detect a predisposition toward aggressive disease and lend insight into the natural history of clinically low risk prostate cancer with time. Therefore, we aimed to characterize the stability of the GPS score on serial biopsies and examine the relationships of multiple GPS tests with Gleason upgrading and the subsequent management strategy.

MATERIALS AND METHODS

Study Population

Study participants were retrospectively selected from the UCSF UODB (Urologic Outcomes Database). The study was approved by the Institutional Review Board to gather diagnostic, surgical, pathological and outcomes data on men treated for prostate cancer at UCSF. The date of the first positive biopsy was considered the date of enrollment on AS. All patients were followed prospectively and no end of study date was defined for this cohort.

Eligibility criteria included diagnosis in 2000 or later, clinical stage cT1/2, PSA less than 20 ng/ml at diagnosis, multiple GPS tests, and Gleason score 3 + 3 at diagnosis and at first biopsy with the GPS. While GPS testing was done at urologist and patient discretion, it was routinely offered to most patients at low risk starting in 2014.

Demographic, clinical and pathological data were obtained from the UODB. All pathological data were obtained by reviewing biopsy and surgical pathology reports. Patients without serial GPS testing and those not consented for research were excluded from study.

Molecular Assay

GPS testing was performed on biopsy specimens obtained during routine clinical care. An experienced UCSF genitourinary pathologist reviewed each biopsy specimen to determine primary and secondary Gleason grades, and selected 1 representative block with the greatest volume of the highest grade tumor. The specimen was formalin fixed and submitted elsewhere for histological evaluation, processing and scoring.

Exposures and Outcomes

The independent variables were continuous (raw) GPS scores at the first and second biopsies. The GPS score ranges from 0 to 100, representing least to most aggressive disease. Age, clinical characteristics at diagnosis such as PSA, PSA density, the percent of positive biopsy cores, the clinical CAPRA (Cancer of the Prostate Risk Assessment) score, the clinical site of diagnostic biopsy, the Gleason score at each GPS biopsy, the GPS score difference and pathological features at prostatectomy were reported.

The outcome variables were upgrading to 3 + 4 or greater at second biopsy and active treatment. Patients were censored at the date of the last clinical encounter, including PSA testing, biopsy, imaging, active treatment or another visit.

Statistical Analysis

Independent variables are described using frequency tables for categorical variables, and the mean ± SD and median (IQR) for continuous variables. We compared GPS scores with the Pearson R correlation coefficient. Cox proportional hazards regression models were evaluated to determine the risk of upgrading for each GPS exposure variable. The base model contained patient age, PSA density, the percent of positive biopsy cores and the clinical site of diagnostic biopsy (UCSF vs elsewhere). Additional models included the base model plus the GPS score at first biopsy and the base model plus the GPS score at second biopsy. We used Cox proportional hazards regression models adjusted for age, PSA density, the percent of positive biopsy cores, the clinical site, the GPS score and the GPS score difference to identify factors associated with the risk of active treatment. LR testing was done to evaluate whether there was an incremental benefit to adding a GPS at second biopsy to an adjusted model containing the GPS at first biopsy. Statistical analyses were performed

using SAS®, version 9.4 with $p < 0.05$ considered statistically significant.

RESULTS

Figure 1 shows a CONSORT (Consolidated Standards of Reporting Trials) diagram illustrating study cohort selection. Of the 1,465 men on AS with low risk disease at diagnosis 452 elected GPS testing. A total of 133 men (29.4%) had valid GPS results on 2 biopsies, of whom 111 with Gleason 3 + 3 at the first GPS comprised the final analysis population. Mean age at diagnosis was 61 ± 7.6 years and median PSA at diagnosis was 5.3 ng/ml (IQR 4.1–6.7). Patients were followed for 64 months (IQR 44–94). Median time between the first and second biopsies was 14 months (IQR 12–23). Table 1 lists clinical and demographic data.

There was no Gleason score upgrade at second biopsy in 62 men (56%). Of the 49 men with upgrading at second biopsy 42 (38%) had upgrading to Gleason 3 + 4 and 7 (6%) had upgrading to 4 + 3. The mean GPS at first and second biopsies was 23.0 ± 9.7 and 25.1 ± 9.9, respectively. The mean change in the GPS was 2.1 ± 10.3. There was moderate correlation between GPS findings at first and second biopsies (Pearson r = 0.45, fig. 2). The mean GPS change was 4.51 ± 10.6 vs 0.26 ± 9.7 in cases with vs without upgrading. Only 13 men (12%) had a GPS decrease greater than 1 SD.

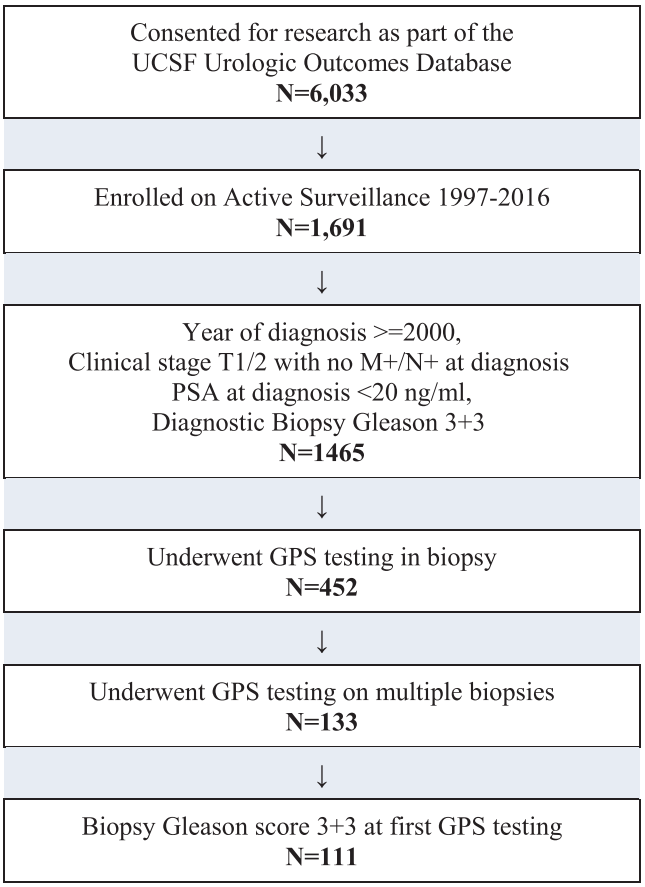

Figure 1. CONSORT diagram of study inclusion

In the base Cox proportional hazards regression model including the GPS at first biopsy a higher GPS at first biopsy was associated with a risk of upgrading at second biopsy when adjusting for age, PSA density, percent of positive cores and clinical site of diagnostic biopsy (HR 1.04, 95% CI 1.00–1.07, p = 0.03, table 2). The GPS at second biopsy was not associated with a biopsy upgrade when added to the base model (p = 0.13). To determine whether biopsy timing had an effect on the GPS (ie a more significant change in men with biopsies done at greater intervals) we examined scores based on the interval between biopsies. Although we observed a weak but significant correlation between the GPS and time, the GPS increased and decreased, creating wide variability which was not significant (Wilcoxon test p = 0.22).

Cox proportional hazards regression models were used to examine the effect of the GPS as well as a difference in the GPS on the risk of active treatment (table 2). In the base model plus the GPS at first biopsy the GPS (HR 1.09, 95% CI 1.04–1.14, $p < 0.01$) and the GPS difference (HR 1.26, 95% CI 1.05–1.52, p = 0.01) were associated with a risk of treatment adjusted for age, PSA density, percent of positive cores and clinical site of diagnostic biopsy. In the base model plus the GPS at second biopsy only the GPS (HR 1.09, 95% CI 1.04–1.14, $p < 0.01$) was associated with higher risk of undergoing active treatment. Each GPS was associated with a risk of treatment in a final adjusted model including scores at the first and second biopsies

Table 1. *Demographic and clinical characteristics in 111 men enrolled on AS of prostate cancer at UCSF with 2 serial scores available*

Mean ± SD age at diagnosis	61 ± 7.6	
Median at diagnosis (IQR):		
PSA (ng/ml)	5.3	(4.1–6.7)
PSA density (ng/ml/cc)	0.13	(0.10–0.17)
GPS biopsy 1:		
No. Gleason grade 3 + 3 (%)	111	(100)
Median % pos cores (IQR)	20	(10–29)
No. clinical CAPRA risk group (%):		
Low (0–2)	100	(90)
Intermediate (3–5)	11	(10)
No. pts meeting UCSF AS criteria (%):		
No	23	(21)
Yes	88	(79)
No. active treatment (%):		
None	68	(61)
Radical prostatectomy	37	(33)
Radiation therapy	5	(5)
Androgen deprivation therapy	1	(1)
Median mos followup (IQR)	64	(44–94)

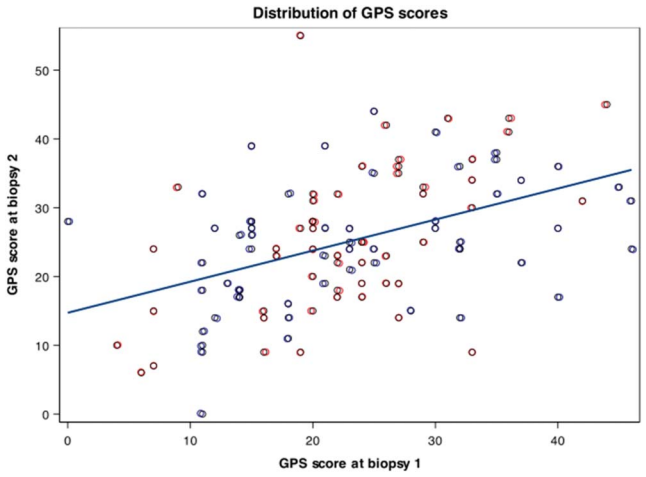

Figure 2. GPS distribution at first (blue) and second (red) biopsies (Pearson correlation coefficient 0.45) in 111 men enrolled on AS of prostate cancer at UCSF.

(LR p <0.01). In models including only the GPS at first biopsy and only the GPS at second biopsy there was no incremental benefit to including serial scores in a single model (partial LR chi-square test p <0.01).

DISCUSSION

In this study we investigated serial GPS testing in men with low grade prostate cancer on AS to understand the impact of the absolute GPS result on biopsy upgrading and time to active treatment. We found that GPS results at first and second biopsies moderately correlated and showed good stability with time. While the GPS at first biopsy was associated with an upgrade at second biopsy, scores at the second biopsy were not so associated. The GPS results at first and second biopsies were associated with active treatment.

Our findings suggest that the GPS is relatively stable with time since the change was less than 10%. This supports the notion that a single GPS result may be more informative than the interval change in men on AS. This also suggests that clinicians may want to pay more attention to the absolute GPS rather than to the clinical category it is placed in at the time of testing (ie very low, low or intermediate risk). However, our cohort had too little variance in clinical risk to directly evaluate an association between risk category and an upgrade at second biopsy.

The GPS at first biopsy was associated with a Gleason upgrade. Absolute scores were also relatively stable even in men with a biopsy upgrade, suggesting that the initial score accurately assessed tumor biology in most patients (ie low false-positive and false-negative rates). At approximately 1 year stable GPS results may be expected in cases in which changes in Gleason findings reflect sampling error rather than genuine disease progression.

Knudsen et al determined that adequate expression levels can be obtained even in limited biopsy samples and there is a high degree of concordance of genomic expression between biopsy and RP samples (r=0.96).[14] These conclusions appear to address concerns of overemphasis on a single biopsy interpretation. The GPS was previously shown to adequately stratify risk and predict upgrading and adverse pathology at RP.[11,12,15] In a study of 314 men with 3 + 3 disease in the same cohort a 5-unit increase in the GPS was associated with a 28% increased risk of upgrading.[15] In men with low to intermediate risk prostate cancer who were candidates for AS a retrospectively tested GPS predicted high grade and high stage disease at RP.[11] Other studies have reinforced the usefulness of the GPS by showing that it predicts time to biochemical recurrence and time to metastasis after treatment.[12]

We found that patients with a Gleason upgrade who ultimately received treatment had higher GPS findings. While we could not definitively assess the impact on treatment strategy without further analyses of the decision making process, it is possible that clinicians may not have interpreted a large change in the GPS on serial testing as a sign of true biological progression. One group observed that

Table 2. *Cox proportional hazards regression models of upgrade risk at GPS 2 and transition to active treatment in 111 men enrolled on AS of prostate cancer at UCSF*

	HR (95% CI)	p Value
Upgrade risk at GPS 2		
Base model + GPS 1:		
1st Biopsy GPS/5-unit increase	1.04 (1.00–1.07)	0.03
Age at diagnosis	1.00 (0.96–1.05)	0.92
PSA density at diagnosis (logarithm)	1.59 (0.82–3.08)	0.17
% GPS 1 pos biopsy cores	1.04 (1.01–1.06)	<0.01
Diagnostic biopsy at UCSF vs elsewhere	1.36 (0.69–2.67)	0.37
Base model + GPS 2:		
2nd Biopsy GPS/5-unit increase	1.02 (0.99–1.05)	0.13
Age at diagnosis	1.01 (0.97–1.06)	0.60
PSA density at diagnosis (logarithm)	1.59 (0.80–3.17)	0.19
% GPS 1 pos biopsy cores	1.04 (1.02–1.06)	<0.01
Diagnostic biopsy at UCSF vs elsewhere	1.48 (0.75–2.94)	0.26
Transition to active treatment		
Base model + GPS 1:		
1st Biopsy GPS/5-unit increase*	1.09 (1.04–1.14)	<0.01
GPS difference	1.26 (1.05–1.52)	0.01
Age at diagnosis	0.70 (0.93–1.01)	0.10
PSA density at diagnosis (logarithm)	1.06 (0.53–2.11)	0.87
% GPS 1 Pos biopsy cores	1.01 (0.99–1.03)	0.34
Diagnostic biopsy done at UCSF (vs elsewhere)	1.48 (0.77–2.84)	0.24
Base model + GPS 2:		
2nd Biopsy GPS/5-unit increase*	1.09 (1.04–1.14)	<0.01
GPS difference	0.83 (0.68–1.00)	0.05
Age at diagnosis	0.97 (0.93–1.01)	0.11
PSA density at diagnosis (logarithm)	1.06 (0.53–2.11)	0.87
% Pos biopsy cores at GPS 1	1.01 (0.99–1.03)	0.34
Diagnostic biopsy done at UCSF (vs elsewhere)	1.48 (0.77–2.83)	0.24

* Full/reduced model likelihood chi-square p = 0.0042/0.0004.

physicians changed treatment recommendations after Prolaris® genetic testing in 65% of patients.[16] Eure et al reported that the rates of active surveillance uptake (62% vs 40%) and persistence on AS at 1 year (55% vs 35%) were higher among men who underwent the GPS test compared to those without GPS testing.[17] These findings support the notion that genetic testing impacts clinical decisions about AS and treatment. In the current study it appeared that the initial GPS test was the most informative one and serial testing may have more limited usefulness. Therefore, adding routine serial genomic testing to current AS regimens could raise costs without providing commensurate benefits.

Our study has several limitations. Given the limited sample size, our observations should be interpreted cautiously. As an observational study our findings were based solely on patients whose biopsies were sent for serial GPS testing. Therefore, our findings are conditional on unknown factors which contributed to this decision, although genomic testing had been incorporated into the surveillance strategy at UCSF for many patients.

Also, we were unable to investigate the association between a change in the GPS and adverse pathology at RP in this cohort due to the small number of men with 2 GPS tests who underwent surgery. However, we noted this correlation in a larger cohort with a single test.[11]

Finally, the decision to move from AS to definitive treatment can be complex, involving individual demographics, cancer characteristics, patient preference and physician judgment, which may be better explored by analyses of patient and provider decision making. Our study had limited ability to investigate such aspects, although shared decision making is uniformly endorsed at UCSF.

CONCLUSIONS

GPS scores were relatively stable with time among patients with favorable risk prostate cancer on AS who underwent serial biopsy and GPS testing. The initial test is the most informative one and serial testing seems to have limited benefit.

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REFERENCES

1. Loeb S, Walter D, Curnyn C et al: How active is active surveillance? Intensity of followup during active surveillance for prostate cancer in the United States. J Urol 2016; **196:** 721.
2. Cooperberg MR and Carroll PR: Trends in management for patients with localized prostate cancer, 1990-2013. JAMA 2015; **314:** 80.
3. Womble PR, Montie JE, Ye Z et al: Contemporary use of initial active surveillance among men in Michigan with low-risk prostate cancer. Eur Urol 2015; **67:** 44.
4. Hamdy FC, Donovan JL, Lane JA et al: 10-Year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. N Engl J Med 2016; **375:** 1415.
5. Tosoian JJ, Mamawala M, Epstein JI et al: Intermediate and longer-term outcomes from a prospective active-surveillance program for favorable-risk prostate cancer. J Clin Oncol 2015; **33:** 3379.
6. Simpkin AJ, Tilling K, Martin RM et al: Systematic review and meta-analysis of factors determining change to radical treatment in active surveillance for localized prostate cancer. Eur Urol 2015; **67:** 993.
7. Lang MF, Tyson MD, Alvarez JR et al: The influence of psychosocial constructs on the adherence to active surveillance for localized prostate cancer in a prospective, population-based cohort. Urology 2017; **103:** 173.
8. Inoue LY, Lin DW, Newcomb LF et al: Comparative analysis of biopsy upgrading in four prostate cancer active surveillance cohorts. Ann Intern Med 2018; **168:** 1.
9. Newcomb LF, Thompson IM Jr, Boyer HD et al: Outcomes of active surveillance for clinically localized prostate cancer in the prospective, multi-institutional Canary PASS cohort. J Urol 2016; **195:** 313.
10. Leapman MS, Nguyen HG and Cooperberg MR: Clinical utility of biomarkers in localized prostate cancer. Curr Oncol Rep 2016; **18:** 30.
11. Klein EA, Cooperberg MR, Magi-Galluzzi C et al: A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol 2014; **66:** 550.
12. Cullen J, Rosner IL, Brand TC et al: A biopsy-based 17-gene genomic prostate score predicts recurrence after radical prostatectomy and adverse surgical pathology in a racially diverse population of men with clinically low-and intermediate-risk prostate cancer. Eur Urol 2015; **68:** 123.
13. National Comprehensive Cancer Network®: Prostate Cancer, v 3.2016. Available at https://www.nccn.org/professionals/physician_gls/PDF/prostate.pdf. Accessed March 14, 2019.
14. Knudsen BS, Kim HL, Erho N et al: Application of a clinical whole-transcriptome assay for staging and prognosis of prostate cancer diagnosed in needle core biopsy specimens. J Mol Diagn 2016; **18:** 395.
15. Kornberg Z, Cowan JE, Westphalen AC et al: Genomic prostate score, PI-RADSv2, and progression in men with prostate cancer on active surveillance. J Urol 2019; **201:** 300.
16. Crawford ED, Scholz MC, Kar AJ et al: Cell cycle progression score and treatment decisions in prostate cancer: results from an ongoing registry. Curr Med Res Opin 2014; **30:** 1025.
17. Eure G, Germany R, Given R et al: Use of a 17-gene prognostic assay in contemporary urologic practice: results of an interim analysis in an observational cohort. Urology 2017; **107:** 67.

EDITORIAL COMMENT

This study addresses the important emerging concept of serial biological monitoring in the treatment of men on active surveillance. Because the molecular changes that drive cancer progression occur before histology changes, and because histological change precedes visibility on magnetic resonance imaging, measurement of these molecular changes may be a harbinger of tumor progression which dictates more frequent monitoring or early intervention. The study provides for the first time prospective information on how gene expression scores as measured by an Oncotype (Genomic Health) change during the initial months in men treated with active surveillance. Most men with National Comprehensive Cancer Network® very low or low risk disease who have upgrading on a second biopsy within a year of initial diagnosis are likely to have been under sampled rather than have true progression. Thus, it is not surprising that mean scores on serial GPS tests did not change in the short mean of 14 months between them in this cohort, suggesting that the GPS has a low false-positive rate. Serial testing at longer intervals and with longer clinical followup will be needed to determine if there is real clinical utility to this approach.

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A 17-Gene Genomic Prostate Score as a Predictor of Adverse Pathology in Men on Active Surveillance

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Abbreviations and Acronyms

AS = active surveillance

CAPRA = Cancer of the Prostate Risk Assessment

GPS = Genomic Prostate Score

IPCW = inverse probability of censoring weighting

PCa = prostate cancer

PCSM = PCa specific mortality

PSA = prostate specific antigen

RP = radical prostatectomy

UCSF = University of California-San Francisco

Purpose: The GPS (Oncotype Dx® Genomic Prostate Score) test is a RNA expression assay which can be performed on prostate biopsies. We sought to determine whether the GPS was associated with an increased risk of adverse pathology findings in men enrolled on active surveillance who later underwent radical prostatectomy.

Materials and Methods: We identified all patients on active surveillance at University of California-San Francisco who had Gleason score 3 + 3 or low volume (33% or fewer positive cores) Gleason score 3 + 4 prostate cancer, GPS testing at diagnostic or confirmatory biopsy, clinical stage T1/T2, prostate specific antigen less than 20 and a clinical CAPRA (Cancer of the Prostate Risk Assessment) score less than 6. The primary outcome was adverse pathology, defined as Gleason score 4 + 3 or greater, stage pT3a or greater, or pN1. The secondary outcome was biochemical recurrence, defined as 2 consecutive prostate specific antigen measurements greater than 0.05 ng/ml following radical prostatectomy.

Results: Of the 215 men 179 (83%) were at low risk and 36 (17%) were at intermediate risk by CAPRA scoring. The median GPS was 26.4 (IQR 18.8-34.6). On multivariate analysis a higher GPS was associated with an increased risk of adverse pathology at delayed radical prostatectomy (HR/5 units 1.16, 95% CI 1.06−1.26, p <0.01). A higher GPS was also associated with an increased risk of biochemical recurrence (HR/5 units 1.10, 95% CI 1.00−1.21, p=0.04).

Conclusions: In patients who undergo radical prostatectomy after a period on active surveillance, as in those who undergo immediate prostatectomy, a higher GPS is associated with an increased risk of adverse pathology. The GPS is also associated with biochemical recurrence following radical prostatectomy in such patients.

Key Words: prostatectomy; genomics; pathology, clinical; watchful waiting; prostate-specific antigen

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In men with low or intermediate risk PCa the decision to enroll in AS in lieu of immediate primary treatment with RP or radiation therapy can be challenging. While clinical risk stratification tools such as the CAPRA score offer excellent accuracy to predict long-term clinical outcomes based on clinical characteristics,[1] they are imperfect to predict indolent disease.[2,3] In recent years additional genomic and imaging modalities have been developed to further guide patients considering definitive treatment of prostate cancer.

The Oncotype DX® GPS test is a RNA based expression assay of 12 PCa related genes normalized to 5 housekeeping genes. The test can be performed on needle core biopsy tissue from men with PCa. The GPS ranges from 0 to 100 with higher scores indicating a greater genomic risk of aggressive disease. Independent of clinical characteristics (PSA, Gleason grade, tumor volume, stage and patient age) the GPS has been validated as a predictor of PCSM and time to metastasis following surgical management.[4] In men with low or intermediate risk PCa who potentially qualify for AS but elect primary treatment with RP higher GPS scores are also associated with an increased risk of adverse pathology.[5] In a recent retrospective clinical cohort study GPS was found to predict biopsy upgrading in men with Gleason 3 + 3 PCa on AS.[6]

To further evaluate the GPS test in men with low or intermediate risk PCa on AS we sought to determine whether a higher GPS score is associated with an increased risk of adverse pathology and/or biochemical recurrence among men who underwent delayed RP after an initial period of AS.

METHODS

We evaluated the association between GPS and adverse pathological features at surgery and/or biochemical recurrence in men enrolled on AS at UCSF between 2001 and 2016 who underwent delayed RP after at least 6 months on AS. Participants were diagnosed with Gleason 3 + 3 or low volume 3 + 4 cancer (ie 33% or fewer positive cores), organ confined disease, PSA less than 20 ng/ml and a clinical CAPRA risk of 0 to 5.[7] Genomic testing was done on diagnostic or confirmatory biopsy within 24 months of diagnosis. All patients were enrolled in the UCSF Urology Outcomes Database under Institutional Review Board supervision and provided informed consent to use their data and tissue for research.

Genomic testing of participants was initiated according to a research protocol or as part of clinical care. A protocol was prespecified to identify study eligible patients, define independent and outcome variables, delineate specimen handling and review, determine data locking and sharing, describe statistical methods of data analysis and calculate sample size and power. UCSF staff requested biospecimens at UCSF or from elsewhere for eligible men who had not undergone prior GPS testing and they received the biopsy tissue of 150 patients. A single genitourinary pathologist (JPS) at UCSF who was blinded to clinical information reviewed each case and selected the tissue, which was sent elsewhere for processing.

Also included in study were an additional 65 patients on AS who met study clinical criteria, underwent GPS testing prospectively as part of routine clinical care and subsequently underwent delayed RP. Again a genitourinary pathologist at UCSF reviewed and selected the tissue, which was sent elsewhere for testing. However, no additional specimen collection or pathology review was completed in these cases. Of these cases 45 were reviewed by the same lead pathologist (JPS) who reviewed the joint study. The remaining 20 cases were reviewed by another genitourinary pathologist in the same group.

The main variable of interest was the GPS at diagnostic or confirmatory biopsy. Related independent variables were described, including patient age in years at diagnosis, race/ethnicity, serum PSA in ng/ml, PSA density, Gleason grade (3 + 3 or 3 + 4), percent of positive biopsy cores, clinical stage (T1 or T2), year of diagnosis, clinical site of GPS biopsy (UCSF or elsewhere), biopsy timing (diagnostic or confirmatory) and whether GPS testing was done as part of the research protocol or routine clinical care by the lead or another pathologist. The primary outcome was adverse pathology, a binary variable which was defined as the presence of pathological Gleason 4 + 3 or greater, or pT3 or pN1 disease at RP. The secondary outcome was biochemical recurrence after RP, defined as 2 consecutive PSA values of 0.05 ng/ml or greater.

Time from diagnostic/confirmatory biopsy to RP varied among patients. Thus, unadjusted rates of adverse pathology at RP as well as post-surgical biochemical recurrence were estimated with life tables. We modeled the association of GPS with the risk of adverse pathology using Cox proportional hazards regression with IPCW to adjust for possible dependent censoring.[8] The model was adjusted a priori for the CAPRA component variables (age, PSA, clinical T stage, Gleason grade and percent of positive biopsy cores), PSA density, race and clinical site of GPS biopsy (UCSF or elsewhere). The final model included age at diagnosis, the clinical CAPRA score, PSA density at the time of GPS, whether the GPS was derived from the diagnostic or the confirmatory biopsy, clinical site of the GPS biopsy and whether the GPS was done as part of the research protocol or as clinical care by the lead or another pathologist.

The effect of the GPS on the risk of adverse pathology was evaluated further on stratified analysis using similarly adjusted Cox proportional hazards regression with IPCW to explore possible selection bias. Stratified models were applied to characterize the testing cohort (research or clinical) and the clinical site of the GPS biopsy (UCSF or elsewhere). The association between the GPS and the risk of biochemical recurrence following RP was assessed by Cox proportional hazards regression without IPCW (time 0 at RP) adjusted for the same variables used to assess the primary outcome. Two-sided $p < 0.05$ was considered statistically significant and all analyses were performed with SAS® 9.4 for Windows®.

RESULTS

Of the 1,662 men enrolled on AS who were consented for research as part of the UCSF Urology Outcomes Database 378 underwent delayed RP and met study inclusion criteria based on clinical characteristics. GPS testing was done on the diagnostic or the confirmatory biopsy in 215 of these men, who comprised the study cohort (fig. 1).

Mean ± SD age of the study cohort was 60.7 ± 6.8 years. Of the men 154 (72%) had GS 3 + 3 PCa while 61 (28%) had GS 3 + 4 PCa on GPS biopsy (table 1). Of the cohort 83% were at low risk by clinical CAPRA scoring. Of the GPS scores 68% were derived from diagnostic biopsies and the median GPS was 26.4 (IQR 18.8-34.6). A total of 125 men experienced a biopsy upgrade while on AS at a median of 18 months (IQR 12-35), of whom 121 (56%) had adverse pathology at delayed RP. Median time from first GPS to RP was 20 months (IQR 14-37). Figure 2 shows a waterfall plot of GPS scores with adverse pathology results.

On multivariate Cox proportional hazards regression with IPCW the GPS was independently associated with an increased risk of adverse pathology at RP (HR/5 units 1.16, 95% CI 1.06–1.26, $p < 0.01$, table 2). Older age at diagnosis (HR per year 1.07, 95% CI 1.03–1.11) and whether GPS testing was performed as part of routine clinical care with Gleason grading done by the lead pathologist (HR 2.42, 95% CI 1.41–4.14) or by another pathologist

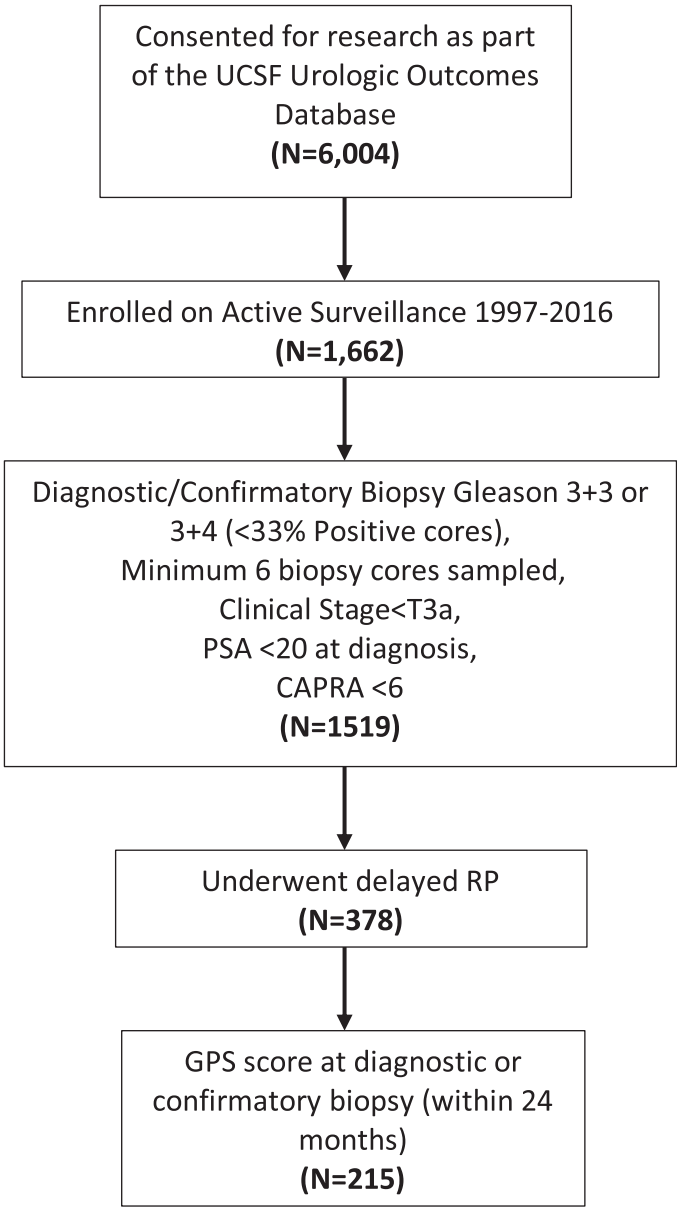

Figure 1. Study inclusion CONSORT (Consolidated Standards of Reporting Trials) diagram

Table 1. *Disease characteristics of 215 men enrolled on active surveillance who later underwent delayed radical prostatectomy at UCSF*

Mean ± SD age at diagnosis	60.7 ± 6.8	
No. race/ethnicity (%):		
Asian/Pacific Islander	5	(2)
Latino	1	(0)
African American	5	(2)
Caucasian	189	(89)
Other	15	(6)
Median ng/ml PSA at diagnosis (IQR)	5.3	(4.2–7.0)
No. clinical T stage (%):		
T1c	145	(67)
T2	6	(3)
T2a	52	(24)
T2b	6	(3)
T2c	6	(3)
No. total biopsy Gleason score (%):		
3 + 3	154	(72)
3 + 4	61	(28)
No. CAPRA clinical risk group (%):		
Low (0–2)	179	(83)
Intermediate (3–5)	36	(17)
Median GPS (IQR)	26.4 (18.8–34.6)	
No. GPS biopsy timing (%):		
Diagnostic	147	(68)
Confirmatory	68	(32)
No. GPS biopsy source (%):		
UCSF	121	(56)
Elsewhere	94	(44)
No. GPS testing cohort (%):		
Research case reviewed by lead pathologist	150	(70)
Clinical care reviewed by lead pathologist	45	(21)
Clinical care reviewed by other pathologist	20	(9)
No. followup biopsy additional GPS (%):		
No	170	(79)
Yes	45	(21)
No. Gleason score at RP (%):		
3 + 3	40	(19)
3 + 4	130	(60)
4 + 3	33	(15)
4 + 4	6	(3)
4 + 5	4	(2)
5 + 4	2	(1)
No. pathological T stage (%):		
T2	123	(57)
T3a	78	(36)
T3b	8	(4)
T4	6	(3)
No. pathological N stage (%):		
NX	154	(72)
N0	58	(27)
N1	3	(1)
No. CAPRA Postsurgical Score risk group at RP (%):		
Low (0–2)	115	(53)
Intermediate (3–5)	91	(42)
High (6–10)	9	(4)
No. adverse pathology characteristic (%):		
pT4	6	(5)
pN1	3	(2)
Seminal vesicle invasion	6	(5)
Gleason score 8 or greater	9	(7)
Multifocal extracapsular extension	59	(49)
Unifocal extracapsular extension	12	(10)
Gleason score 4 + 3	14	(12)
Gleason score 3 + 4 with tertiary pattern 5	12	(10)

(HR 2.57, 95% CI 1.45–4.55, all p <0.01) were also associated with an increased risk of adverse pathology. GPS testing performed on the confirmatory biopsy was associated with a decreased risk of adverse pathology (HR 0.53, 95% CI 0.34–0.82).

The GPS testing cohort (research vs clinical) was independently associated with a risk of adverse pathology, prompting further analysis of this relationship. In similarly adjusted models stratified by testing cohort we found that the GPS remained significant in 150 men in the research cohort (HR/5 units 1.14, 95% CI 1.03–1.26, p=0.01) and in 65 in the clinical cohort (HR/5 units 1.26, 95% CI 1.05–1.52, p=0.01.) In models stratified by the GPS biopsy site (UCSF vs elsewhere) the GPS remained significant in 121 men who underwent biopsy at UCSF (HR/5 units 1.14, 95% CI 1.25–1.61, p <0.01) but not in the 94 with biopsies elsewhere (HR/5 units 1.04, 95% CI 0.91–1.20, p=0.57.) However, in the full multivariate model the clinical site of biopsy (UCSF or elsewhere) was not significantly associated with adverse pathology.

Biochemical recurrence was evaluated as a secondary outcome. Median time to recurrence was 17 months (IQR 8-26). Three years after RP recurrence developed in 52 men according to our strict definition of biochemical recurrence (2 × PSA 0.05 ng/ml or greater). On Cox proportional hazards regression the GPS was independently associated with biochemical recurrence following delayed RP (HR/5 units 1.10, 95% CI 1.00–1.21, p=0.04, table 2). No other variable in the model was associated with an increased risk of biochemical recurrence following RP.

DISCUSSION

In men who elect AS after the diagnosis of PCa the burden of monitoring disease is significant. Prostate biopsy, which remains the mainstay of AS, can be uncomfortable, costly and a potential cause of infection.[9,10] Although the risk of metastasis and PCSM is low, it is not insignificant in men with PCa who are conservatively treated.[11,12] Therefore, identifying men at the time of diagnosis or shortly thereafter who are likely to experience progression on AS and, thus, who would benefit from earlier definitive treatment is important in patients with low and intermediate risk disease.

With this analysis we present an assessment of the prognostic capabilities of a commercially available genomic assay in men on AS. Our findings show that when controlling for clinical variables, higher GPS scores were independently associated with an increased risk of adverse pathology in men who underwent delayed RP. Age and the GPS testing group were the only other factors, including the CAPRA score, which were associated with adverse pathology. Men with a higher GPS score were also at increased risk for biochemical recurrence following delayed RP.

These findings build on our previously published data showing that higher GPS scores were associated with adverse pathology in men who immediately

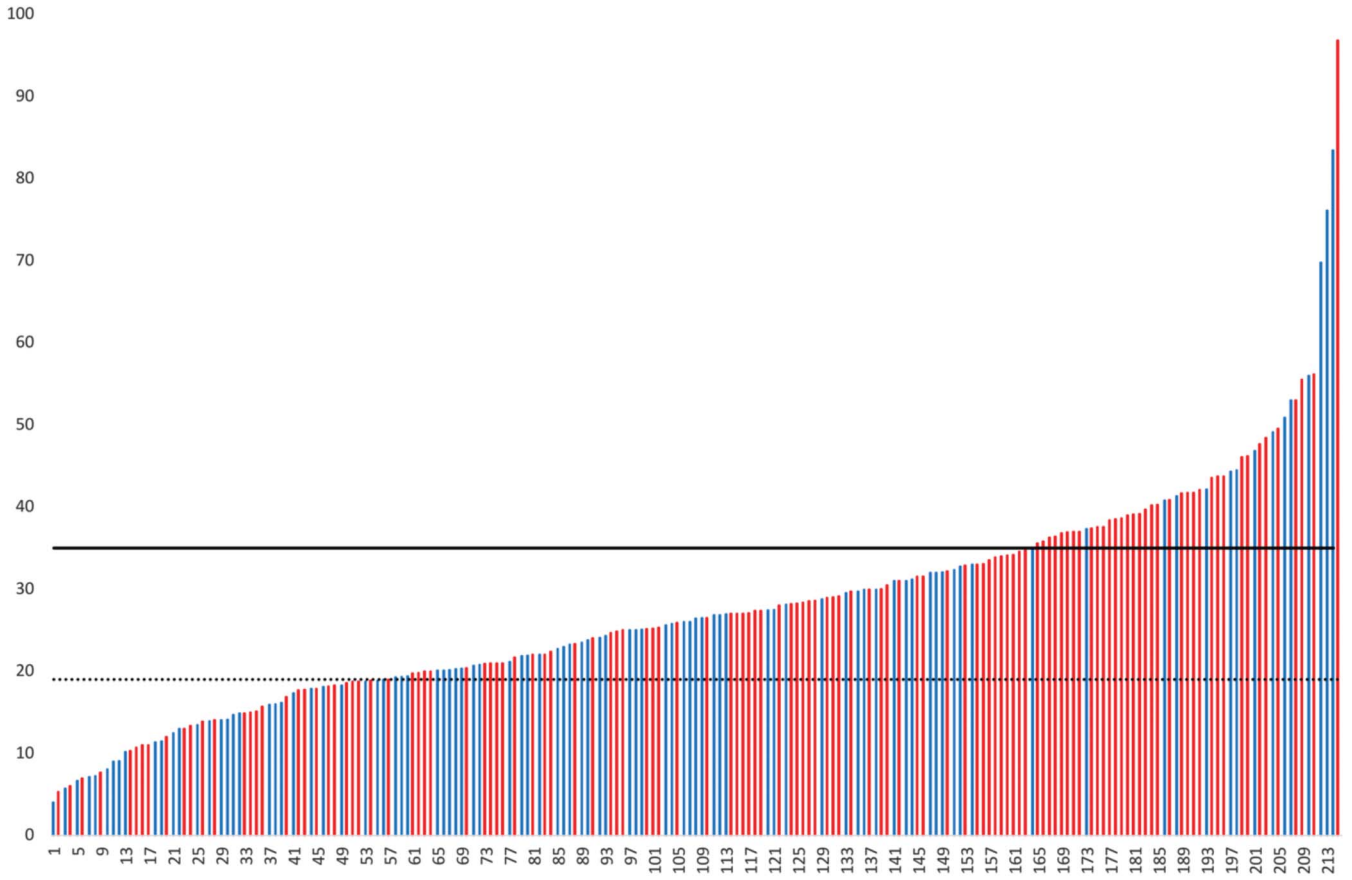

Figure 2. Waterfall plot shows GPS and adverse surgical pathology (red bars) outcome in 215 men enrolled on AS who later underwent delayed RP at UCSF. Blue bars indicate favorable pathology. Dotted horizontal line indicates 25th percentile. Solid horizontal line indicates 75th percentile.

elected RP and with an increased risk of biopsy upgrading in men with GS 3 + 3 PCa on AS.[5,6] In the current study the GPS was independently associated with adverse pathology when adjusting for the clinical CAPRA score and other factors. Considering these results, it is reasonable to use the GPS in conjunction with known clinical risk factors associated with aggressive disease to counsel select patients who are considering AS. However, identifying logical cutoffs with which to guide treatment remains challenging for clinicians and patients alike. This is evident in figure 2, which demonstrates that no lower limit of the GPS ensured no adverse pathology and no upper limit of the GPS ensured adverse pathology on delayed RP.

Notably alternatives to GPS may also be performed on paraffin fixed prostate biopsy samples. The Prolaris® cell cycle progression score was

Table 2. *Inverse probability of censoring weighted multivariable Cox proportional hazards regression of GPS on risk of adverse surgical pathology and multivariable Cox proportional hazards regression of GPS on risk of biochemical recurrence after surgery in 215 men on active surveillance who later underwent delayed radical prostatectomy at UCSF*

	Adverse Pathology Risk		Biochemical Recurrence Risk	
	p Value	HR (95% CI)	p Value	HR (95% CI)
GPS/5 units	<0.01	1.16 (1.06–1.26)	0.04	1.10 (1.00–1.21)
At diagnosis:				
Age	<0.01	1.07 (1.03–1.11)	0.41	0.98 (0.94–1.02)
Clinical CAPRA score	0.60	0.92 (0.66–1.27)	0.19	1.29 (0.88–1.90)
Log PSA density at GPS	0.06	1.70 (0.97–2.96)	0.65	0.88 (0.49–1.57)
GPS at confirmatory vs diagnostic biopsy	<0.01	0.53 (0.34–0.82)	0.08	1.80 (0.94–3.45)
Biopsy source (UCSF vs elsewhere)	0.77	0.93 (0.58–1.49)	0.59	0.86 (0.50–1.49)
GPS clinical testing group vs research protocol:				
Lead pathologist	<0.01	2.42 (1.41–4.14)	0.14	0.60 (0.28–1.30)
Other pathologist	—	2.57 (1.45–4.55)	—	0.20 (0.03–1.46)

recently validated as a predictor of PCSM in a large cohort of men with PCa who were conservatively treated with AS.[13] Using the cell cycle progression score Lin et al were able to identify cutoffs with high negative predictive value for PCSM in men with low and intermediate risk PCa. The Decipher® genomic classifier for biopsy tissue, which is to our knowledge the only other commercially available biopsy assay in this space, was validated in a cohort of men who underwent primary treatment with RP or radiation therapy as a predictor of metastasis and PCSM, although it has not yet been examined in an AS population.[14,15] Because specific outcomes and populations differ in the validation studies of these assays, prospective head-to-head trials would be beneficial to help patients and clinicians navigate the market of commercially available genomic assays. It also should be noted that existing guidelines stress that AS is preferred in most men with clinically low risk disease, genomic testing is optional in select patients, and PSA and biopsy monitoring remain the mainstay of observation in men on AS.[9,16]

To report on all UCSF men on AS who underwent delayed RP and had a GPS score we combined data on those tested retrospectively for research with data on those who were prospectively tested as part of clinical care. Median GPS scores were similar in these groups (research group 26.5 vs clinical group 26). We observed that the GPS was associated with the risk of adverse pathology in the research and clinical cohorts.

Limitations to this study must be acknowledged, such as its retrospective nature. Because the majority of patients were Caucasian, generalizability to a more diverse population may be limited. The subset of men on AS who elected RP may not be representative of typical patients on AS and the duration of surveillance was relatively short in most men in this study. We analyzed the GPS as a continuous variable in the setting of a multivariable clinical model, as was done in the original validation studies.[14,15] The GPS score is presented in a different and more limited context of the National Comprehensive Cancer Network® risk groups in current clinical reports.[16] Our findings do not necessarily support its use as presented in those reports.

CONCLUSIONS

In men with low and intermediate risk PCa who enroll in AS and go on to delayed RP a higher GPS at baseline is independently associated with an increased risk of adverse pathology and biochemical recurrence following definitive treatment. The GPS may be used in conjunction with clinical characteristics to help guide treatment decisions for patients considering AS.

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REFERENCES

1. Cooperberg MR, Broering JM and Carroll PR: Risk assessment for prostate cancer metastasis and mortality at the time of diagnosis. J Natl Cancer Inst 2009; **101:** 878.

2. Wang SY, Cowan JE, Cary KC et al: Limited ability of existing nomograms to predict outcomes in men undergoing active surveillance for prostate cancer. BJU Int 2014; **114:** E18.

3. Cooperberg MR, Carroll PR and Klotz L: Active surveillance for prostate cancer: progress and promise. J Clin Oncol 2011; **29:** 3669.

4. Van Den Eeden SK, Lu R, Zhang N et al: A biopsy-based 17-gene genomic prostate score as a predictor of metastases and prostate cancer death in surgically treated men with clinically localized disease. Eur Urol 2018; **73:** 129.

5. Klein EA, Cooperberg MR, Magi-Galluzzi C et al: A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol 2014; **66:** 550.

6. Kornberg Z, Cowan JE, Westphalen AC et al: Genomic prostate score, PI-RADSv2, and progression in men with prostate cancer on active surveillance. J Urol 2019; **201:** 300.

7. Cooperberg MR, Pasta DJ, Elkin EP et al: The University of California, San Francisco Cancer of the Prostate Risk Assessment Score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. J Urol 2005; **173:** 1938.

8. Robins JM and Finkelstein DM: Correcting for noncompliance and dependent censoring in an AIDS clinical trial with inverse probability of censoring weighted (IPCW) log-rank tests. Biometrics 2000; **56:** 779.

9. Chen RC, Rumble RB, Loblaw DA et al: Active surveillance for the management of localized prostate cancer (Cancer Care Ontario Guideline): American Society of Clinical Oncology clinical practice guideline endorsement. J Clin Oncol 2016; **34:** 2182.

10. Borghesi M, Ahmed H, Nam R et al: Complications after systematic, random, and image-guided prostate biopsy. Eur Urol 2017; **71:** 353.

11. Hamdy FC, Donovan JL, Lane JA et al: 10-Year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. N Engl J Med 2016; **375:** 1415.

12. Klotz L, Vesprini D, Sethukavalan P et al: Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol 2015; **33:** 272.

13. Lin DW, Crawford ED, Keane T et al: Identification of men with low-risk biopsy-confirmed prostate cancer as candidates for active surveillance. Urol Oncol Semin Original Invest 2018; **36:** 310.e317.

14. Klein EA, Haddad Z, Yousefi K et al: Decipher genomic classifier measured on prostate biopsy predicts metastasis risk. Urology 2016; **90:** 148.

15. Nguyen PL, Haddad Z, Ross AE et al: Ability of a genomic classifier to predict metastasis and

prostate cancer-specific mortality after radiation or surgery based on needle biopsy specimens. Eur Urol 2017; **72:** 845.

16. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology. Prostate Cancer, version 3.2018. Available at https://www.nccn.org/professionals/physician_gls/default.aspx. Accessed April 12, 2019.

EDITORIAL COMMENTS

Tissue based genomic markers have emerged as useful tests to select patients for treatment vs observation with evidence supporting the claim of predicting adverse oncologic end points. The authors previously validated 1 such signature, the GPS, for predicting adverse pathology in a cohort of men with low/intermediate risk prostate cancer who underwent immediate radical prostatectomy (reference 5 in article). Building on this work, in the current study they investigated the same signature for predicting adverse cancer in men who underwent initial observation followed by delayed surgery. The study concludes that the GPS score is an independent predictor of adverse pathology in men with delayed therapy, making it helpful for treatment decision making.

While I agree that these tests add prognostic value, we have much to learn about how to best use them in the clinic. For instance, it is not clear which patients benefit from these tests vs those who do not. Furthermore, we need a better understanding of the correlation between serial test results and tumor progression. Finally, evidence has emerged that genomic testing may be impacted by tumor heterogeneity and you may see different results between different cores in the same biopsy.[1,2] However, to our knowledge the impact of this on genomic risk classification using these tests remains unknown.

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REFERENCES

1. Wei L, Wang J, Lampert E et al: Intratumoral and intertumoral genomic heterogeneity of multifocal localized prostate cancer impacts molecular classifications and genomic prognosticators. Eur Urol 2017; **71:** 183.

2. Salami SS, Hovelson DH, Kaplan JB et al: Transcriptomic heterogeneity in multifocal prostate cancer. JCI Insight 2018; **3:** 3.

The reporting of long-term outcomes in prospective AS cohorts and randomized trials of active monitoring vs treatment have helped increase AS utilization in men with favorable risk prostate cancer. Active surveillance of men with very low risk disease is the standard management strategy and it should be offered as an option to men at low and favorable intermediate risk. It is important to note that men with high volume, low risk prostate cancer and those at favorable intermediate risk are under studied compared to men with low volume, low grade disease. They are at higher risk for disease reclassification and poor oncologic outcomes if AS is pursued.[1,2] Newer technologies such as genomics and imaging may help physicians more safely extend the scope of AS in practice.

Expression based biomarkers such as the Oncotype Dx Prostate GPS score, the Decipher GC score and the Prolaris cell cycle progression score have all been shown to be independently prognostic of oncological outcomes in prostate cancer.[3] Kornberg et al evaluated the GPS in men from UCSF who initially chose AS but subsequently underwent radical prostatectomy. Building on their previous work the authors found that an increasing GPS score is an independent predictor of adverse pathology at prostatectomy and subsequent ultrasensitive biochemical failure. Although not explored in this study, the authors previously reported that the GPS score has independent prognostic value even when magnetic resonance imaging findings are considered.[4]

Unanswered questions remain, including what the frequency of genomic testing should be and whether favorable genomic scores can allow for a reduction in the frequency of surveillance biopsies. Currently, although not the standard of care, I find molecular testing to be a valuable tool for counseling men at favorable risk in my practice regarding surveillance.

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REFERENCES

1. Tosoian JJ, Mamawala M, Patel HD et al: Tumor volume on biopsy of low risk prostate cancer managed with active surveillance. J Urol 2018; **199:** 954.
2. Balakrishnan AS, Cowan JE, Cooperberg MR et al: Evaluating the safety of active surveillance: outcomes of deferred radical prostatectomy after an initial period of surveillance. J Urol 2019; **202:** 506.
3. Loeb S and Ross AE: Genomic testing for localized prostate cancer: where do we go from here? Curr Opin Urol 2017; **27:** 495.
4. Kornberg Z, Cowan JE, Westphalen AC et al: Genomic Prostate Score, PI-RADS™ version 2 and progression in men with prostate cancer on active surveillance. J Urol 2019; **201:** 300.

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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Obesity at Diagnosis and Prostate Cancer Prognosis and Recurrence Risk Following Primary Treatment by Radical Prostatectomy

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Abstract

Background: The association of obesity at diagnosis with prostate cancer progression is uncertain. This study aimed to examine the relationship between body mass index (BMI; 18.5–<25, 25–<30, 30–<35, ≥35 kg/m^2) and prognostic risk at diagnosis, compare the concordance between prognostic risk assessed at diagnostic biopsy versus pathologic risk assessed at surgery across BMI categories, and investigate the association between obesity and prostate cancer recurrence and all-cause death.

Methods: We examined men enrolled in CaPSURE who underwent radical prostatectomy between 1995 and 2017. Multiple imputation methods were used to handle missing data and reported along with complete case findings.

Results: Participants (n = 5,200) were followed for a median of 4.5 years; 685 experienced recurrence. Obesity was associated with higher prognostic risk at time of diagnosis ($OR_{obese} = 1.5$; $OR_{very\ obese} = 1.7$) and upward reclassification of disease between biopsy and surgery, driven by change in tumor stage ($OR_{obese} = 1.3$; $OR_{very\ obese} = 1.6$). We observed an association between BMI and recurrence with adjustment for disease severity using diagnostic factors ($HR_{very\ obese} = 1.7$); this association disappeared when adjusting for disease severity factors obtained at surgery.

Conclusions: Our findings suggest that residual confounding may partially explain the conflicting evidence regarding obesity's influence on prostate cancer progression. Assessing T-stage via digital rectal exam may be complicated in larger men, potentially affecting clinical treatment decisions. A strong association with all-cause mortality demonstrates healthier BMI at diagnosis may still improve overall survival.

Impact: Patients with greater BMI are prone to more advanced disease at diagnosis and may be more likely to have their tumor stage underestimated at diagnosis.

Introduction

Although prostate cancer is the second leading cause of cancer death among men in the United States, the severity of the disease varies considerably (1–4). Much research has focused on identifying patient characteristics that predict prostate cancer mortality in an effort to target resources and avoid unnecessary interventions and the associated harms, while decreasing health care spending (5). The relationship between obesity and prostate cancer outcomes is one area of active research and much debate. Biological responses to increased adiposity—such as changes in insulin-like growth factor, insulin, sex hormones, and adipokine signaling molecule concentrations—have been shown to promote prostate tumor growth in preclinical studies and have been associated with increased risk of prostate cancer progression and mortality in some, but not all, epidemiologic studies (6–10). Specifically, while an increasing number of studies have found an association between obesity and increased risk of advanced prostate cancer and poorer outcomes following diagnosis, a few studies have found no evidence for these associations, leading to inconclusive evidence to recognize obesity as a formal risk factor for prostate cancer progression. For example, a literature review published in 2017 reviewed 5 recently published reports on the association between body mass index (BMI) and prostate cancer recurrence with different conclusions (11). Two of these studies were conducted on the same sample of men, and the vastly different findings [hazard ratios (HR) point estimates 2.83 vs. 0.83] were partially attributed to differences in covariate adjustments (12, 13). A 2013 meta-analysis noted similar contradicting evidence (14).

In addition to a role in the biology of prostate cancer, adiposity may directly influence the efficacy of clinical screening and risk assessment using standard criteria applied to the population. Namely, the physical increases in blood volume and prostate gland size that occur with obesity may dilute prostate-specific antigen (PSA) levels and lessen the likelihood of finding small

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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tumors on biopsy (6, 15, 16). Additionally, careful digital rectal exam may be more difficult in obese patients. As a result, due to the mechanism by which information is obtained (i.e., via physical exam and needle biopsy in diagnostic setting versus via surgical removal and subsequent pathologic evaluation of entire prostate gland), clinical assessment may underestimate true disease severity, particularly among obese versus normal weight men, leading to undertreatment of obese men and an observed increase in risk of prostate cancer progression or death (17–21).

The objectives of this study were to (1) investigate the relationship between BMI and prognostic risk at diagnosis; (2) compare the concordance between prognostic risk factors (clinical Gleason score, stage) assessed at diagnostic biopsy versus pathologic risk at surgery (pathologic Gleason score, stage) across different BMI groups; and (3) investigate the association between obesity and outcomes (i.e., prostate cancer recurrence, all-cause mortality) following radical prostatectomy adjusting for prognostic versus pathologic risk. We hypothesized that obesity would be associated with higher prognostic risk at the time of diagnostic biopsy; obese men would have greater discordance between their prognostic risk assessed at the time of biopsy versus surgery (i.e., obese men would experience more misclassification of their disease severity at diagnosis compared with normal weight men and be more likely to experience an upgrade or upstage from biopsy to surgery); and that obesity would be associated with increased risk of prostate cancer progression, independent of prognostic risk at diagnosis but not of pathologic risk at surgery. To address these objectives, we utilized a unique data source, the Cancer of the Prostate Strategic Urologic Research Endeavor (CaPSURE). With over 20 years of follow-up completed, CaPSURE offers a substantial number of participants and nearly 700 recurrence events, larger than most of the prior published studies on this topic.

Materials and Methods

Study design

Data for this project were obtained from CaPSURE (22, 23). CaPSURE is a longitudinal observational registry that includes 15,310 men diagnosed with biopsy-proven prostate adenocarcinoma. Participants were recruited by participating urologists at 43 academic- and community- based urology practices across the United States, between 1995 and 2018. Data on clinical features including prognostic and pathologic factors (stage, Gleason score, PSA, etc.), treatments, and recurrences were reported by participating urologists. All participants provided written informed consent following institutional review board (IRB) approval. The study was conducted in accordance with the Belmont Report and U.S. Common Rule under local IRB supervision. Patients were followed until death or withdrawal from the study. Additional study details have been provided previously (22, 23).

Of the 15,310 CaPSURE participants, we excluded those without a primary treatment within 9 months ($n = 1,128$) and patients diagnosed prior to 1995 ($n = 2,369$). We further excluded patients without radical prostatectomy as their primary treatment ($n = 6,590$) and those diagnosed with metastasis ($n = 7$). Due to the well-documented imbalance in both disease and mortality hazard of underweight individuals, participants with a BMI <18.5 kg/m^2 (underweight) were also excluded from this analysis ($n = 16$) rather than being included in the normal weight category (24, 25). This left a total of 5,200 CaPSURE participants who met inclusion criteria (Fig. 1); 3,230 (62%) of which had complete records. The remaining 1,970 (38%) had missing data on at least one variable of interest, with the majority of these missing BMI ($n = 1,353$; see Missing data section).

Obesity measures

Self-reported height and weight from the baseline questionnaire completed at diagnosis were used to calculate BMI. BMI was categorized as normal weight (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), obese (30 to <35 kg/m^2), and very obese (≥35 kg/m^2; ref. 26). We also examined obesity as a binary variable (≥30 kg/m^2).

Outcome measures

Disease severity at time of diagnosis was defined using a well-validated tool, the Cancer of the Prostate Risk Assessment (CAPRA), categorized as low (0–2), intermediate (3–5), or high

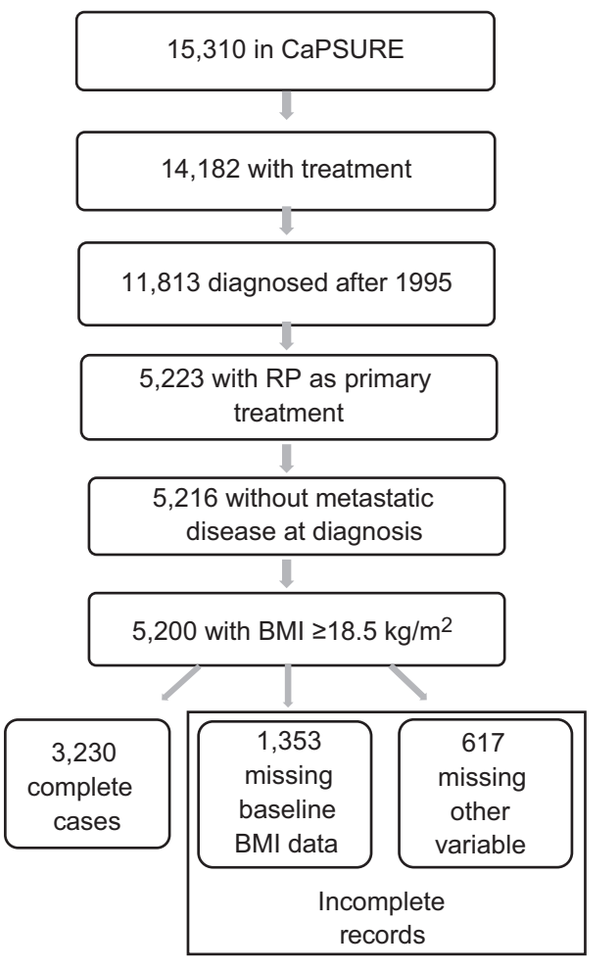

Figure 1.
Patient flow chart showing inclusion of men with prostate cancer from CaPSURE cohort. BMI, body mass index; CaPSURE, Cancer of the Prostate Strategic Urologic Research Endeavor; RP, radical prostatectomy.

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score (≥6; refs. 27–30). CAPRA uses age, stage, PSA, Gleason score, and percentage of positive biopsy cores to predict prognostic risk. Upward reclassification of disease risk was defined as an increase between the diagnostic and surgical values for either the Gleason score (change from <7 to ≥7) or T-stage (change from T1 or T2 to ≥T3). (Note that we use the term "T-stage" to refer to the T category of the TNM staging criteria.) Prostate cancer recurrence was defined as a PSA level ≥0.2 ng/mL at two consecutive visits following radical prostatectomy, or a need for a secondary treatment at least 6 months after radical prostatectomy (31–33). The date of recurrence was defined as the date of second PSA level ≥0.2 ng/mL or the start date of second treatment. Time to event was thus measured from date of radical prostatectomy to date of recurrence. Patients without documentation of recurrence were censored at the date of last follow-up or death.

Mortality data were obtained from physician report, state death certificates, and queries to the National Death Index (NDI). Timing of the last NDI request allowed for follow-up through April 2017. The date of death was obtained from the death certificate, and time to event was thus measured from date of radical prostatectomy to date of death. Patients without documented death were censored at the date of last follow-up.

Data analysis

Multivariable ordinal logistic regression was used to investigate the association between BMI and disease severity at time of diagnosis, with the categorized CAPRA (i.e., disease severity) score as the outcome. A likelihood ratio test was used to test the proportional odds assumption. Multivariable logistic regression was used to investigate the association between BMI and upward reclassification of disease score and stage between diagnostic biopsy and surgical assessment. These models were both adjusted for age at diagnosis, race (white, black, other), smoking status (yes/no; reported at diagnosis), comorbidities (reported history (yes/no) of heart disease, hypertension, diabetes, and/or stroke), and type of CaPSURE site (academic, veteran, community-based). Odds ratios (OR) and associated 95% confidence intervals (CI) were reported.

Stratified Cox proportional hazards multivariable regression was used to investigate associations between BMI at date of diagnosis in relation to risk of prostate cancer recurrence and mortality. Models were stratified by the CaPSURE site to account for the hierarchical structure of the data. Stratified Cox models allowed the unspecified baseline hazard to vary across the stratified variable (here, CaPSURE site), and are a common way to deal with clustering (34). HR and associated 95% CI were estimated relative to the normal weight group (BMI 18.5 to <25kg/m^2) or the nonobese group (18.5 to <30 kg/m^2). Covariates for multivariable analyses were determined *a priori* and included age at diagnosis, race, smoking status (reported at diagnosis), surgical approach (open, robotic, other), comorbidities, PSA (log-transformed continuous), and prognostic factors (Gleason score, T-stage, N-stage) obtained from diagnostic or surgical assessment (32, 35–37). Fine-Gray models were also fit to assess sensitivity to competing events when modeling recurrence. Proportional hazards assumptions were investigated graphically using log-minus-log plots and statistically using the Schoenfeld test. Analysis was performed in Stata version 15.1.

Missing data

BMI data were missing for 1,353 (26%) participants. Due to the high frequency of missing data on our primary predictor (BMI), we chose to use multiple imputation to handle missing data. Multiple imputation assumes data are missing at random. To assess the possibility that unobserved BMI data were missing *not* at random (which would suggest multiple imputation would not be appropriate), we pulled height and weight values from medical record data at one site on or near the date of diagnosis, for records with missing self-reported BMI (Fig. 1). Using these data, we compared the distribution of the recovered (i.e., missing on self-report) BMI values to the distribution of reported BMI. Results suggested it was plausible data were missing at random (Supplementary Table S1).

Two methods for handling unobserved data were then used. First, we applied a complete case analysis, excluding from the analysis any individual with incomplete data (38). Second, we performed multiple imputation via chained equations using the *chained* command in Stata, under the assumption that data are missing at random (39). Multiple imputation via chained equations is a multistep process that first generates n (here, 50) complete plausible data sets using estimation (and reestimation). Analyses are then run on each imputed data set and results are pooled using Rubin's Rules (40). Our imputation model included fully observed variables (age at diagnosis, race, surgical approach, death, time from surgery to recurrence, time from surgery to death, type of institution patient was treated at, and the CaPSURE site) and variables with incomplete values (BMI; patients' smoking, marital, and insurance status, education level, and income; PSA at diagnosis; total Gleason score, T-stage, and N-stage at biopsy and surgery; CAPRA; smoking status; presence of extracapsular extension, positive surgical margins, and seminal vesicle involvement at radical prostatectomy). The numbers of complete values and missing and imputed values for incomplete variables are shown in Supplementary Table S2.

Results

Of the 5,200 CaPSURE participants who met inclusion criteria, 3,230 were complete cases; most incomplete records were considered incomplete due to missing BMI data ($n = 1,353$) and were subsequently excluded from complete case analyses. The remaining 617 records were missing data for at least one variable used in at least one model, and therefore, were only excluded from some of the complete case analyses.

Baseline patient and clinical characteristics are presented in Table 1 by BMI category. Overall, patients were followed for a median of 4.5 years (IQR: 2.1–8.3) after radical prostatectomy. There were 685 patients with documented recurrence a median of 1.8 years (IQR: 1.0–3.5) post-radical prostatectomy. Most patients recurred via elevated PSA value post-radical prostatectomy ($n = 510$), rather than need for secondary treatment ($n = 175$). A total of 671 deaths were observed during the follow-up period a median of 8.6 years (IQR: 5.1–11.6) post-radical prostatectomy.

Clinical presentation and reclassification from biopsy to surgery

Adjusted imputation analysis of the association between BMI and clinical disease severity indicated that obese ($OR_{obese} = 1.5$;

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Table 1. Baseline patient and clinical characteristics of 5,200 CaPSURE patients who underwent radical prostatectomy

	BMI at diagnosis				
	Normal weight (18.5 to <25 kg/m²) *n* (%) Mean ± SD	**Overweight (25 to <30 kg/m²)** *n* (%) Mean ± SD	**Obese (30 to <35 kg/m²)** *n* (%) Mean ± SD	**Very obese (≥35 kg/m²)** *n* (%) Mean ± SD	**Missing** *n* (%) Mean ± SD
N (%)	937 (18)	1,998 (38)	719 (14)	193 (4)	1,353 (26)
Race					
White	861 (92)	1,809 (91)	635 (88)	169 (88)	1,099 (81)
Black	52 (6)	134 (7)	59 (8)	18 (9)	186 (14)
Other	24 (3)	55 (3)	25 (3)	6 (3)	68 (5)
Age at diagnosis (yr)	61.8 ± 7.2	61.3 ± 6.8	61.1 ± 6.5	59.1 ± 6.3	60.1 ± 7.2
Current smoker	119 (13)	189 (9)	51 (7)	16 (8)	10 (1)
Surgical approach					
Open	769 (82)	1,677 (84)	554 (77)	147 (76)	1,042 (77)
Robotic	124 (13)	232 (12)	118 (16)	28 (15)	249 (18)
Other	44 (5)	89 (4)	47 (7)	18 (9)	62 (5)
Comorbidity	355 (38)	1,024 (51)	460 (64)	144 (75)	37 (3)
Heart disease	122 (13)	266 (13)	86 (12)	31 (16)	7 (1)
Hypertension	246 (26)	824 (41)	401 (56)	121 (63)	28 (2)
Diabetes	42 (4)	129 (7)	93 (13)	35 (18)	8 (1)
Stroke	45 (5)	85 (4)	30 (4)	8 (4)	2 (<1)
PSA (ng/dL)[a,b]	6.9 ± 4.8	6.9 ± 7.1	6.8 ± 47	6.2 ± 3.9	7.1 ± 5.2
≤6.0	523 (56)	1,178 (59)	399 (56)	116 (60)	705 (52)
>6.0 to ≤10	262 (28)	515 (26)	185 (26)	51 (26)	398 (29)
>10 to ≤20	101 (11)	193 (10)	82 (11)	14 (7)	164 (12)
>20 to ≤30	19 (2)	30 (2)	9 (1)	3 (2)	26 (2)
>30	7 (<1)	26 (1)	5 (<1)	0 (0)	10 (<1)
Total Gleason[a,b]	6.3 ± 0.8	6.3 ± 0.8	6.4 ± 0.8	6.4 ± 0.8	6.4 ± 0.8
<7	638 (68)	1,360 (68)	435 (61)	113 (59)	836 (62)
7	238 (25)	509 (25)	218 (30)	67 (35)	428 (32)
>7	51 (5)	95 (5)	56 (8)	11 (6)	79 (6)
T stage[a,b]					
T1	515 (55)	1,110 (56)	446 (62)	108 (56)	786 (58)
T2	378 (40)	795 (40)	237 (33)	72 (37)	477 (35)
T3	10 (1)	15 (1)	4 (<1)	2 (1)	14 (1)
T4	—	1 (<1)	—	—	—
≥34% positive cores[a]	301 (32)	624 (31)	250 (35)	72 (37)	458 (34)
Positive surgical margins	214 (23)	466 (23)	197 (27)	60 (31)	350 (26)
Site type					
Academic	116 (12)	195 (10)	68 (9)	27 (14)	110 (8)
Community	801 (85)	1,760 (88)	629 (87)	166 (86)	1,206 (89)
Veteran	20 (2)	43 (2)	22 (3)	—	37 (3)

Abbreviations: PSA, prostate-specific antigen; T-stage, tumor stage; yr, year.

[a]Obtained at diagnostic biopsy.

[b]$n = 179$ with unknown PSA; $n = 66$ with unknown Gleason score; $n = 225$ with unknown stage; $n = 242$ with unknown % positive cores.

95% CI, 1.2–1.8) and very obese ($OR_{very\ obese} = 1.7$; 95% CI, 1.2–2.3) patients were more likely to have higher CAPRA scores at time of diagnosis, compared with their normal weight peers (Table 2). The association remained when we dichotomized obesity ($OR_{BMI\geq30} = 1.4$; 95% CI: 1.2–1.6). Results for the complete case analysis were similar (Table 2).

Overall, we detected a statistically significant association between BMI and upward reclassification among only the obese and very obese categories of BMI in the imputed analysis ($OR_{overweight} = 1.1$; 95% CI, 0.9–1.3; $OR_{obese} = 1.3$; 95% CI, 1.0–1.6; $OR_{very\ obese} = 1.6$; 95% CI, 1.1–2.1). This association persisted when we dichotomized BMI ($OR_{BMI\geq30} = 1.3$; 95% CI, 1.1–1.5). There was a small positive, but not statistically significant association between obesity and upward reclassification of Gleason score (results shown in Table 3), suggesting the overall association was mainly driven by the upward reclassification of T-stage (results for T-stage reclassification: $OR_{overweight} = 1.2$; 95% CI: 0.9–1.5; $OR_{obese} = 1.4$; 95% CI, 1.1–1.8; $OR_{very\ obese} = 1.7$; 95% CI, 1.1–2.5). Results from the complete case analysis were similar (Table 3). Using the complete case data, we observed 550 subjects reclassified from a T1 or T2 to T-stage ≥3; 154 (28%) of these men were obese or very obese. More specifically, 14% of normal weight men were reclassified versus 16% of overweight, 19% of obese men, and 22% of very obese men ($P_{chi\text{-}sqaured} = 0.027$). We further investigated the association between BMI at date of diagnosis and upward reclassification of disease using a mixed-effects model to account for clustering at the site level (using clinical site in place of type of site) and the results were similar (data from the mixed-effects model not shown).

Recurrence and all-cause mortality

When we used the prognostic risk measures from diagnostic biopsy to adjust for disease severity to assess the association between BMI and various outcomes, we found some evidence that very obese (≥35 kg/m²) patients were at greater risk of recurrence ($HR_{very\ obese} = 1.7$; 95% CI, 1.1–2.5; *P*-trend = 0.066) and all-cause mortality ($HR_{very\ obese} = 1.7$; 95% CI,

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Table 2. Results of ordinal logistic regression for the association between BMI and clinical disease severity (CAPRA) at time of diagnosis within imputed and complete case data sets[a]

	Multiple imputation analysis		Complete case analysis	
	Crude OR (95% CI)	Adjusted[b] OR (95% CI)	Crude OR (95% CI)	Adjusted[b] OR (95% CI)
BMI category				
Normal weight	Ref	Ref	Ref	Ref
Overweight	1.09 (0.92–1.29)	1.13 (0.95–1.34)	1.05 (0.88–1.25)	1.07 (0.89–1.28)
Obese	1.37 (1.12–1.68)	1.48 (1.20–1.82)	1.39 (1.12–1.72)	1.43 (1.14–1.79)
Very obese	1.47 (1.08–1.99)	1.66 (1.21–2.28)	1.54 (1.10–2.15)	1.68 (1.19–2.38)
Obese (kg/m^2)				
18.5 to <30	Ref	Ref	Ref	Ref
≥30	1.31 (1.13–1.52)	1.39 (1.19–1.62)	1.38 (1.17–1.62)	1.41 (1.19–1.67)

Abbreviations: CAPRA, Cancer of the Prostate Risk Assessment; OR, odds ratio.
[a]ORs are estimated from ordinal logistic regression analysis for a one-category increase in CAPRA score (categorized as 0–2, 3–5, or ≥6).
[b]Adjusted for age at diagnosis, race, smoking status, comorbidities, and site type.

1.1–2.7; *P*-trend = 0.001) in the imputed analysis (Table 4). Associations remained when we used the dichotomized version of BMI ($OR_{BMI\geq30;\ recurrence}$ = 1.2; 95% CI, 1.0–1.5; $OR_{BMI\geq30;\ mortality}$ = 1.5; 95% CI, 1.2–1.8). Similar results were observed in the complete case analysis (Table 4).

When we adjusted for disease severity based on pathologic risk factors from surgery (rather than prognostic risk from diagnostic biopsy), the associations between BMI and recurrence were positive but no longer statistically significant, even for the most obese patients ($HR_{very\ obese}$ = 1.3; 95% CI, 0.9, 2.0; *P*-trend = 0.495). This was also observed using the dichotomized version of BMI ($HR_{BMI\geq30}$ = 1.2; 95% CI, 0.9–1.4). The association between obesity and all-cause mortality remained after adjustment for prognostic risk factors at surgery using both the categorical ($HR_{very\ obese}$ = 1.7; 95% CI, 1.1–2.6; *P*-trend = 0.0012) and binary ($HR_{BMI\geq30}$ = 1.5; 95% CI, 1.2–1.8) versions of BMI. In the complete case analysis, there was evidence of an overall association between BMI and all-cause mortality (*P*-trend = 0.008), though there was no statistically significant association observed within any single BMI category ($HR_{overweight}$ = 0.8; 95% CI, 0.6–1.1; HR_{obese} = 1.2; 95% CI, 0.8–1.6; $HR_{very\ obese}$ = 1.5; 95% CI, 0.9–2.5); however, the binary version of BMI did capture this association ($HR_{BMI\geq30}$ = 1.4; 95% CI, 1.1–1.8). The rest of the

Table 3. Association of BMI and odds of upward reclassification of disease status between clinical and surgical assessment within imputed and complete case data sets

	Reclassification events/total *N*[a]	Multiple imputation analysis		Complete case analysis	
		Crude OR (95% CI)	Adjusted[b] OR (95% CI)	Crude OR (95% CI)	Adjusted[b] OR (95% CI)
Overall upward reclassification (Gleason score or T-stage)					
BMI category					
Normal weight	272/937	Ref	Ref	Ref	Ref
Overweight	603/1,998	1.06 (0.90–1.24)	1.09 (0.92–1.28)	1.06 (0.89–1.25)	1.09 (0.91–1.29)
Obese	245/719	1.22 (1.00–1.49)	1.28 (1.04–1.57)	1.26 (1.03–1.56)	1.32 (1.07–1.64)
Very obese	77/193	1.47 (1.07–2.01)	1.55 (1.12–2.13)	1.62 (0.18–2.24)	1.68 (1.21–2.34)
P-trend		0.035	0.013	0.006	0.003
Obese (kg/m^2)					
18.5 to <30	875/2,935	Ref	Ref	Ref	Ref
≥30	322/912	1.22 (1.05–1.42)	1.25 (1.07–1.46)	1.28 (1.10–1.50)	1.31 (1.12–1.54)
Upward reclassification of Gleason score					
BMI category					
Normal weight	184/886	Ref	Ref	Ref	Ref
Overweight	400/1,888	1.01 (0.84–1.22)	1.03 (0.85–1.24)	1.03 (0.84–1.25)	1.05 (0.86–1.29)
Obese	160/683	1.12 (0.89–1.39)	1.14 (0.91–1.43)	1.17 (0.92–1.48)	1.22 (0.95–1.56)
Very obese	48/185	1.28 (0.91–1.80)	1.28 (0.90–1.81)	1.34 (0.93–1.93)	1.34 (0.92–1.96)
P-trend		0.415	0.417	0.274	0.245
Obese (kg/m^2)					
18.5 to <30	584/2,774	Ref	Ref	Ref	Ref
≥30	208/868	1.14 (0.96–1.34)	1.14 (0.96–1.35)	1.19 (0.99–1.42)	1.20 (0.99–1.44)
Upward reclassification of T-stage					
BMI category					
Normal weight	118/818	Ref	Ref	Ref	Ref
Overweight	278/1,769	1.13 (0.91–1.40)	1.16 (0.94–1.45)	1.11 (0.88–1.40)	1.12 (0.88–1.42)
Obese	116/631	1.32 (1.02–1.71)	1.39 (1.07–1.80)	1.34 (1.01–1.77)	1.37 (1.02–1.82)
Very obese	38/170	1.53 (1.04–2.25)	1.66 (1.11–2.46)	1.71 (1.13–2.57)	1.81 (1.18–2.75)
P-trend		0.063	0.022	0.029	0.018
Obese (kg/m^2)					
18.5 to <30	396/2,587	Ref	Ref	Ref	Ref
≥30	154/801	1.25 (1.04–1.51)	1.29 (1.07–1.56)	1.32 (1.07–1.62)	1.34 (1.08–1.65)

Abbreviation: OR, odds ratio estimated from logistic regression analysis.
[a]Reclassification events and total *N* reported based on complete case data set.
[b]Adjusted for age at diagnosis, race, smoking status, comorbidities, and site type.

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Table 4. Association between BMI and prostate cancer outcome using clinical and surgical assessments within imputed and complete case data sets

	Multiple imputation analysis[a]			Complete case analysis[b]		
	Crude analysis HR (95% CI)	Clinical adjustment[c] HR (95% CI)	Surgical adjustment[d] HR (95% CI)	Crude analysis HR (95% CI)	Clinical adjustment[c] HR (95% CI)	Surgical adjustment[d] HR (95% CI)
Prostate cancer recurrence						
BMI						
Normal weight	Ref	Ref	Ref	Ref	Ref	Ref
Overweight	1.07 (0.86–1.32)	1.07 (0.86–1.34)	1.04 (0.84–1.29)	1.04 (0.84–1.30)	1.04 (0.82–1.30)	1.01 (0.81–1.29)
Obese	1.19 (0.92–1.54)	1.22 (0.93–1.59)	1.15 (0.88–1.50)	1.15 (0.88–1.50)	1.16 (0.87–1.54)	1.07 (0.80–1.43)
Very obese	1.51 (1.03–2.20)	1.66 (1.10–2.49)	1.32 (0.87–2.00)	1.51 (1.03–2.20)	1.68 (1.12–2.53)	1.24 (0.78–1.95)
P-trend	0.138	0.066	0.495	0.151	0.066	0.819
Obese (kg/m^2)						
18.5 to <30	Ref	Ref	Ref	Ref	Ref	Ref
≥30	1.20 (0.99–1.45)	1.23 (1.01–1.51)	1.15 (0.94–1.41)	1.19 (0.98–1.45)	1.23 (0.99–1.52)	1.09 (0.87–1.36)
All-cause mortality						
BMI						
Normal weight	Ref	Ref	Ref	Ref	Ref	Ref
Overweight	0.78 (0.64–0.95)	0.89 (0.72–1.10)	0.90 (0.73–1.12)	0.75 (0.61–0.92)	0.79 (0.62–1.00)	0.81 (0.62–1.05)
Obese	1.01 (0.79–1.30)	1.29 (0.98–1.70)	1.30 (0.98–1.72)	1.00 (0.77–1.31)	1.26 (0.94–1.69)	1.15 (0.83–1.59)
Very obese	1.08 (0.73–1.61)	1.74 (1.12–2.70)	1.70 (1.12–2.60)	1.14 (0.75–1.72)	1.76 (1.14–2.72)	1.52 (0.93–2.49)
P-trend	0.020	0.001	0.001	0.008	<0.001	0.008
Obese (kg/m^2)						
18.5 to <30	Ref	Ref	Ref	Ref	Ref	Ref
≥30	1.21 (1.00–1.47)	1.47 (1.19–1.82)	1.47 (1.18–1.82)	1.25 (1.01–1.54)	1.59 (1.27–1.99)	1.41 (1.11–1.80)

Abbreviations: BMI, body mass index; HR, hazards ratio estimated from stratified Cox proportional hazards regression analysis; IQR, interquartile range.
[a]Prostate cancer recurrence: $n = 685$ events; median (IQR) time to event: 1.8 (1.0–3.5). All-cause mortality: $n = 671$ events; median (IQR) time to event: 8.6 (5.1–11.6).
[b]Prostate cancer recurrence: $n = 523$ events; median (IQR) time to event: 1.8 (1.0–3.6). All-cause mortality: $n = 496$ events; median (IQR) time to event: 8.8 (5.2–11.8).
[c]Adjusted for age at diagnosis, race, smoking status, comorbidities, surgical approach, PSA, clinical Gleason score, clinical T-stage, clinical N-stage, and clinical site.
[d]Adjusted for age at diagnosis, race, smoking status, comorbidities, surgical approach, PSA, pathologic Gleason score, pathologic T-stage pathologic N-stage, and clinical site.

findings were similar under the complete case analysis (Table 4).

We further considered adjustment for the presence of positive surgical margins, but no meaningful change in the estimates were observed. We also further analyzed the association between BMI at date of diagnosis and prostate cancer recurrence while accommodating competing risks (i.e., death) by fitting Fine-Gray models, and the results did not materially differ from those reported from our simple stratified Cox model (data not shown).

Discussion

In this report, we attempted to elucidate the apparent discrepancies seen in the literature regarding the association between BMI and prostate cancer recurrence. Although counterexamples can be found, results from our models adjusting for measures of disease severity using prognostic risk factors from diagnostic biopsy are consistent with much of the literature that also used covariate data from the diagnostic biopsy, suggesting that BMI at diagnosis is independently associated with an increased risk of recurrence (41–45). Next, when we instead used pathologic risk measures from surgery to adjust for disease severity, we observed no association, consistent with two reports in the literature that also adjusted for surgical measures (46, 47). A recent report contradicted this finding using a more stringent definition of recurrence (PSA >0.2 ng/mL on 2 consecutive visits; ref. 48). Overall, these results support the conclusion that there may be residual confounding in studies examining BMI in relation to prostate cancer recurrence when analyses adjust for prognostic factors (e.g., stage and score) assessed via diagnostic biopsy versus using pathologic stage and score assessed from surgery. This may also explain apparent discrepancies in the literature.

Once surgical measurements were used to characterize disease severity, the independent associations of BMI with risk of recurrence was attenuated. This is not to say that obesity does not influence disease. In fact, we observed an increased CAPRA score (an indicator of disease severity) at time of diagnosis among more obese versus normal BMI men, consistent with more than a 2-fold increase in high prognostic risk disease for the very obese patients. This can have important implications for clinicians, suggesting patients with greater BMI are more likely to present with greater disease severity. Obese patients may present with worse prognostic risk disease due to later detection due to the physical presence of fat affecting sex hormones, adipokines signaling molecules, and insulin-like growth factor, which act to promote more aggressive disease (6–9). This is also consistent with prior reports that have found that prostate volume, which increases with body size, can lead to difficulties in finding cancer (49, 50). Trends in Table 1 suggest increased presence of positive biopsy cores and slightly younger age may be partially driving increased CAPRA scores among the most obese men. Consequently, these results suggest that assessing tumor stage via digital rectal exam may be more difficult in larger men, which can affect clinical decisions regarding the type and urgency of subsequent treatment and highlights the need for additional research on the potential benefits of alternative screening or prognostication methods. Such tailored approaches may help address the difficulties in detecting and staging disease in more obese patients (e.g., different PSA thresholds for different categories of BMI, or PSA with different imaging follow-up), as has been suggested by other authors (15, 50, 51). Further, our findings indicate that obesity remains a predictor of all-cause mortality, regardless of whether we adjusted for prognostic factors at diagnostic biopsy or pathologic measures

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obtained at surgery, consistent with our stated hypothesis. Given that most men with prostate cancer will die of a cause other than prostate cancer, these results underscore the importance of monitoring and reducing obesity among all men, including those with prostate cancer.

The analysis for the association between BMI and upward reclassification of disease showed an increased risk in reclassification for obese men. This association appears to be driven by a change in T-stage between diagnostic biopsy and pathology, determined after surgical removal of the prostate. These results are consistent with our hypothesis and suggest that assessing tumor stage via digital rectal exam may be more difficult—and in some cases, imaging may be less ideal—in larger men, which can affect clinical decisions regarding the type and urgency of subsequent treatment. Specifically, the reclassification of T-stage for 18% and 22% of reclassified obese and very obese men, respectively, resulted in a change in stage that likely would have affected treatment decisions (i.e., T1 or T2 reclassified to T3 or T4), compared with only 14% of normal weight men.

In this study, we examined the extent and potential impact of the missing data on our reported estimates, with particular interest in the relatively large amount of missing BMI data. Results from our imputation analysis suggest that our estimates were not greatly affected by the missing data, to the extent that our missing at random assumption is true. Although we were unable to identify any systematic issues that resulted in a large number of missing BMI values, we were also unable to identify characteristic differences between those patients who reported BMI and those who did not (Supplementary Table S3). Further, where we were able to obtain data from patient charts to assess patterns of missingness, we gained confidence in the plausibility that our data were missing at random (Supplementary Table S1). Therefore, where our results differ, we put more stock in the results of the multiply imputed data, due to the potential bias that may arise in complete case analysis if data are not missing completely at random. In particular, results from our multiple imputation analysis were consistent with the complete case for all but one analysis, when examining the association between BMI and all-cause mortality. In that analysis, results from the imputation analysis were more consistent with the hypothesis that BMI increases the risk of all-cause death. However, because it is not possible to rule out that missing data are missing not at random, the slight difference in the complete case analysis and multiple imputation results should be interpreted cautiously.

Several limitations should be considered when interpreting these results. First, while there was a fair amount of missing data in BMI, great effort was made to assess the impact of these missing data and to use advanced analytical techniques to guide inferences. Second, as patients managed by modalities other than surgery do not have comprehensive pathologic review conducted on their tumors, this analysis was unable to incorporate patients who had undergone other forms of primary treatment, including radiation, watchful waiting, or active surveillance, although radical prostatectomy was the most common form of primary treatment in CaPSURE. Given our findings, it may be of value for clinicians to take into account BMI when contemplating these other forms of treatment. Third, we recognize that BMI has been criticized for its inability to distinguish between different fat distributions within the body and may be less reflective of obesity in aging populations; however, it is the most readily understood and widely used metric for measuring obesity. Fourth, we recognize that non-obese men may have different risk factors (other than BMI) for advanced grade and stage that increase their risk of recurrence, which could act to attenuate the association between obesity and prostate cancer recurrence. However, men in this study predominantly had localized disease, as they underwent radical prostatectomy as primary treatment. Therefore, this is unlikely to explain our null findings after adjustment for pathologic factors obtained at surgery. Regardless, caution should be taken in generalizing our results to men diagnosed with advanced disease. Fifth, limited follow-up time and number of prostate cancer deaths precluded analysis of the association between BMI and prostate cancer–specific mortality. Finally, due to the large concentration of white men in this study, care should be taken when generalizing these results to non-white populations.

Overall, we observed that patients with greater BMI are prone to more advanced disease at time of diagnosis and may be more likely to have their tumor stage underestimated at diagnostic biopsy. Further, results for BMI and the outcome of recurrence varied based on the type of measures used to adjust for disease severity (diagnostic biopsy vs. surgical pathology), which may help explain some of the discrepancy observed in the literature. These findings have important methodological implications, suggesting that surgical measures of disease severity may more accurately capture true disease status, particularly among obese men. Important clinical implications of these findings include the need for potentially different prognostic risk classifications and more accurate screening approaches for obese men, to best inform treatment decisions and aid earlier disease detection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.S. Langlais, S.A. Kenfield, J.M. Chan
Development of methodology: C.S. Langlais, P. Carroll, J.M. Chan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.E. Cowan, J.M. Broering, P. Carroll
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.S. Langlais, J. Neuhaus, S.A. Kenfield, E.L. Van Blarigan, M.R. Cooperberg, P. Carroll, J.M. Chan
Writing, review, and/or revision of the manuscript: C.S. Langlais, J.E. Cowan, J. Neuhaus, S.A. Kenfield, E.L. Van Blarigan, J.M. Broering, M.R. Cooperberg, P. Carroll, J.M. Chan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.S. Langlais, P. Carroll
Study supervision: J.M. Chan
Other (data management): J.E. Cowan

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.
2. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends–an update. Cancer Epidemiol Biomarkers Prev 2016;25:16–27.
3. American Cancer Society. Cancer facts and figures 2018 [cited 2019 Jan 20]. Available from: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2018/cancer-facts-and-figures-2018.pdf.
4. Negoita S, Feuer EJ, Mariotto A, Cronin KA, Petkov VI, Hussey SK, et al. Annual report to the nation on the status of cancer, part II: recent changes in prostate cancer trends and disease characteristics. Cancer 2018;124:2801–14.
5. Chang AJ, Autio KA, Roach M 3rd, Scher HI. High-risk prostate cancer-classification and therapy. Nat Rev Clin Oncol 2014;11:308–23.
6. Allott EH, Masko EM, Freedland SJ. Obesity and prostate cancer: weighing the evidence. Eur Urol 2013;63:800–9.
7. Schnoeller T, Jentzmik F, Rinnab L, Cronauer MV, Damjanoski I, Zengerling F, et al. Circulating free testosterone is an independent predictor of advanced disease in patients with clinically localized prostate cancer. World J Urol 2013;31:253–9.
8. Huang CY, Yu HS, Lai TY, Yeh YL, Su CC, Hsu HH, et al. Leptin increases motility and integrin up-regulation in human prostate cancer cells. J Cell Physiol 2011;226:1274–82.
9. Cox ME, Gleave ME, Zakikhani M, Bell RH, Piura E, Vickers E, et al. Insulin receptor expression by human prostate cancers. Prostate 2009;69:33–40.
10. Cao Y, Ma J. Body mass index, prostate cancer-specific mortality, and biochemical recurrence: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2011;4:486–501.
11. Bandini M, Gandaglia G, Briganti A. Obesity and prostate cancer. Curr Opin Urol 2017;27:415–21.
12. Vidal AC, Howard LE, Moreira DM, Castro-Santamaria R, Andriole GL Jr, Freedland SJ. Obesity increases the risk for high-grade prostate cancer: results from the REDUCE study. Cancer Epidemiol Biomarkers Prev 2014;23:2936–42.
13. Shiota M, Yokomizo A, Takeuchi A, Imada K, Kiyoshima K, Inokuchi J, et al. The feature of metabolic syndrome is a risk factor for biochemical recurrence after radical prostatectomy. J Surg Oncol 2014;110:476–81.
14. Liu L, Nishihara R, Qian ZR, Tabung FK, Nevo D, Zhang X, et al. Association between inflammatory diet pattern and risk of colorectal carcinoma subtypes classified by immune responses to tumor. Gastroenterology 2017;153:1517–30.e14.
15. Banez LL, Hamilton RJ, Partin AW, Vollmer RT, Sun L, Rodriguez C, et al. Obesity-related plasma hemodilution and PSA concentration among men with prostate cancer. JAMA 2007;298:2275–80.
16. Freedland SJ, Platz EA, Presti JC Jr, Aronson WJ, Amling CL, Kane CJ, et al. Obesity, serum prostate specific antigen and prostate size: implications for prostate cancer detection. J Urol 2006;175:500–4; discussion 504.
17. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;371:569–78.
18. MacInnis RJ, English DR. Body size and composition and prostate cancer risk: systematic review and meta-regression analysis. Cancer Causes Control 2006;17:989–1003.
19. Bergstrom A, Pisani P, Tenet V, Wolk A, Adami HO. Overweight as an avoidable cause of cancer in Europe. Int J Cancer 2001;91:421–30.
20. Engeland A, Tretli S, Bjorge T. Height, body mass index, and prostate cancer: a follow-up of 950000 Norwegian men. Br J Cancer 2003;89:1237–42.
21. Andersson SO, Wolk A, Bergstrom R, Adami HO, Engholm G, Englund A, et al. Body size and prostate cancer: a 20-year follow-up study among 135006 Swedish construction workers. J Natl Cancer Inst 1997;89:385–9.
22. Lubeck DP, Litwin MS, Henning JM, Stier DM, Mazonson P, Fisk R, et al. The CaPSURE database: a methodology for clinical practice and research in prostate cancer. CaPSURE research panel. Cancer of the Prostate Strategic Urologic Research Endeavor. Urology 1996;48:773–7.
23. Cooperberg MR, Broering JM, Litwin MS, Lubeck DP, Mehta SS, Henning JM, et al. The contemporary management of prostate cancer in the United States: lessons from the Cancer of the Prostate Strategic Urologic Research Endeavor (CapSURE), a national disease registry. J Urol 2004;171:1393–401.
24. Allison DB, Faith MS, Heo M, Kotler DP. Hypothesis concerning the U-shaped relation between body mass index and mortality. Am J Epidemiol 1997;146:339–49.
25. Aune D, Sen A, Prasad M, Norat T, Janszky I, Tonstad S, et al. BMI and all cause mortality: systematic review and non-linear dose-response meta-analysis of 230 cohort studies with 3.74 million deaths among 30.3 million participants. BMJ 2016;353:i2156.
26. Centers for Disease Control and Prevention. Defining adult overweight and obesity [cited 2017 Sep]. Available from: https://www.cdc.gov/obesity/adult/defining.html.
27. Cooperberg MR, Pasta DJ, Elkin EP, Litwin MS, Latini DM, Du Chane J, et al. The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. J Urol 2005;173:1938–42.
28. Cooperberg MR, Freedland SJ, Pasta DJ, Elkin EP, Presti JC Jr, Amling CL, et al. Multiinstitutional validation of the UCSF cancer of the prostate risk assessment for prediction of recurrence after radical prostatectomy. Cancer 2006;107:2384–91.
29. May M, Knoll N, Siegsmund M, Fahlenkamp D, Vogler H, Hoschke B, et al. Validity of the CAPRA score to predict biochemical recurrence-free survival after radical prostatectomy. Results from a European multicenter survey of 1,296 patients. J Urol 2007;178:1957–62; discussion, 1962.
30. Zhao KH, Hernandez DJ, Han M, Humphreys EB, Mangold LA, Partin AW. External validation of University of California, San Francisco, Cancer of the Prostate Risk Assessment score. Urology 2008;72:396–400.
31. Bassett WW, Cooperberg MR, Sadetsky N, Silva S, DuChane J, Pasta DJ, et al. Impact of obesity on prostate cancer recurrence after radical prostatectomy: data from CaPSURE. Urology 2005;66:1060–5.
32. Kane CJ, Bassett WW, Sadetsky N, Silva S, Wallace K, Pasta DJ, et al. Obesity and prostate cancer clinical risk factors at presentation: data from CaPSURE. J Urol 2005;173:732–6.
33. Anast JW, Sadetsky N, Pasta DJ, Bassett WW, Latini D, DuChane J, et al. The impact of obesity on health related quality of life before and after radical prostatectomy (data from CaPSURE). J Urol 2005;173:1132–8.
34. Glidden DV, Vittinghoff E. Modelling clustered survival data from multicentre clinical trials. Stat Med 2004;23:369–88.
35. Kenfield SA, Stampfer MJ, Chan JM, Giovannucci E. Smoking and prostate cancer survival and recurrence. JAMA 2011;305:2548–55.
36. Giovannucci E, Rimm EB, Liu Y, Leitzmann M, Wu K, Stampfer MJ, et al. Body mass index and risk of prostate cancer in U.S. health professionals. J Natl Cancer Inst 2003;95:1240–4.
37. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 2000;92:2009–17.

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38. Vach W. Some issues in estimating the effect of prognostic factors from incomplete covariate data. Stat Med 1997;16:57–72.
39. Harel O, Mitchell EM, Perkins NJ, Cole SR, Tchetgen Tchetgen EJ, Sun B, et al. Multiple imputation for incomplete data in epidemiologic studies. Am J Epidemiol 2018;187:576–84.
40. Rubin DB. Inference and missing data. Biometrika 1976;63:581–92.
41. Davies BJ, Smaldone MC, Sadetsky N, Dall'era M, Carroll PR. The impact of obesity on overall and cancer specific survival in men with prostate cancer. J Urol 2009;182:112–7; discussion, 117.
42. Freedland SJ, Sun L, Kane CJ, Presti JC Jr, Terris MK, Amling CL, et al. Obesity and oncological outcome after radical prostatectomy: impact of prostate-specific antigen-based prostate cancer screening: results from the Shared Equal Access Regional Cancer Hospital and Duke Prostate Center databases. BJU Int 2008;102:969–74.
43. Strom SS, Kamat AM, Gruschkus SK, Gu Y, Wen S, Cheung MR, et al. Influence of obesity on biochemical and clinical failure after external-beam radiotherapy for localized prostate cancer. Cancer 2006;107:631–9.
44. Strom SS, Wang X, Pettaway CA, Logothetis CJ, Yamamura Y, Do KA, et al. Obesity, weight gain, and risk of biochemical failure among prostate cancer patients following prostatectomy. Clin Cancer Res 2005;11:6889–94.
45. Gong Z, Agalliu I, Lin DW, Stanford JL, Kristal AR. Obesity is associated with increased risks of prostate cancer metastasis and death after initial cancer diagnosis in middle-aged men. Cancer 2007;109:1192–202.
46. Siddiqui SA, Inman BA, Sengupta S, Slezak JM, Bergstralh EJ, Leibovich BC, et al. Obesity and survival after radical prostatectomy: a 10-year prospective cohort study. Cancer 2006;107:521–9.
47. Spangler E, Zeigler-Johnson CM, Coomes M, Malkowicz SB, Wein A, Rebbeck TR. Association of obesity with tumor characteristics and treatment failure of prostate cancer in African-American and European American men. J Urol 2007;178:1939–44; discussion, 1945.
48. Maj-Hes AB, Mathieu R, Ozsoy M, Soria F, Moschini M, Abufaraj M, et al. Obesity is associated with biochemical recurrence after radical prostatectomy: a multi-institutional extended validation study. Urol Oncol 2017;35:460e461–e8.
49. Rundle A, Wang Y, Sadasivan S, Chitale DA, Gupta NS, Tang D, et al. Larger men have larger prostates: detection bias in epidemiologic studies of obesity and prostate cancer risk. Prostate 2017;77:949–54.
50. Wallner LP, Morgenstern H, McGree ME, Jacobson DJ, St Sauver JL, Jacobsen SJ, et al. The effects of body mass index on changes in prostate-specific antigen levels and prostate volume over 15 years of follow-up: implications for prostate cancer detection. Cancer Epidemiol Biomarkers Prev 2011;20:501–8.
51. Aref AT, Vincent AD, O'Callaghan ME, Martin SA, Sutherland PD, Hoy AJ, et al. The inverse relationship between prostate specific antigen (PSA) and obesity. Endocr Relat Cancer 2018;25:933–41.

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Cancer Epidemiology, Biomarkers & Prevention

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Obesity at Diagnosis and Prostate Cancer Prognosis and Recurrence Risk Following Primary Treatment by Radical Prostatectomy

Crystal S. Langlais, Janet E. Cowan, John Neuhaus, et al.

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SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) (Last, First, Middle Initial) a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS (X) d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER (X) e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
NONE if there is a patent(s) add the names of the inventors	Add the patent title NONE	add provisional patent and PCT number	☐	☐	☐	☐	☐	☐

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(2) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
N/A		N/A.	
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER (Include ZIP Code)	(c) ADDRESS OF EMPLOYER (Include ZIP Code)		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS (Include ZIP Code) b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES (YYYYMMDD) f.	
			(1) CLAUSE NUMBER	(2) DATE (YYYYMM)		(1) AWARD	(2) ESTIMATED COMPLETION
Spectrum Health System	15 Michigan Street NE, Suite 750 Grand Rapids, MI-49503-2508	10694sc			Screen, Recruit, Consent and provide onsite coordination in the conduct of the RCT associated with Aim 2B of the DOD sponsored study that evaluates DSI for men newly diagnosed with Prostate Cancer		

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate)* ☐ SMALL BUSINESS ☑ NON-PROFIT ORGANIZATION

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL (Last, First, Middle Initial)	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Sippy, Renuka	Contracts and Grants Officer	Renuka Sippy	2020/01/03

DD FORM 882 INSTRUCTIONS

GENERAL

This form is for use in submitting INTERIM and FINAL invention reports to the Contracting Officer and for use in reporting the award of subcontracts containing a "Patent Rights" clause. If the form does not afford sufficient space, multiple forms may be used or plain sheets of paper with proper identification of information by item number may be attached.

An INTERIM report is due at least every 12 months from the date of contract award and shall include (a) a listing of "Subject Inventions" during the reporting period, (b) a certification of compliance with required invention identification and disclosure procedures together with a certification of reporting of all "Subject Inventions," and (c) any required information not previously reported on subcontracts containing a "Patent Rights" clause.

A FINAL report is due within 6 months if contractor is a small business firm or domestic nonprofit organization and within 3 months for all others after completion of the contract work and shall include (a) a listing of all "Subject Inventions" required by the contract to be reported, and (b) any required information not previously reported on subcontracts awarded during the course of or under the contract and containing a "Patent Rights" clause.

While the form may be used for simultaneously reporting inventions and subcontracts, it may also be used for reporting, promptly after award, subcontracts containing a "Patent Rights" clause.

Dates shall be entered where indicated in certain items on this form and shall be entered in six or eight digit numbers in the order of year and month (YYYYMM) or year, month and day (YYYYMMDD). Example: April 1999 should be entered as 199904 and April 15, 1999 should be entered as 19990415.

1.a. Self-explanatory.

1.b. Self-explanatory.

1.c. If "same" as Item 2.c., so state.

1.d. Self-explanatory.

2.a. If "same" as Item 1.a., so state.

2.b. Self-explanatory.

2.c. Procurement Instrument Identification (PII) number of contract (DFARS 204.7003).

2.d. through 5.e. Self-explanatory.

5.f. The name and address of the employer of each inventor not employed by the contractor or subcontractor is needed because the Government's rights in a reported invention may not be determined solely by the terms of the "Patent Rights" clause in the contract.

Example 1: If an invention is made by a Government employee assigned to work with a contractor, the Government rights in such an invention will be determined under Executive Order 10096.

Example 2: If an invention is made under a contract by joint inventors and one of the inventors is a Government employee, the Government's rights in such an inventor's interest in the invention will also be determined under Executive Order 10096, except where the contractor is a small business or nonprofit organization, in which case the provisions of 35 U.S.C. 202(e) will apply.

5.g.(1) Self-explanatory.

5.g.(2) Self-explanatory with the exception that the contractor or subcontractor shall indicate, if known at the time of this report, whether applications will be filed under either the Patent Cooperation Treaty (PCT) or the European Patent Convention (EPC). If such is known, the letters PCT or EPC shall be entered after each listed country.

6.a. Self-explanatory.

6.b. Self-explanatory.

6.c. Self-explanatory.

6.d. Patent Rights Clauses are located in FAR 52.227.

6.e. Self-explanatory.

6.f. Self-explanatory.

7. Certification not required by small business firms and domestic nonprofit organizations.

7.a. through 7.d. Self-explanatory.