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ORIGINAL ARTICLE

A novel prostate cancer subtyping classifier based on luminal and basal phenotypes

Adam B. Weiner MD[1] | Yang Liu PhD[2] | Alex Hakansson BS[2] | Xin Zhao MS[2] | James A. Proudfoot MS[2] | Julian Ho PhD[2] | JJ H. Zhang MD[1] | Eric V. Li MD[3] | R. Jeffrey Karnes MD[4] | Robert B. Den MD[5] | Amar U. Kishan MD[6] | Robert E. Reiter MD, MBA[1] | Anis A. Hamid MBBS[7] | Ashely E. Ross MD, PhD[3] | Phuoc T. Tran MD, PhD[8] | Elai Davicioni PhD[2] | Daniel E. Spratt MD[9] | Gerhardt Attard MD, PhD, FRCP[10] | Tamara L. Lotan MD[11] | Melvin Lee Kiang Chua MBBS, PhD, FRCR[12,13] | Christopher J. Sweeney MBBS[7] | Edward M. Schaeffer MD, PhD[3]

[1]Department of Urology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA

[2]Veracyte Inc, San Diego, California, USA

[3]Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

[4]Department of Urology, Mayo Clinic, Rochester, Minnesota, USA

[5]Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

[6]Department of Radiation Oncology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA

[7]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

[8]Department of Radiation Oncology, University of Maryland, Baltimore, Maryland, USA

[9]Department of Radiation Oncology, University Hospitals Seidman Cancer Center, Case Comprehensive Cancer Center, Cleveland, Ohio, USA

[10]Cancer Institute, University College London, London, UK

[11]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[12]Division of Radiation Oncology, National Cancer Centre, Singapore, Singapore

[13]Division of Medical Sciences, National Cancer Centre, Singapore, Singapore

Correspondence
Edward M. Schaeffer, 676 N St Clair St, Ste 2300 Chicago, IL 60611, USA.
Email: e-schaeffer@northwestern.edu

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Abstract

Background: Prostate cancer (PCa) is a clinically heterogeneous disease. The creation of an expression-based subtyping model based on prostate-specific biological processes was sought.

Methods: Unsupervised machine learning of gene expression profiles from prospectively collected primary prostate tumors (training, n = 32,000; evaluation, n = 68,547) was used to create a prostate subtyping classifier (PSC) based on basal versus luminal cell expression patterns and other gene signatures relevant to PCa

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biology. Subtype molecular pathways and clinical characteristics were explored in five other clinical cohorts.

Results: Clustering derived four subtypes: luminal differentiated (LD), luminal proliferating (LP), basal immune (BI), and basal neuroendocrine (BN). LP and LD tumors both had higher androgen receptor activity. LP tumors also had a higher expression of cell proliferation genes, MYC activity, and characteristics of homologous recombination deficiency. BI tumors possessed significant interferon γactivity and immune infiltration on immunohistochemistry. BN tumors were characterized by lower androgen receptor activity expression, lower immune infiltration, and enrichment with neuroendocrine expression patterns. Patients with LD tumors had less aggressive tumor characteristics and the longest time to metastasis after surgery. Only patients with BI tumors derived benefit from radiotherapy after surgery in terms of time to metastasis (hazard ratio [HR], 0.09; 95% CI, 0.01–0.71; $n = 855$). In a phase 3 trial that randomized patients with metastatic PCa to androgen deprivation with or without docetaxel ($n = 108$), only patients with LP tumors derived survival benefit from docetaxel (HR, 0.21; 95% CI, 0.09–0.51).

Conclusions: With the use of expression profiles from over 100,000 tumors, a PSC was developed that identified four subtypes with distinct biological and clinical features.

Plain language summary

- Prostate cancer can behave in an indolent or aggressive manner and vary in how it responds to certain treatments.
- To differentiate prostate cancer on the basis of biological features, we developed a novel RNA signature by using data from over 100,000 prostate tumors—the largest data set of its kind.
- This signature can inform patients and physicians on tumor aggressiveness and susceptibilities to treatments to help personalize cancer management.

KEYWORDS
biomarkers, gene expression, gene expression profiling, genetics, humans, pathology, prognosis, prostatic neoplasms, tumor

INTRODUCTION

Prostate cancer (PCa) results in more than 375,000 deaths worldwide on an annual basis and represents a clinically and biologically diverse disease process.[1–5] A substantial heterogeneity of prognosis exists even within subgroups of PCa defined by clinicopathologic features.[6,7] As we move beyond clinicopathologic characteristics, advanced PCa care has entered an era of precision oncology in which individualized treatment courses might be determined on the basis of tumor molecular characteristics.[8] Efforts to molecularly characterize earlier stage disease include landmark work from The Cancer Genome Atlas (TCGA), which defined several tumor subtypes.[2] However, this work was limited by a small sample size without broad representation of clinicopathologic features, and was largely based on tumors from patients of European ancestry. Additionally, with advances in our understanding of identifiable factors and biological processes defining molecular subtypes for PCa,[9–11] new efforts to comprehensively subclassify PCa using large, diverse cohorts are warranted.

Within breast cancer, a molecular subtyping method (formerly Prediction Analysis of Microarray 50 [PAM50]) based on molecular features that distinguish basal from luminal cell of origin is widely used in clinical practice for patients with early-stage, hormone receptor–positive tumors and helps predict treatment susceptibility.[12,13] We hypothesized that molecular signatures using PCa-specific cell-of-origin features could delineate molecular subtypes with distinct biological processes and treatment susceptibilities.

To that end, here we used gene expression profiles from over 100,000 primary prostate tumors collected prospectively to create and characterize an expression-based prostate subtyping classifier

(PSC) on the basis of molecular features defining cell of origin and pathways specific to PCa. We then associated PSC subtypes with previously validated signatures for cancer biological processes and began to evaluate differential treatment susceptibilities using multiple independent cohorts in exploratory analyses.

MATERIALS AND METHODS

Patients

Seven independent tumor and patient cohorts were used for the development and characterization of a PSC in exploratory analyses with respect to genomic signatures, molecular pathways, and clinical end points. The first two cohorts were based on deidentified gene expression profiles obtained prospectively from clinical usage of the Decipher prostate genomic classifier between October 2017 and February 2022 (Veracyte Inc, San Diego, California). These tumors were divided into two cohorts: a model training cohort (n = 32,000) and an evaluation cohort (n = 68,547; Tables S1 and S2). For all analyses after model creation, the training and evaluation cohorts were combined into the Decipher Genomics Resource for Intelligent Discovery (GRID) cohort. Ordering criteria for the genomic classifier exclude prior treatment with hormone therapies or radiotherapy. These tumor samples were obtained by either prostate biopsy or radical prostatectomy. All tumors were prospectively gathered in the GRID (NCT02609269).[14]

Data were also obtained from large, retrospective cohorts from the Johns Hopkins Medical Institute (JHMI; n = 498; Table S3). This cohort included patients who underwent radical prostatectomy with no additional treatments until the end of follow-up or metastatic recurrence. Details on this cohort can be found in prior work.[15] Records from another retrospective cohort were obtained from individual patient data generated in a prior meta-analysis with long-term follow-up (META855; n = 855; Table S4).[16] This was used to test the model's associations with time to metastasis after radical prostatectomy with or without adjuvant radiation. In the E3805 CHAARTED trial, patients with metastatic, hormone-sensitive PCa were randomized to androgen deprivation therapy (ADT) with or without six cycles of docetaxel.[17] Results from this phase 3 trial showed that the addition of docetaxel improves overall survival. A subset of tumors from patients in this trial underwent gene expression profiling (CHAARTED cohort; n = 108; Table S5) and comprised a fifth cohort.[18] From this subset, PSC subtypes were correlated with response to docetaxel. A retrospective cohort of Asian patients from the National Cancer Centre Singapore (NCCS) was assessed to compare the frequency of PSC subtypes between Asian patients and White and Black patients from the JHMI (Table S6).[19]

Finally, data from patients with PCa in TCGA (n = 491; Table S7) were downloaded from the cBioPortal for a seventh cohort.[20,21] Serum prostate-specific antigen (PSA) values for TCGA were downloaded from the Broad Institute.[22] Data from this cohort were used to associate the PSC with genomic change characteristics of homologous recombination deficiency (HRD). Immunohistochemistry data from Saltz et al. (n = 330)[23] were used to associate the PSC with percent tumor-infiltrating lymphocyte fraction.

Expression data

The expression assay data for the two GRID cohorts, the META855 cohort, and the CHAARTED cohort were derived from the Human Exon 1.0 ST oligonucleotide microarray (Thermo Fisher, Santa Clara, California) to measure the expression of 46,050 genes and noncoding RNA transcripts. Microarray processing was performed in a Clinical Laboratory Improvement Amendments-certified clinical operations laboratory (Veracyte Inc). Microarrays were normalized by using single-channel array normalization.[24] Expression data from the JHMI, NCCS, META855, TCGA, and CHAARTED cohorts were quantile matched to the GRID cohorts on the basis of pathologic stage, Gleason group, and age.[25]

Model creation

All machine-learning methods for tumor clustering, model development, and model training and citations for expression signatures used for clustering can be found in Supplemental Methods, Tables S8–S11, and Figures S1 and S2. We selected an a priori list of genes and gene signatures on the basis of processes relevant to PCa biology and treatment susceptibilities including a signature for a luminal versus basal cell of origin.[26] This was done as opposed to using the complete list of gene expression levels to achieve the primary objective of creating a signature that could classify tumors in clinically meaningful ways. An unsupervised analysis using a previously validated expression signature for basal versus luminal benign prostate cells was used for the initial clustering among these selected signatures and genes (Table S9). A radar plot was created to visually compare PSC subtypes based on an ad hoc grouping of expression-based signatures categorized as reflections of cancer hallmarks (Supplemental Data).[27]

Statistical tests

Time-to-event end points were shown graphically using the Kaplan–Meier method. Multivariable Cox regressions were used to compare time to failures. Within the JHMI cohort, time to metastasis after radical prostatectomy was assessed by adjusting for grade group, log-transformed serum PSA, patient-defined race, and stage at prostatectomy. Time to metastasis in patients who did and did not receive radiotherapy after prostatectomy was assessed in META855 by adjusting for age at diagnosis, grade group, PSA, and stage at prostatectomy. In the CHAARTED cohort, overall survival was assessed by adjusting for age, Eastern Cooperative Oncology Group (ECOG) functional status, prior local treatment, disease volume, and docetaxel receipt. These Cox regressions were repeated for comparison

to the PAM50 model. For the analysis in CHAARTED, this was limited to patients with non–luminal A and luminal A tumors because of prior work that suggested this group derived more benefit from docetaxel.[18] The statistical significance of differences in continuous and categorical variables between groups was assessed using Kruskal–Wallis and Pearson X^2 tests, respectively. Given the exploratory nature of our work, no adjustments for multiple hypothesis testing were performed, all tests were two sided, and all analyses were performed using R version 3.6.2.

RESULTS

PSC model definition

A panel of eight genes and 13 gene expression signatures relevant to PCa carcinogenesis from expert curation of the literature was used as seed features in an unsupervised hierarchical clustering solution for a training cohort consisting of 32,000 genome-wide expression profiles from biopsy and radical prostatectomy samples spanning the clinical spectrum of localized disease (Supplemental Results and Figures S3 and S8). The categories of seed features included those related to androgen receptor (AR) activity, basal–luminal cell of origin, tumor cell proliferation, and the tumor-immune microenvironment. Four distinct prostate subtypes were arrived at in the training cohort: luminal differentiated (LD), luminal proliferating (LP), basal immune (BI), and basal neuroendocrine (BN). The final PSC model (Figure 1A) showed substantial luminal and basal class change as compared to the PAM50 breast cancer model in the combined training and evaluation GRID cohort (Figure 1B).[12,13] Distinct PCa-specific findings within the subtypes were also noted. The BN subtype showed a high frequency of RB loss, whereas a low frequency of PTEN loss was seen in the BI subtype. The lowest levels of a p53 mutation signature were observed in the LD class (Figure 1C). ERG fusion was relatively stable across different subtypes, whereas BN tumors were significantly enriched for an SPOP mutation signature (Figure 1C).[28] Intriguingly, the BN class also had increased expression of neuroendocrine biology signatures for PCa (Figure 1D and Figure S9).[29] TCGA subtypes based on multiomic analyses including DNA, RNA, and protein expression data in 333 tumors did not show an obvious association with the PSC but was limited by sample size (Figure S10).[2]

Molecular pathways defining subtypes

PSC classes were compared on the basis of signatures that represented relevant molecular pathways in PCa (Figure 2A). In general, the luminal subtypes tended to harbor higher levels of androgen receptor transcriptional activity and expression of prostate-specific membrane antigen (PSMA; *FOLH1*) as well as prostate luminal genes such as *KLK2*, *KLK3* (PSA), and *KLK4*. The hallmark of cancer MYC activity signature 1 (enriched with genes related to cell cycle and DNA repair processes) was higher in LP tumors, whereas MYC activity signature 2 (enriched with RNA processing and ribosomal biogenesis) was higher in BN tumors (Table S9). LP tumors had higher

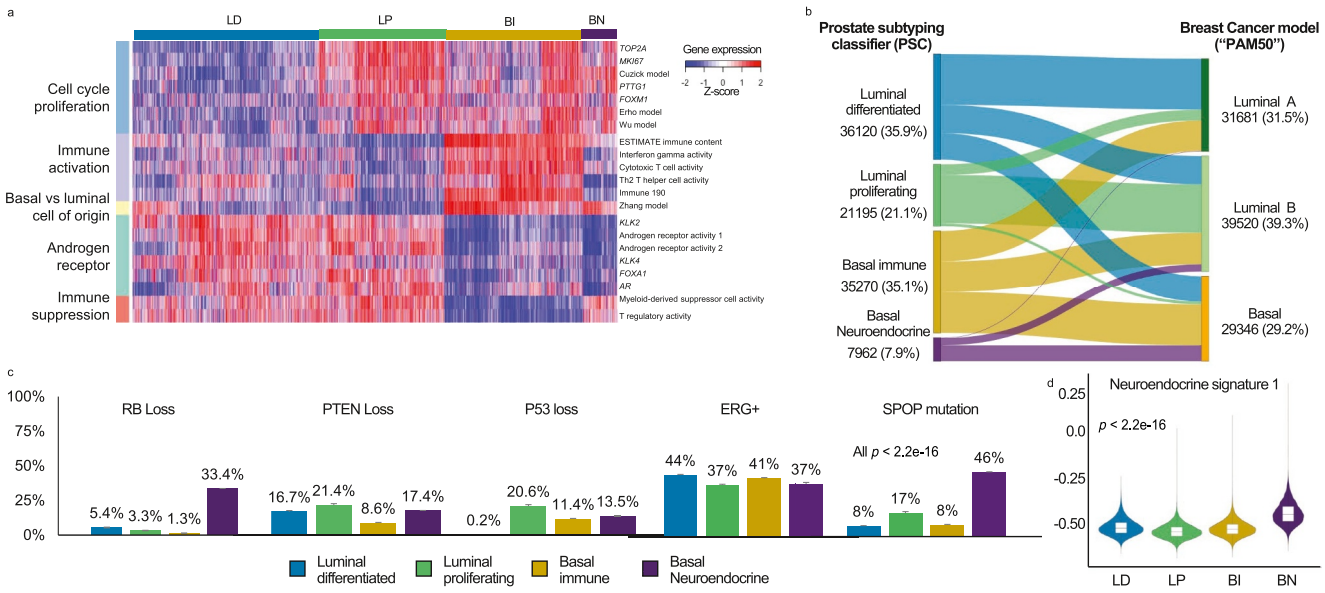

FIGURE 1 Prostate subtyping classifier compared to other subtypes. (A) The final prostate subtyping classifier groupings are differentiated by the biomarkers used to cluster tumors for model development. (B) There was substantial class change between the breast cancer model PAM50 and the prostate subtyping classifier model. (C) Subtypes differed by relevant copy number losses and mutations on the basis of previously validated expression signatures. (D) The basal neuroendocrine subtype resembled phenotypically neuroendocrine tumors on the basis of expression patterns. *p* values are from Kruskal–Wallis tests. All data here are from the Genomics Resource for Intelligent Discovery cohort. BI indicates basal immune; BN, basal neuroendocrine; LD, luminal differentiated; LP, luminal proliferating.

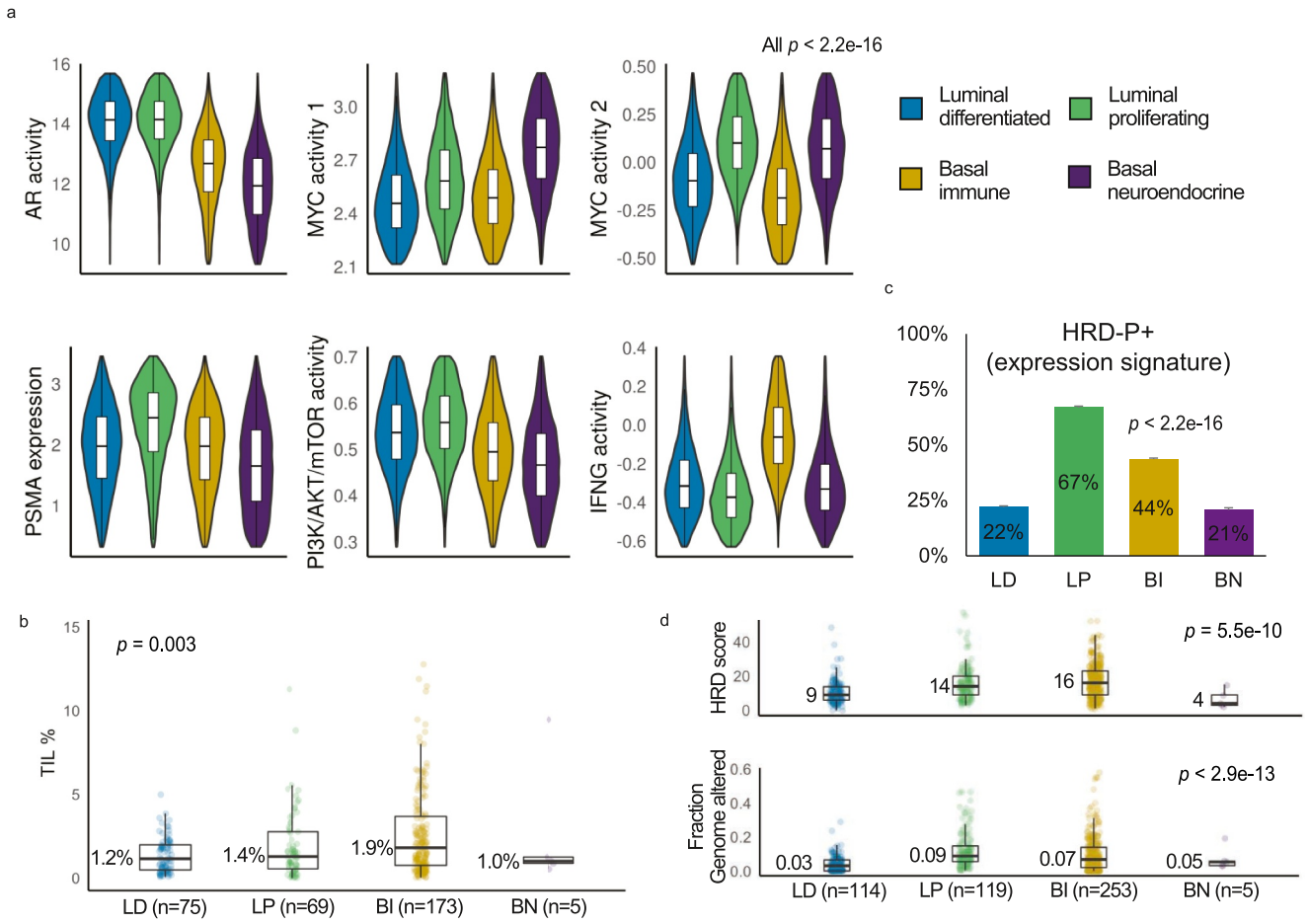

FIGURE 2 Molecular pathways defining the prostate subtyping classifier. (A) The prostate subtyping classifier model differentiated tumors on the basis of relevant molecular pathways in the Genomics Resource for Intelligent Discovery (GRID) cohort (Kruskal–Wallis test). (B) The percentage of tumor samples occupied by tumor-infiltrating lymphocytes was highest in the basal immune tumors on the basis of immunohistochemistry data from The Cancer Genome Atlas (Kruskal–Wallis test; numbers are medians). (C,D) Luminal proliferating and basal immune tumors characteristically resembled tumors with homologous recombination deficiency on the basis of (C) an expression signature in the GRID cohort (X^2 test) and (D) genomic characteristics from The Cancer Genome Atlas (p values are from Kruskal–Wallis tests; numbers are medians). AR indicates androgen receptor; BI, basal immune; BN, basal neuroendocrine; HRD, homologous recombination deficiency; HRD-P+, homologous recombination deficiency prostate cancer signature; IFNG, interferon γ; LD, luminal differentiated; LP, luminal proliferating; PSMA, prostate-specific membrane antigen; TIL, tumor-infiltrating lymphocytes.

levels of the hallmark for PI3K/mTOR activity. BI tumors tended to have more interferon γ activity, and accordingly immunohistochemistry analyses in TCGA showed that these tumors tended to have a greater proportion of tumor-infiltrating lymphocytes (Figure 2B). LP and BI tumors tended to have more tumors with characteristics suggestive of HRD on the basis of a previously validated expression signature (Figure 2C)[30] and DNA-level genomic changes observed in TCGA (Figure 2D).

PSC and clinicopathologic correlates

Consistent with prior work that demonstrated differences in tumor expression profiles by race,[31] Asian and Black patients tended to harbor more BN tumors compared to White patients (Figure S11). Similarly, serum PSA values (at diagnosis or preoperative) showed variance across PSC class, with BN harboring a lower PSA distribution whereas both luminal subtypes had higher baseline PSA distribution (Figure S12a). Patients with LD tumors tended to harbor a lower frequency of non–organ-confined disease, and a higher proportion of LD had lower grade disease (Figure S12b,c). On the basis of three commercially available prognostic test scores,[32] LD tumors had the lowest median scores (genomic risks 1–3; Table S6) and were characterized by cancer hallmarks associated with a higher degree of cellular differentiation, more similar to nonneoplastic luminal cells (Supplemental Data),[27] which suggested that overall, LD tumors had the least aggressive tumor biology (Figure 3). Accordingly, in the JHMI cohort, patients with LD tumors experienced a favorable time to metastasis after radical prostatectomy compared to patients with LP and BI tumors (Figure 4A and Table S12).

Notably, LP tumors had the highest levels of genomic risk,[33] a signature for cell cycle proliferation genes (Figure 3A and Table S8).[33]

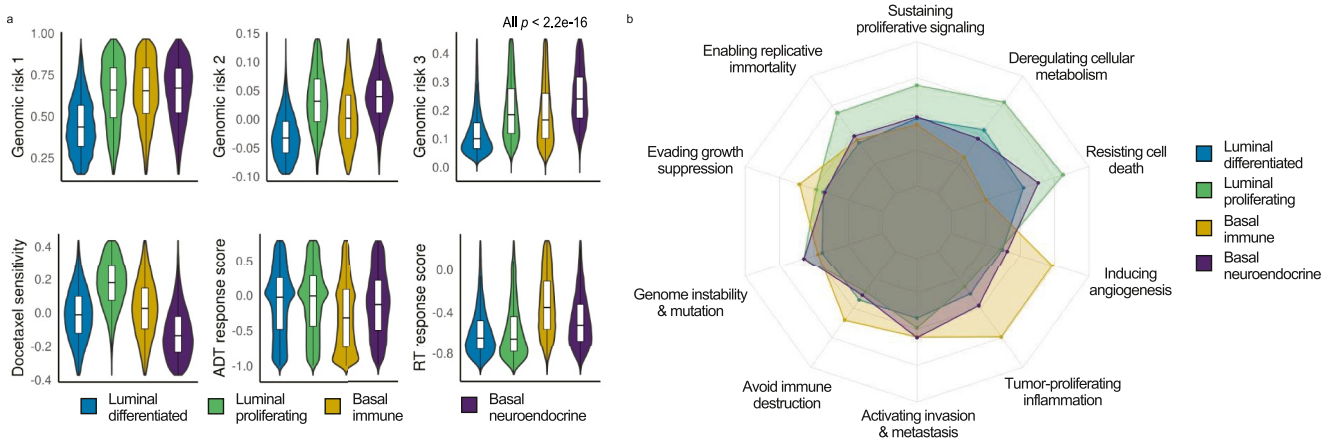

FIGURE 3 Clinical transcriptomic biomarkers and prostate subtyping classifier. (A) Luminal differentiated tumors tended to harbor lower gene expression risk scores. Luminal proliferating tumors were predicted to be more responsive to taxane chemotherapy compared to the other subtypes, whereas basal immune tumors were predicted to be the least responsive to androgen deprivation therapy. Basal immune tumors were predicted to be the most responsive to radiotherapy (*p* values are from Kruskal–Wallis tests). (B) A radar plot visually compares tumors on the basis of cancer hallmarks. Plotted are the means or inverse means of the percentile ranks of various signatures (Supplemental Data) with the center to outer edge representing the 0th to 100th percentile. All data here are from the Genomics Resource for Intelligent Discovery cohort. ADT indicates androgen deprivation therapy; BI, basal immune; BN, basal neuroendocrine; LD, luminal differentiated; LP, luminal proliferating; RT, radiotherapy.

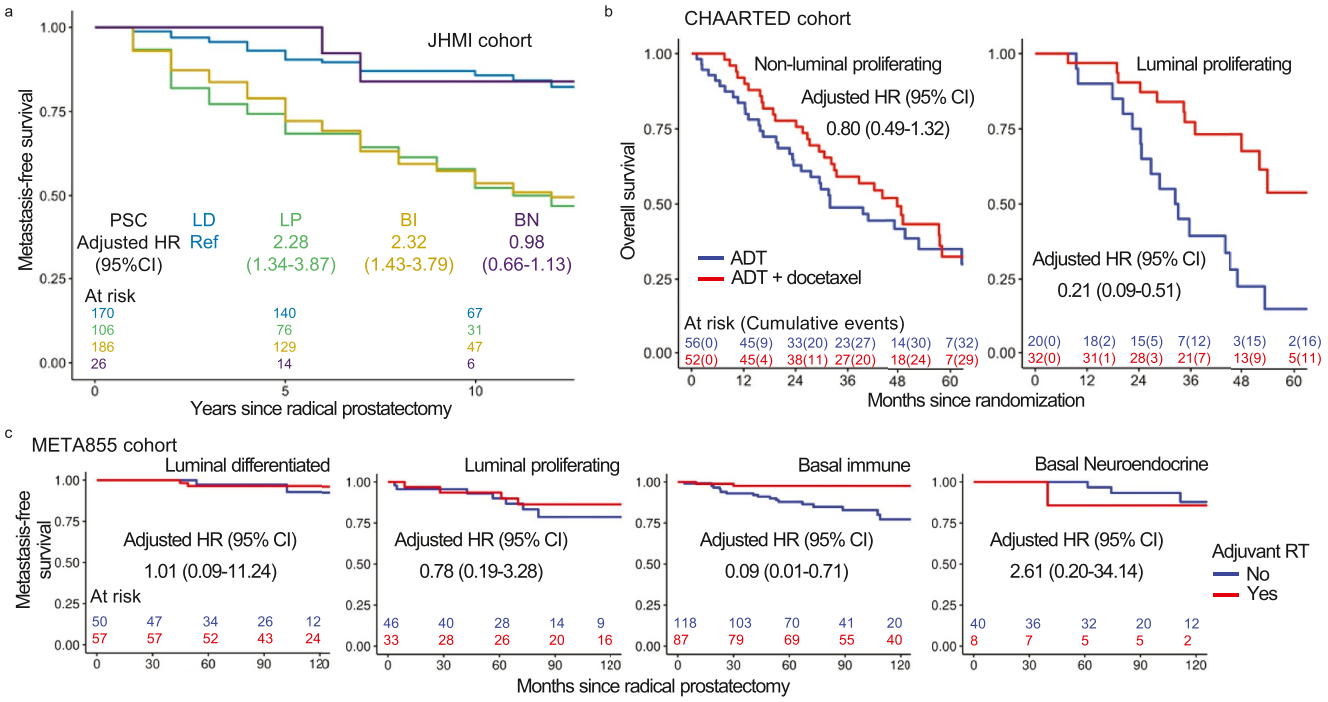

FIGURE 4 Clinical outcomes associated with the prostate subtyping classifier. (A) Patients with luminal differentiated and basal neuroendocrine tumors experienced the longest time to metastatic recurrence after radical prostatectomy. Note that in the Johns Hopkins Medical Institute natural history cohort (no hormone therapy or radiotherapy before metastatic onset), patients with luminal differentiated tumors did not differ from those with basal neuroendocrine tumors significantly, but few patients in this cohort were classified with basal neuroendocrine tumors with only two metastatic events. (B) In patients with metastatic hormone-sensitive prostate cancer who were randomized to androgen deprivation with or without docetaxel, patients with luminal proliferating tumors benefited from chemotherapy whereas those with other prostate subtyping classifier subtypes did not. (C) Patients with basal immune tumors benefited from adjuvant radiotherapy after prostatectomy in a retrospective cohort whereas patients with other prostate subtyping classifier subtypes did not (all hazard ratios are from the multivariable Cox regressions in Tables S10–S12). ADT indicates androgen deprivation therapy; BI, basal immune; BN, basal neuroendocrine; HR, hazard ratio; JHMI, Johns Hopkins Medical Institute; LD, luminal differentiated; LP, luminal proliferating; PSC, prostate subtyping classifier; RT, radiotherapy.

Consistent with this finding, LP tumors were associated with the greatest predicted sensitivity to docetaxel chemotherapy (a microtubule inhibitor) on the basis of an in vitro drug response expression signature derived from the Cancer Cell Line Encyclopedia and NCI-60 resources (Figure 3A, Table S8, and Supplemental Methods). Clinical support for these in silico findings were obtained in an exploratory analysis of the CHAARTED phase 3 trial that randomized patients with metastatic, hormone-sensitive PCa to ADT or ADT with docetaxel. Patients with LP tumors experienced greater overall survival benefit from the addition of docetaxel compared to the other PSC types (Figure 4B and Table S13).

BI tumors were associated with the lowest predicted response to ADT but were notably predicted to be more responsive to radiotherapy compared to the other PSC classes on the basis of the postoperative radiation therapy outcomes score (Figure 3 and Table S8). In the META855 cohort,[16] only patients with BI tumors appeared to benefit from radiotherapy after radical prostatectomy in terms of time to metastasis (Figure 4C and Table S14a–c).

In broad comparison, the outcomes for patients stratified by PAM50 subgroups in the JHMI, CHAARTED, and META855 cohorts produced less significant or nonsignificant hazard ratios compared to those of the PSC (Tables S15–S17). In particular, the observed treatment effects are larger with the PSC model as compared to PAM50. For example, the hazard ratio for the docetaxel treatment effect in PSC LP tumors is 0.21 (95% CI, 0.09–0.51; $p < .001$), whereas with the cognate PAM50 luminal B tumors it is 0.44 (95% CI, 0.24–0.81; $p = .008$). This suggests that the PSC is identifying a group of patients with greater sensitivity to the addition of docetaxel than does PAM50. Similar results were observed after radical prostatectomy (JHMI cohort) and radiation after surgery (META855 cohort).

DISCUSSION

PCa is a heterogeneous disease even in the early localized stage. For localized PCa, there are multiple seemingly equivalent therapies (surgery and radiation) but clinical experience informs us that not all patients benefit similarly from these treatments.[6,7] Similarly, in more advanced PCa there are now a number of Food and Drug Administration–approved systemic therapies without the needed evidence guiding preferential use or sequencing of these agents.[34] Efforts to subclassify tumors on the basis of relevant biological processes could provide meaningful avenues to personalize treatment. Prior subclassifying methods have been limited in clinical application or simply prognosticated tumor aggressiveness.[2,35–37] Here, we use an unsupervised machine-learning approach that leverages gene expression profiles from over 100,000 tumors to create and evaluate a PCa classification that, similar to other epithelial tumors, can be defined to a large degree by a tumor's similarity to the biology of normal basal or luminal cells. This fundamental aspect of PCa tumor biology may be clinically relevant to disease progression after surgery, sensitivity to radiation, and response to systemic treatments. The PSC may therefore provide a clinically implementable means to advance personalized PCa management using a currently available clinical test.

Previous work classified PCa on the basis of cell of origin by using a model created for breast cancer.[38,39] As part of the current PSC development, we used a previously validated expression signature for basal versus luminal benign prostate cells (Zhang et al.)[26] and subsequently showed substantial class change between the breast- and prostate-specific models. Furthermore, gene signatures used in combination with the basal versus luminal signature were used as seed features to create the PSC model via an unsupervised approach and included processes relevant to both PCa biology and currently used or investigated treatments (Table S9). Many treatments active in metastatic PCa have been tested in the adjuvant PCa setting. Future attempts to move other late-stage treatments to earlier lines of care, prevent relapse, and cure more men could be informed by PSC subgroups and biologically directed precision treatments.

Although the subclassification of early-stage PCa on the basis of molecular processes can help characterize tumors, there is often limited translatability to clinical practice.[40] Many commonly used gene expression risk scores use RNA expression to prognosticate tumor aggressiveness but do not predict treatment responses.[35–37] The PSC stratifies tumors not only by innate aggressiveness but also by other aspects of tumor biology that may predict response to treatments commonly used for PCa. Within the META855 cohort, only patients with BI tumors derived a benefit from the addition of radiotherapy after prostatectomy. It is unknown whether this is because of BI tumors being more likely to have local recurrences and benefit from adjuvant prostate radiation or greater inherent sensitivity to radiation. The latter is consistent with the in silico prediction of being more responsive to radiotherapy based on a previously validated signature (Table S8). By using specimens from the ECOG-ACRIN E3805 CHAARTED trial, only patients with LP tumors appeared to benefit from the addition of docetaxel to ADT in the setting of metastatic hormone-sensitive PCa. PSC subtypes also differed by immune content, HRD characteristics, and expression of PSMA, PI3K/AKT/mTOR, and MYC activity. Each of these measures may prove relevant in helping select given patients for the various systemic therapies currently used for metastatic PCa.[41–44] Tumor biology classification schemes such as the PSC may have the potential to provide granular predictions related to differential treatment efficacy, but much additional work to validate the hypothesis generating the results presented here is still needed.

Accordingly, limitations to this work include the need for additional external clinical validation. Data from the five clinical cohorts evaluated in exploratory analyses here provide some assessment of the clinical implications for the PSC. Among the clinical cohorts, some PSC subtypes were uncommon, which potentially led to small sample sizes that could not detect significant differences in outcomes. Future work should apply the PSC to prospective cohorts with varying treatment arms to assess differential treatment efficacy on the basis

of subtype. Exploratory analysis from the E3805 CHAARTED trial is limited by sample size as previously described in Hamid et al.[18] The main limitations include the bias related to the partial sample of the trial patients whose tumors were profiled (e.g., the distribution of the burden of metastatic disease) and the potential molecular differences between the metastatic tumors and the primary tumors that were analyzed. The GRID cohort is based on the commercial use of a prognostic genomic classifier for PCa and was skewed toward more unfavorable-risk tumors. Thus, generalizability to favorable-risk tumors may be limited. However, favorable-risk tumors less often require additional treatments beyond surveillance after standard definitive treatments. Additionally, important demographic information such as race is not well annotated in the GRID cohort.

In conclusion, by using the largest collection of prostate tumors with expression data, we introduce a method to classify PCa on the basis of prostate-specific luminal versus basal cell-of-origin biology. The PSC differentiates tumors on the basis of molecular processes relevant to PCa biology and potential treatment susceptibilities, which may broaden its application as a tool for personalizing treatment. Evaluation of the PSC in prospective cohorts will confirm its ability to predict differential treatment efficacy.

AUTHOR CONTRIBUTIONS

Adam B. Weiner: Conceptualization, formal analysis, investigation, methodology, software, validation, visualization, and writing–original draft. **Yang Liu:** Conceptualization, formal analysis, investigation, methodology, software, validation, visualization, and writing–original draft. **Alex Hakansson:** Formal analysis, investigation, methodology, software, validation, visualization, and writing–original draft. **Xin Zhao:** Formal analysis, investigation, methodology, software, validation, and writing–original draft. **James A. Proudfoot:** Formal analysis, investigation, methodology, and writing–original draft. **Julian Ho:** Formal analysis, investigation, and methodology. **JJ H. Zhang:** Formal analysis, investigation, and writing–original draft. **Eric V. Li:** Formal analysis, investigation, and writing–original draft. **R. Jeffrey Karnes:** Formal analysis, investigation, and resources. **Robert B. Den:** Formal analysis, investigation, and resources. **Amar U. Kishan:** Formal analysis and investigation. **Robert E. Reiter:** Formal analysis and investigation. **Anis A. Hamid:** Formal analysis and investigation. **Ashely E. Ross:** Formal analysis and investigation. **Phuoc T. Tran:** Formal analysis, investigation, and resources. **Elai Davicioni:** Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, visualization, writing–original draft, and supervision. **Daniel E. Spratt:** Formal analysis, investigation, and resources. **Gerhardt Attard:** Formal analysis and investigation. **Tamara L. Lotan:** Data curation, formal analysis, investigation, resources, and funding acquisition. **Melvin Lee Kiang Chua:** Data curation, formal analysis, investigation, and resources. **Christopher J. Sweeney:** Data curation, formal analysis, investigation, resources, supervision, and project administration. **Edward M. Schaeffer:** Conceptualization, formal analysis, investigation, resources, writing–original draft, funding acquisition, supervision, and project administration. All authors contributed to writing–review and editing.

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CONFLICT OF INTEREST STATEMENT

Yang Liu, Alex Hakansson, Xin Zhao, James A. Proudfoot, Julian Ho, and Elai Davicioni are employees of Veracyte Inc. Yang Liu holds a patent and stock from Veracyte Inc. Xin Zhao holds stock from Veracyte Inc. R. Jeffrey Karnes holds intellectual property. Amar U. Kishan has been a consultant for Boston Scientific, ViewRay Technologies, Varian Medical Systems, and Janssen Biotech, has received grants from Janssen Biotech and Point Biopharma, and holds stock from ViewRay Technologies. Robert E. Reiter has been a consultant for Lantheus Medical Imaging. Ashely E. Ross has been a consultant for Veracyte Inc. Phuoc T. Tran holds a patent from Natsar Pharm and has been a consultant for Noxopharm, RefleXion Medical, Natsar Pharm, Regeneron Pharmaceuticals, Myovant, AstraZeneca, and Janssen Biotech. Elai Davicioni holds a patent from Veracyte Inc. Daniel E. Spratt has been a consultant for Boston Scientific, Pfizer, Siemens, Janssen Biotech, Bayer, AstraZeneca, Novartis, Elekta Instruments, and Varian Medical Systems. Gerhardt Attard holds a patent with Institute of Cancer Research, has been a consultant for Janssen Biotech and Novartis, and has received grants from Astellas Pharma and Veracyte. Tamara L. Lotan has received grants from AIRA Matrix, Myriad Genetic Laboratories, Roche, and Deep Bio. Melvin Lee Kiang Chua holds a patent with Digital Life Line and has been a consultant for Bayer, Merck Sharp & Dohme, Astellas Pharma, Janssen Pharmaceuticals, and Varian Medical Systems. Christopher J. Sweeney has been a consultant for Astellas Pharma, Pfizer, Cell-Centric, Bayer HealthCare Pharmaceuticals, Point Biopharma, Sanofi US Services, AstraZeneca, Janssen Biotech, and Genentech. Edward M. Schaeffer has been a consultant for Pinnacle, Astellas Pharma, Pfizer Canada, and Lantheus Medical Imaging. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article were provided by Veractye Inc by permission. Data will be shared upon request to the corresponding author with the permission of Veracyte Inc.

ORCID

Adam B. Weiner https://orcid.org/0000-0002-1912-042X
Eric V. Li https://orcid.org/0000-0002-5506-5755
Anis A. Hamid https://orcid.org/0000-0002-7193-9723
Daniel E. Spratt https://orcid.org/0000-0002-5973-4741

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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