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TITLE: Predicting Response to Intense Neoadjuvant
Androgen Deprivation Therapy Through
Spatial Genomics and Imaging Heterogeneity

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14. ABSTRACT Objective: Patients enrolled in our clinical study of GnRH agonist plus enzalutamide for six months prior to radical prostatectomy have shown a diversity of responses, including differential responses within the same patient (i.e. one region of tumor responds and another region does not). Based on these initial clinical outcomes, we hypothesize that the specific genomic alterations that arise during tumor development can distinguish exceptional from poor responders to this intense neoadjuvant ADT. We will also test the hypothesis that patients who fail to respond to this intense therapy do so through either pre-existing genomic alterations, adapt to survive with lower levels of androgen receptor activity, or a combination of both. Impact: Further understanding of the genetics of prostate cancer underlying response to treatment is critical to guide patient-specific treatments based on genetic status to improve overall survival. By understanding the genetic factors responsible for either exceptional or failed response to treatment, we will define the biology of lethal prostate cancer to reduce death, while also developing treatments that improve outcomes for men with lethal prostate cancer.					
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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	11
5. Changes/Problems.....	12
6. Products.....	14
7. Participants & Other Collaborating Organizations.....	16
8. Special Reporting Requirements.....	18
9. Appendices.....	19

1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Although a large number of prostate cancer patients exhibit a non-aggressive (indolent) form of disease, among those with aggressive disease, treatment options include radiation therapy targeted to the pelvis or radical prostatectomy survey to remove the prostate. Given that newer agents such as abiraterone and enzalutamide have shown promise in the metastatic setting, there is increasing interest in treatment with hormone therapy (neoadjuvant androgen deprivation therapy followed by surgery) while definitive therapy may still be curative. Although several factors, including age, family history, PSA levels, and, increasingly, genetic or genomic test are considered before deciding on surgery, there is currently no clear indication what can predict how a patient might respond to neoadjuvant therapy. This study uses correlative samples from a clinical trial of neoadjuvant enzalutamide at the National Cancer Institute, in which men enrolled on the study receive multiparametric MRI (mpMRI) screening and undergo a pre-treatment targeted biopsy to regions identified on mpMRI. A second mpMRI is performed after treatment, before surgery. The first objective of this study is to ask whether genetic features identified in a pre-treatment biopsy would indicate whether a tumor is likely to respond to treatment. The second objective is to use mpMRI imaging with tissue analysis to create a prostate cancer profile that would allow clinicians to better screen patients prior to treatment.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Prostate cancer, androgen deprivation therapy, ADT, neoadjuvant, genomics, tumor heterogeneity, evolution, diversity, immunohistochemistry, enzalutamide

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project? *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

3.1 Major Goals (From Statement of Work):

Research-Specific Tasks:

Specific Aim 1: Assess genetic features of exceptional response and resistance to neoadjuvant anti-androgen therapies			Status:
Major Task 1: Biopsy Tumor Mapping and DNA Analysis	Original Timeline	NCI	In Progress
Subtask 1: IRB approval and HRPO review/approval	1-4	Dr. Wilkinson	Complete
Subtask 2: Acquisition of ≥ 37 biopsy and corresponding	5-18	Dr.	Complete

RP specimens from patients enrolled in neoadjuvant enzalutamide trial		Wilkinson	
Subtask 3: IHC and tumor mapping on cases	5-18	Dr. Wilkinson	Complete
<i>Milestone Achieved: Tumor mapping for Specific Aim 1</i>	18		
Subtask 4: Tissue microdissection and sequencing analysis	5-20	Dr. Wilkinson	In Progress
<i>Milestones Achieved: Identification of genomic marks that predict response to anti-androgen therapy; publication of 1-2 peer-reviewed papers</i>	5-20		
Specific Aim 2: Correlate mpMRI features to multiplex histopathologic analysis of primary prostate disease			
Major Task 2: Mapping RP to corresponding biopsy, IHC	Months	NCI	In Progress
Subtask 1: Correlate immunohistologic profile of targeted pre-treatment biopsy with mpMRI lesions	5-18	Dr. Wilkinson	In Progress
Subtask 2: Predict efficacy of neoadjuvant ADT by tuning mpMRI to histologic features of RP specimens	12-24	Dr. Wilkinson	In Progress
<i>Milestones Achieved: Correlate mpMRI tumor burden with histological tumor burden; publication of 1-2 peer-reviewed papers</i>	5-24	Dr. Wilkinson	

What was accomplished under these goals? For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activities:

The framework of this proposal relied upon a correlative study to a then-ongoing clinical trial examining men with intermediate and high-risk features of prostate cancer that were treated with enzalutamide + GnRH antagonist for 6 months. The trial completed accrual in late 2019. In this award year, we have worked with radiologist Dr. Baris Turkbey to map tumor burden and correlate pre-treatment targeted biopsies with post-treatment residual tumor. We then performed IHC to characterize tumor of pre-treatment biopsies, and conducted laser capture microdissection to isolate ultra-pure tumor populations for whole exome sequencing and RNAseq.

Specific Objectives:

The objectives of the project are to 1) Assess genetic features of exceptional response

and resistance to neoadjuvant anti-androgen therapies; and 2) Correlate mpMRI features to multiplex histopathologic analysis of pre- and post-treatment tissue.

The first objective seeks to examine tumor specimens from patients enrolled in a clinical trial of neoadjuvant androgen deprivation therapy, where pre-treatment targeted biopsy specimens and post-treatment radical prostatectomy are analyzed to determine genomic features unique to tumor samples that exhibit exceptional response to therapy (measured by a residual cancer burden/RCB of $< 0.05\text{cc}$), or incomplete/non-responders ($\text{RCB} > 0.05\text{cc}$). By identifying features unique to these separate cohorts, we are able to better predict whether a tumor sample is likely to respond to neoadjuvant androgen deprivation therapy.

The second objective aims to correlate histology of pre-treatment biopsies with mpMRI lesions. While mpMRI is a non-invasive method to detect prostate lesions, the resolution provided is inadequate to detect minor lesions. By using our histopathology generated from this trial, we have been able to compare histology with mpMRI lesions in an attempt to better define these lesions. This objective aims to develop a novel multiplex immunofluorescence staining panel to stain six proteins on one slide, which will then be scanned to determine distinct staining patterns. We would then register each stain to its corresponding mpMRI lesion, which would allow detection of potential subclonal tumor populations that are not visible on mpMRI imaging alone.

Significant Results:

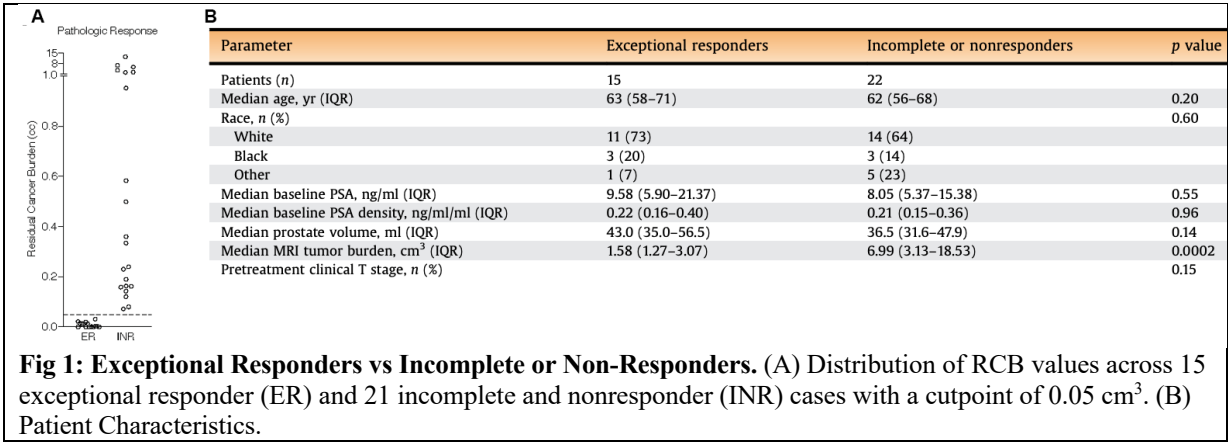
When mapping pre-treatment biopsy to post-treatment residual cancer burden, we identified, using a combination of genomics, histology, and mpMRI imaging, specific lesions that were targeted by biopsies and their response following treatment. This identification allowed us to classify tumors as “Exceptional Responders (ER)” or “Incomplete/Non-Responders (INR),” based on the residual tumor burden. Using this methodology, we identified 62 mpMRI-visible lesions that were sampled by biopsy (up to 4 lesions per patient). 9 of these lesions had no visible tumor, while 5 had too little tumor to be considered for molecular analysis, resulting in 48 lesions subject to histological and genomic analysis. Of the lesions sampled, 25 exhibited exceptional response ($\text{RCB} < 0.05\text{cc}$), while 23 were Incomplete/Non-Responders ($\text{RCB} > 0.05\text{cc}$) (Fig 1a). Importantly, between ER and INR samples, no significant differences were seen in baseline age ($p = 0.20$), baseline median PSA ($p = 0.55$), or baseline median prostate volume ($p = 0.14$). Of baseline characteristics, the only significant baseline measurement was median MRI tumor volume ($p = 0.0002$) (Fig 1b).

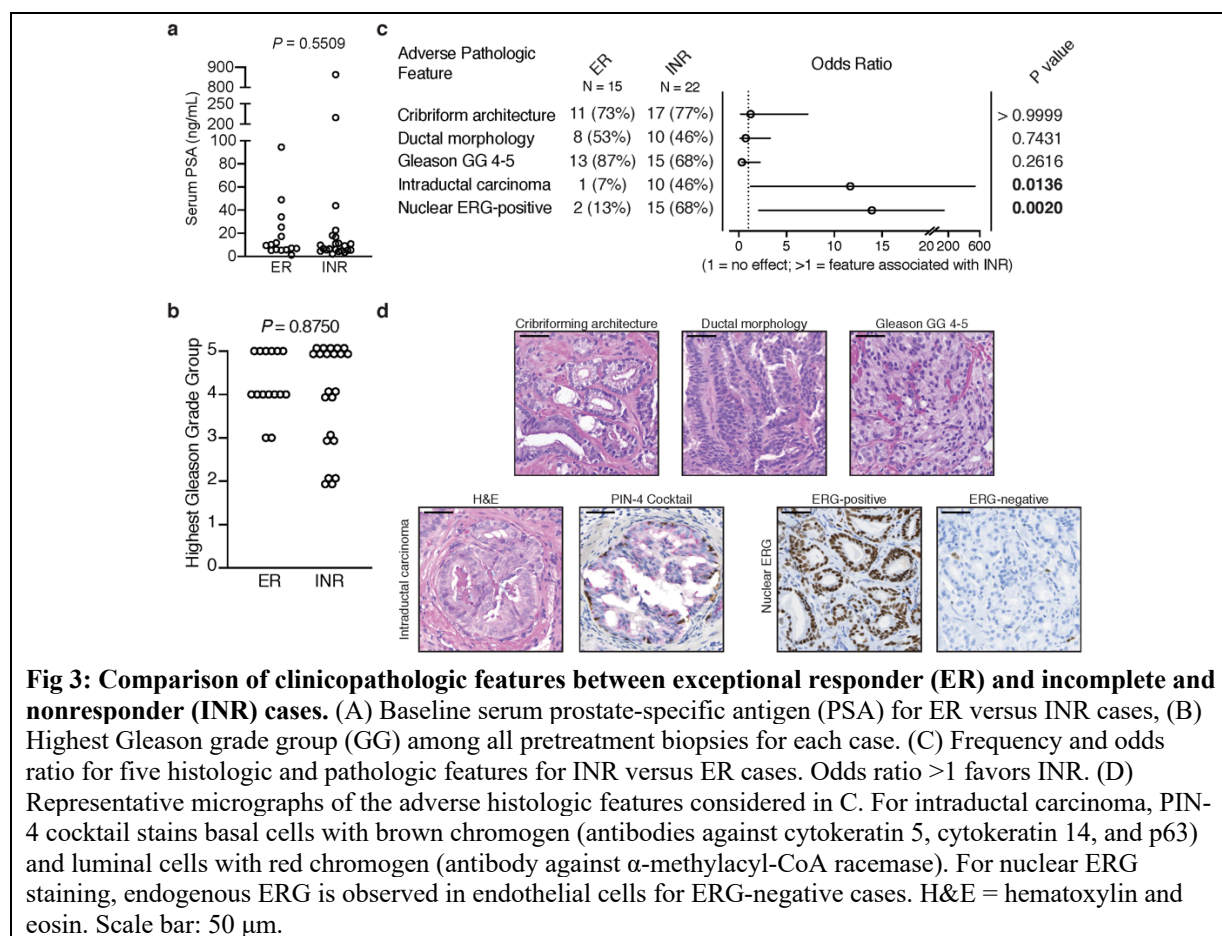
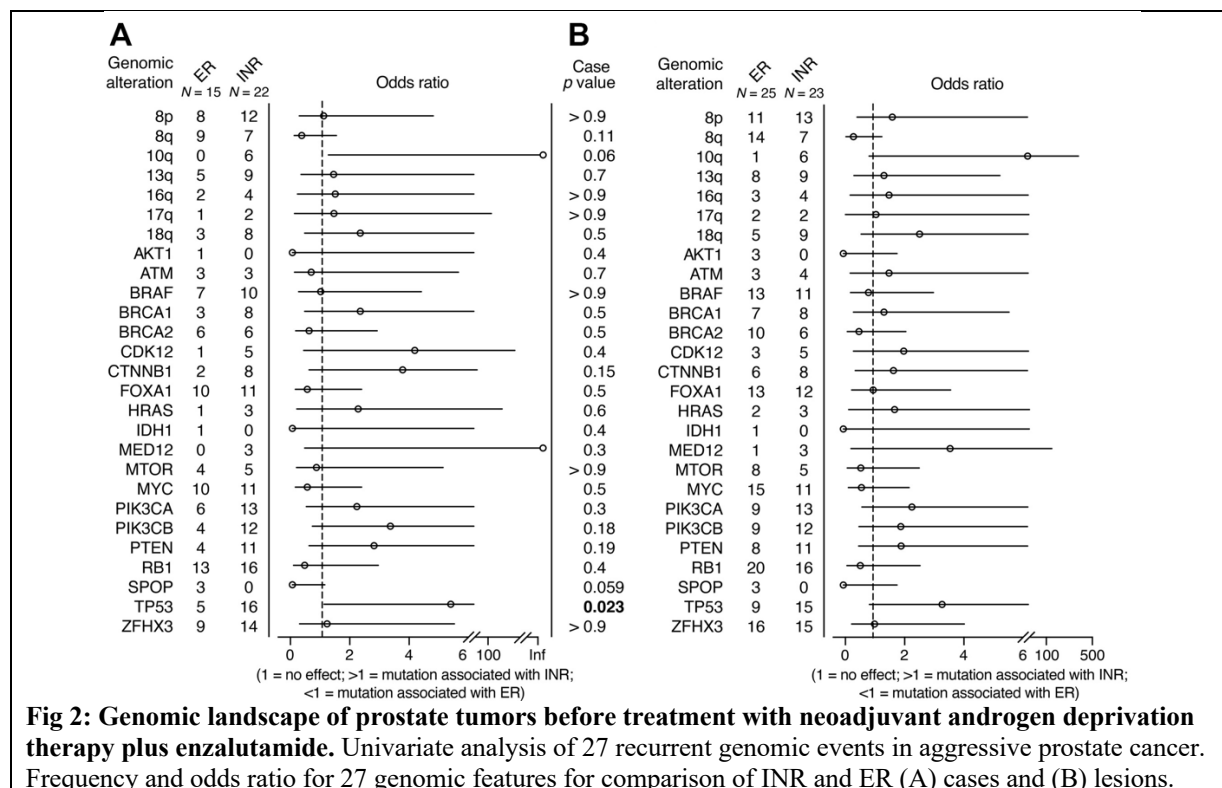
Using a panel of 27 common genomic alterations in prostate cancer, we examined specific characteristics between the exceptional vs incomplete/non-responder patients. At a patient level (using the index lesion for each patient), this panel showed significant alterations in genomic TP53 status between the two cohorts ($p = 0.0233$) (Fig 2a). Although not significantly different between the two cohorts at a patient level, when examining these alterations at the lesion level, loss of chromosome 10q (containing the *PTEN* locus) was significantly altered between the two cohorts ($p = 0.044$) (Fig 2b). Taken together, we see very few genomic characteristics indicative of aggressive prostate cancer significantly altered between the two cohorts at baseline that would predict response to therapy.

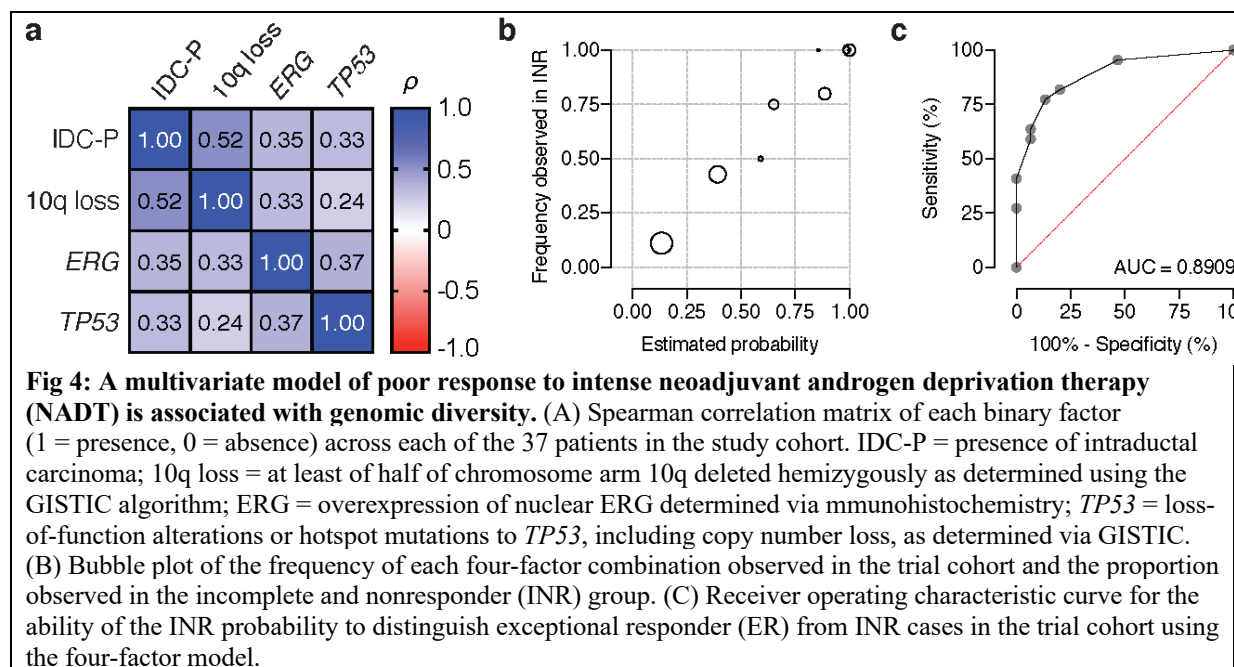
To further examine potential features predictive of therapeutic response, we examined adverse pathologic and histologic features. At baseline, no significant differences in median serum PSA ($p = 0.5509$) or highest Gleason Grade (GG) group ($p = 0.8750$) were identified between the ER and INR cohorts (Fig 3a-b). Additionally, no significant differences were seen in cribriforming architecture, ductal morphology, or Gleason Grade 4-5. However, the presence of intraductal carcinoma of the prostate (IDC) and nuclear ERG were significantly enriched in

incomplete/non-responders ($p = 0.013$ and $p = 0.0020$, respectively) (Fig 3c-d). When combined with the genomic features highlighting *TP53* alterations, these features suggest a combination of genomic and histologic analysis is predictive of response to therapy.

Taken together, these genomic alterations highlight significant differences in tumor response to ADT, which, when combined with histology patterns, was used to develop a 4-factor model to predict response. This model (presence of the *TMPRSS2:ERG*-fusion/nuclear ERG expression, deletion of at least half of chromosome 10q, loss-of-function alterations or hotspot mutations to *TP53*, and the presence of intraductal carcinoma (IDC-P)) allows up to 16 possible combinations, of which 9 were present in our dataset. Each combination is associated with a probability of being an INR case, with an AUC of 0.8909 ($P < 0.0001$), and a cutoff of 60% offered the best discrimination within the test cohort, classifying 30 of 37 cases correctly ($P = 0.0002$) (Fig 4).







What opportunities for training and professional development did the project provide?

How were the results disseminated to communities of interest? *If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project has provided training and professional development activities, as summarized by major tasks:

Training-specific tasks:

Major Task 1: Broaden Knowledge in Prostate Cancer Bioinformatics.

NCI has routine bioinformatics seminars through the Bioinformatics Training and Education Program (BTEP). These courses include topics ranging from Whole Genome Sequencing to single-cell RNAseq and the interpretation/analysis of data. These seminars have continued in a digital format during COVID-19, and have been attended as part of the Training-Specific Tasks (Major Task 1: Broaden Knowledge in Prostate Cancer Bioinformatics).

Major Task 2: Enhance Familiarity with Prostate Cancer Biology, Pathology, Oncology, and Basic Cancer Biology

Before the COVID-19 pandemic, routine weekly seminars on oncology were offered throughout NCI, including Grand Rounds, distinguished invited speakers, departmental invited speakers, and departmental seminars. These have largely shifted to a digital format, but continue in a routine manner. As part of this project, I routinely attend these seminars and our weekly journal club. In addition, I have attended the Prostate Cancer Foundation's Scientific Retreat in 2019 and 2020, as well as GU-ASCO in 2020. As part of these meetings, I presented my research at PCF in 2019 and at GU-ASCO in 2020, and participated in activities geared to "young investigators" including networking sessions, mentorship lectures, and discussions about transitioning to a tenure-track position in academia. Together, these conferences and seminars have increased my knowledge of cancer biology and prostate cancer oncological sciences.

Major Task 3: Development of Key Professional Skills

As part of my attendance at scientific conferences, I have presented my research in poster sessions at PCF in 2019, and GU ASCO in 2020. These presentations allow development of presentation skills, while also encouraging active discussion regarding the interpretation of data and hypotheses leading to future directions. In addition, I routinely attend our departmental seminar series, and presented my research in an hour-long presentation in July 2020.

To further develop skills as a future independent investigator, I have participated in grant writing workshops offered by NCI and the American Society for Cell Biology. I plan to enroll in a formal grant writing workshop offered by NCI in early 2021. I have submitted a K99/R00 application in February 2020, and will submit a resubmission in November 2020. In 2021, I plan to submit a DOD Idea Development award and potentially a K22 application, pending results of the K99/R00 submission.

I continue to develop my professional and leadership skills through active participation in scientific societies. I have maintained an active Associate Membership in AACR since 2015, and submitted an abstract for the AACR Prostate Cancer meeting and AACR regular meeting. AACR Prostate was canceled due to COVID-19, while the abstract submitted to AACR was withdrawn due to concerns of the COVID-19 pandemic. I have also maintained active membership in the American Society for Cell Biology (ASCB) since 2013. I am currently (2019-2021) serving as the co-chair of the student/postdoc leadership committee (COMPASS). I was on the Public Policy Committee from 2016-2019, the Governing Taskforce in 2019, the Nomination Committee in 2020, the Finance Committee in 2020, and am serving on the Honorific Awards Taskforce for 2020-2021. Through my participation in these scientific societies, I am developing leadership skills and networking with leaders in the cell biology community.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Results have been disseminated to communities through presentations at scientific societies and manuscript publications. This research was presented as a poster at the 2019 Prostate Cancer Foundation Retreat, 2019 ASCB, 2020 GU ASCO, and was anticipated to be shared at AACR Prostate Cancer and AACR, prior to their cancelation due to the COVID-19 pandemic.

Scientific publications are also being prepared to disseminate this data to the broader scientific community. Of note, one first-author manuscript was published in *Nature Communications* in 2020 (Wilkinson and Harmon, et al). An additional first-author manuscript was recently published in *European Urology* (Wilkinson et al, 2021). A co-authored manuscript was published reporting the results of the clinical trial at *Clinical Cancer Research*.

What do you plan to do during the next reporting period to accomplish the goals and objectives?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

For Research-Specific tasks during the next reporting period, we will continue to move forward to meet the second major task, “Mapping RP to corresponding biopsy, IHC.” Due to the COVID-19 pandemic, this task was delayed due to capacity limits and NIH shutdown procedures. We will begin to develop the multiplex immunofluorescence assay and work with pathologists and radiologists to correlate protein staining with mpMRI lesions.

For Training-Specific Tasks and Mentoring-Specific Tasks, we will continue to attend seminars related to the goals of the project, present data at national conferences, and attend grant writing workshops in preparation for new career development awards. Meetings with Drs. Sowalsky and Kelly (mentor and co-mentor, respectively) will continue, as well as meeting with other personnel related to the project.

4. IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project? *If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The major impact of the discipline of this project is the enhanced understanding of tumor heterogeneity and diversity, as well as the concept of tumor evolution. Our work highlights that more-evolved tumors correlate with a worse response to neoadjuvant androgen deprivation therapy, which has implications on our understanding of the underlying biology of primary

prostate tumors. By better researching the diversity and evolution of these aggressive localized tumors, we are able to better predict whether patients will respond to therapy.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS:

The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them:

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the COVID-19 pandemic, progress on specific tasks for research-specific tasks was significantly slowed in March 2020. Data analysis on samples collected prior to the project start was continued, allowing us to achieve a majority of the subtasks for Major Task 1 under the research-specific tasks. As we return to the lab, we will begin to resume the remaining research-specific task (Major Task 2: Mapping RP to corresponding biopsy, IHC). The completion of this task requires significant interaction with other experts, so appropriate measures will be taken to ensure the safety of all lab personnel and collaborators.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Nothing to report.

Significant changes in use or care of human subjects:

Nothing to report.

Significant changes in use or care of vertebrate animals:

Nothing to report.

Significant changes in use of biohazards and/or select agents:

Nothing to report.

6. PRODUCTS:

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

Publications, conference papers, and presentations

Report only the major publication(s) resulting from the work under this award.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2020). Multiple primary prostate tumors exhibit divergent responses to intense neoadjuvant androgen deprivation therapy. 2020 AACR Prostate Cancer meeting. Denver, CO.

*Conference was canceled due to COVID-19 pandemic.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2020). Multiple primary tumors with differential drug sensitivity. 2020 GU ASCO Symposium. San Francisco, CA.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2019). Multiple primary tumors with differential drug sensitivity. 2019 ASCB Annual Meeting. Washington, DC.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2019). Integrated radiologic, genomic and pathologic analysis revealed intratumoral divergent responses to neoadjuvant anti-androgen therapy. 26th Annual Prostate Cancer Foundation Scientific Retreat. Carlsbad, CA.

S. Harmon, **S. Wilkinson**, H. Ye, F. Karzai, N. V. Carrabba, R. Atway, N. Terrigino, J. Bright, R. Lake, D. VanderWeele, P. Choyke, P. Pinto, W. L. Dahut, A. G. Sowalsky, B. Turkbey. (2019). Baseline multiparametric MRI characteristics of exceptional pathologic response to neoadjuvant enzalutamide for high-risk, localized prostate cancer. 2019 RSNA Annual Meeting. Chicago, IL.

Journal publications: *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Authors: **S. Wilkinson**, H. Ye, F. Karzai, S. Harmon, N. T. Terrigino, D. VanderWeele, J. Bright, R. Atway, S. Trostel, N. Carrabba, N. Whitlock, S. Walker, R. Lis, H. Sater, B. Capaldo, R. Madan, J. Gulley, G. Chun, M. Merino, P. Pinto, D. Salles, H. Kaur, T. Lotan, D. Venson, P. Choyke, B. Turkbey, W. L. Dahut, A. G. Sowalsky.

Title: Nascent prostate cancer heterogeneity drives evolution and resistance to intense hormonal therapy
Journal: European Urology
Issue: 2021 Mar 27;S0302-2838(21)00207-4. doi: 10.1016/j.eururo.2021.03.009. Online ahead of print.
Acknowledgement of federal support: Yes

Authors: **S. Wilkinson***, S. Harmon*, N. T. Terrigino, K. Karzai, P. A. Pinto, R. A. Madan, D. J. VanderWeele, R. Lake, R. Atway, J. R. Bright, N. V. Carrabba, S. Y. Trostel, R. T. Lis, G. Chun, J. L. Gulley, M. J. Merino, P. L. Choyke, H. Ye, W. L. Dahut, B. Turkbey, A. G. Sowalsky
Title: A case report of multiple primary prostate tumors with differential drug sensitivity
Journal: Nature Communications
Issue: 11, 837. DOI: 10.1038/s41467-020-14657-7.
Acknowledgement of federal support: Yes

Books or other non-periodical, one-time publications:

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers, and presentations:

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk () if presentation produced a manuscript.*

Nothing to report.

Website(s) or other Internet site(s):

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

Technologies or techniques:

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

Inventions, patent applications, and/or licenses:

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

Other Products:

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Scott Wilkinson
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-2613-8425
Nearest person month worked:	12 months
Contribution to Project:	PI, Performed experiments, attended workshops and trainings

Funding Support:	DOD; W81XWH1910712
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Name:	
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Organization Name: UCLA Medical Center

Location of Organization: Los Angeles, California

Partner’s Contribution: Collaboration

8. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

ARTICLE



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OPEN

A case report of multiple primary prostate tumors with differential drug sensitivity

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Localized prostate cancers are genetically variable and frequently multifocal, comprising spatially distinct regions with multiple independently-evolving clones. To date there is no understanding of whether this variability can influence management decisions for patients with prostate tumors. Here, we present a single case from a clinical trial of neoadjuvant intense androgen deprivation therapy. A patient was diagnosed with a large semi-contiguous tumor by imaging, histologically composed of a large Gleason score 9 tumor with an adjacent Gleason score 7 nodule. DNA sequencing demonstrates these are two independent tumors, as only the Gleason 9 tumor harbors single-copy losses of *PTEN* and *TP53*. The *PTEN/TP53*-deficient tumor demonstrates treatment resistance, selecting for subclones with mutations to the remaining copies of *PTEN* and *TP53*, while the Gleason 7 *PTEN*-intact tumor is almost entirely ablated. These findings indicate that spatiogenetic variability is a major confounder for personalized treatment of patients with prostate cancer.

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Localized prostate cancers are uniquely genetically variable and frequently multifocal. Retrospective DNA sequencing studies have demonstrated that 80–90% of prostate cancers comprise multiple spatially separate (distinct) regions, with occasional development of two independent clones within the same tumor^{1,2}. This multifocality is a well-recognized problem for diagnosis, and to date there has been no understanding of whether this variability can influence personalized management decisions. Conventional management of high-risk localized prostate cancer includes radical prostatectomy or external beam radiation therapy with androgen deprivation therapy (ADT)³. The target of ADT is the transcription factor androgen receptor (AR), which mediates terminal differentiation of luminal epithelium in benign prostatic tissue, but drives cell proliferation in prostatic adenocarcinoma. Observations that AR-targeting agents (such as abiraterone and enzalutamide) improve overall and metastasis-free survival in patients with hormone-sensitive and nonmetastatic disease, respectively, suggests that earlier intense inhibition of AR may further improve outcomes^{4–6}. To this end, the effect of introducing intense androgen deprivation therapies in the neoadjuvant setting has been explored in a series of clinical trials at several cancer centers⁷. Although overall survival benefits associated with neoadjuvant intense ADT are yet unknown, complete pathologic responses were reported in ~10% of patients, and minimal residual disease (MRD) has been observed in ~30% of patients⁸. Surgical specimens from ~90% of patients show treatment effect, with substantial residual disease in ~20% of cases, which are likely due to driver mutations present at baseline, such as mutations to *AR* itself⁹, or selection for subclones with losses to tumor suppressors, including *PTEN* and *TP53*¹⁰. Despite evidence demonstrating repeated sampling of multifocal prostate cancer gives rise to discordant scores on prognostic tissue tests, the treatment outcomes of independent prostate cancer foci in a patient with a polyclonal or polytumor phenotype remain unknown¹¹.

Multiparametric magnetic resonance imaging (mpMRI) has become an established imaging technique for diagnosis and staging of localized prostate cancer, especially in detection and sampling of clinically significant disease while decreasing detection of indolent, low-risk tumors^{12–14}. In our study of neoadjuvant ADT plus enzalutamide, we hypothesized that mpMRI prior to surgery would assess radiographic tumor response compared with baseline imaging, while also providing biopsy targets for molecular and histologic examination of pre-treatment foci. Given the high-risk population for whom neoadjuvant intense ADT is suggested to benefit, patients on our study with larger lesions on mpMRI undergo saturation biopsy of imaging targets, to more accurately assess tumor heterogeneity^{15,16}.

In this report, we describe a patient who presented with high-risk prostate cancer that exhibited differential intratumoral response to a clinical trial of neoadjuvant intense ADT. Histologic and genomic analyses reveal the presence of a polytumor, in which one nodule was mostly sensitive to treatment and a separate, clonally independent nodule was resistant. While comprehensive molecular profiling of multiple specimens from a single patient is uncommon for many biomarker-driven therapies, we propose that success of genomically driven trials and treatment strategies may require complete assessment of larger tumors prior to application of precision therapies.

Results

Identification of two primary prostate tumors at baseline. In our phase 2 clinical study of neoadjuvant ADT and enzalutamide, patients with localized intermediate to high-risk prostate cancer undergo mpMRI, which is used for guiding the acquisition of biopsy tissue. Patients then receive intense hormonal therapy

(goserelin plus enzalutamide) for 24 weeks, followed by a second mpMRI and radical prostatectomy (RP). The final results for this trial have yet not been reported.

In a single case from this trial, a 66-year-old patient's pre-treatment mpMRI (see Supplementary Table 1) identified a semi-contiguous tumor that extended from the right apical-mid peripheral zone to the left distal apical peripheral zone, although imaging alone did not provide sufficient evidence of two independent tumors during the initial mpMRI interpretation (Fig. 1a). Due to the extension of tumor across the midline with differential T2-weighted and *b2000* MRI characteristics in the distal apical portion of the prostate (Supplementary Fig. 1a), the left- and right-sided tumor extent were separately sampled by MR/ultrasound (US)-fusion transrectal biopsy (Fig. 1b; Supplementary Fig. 1b). These biopsies showed markedly differing histologies (Fig. 1c). The left-sided tumor (B1) was Grade Group (GG) 3 (Gleason score 4 + 3 = 7) with intermediately to well-differentiated adenocarcinoma. In contrast, the right-sided tumor (B2/B3) was GG5 (Gleason score 4 + 5 = 9) poorly differentiated carcinoma with a component of intraductal carcinoma (IDC-P) (Supplementary Fig. 1c) and perineural invasion.

The TMPRSS2:ERG fusion, which occurs in 40–50% of prostate cancer patients and results in ERG expression, is an early genomic event during tumor development^{17,18}. *PTEN* loss, represented by significant reduction or complete loss of PTEN expression, is another common alteration in prostate cancer pathogenesis and tends to co-occur after an ETS family fusion^{18,19}. We therefore performed anti-ERG and anti-PTEN immunohistochemistry on serial sections of biopsy tissue (Fig. 1c; Supplementary Fig. 1c). The left-sided tumor was ERG-negative with reduced PTEN in a subset of tumor cells (B1), while the right-sided tumor stained positively for ERG and had homogeneous reduction in PTEN expression in both the IDC-P (B2) and invasive (B3) components.

To determine if the genetic basis of reduced PTEN expression in the left- and right-sided tumors was due to the same genomic event, we performed laser capture microdissection (LCM) of tumor foci and adjacent normal tissue, and then performed whole-exome sequencing (WES) to assess somatic copy number alterations (SCNAs) and point mutations. As shown in Supplementary Fig. 2a and Supplementary Table 2, both foci from the right-sided tumor (B2 and B3) showed an arm-level deletion of chromosome 10q encompassing *PTEN* and a focal deletion in chromosome 17p encompassing *TP53*. However, the left-sided tumor (B1) showed no genomic hits to *PTEN* or *TP53* (Supplementary Fig. 2a), suggesting a noncoding or epigenetic driver of reduced PTEN expression. In addition to these mutually exclusive SCNAs, the B2 and B3 foci harbored shared losses of chromosome 16 not found in the B1 focus, and B1 carried a gain of chromosome 7 not detected in B2 or B3 (Supplementary Fig. 2a).

Examination of somatic point mutations resolved by WES revealed that the vast majority were harbored by only a single tumor focus, although nine point mutations were shared by the B2 and B3 foci (Supplementary Table 2). No mutations were shared by all three foci. Given the relatively shallow coverage from WES, we verified that these mutations were also definitive markers distinguishing the B1 and B2/B3 tumors by performing additional focused sequencing to an average depth of ~50,000×. We observed 29 point mutations that demonstrated mutual exclusivity between the left- and right-sided tumor foci (Supplementary Table 2), confirming the clonal independence of these two tumors.

Identification of independent tumors after treatment. Following baseline imaging and biopsy, the patient received 6 months of neoadjuvant intense ADT with goserelin and enzalutamide per

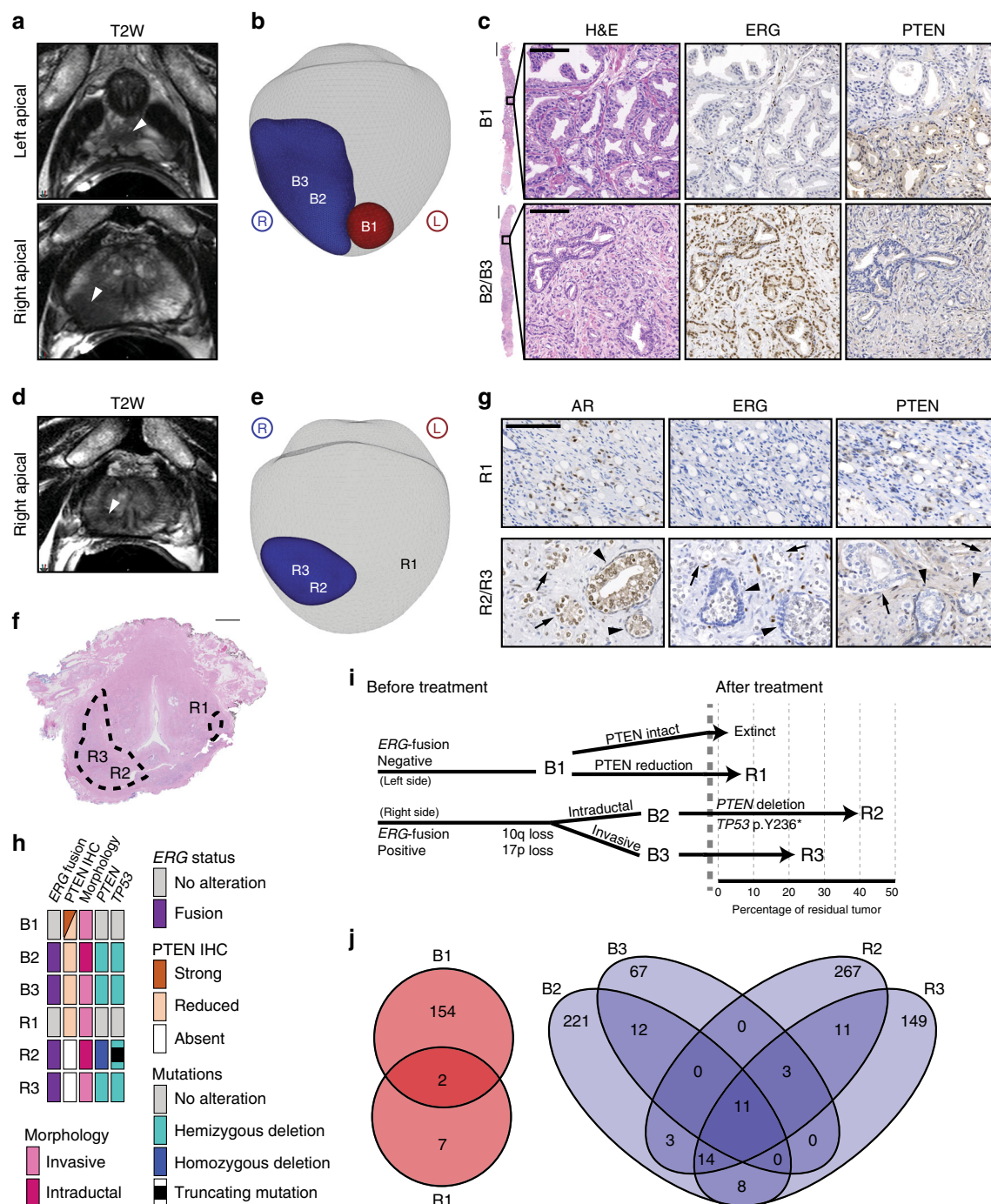


Fig. 1 Molecular evolution of independent prostate tumor nodules following neoadjuvant intense androgen deprivation therapy. **a–g** Pre-treatment (**a–c**) or post-treatment (**d–g**) T2-weighted MRI (**a–d**), volumetric burden (**b–e**), and staining (**c–g**) of a large, semi-contiguous lesion sampled at multiple levels by mpMRI/TRUS fusion-guided biopsy (**c**) or whole-mount pathology (**f, g**) showing the location of biopsied and laser capture microdissected tumor foci. Arrowheads depict lesions on representative slices on MRI (**a, d**) or IDC-P (**g**). Arrows depict invasive carcinoma lesions. Scale bars: whole mount, 5 mm; biopsy, 1000 μ m; insets, 100 μ m. **h** Oncoprint depicting the alterations and histologic phenotypes of the microdissected tumor foci. **i** Phylogenetic tree depicting the relationships between baseline biopsy and prostatectomy tumor foci with respect to the alterations shown in the Oncoprint. Branches were constructed based on shared and unique mutations in each sample. **j** Venn diagram depicting the overlap between tumor foci based on somatic point mutations and large copy number alterations. Source data for the Venn diagram are provided in a source data file. B2/R2 = baseline/residual intraductal carcinoma, and B3/R3 = baseline/residual invasive carcinoma; B2 was intermingled with B3, and R2 was intermingled with R3.

protocol, and then underwent a second mpMRI prior to RP. The post-treatment mpMRI displayed reductions both to total prostate volume (33 cc to 20 cc) and tumor size (2.8–1.7 cm) (Fig. 1d, e). The right-sided tumor exhibited a partial response to therapy and was visible on both MR imaging and microscopic

examination, whereas the left-sided tumor displayed an excellent response and was only detected microscopically (Fig. 1f; Supplementary Fig. 3). Per protocol, the prostatectomy specimen was grossed and sectioned in the same three-dimensional plane as the mpMRI (Supplementary Fig. 3a), and comparison of residual

tumor on the left side revealed that left-sided tumor was likely too hypocellular and scarce to be detected by mpMRI. While diagnostic performance of mpMRI after neoadjuvant treatment is not well-characterized in the literature, mpMRI of untreated prostate cancer is known to have a poor detection rate for clinically insignificant disease^{20,21}.

Because reconstitution of AR nuclear localization and AR activity are common mechanisms of resistance to neoadjuvant intense ADT¹⁰, we examined nuclear AR expression using immunohistochemistry in treatment-resistant tumor foci in whole-mount RP tissues, as well as anti-PTEN and anti-ERG immunohistochemistry (Fig. 1g; Supplementary Fig. 3b). As the right-sided tumor foci (R2 and R3) displayed reduced but positive ERG staining consistent with suppression of AR activity by ADT plus enzalutamide, we confirmed using immunofluorescence that the right-sided tumor was ERG-positive and distinct from the left-sided tumor (R1), which was ERG-negative (Supplementary Fig. 3c). The left-sided tumor, which showed focal PTEN reduction before treatment, displayed homogeneous PTEN reduction in the minimal residual disease, whereas residual tumor on the right side displayed complete loss of PTEN expression in both IDC-P (R2) and invasive tumor (R3) foci.

Taking the same approach as with the biopsy tissue, we performed LCM and WES on residual tumor foci from the right and left sides, sampling tissue from three separate levels (Supplementary Fig. 3a). Residual IDC-P (depicted as R2) on the right side had two copy losses of *PTEN*: the same single-copy loss observed on biopsy plus an additional focal loss at 10q23.31 (Supplementary Fig. 2a, b). The residual invasive tumor (R3) on the right side showed the same single-copy loss of *PTEN*, but no additional loss. Copy number analysis of the residual IDC-P also revealed the same focal loss of chromosome 17p13.1 (Supplementary Table 2) observed on biopsy plus an additional nonsense mutation to *TP53* rendering the IDC-P negative for both *PTEN* and p53. R2 and R3 also harbored the same loss to chromosome 16 detected in the B2 and B3 foci. In contrast, none of these alterations were observed in the residual tumor cells on the left side (R1), which despite being *PTEN*-deficient by IHC did not harbor any detectable SCNAs (Supplementary Fig. 2a).

Evolution of treatment-resistant tumor. In order to visualize the spatial, genomic, and temporal relationships between micro-dissected pre-treatment biopsies and post-treatment residual tumor, we constructed an oncoprint plot and phylogenetic tree (Fig. 1h, i). Using the TMPRSS2:ERG fusion as a putative early driver of tumorigenesis, based on its expression in all right-sided tumor foci (B2, B3, R2, and R3), we modeled that the IDC-P and invasive foci identified in the RP descended from IDC-P and invasive foci sampled on biopsy. Presumably, a small subpopulation of the IDC-P at baseline (B2) also harbored the second loss to *PTEN* and the nonsense mutation to *TP53*, as these were observed in the IDC-P on the right side (R2), which in turn comprised the largest volume of all residual tumor.

Deep focused resequencing of a subset of mutations at ~50,000× coverage confirmed that of 27 point mutations harbored by R2 and/or R3, no somatic mutations were shared by both R1 and R2 and/or R3 (Supplementary Table 4). Moreover, one point mutation detected in R1 was shared by B1, but neither B2 nor B3, and of six SCNAs observed in R1, one was shared by B1 (Fig. 1j). In addition, 21 mutations were confirmed as shared by B2/R2 and B3/R3. Further strengthening the relationship of the IDC-P and invasive post-treatment foci to their pre-treatment counterparts was the observation that when point mutations were observed in either B2 or B3, but both R2 and R3 (Supplementary Table 4), the frequency of the mutation

in the matching histologic tumor was generally greater. Combined with shared and distinct somatic copy number alterations (Supplementary Fig. 2a), these data collectively confirm that the right-sided and left-sided residual tumors were clonally independent and descended from the tumor foci sampled on biopsy (Fig. 1j). The absence of any genomic hits to *PTEN* on the left side suggests alternative mechanisms of suppressing *PTEN* expression, which may include epigenetic silencing. As all three foci of residual tumor displayed reduced or absent *PTEN* expression by IHC (Supplementary Fig. 3b), the left- and right-sided tumors independently underwent phenotypic convergence, selecting for subclones with dysregulated *PTEN*.

Discussion

Here, we describe a patient with high-risk prostate cancer treated by neoadjuvant intense ADT and surgery. Retrospective analysis of imaging and tissue data revealed that there were two potentially lethal-independent cancers present at baseline, and the two tumors responded differently and demonstrated different status for the tumor suppressor genes *PTEN* and *TP53*.

These molecular findings are consistent with large cohort studies of metastatic castration-resistant prostate cancer (mCRPC) previously treated with enzalutamide, in which loss of function of p53 is the most common and enriched alteration relative to untreated primary disease^{22,23}. In contrast, *PTEN* loss was abundant in primary disease and mCRPC, and thus likely contributed to early-stage disease initiation or progression. Treatment of this patient with intense ADT selected for *PTEN*- and p53-null subclones as they represented the majority of residual disease, with cells harboring epigenetically silenced *PTEN* or single-copy losses of *PTEN* and *TP53* displaying less of a growth advantage. Meanwhile, the cells with intact *PTEN* were fully sensitive to intense ADT, as they were not present in the post-treatment specimen.

Multiple lines of histologic and genomic data contributed to the evidence that the left- and right-sided tumors were independent. First, the incidence of the TMPRSS2:ERG fusion is documented to occur early during tumorigenesis, even in pre-cancerous high-grade prostatic intraepithelial neoplasia (PIN), such that it is rarely a subclonal event²⁴. We have previously showed that when low- and high-grade tumor foci were sampled from the same index tumor, they were 100% concordant for ERG expression and when positive harbored the same genomic breakpoints¹⁷. Based on these existing evidence, and morphologic and spatial findings in our study, the left- and right-sided tumors are most likely two juxtaposed independent tumor nodules even though we cannot completely eliminate the possibility that the ERG-positive tumor diverged early from the ERG-negative clone.

Second, our exome sequencing and high-coverage resequencing did not identify somatic variants in common between the left- and right-sided tumor foci, but did confirm the lineage of pre-treatment to post-treatment tumor foci in these tumors. By segmented copy number analysis, the right-sided tumor carried clonal losses to 17p (*TP53*) and 10q (*PTEN*), as well as 16q, which carries multiple putative tumor suppressors²⁵. In contrast, these were absent from all tumor foci on the left side. The left-sided tumor at baseline harbored a subclonal gain to most of chromosome 7, and these chromosome 7 alterations were absent from all right-sided foci. This chromosome 7 gain may have been associated with the *PTEN*-intact portion of that left-sided tumor, which completely responded to treatment, potentially explaining in part why it was not detected in the residual tumor from the left side. A limitation of this analysis was the low cellularity of the left-sided residual tumor, which may explain in part why somatic mutations and copy number alterations were infrequent but still

present, distinguishing the sampled cells from adjacent benign tissue.

Third, the ERG-negative and ERG-positive tumors displayed different behaviors in the context of neoadjuvant intense ADT. We observed that the ERG-negative/*PTEN*-intact subclone was the most sensitive to treatment, and the ERG-positive/*PTEN*-negative IDC-P subclone was the most resistant in this patient. This pattern is consistent with results from a recent trial of neoadjuvant ADT plus abiraterone and enzalutamide, in which IDC-P, ERG positivity, and *PTEN* loss were associated with the highest residual tumor volumes⁷.

Notably, the tumor focus at baseline with IDC-P histology demonstrated the most resistance to therapy, harboring homozygous inactivation of both *TP53* and *PTEN*. Recent reports have also linked prostate ductal and intraductal pathologies to somatic and/or germline alterations affecting the homologous recombination genes *ATM*, *BRCA1*, and *BRCA2*^{26–28}. The patient did not carry somatic or germline alterations to these three genes, although the IDC-P focus at baseline did harbor a deletion to chromosome 11 which encompassed *ATM* (see Supplementary Table 2). As this deletion was not observed in its descendent post-treatment tumor (R2), we conclude that the effects of this alteration on *ATM* expression were not required for the maintenance, resistance or progression of the IDC-P tumor focus.

The previous study of neoadjuvant ADT plus abiraterone and enzalutamide also concluded that no tumor with both ERG positivity and *PTEN* loss responded to the point of MRD⁷. MRD has been defined by either RCB <0.25 cc, or as the greatest dimension of residual tumor <0.5 cm. Using this latter criterion, a meta-analysis of three recent trials demonstrated that MRD was a surrogate for 5-year biochemical recurrence-free survival; 11 out of 11 patients in those trials with MRD ≤ 0.5 cm were recurrence-free with median follow-up of 3.4 years⁸. These patients tended to have lower tumor volumes at baseline, but biopsy GG alone did not predict response. In this study, the response of the left-sided tumor met the criteria for MRD despite both the left- and right-sided tumors harboring predominantly Gleason pattern 4 disease.

Integrating the imaging, histologic, and genomic data available to us, we conclude that this patient harbored a polytumor, defined by two completely independent primary tumor systems in the same organ². Evolutionarily, polyclonal or multiple independent prostate tumors trend toward higher risk, because increased diversity of tumor cells increases fitness and thus may increase aggressiveness and treatment resistance². Evolutionary trajectories that resulted in tumor heterogeneity in this case likely contributed to our observation that two independent prostate tumor nodules with distinct genetic alterations responded differently to neoadjuvant intense ADT.

In previous neoadjuvant clinical trials, it was suggested that multiple clones developed in a subset of higher-risk patients prior to therapy, but the absence of targeted biopsies precluded reconstruction of the evolutionary events leading to heterogeneously resistant tumors^{9,10}. Our analysis of this patient reveals that genomic profiling of a single biopsy would not have predicted the overall response observed following treatment. It is likely that the spatiogenetic variability of localized prostate tumors will continue to be a major confounder for genetically driven trials in localized prostate cancer, and efforts to integrate multiparametric MRI with histopathology to direct genomic studies in the context of neoadjuvant therapies are needed to characterize independent clones at baseline for optimal clinical management.

Methods

Study approval. This clinical and laboratory studies described in this paper were conducted according to the principles of the Declaration of Helsinki. Informed

written consent was received from the study participant prior to inclusion in the study. This clinical study was approved by the National Institutes of Health Institutional Review Board (protocol number 15-c-0124), and was registered with ClinicalTrials.gov (registration number NCT02430480).

Study subject. The patient is a 66-year-old man who presented to the National Institutes of Health Prostate Multidisciplinary Clinic to discuss treatment options for newly diagnosed high-risk prostate cancer (clinical stage T3a). His PSA had risen slowly over the past 8 years (1.65–5.53 ng/ml), and a templated 6-core biopsy with his primary urologist demonstrated Gleason score 9 and Gleason score 7 cancer. Apical and basal biopsy cores showed evidence of perineural invasion, and conventional imaging (CT, bone scan) did not show any evidence of metastatic disease.

mpMRI of the prostate showed a single semi-contiguous lesion encompassing the right apical–mid peripheral zone also affecting the left distal apical peripheral zone with concern for extraprostatic extension (PIRADS score 5/5), and axial T1-weighted MRI of the abdomen showed no nodal disease. Using right apical and left apical MRI targets, MR/ultrasound-fusion-targeted biopsy of the prostate was performed and confirmed the presence of adenocarcinoma in both lobes: Gleason patterns 4 and 5 in the right apex and right mid regions, and Gleason patterns 3 and 4 in the left apex and right apex regions. Treatment options, including RP and EBRT with ADT, were discussed with the patient along with clinical trial options. The patient enrolled in an IRB-approved phase 2 clinical study of neoadjuvant ADT and enzalutamide (ClinicalTrials.gov #NCT02430480).

The patient was administered two injections of goserelin 12 weeks apart with 160 mg enzalutamide orally daily. After 24 weeks of treatment, his total testosterone declined from 193 ng/dl to 26.8 ng/dl, and his PSA declined from 5.53 ng/ml to <0.02 ng/ml. He then received a second multiparametric MRI, which demonstrated substantial reduction in total prostate volume and a decrease in size of the right apical–mid peripheral zone lesion. No disease was noted on the left side, and no extraprostatic extension or nodal disease was observed on MR. A three-dimensional mold was constructed based on the MR, and following robotic-assisted prostatectomy, the prostate was grossed in the mold to create tissue slices in the same plane as the MRI. Pathologic examination revealed extensive residual disease (cellularity corrected volume of residual cancer burden, RCB) of 0.35 cc on the right side corresponding to the MR-visible lesion, with features of intraductal carcinoma. A much smaller, hypocellular region (RCB 0.01 cc) was also observed on the left side. No additional ADT or RT was performed, and 2 years following surgery, PSA levels remain undetectable.

Multiparametric MRI acquisition. MR images were acquired at 3-Tesla (Achieva 3.0T-TX, Phillips Healthcare, Best, Netherlands) using a combination of the anterior half of a 32-channel cardiac SENSE coil (InVivo, Gainesville, FL, USA) and an endorectal coil (BPX-30, Medrad, Pittsburgh, PA, USA) filled with 45 ml of fluorinert (3 M, Maplewood, MN, USA). The mpMRI protocol included acquisition of T2-weighted (T2W) sequences acquired separately in axial, coronal, sagittal plane, two diffusion-weighted imaging (DWI) sequence acquisitions consisting of one 5 b-value acquisition (0–750 s/mm²) for Apparent Diffusion Coefficient (ADC) estimation and one high b-value (2000 s/mm²), and Dynamic Contrast Enhanced (DCE) MRI. DCE MRI images were obtained in 5.6 s intervals following a single dose of gadopentetate dimeglumine 0.1 mmol/kg at 3 ml/s. Axial T1-weighted MR images of the abdomen were also obtained. Full acquisition parameters are listed in Supplementary Table 1.

Multiparametric MRI interpretation. Pre-treatment mpMRI imaging was interpreted by single expert genitourinary radiologist with more than 10 years' experience (B.T.) following the Prostate Imaging–Reporting and Data System version 2 (PI-RADSv2) guidelines. Five distinct regions within the large apical–mid PZ lesion affecting right side of prostate and extending to left side of the prostate in distal component were identified for biopsy targeting. These five targets included: left distal apical PZ, right distal apical PZ, right posterior apical PZ, right anterior apical PZ, and right mid PZ. Post-treatment imaging was interpreted by the same expert radiologist for assessment of residual tumor burden.

MR/ultrasound-fusion biopsy. mpMRI was performed upon study entry and again prior to surgery. Biopsy planning and procedure was completed using a commercially available MRI/TRUS fusion-targeting platform (UroNav, Invivo Corp., Gainesville, Florida). MR/ultrasound-fusion biopsies were targeted to regions identified on the first mpMRI. For each MR target, the urologist (P.P.) acquired a biopsy in the axial and sagittal plane of the patient. The axial and sagittal biopsies of the right-sided tumor were concordant for high-grade cancer, perineural invasion, and intraductal carcinoma. Invasive and intraductal tumor foci were separately isolated, and genomic material was pooled across both biopsies by histology. The axial biopsy of the left-sided tumor-only sampled fibromuscular tissue. The sagittal biopsy of the left-sided tumor was used for this study.

Histology. After sampling, biopsy tissue was placed into formalin and processed into paraffin blocks using standard methods. Following surgery, whole-mount prostate specimens were processed with patient-specific MRI-based 3D-printed

molds for image-pathology correlation²⁹. Briefly, the prostate specimen was serially sectioned within the mold from apex to base at 6-mm intervals corresponding to axial planes on mpMRI images. The mold was placed in formalin overnight, the prostate was sliced for whole mounting, and slices were placed in cassettes for processing into paraffin blocks using standard methods.

Biopsy and prostatectomy tissues were sectioned at 5 µm thickness onto charged slides and stained with hematoxylin and eosin using standard methods. Serial sections of biopsy and RP tissues were stained with anti-AR (Cell Signaling, Cat# 5153S, 1:100 into SignalStain diluent), anti-ERG (Abcam, Cat# ab92513, 1:500 into SignalStain diluent), anti-PTEN (Cell Signaling, Cat# 9188L, 1:100 into SignalStain diluent), and PIN-4 cocktail (Biocare Medical, Cat# PPM225DS, ready-to-use) antibodies. SignalStain diluent was from Cell Signaling. Slides were baked for 15 min at 60 °C, except for PIN-4 staining which was baked overnight at 45 °C, deparaffinized through xylenes and rehydrated through graded alcohols. Antigen retrieval was performed using a NxGen Decloaker (Biocare Medical), for 15 min at 110 °C in Tris-EDTA (Abcam, Cat# ab93684) for PTEN, and for 15 min at 110 °C in Diva Decloaker (Biocare Medical, Cat# DV2004MX) buffers. Sections were blocked with hydrogen peroxide (Sigma Aldrich, Cat# 216763) for 5 min, blocked with Background Sniper (Biocare Medical, Cat# BS966) for 10 min for PIN-4 or VectaStain Elite ABC HRP kit (Vector Laboratories, Cat# PK-6101) for PTEN, AR, and ERG, and incubated with primary antibody for overnight at 4 °C (AR, ERG, PTEN) or 1 h (PIN-4). Secondary labeling was performed using Mach 2 Double Stain for PIN-4 (Biocare Medical, Cat# MRC525) or the VectaStain Elite ABC HRP kit for AR, ERG and PTEN for 30 min. Avidin-biotin complexing was then performed for 30 min for AR, ERG, and PTEN. Colorimetric detection was achieved using DAB Peroxidase HRP (Vector Laboratories, Cat# SK4100) for AR, ERG, and PTEN, or Vulcan Red Fast Chromogen (Biocare Medical, Cat# FR805) and Betazoid DAB (Biocare Medical, Cat# BDB2004) for PIN-4. Counterstaining was performed using Mayer's Hematoxylin Solution (Sigma Aldrich, Cat# MHS16). PIN-4 stained slides were air-dried. AR, ERG, and PTEN-stained slides were dehydrated through graded alcohol and cleared in xylenes. Slides were mounted using Permount (Thermo Fisher).

ERG expression of post-treatment specimens was assessed using Opal Multiplex Immunohistochemistry (Akoya Biosciences, Cat# NEL811001KT). Antigen retrieval was performed using a NxGen Decloaker, for 15 min at 110 °C in Opal AR 9 antigen retrieval buffer (Akoya Biosciences, Cat# AR9001KT). Sections were blocked with 2.5% NGS (ImmPRESS HRP Anti-Rabbit IgG polymer kit, Vector Laboratories, Cat# MP-7451) for 10 min, and incubated with anti-ERG (Abcam, Cat# ab92513) primary antibody at 1:500 for 30 min at room temperature. ImmPRESS HRP anti-rabbit IgG polymer was added for 15 min, then an anti-HERP-conjugated Opal 520 fluorophore (Akoya Biosciences, Cat# FP1487001KT) was applied at 1:500 for 10 min. DAPI (4', 6-diamidino-2-phenylindole, Thermo Fisher, Cat# 1306) was applied at 350 nM for 10 min before slides were mounted using ProLong Glass Antifade Mountant (Thermo Fisher, Cat# P36980).

H&E-, bright-field IHC-, and immunofluorescent IHC-stained slides were scanned using the 20× objective (Plan-Apochromat, NA 0.8) with bright-field or LED fluorescent illumination on an Axio Scan.Z1 (Zeiss) with slide loaders to accommodate standard (25 mm × 75 mm) slides and whole-mount double-wide (50 mm × 75 mm) slides. Immunofluorescent images were uniformly contrast enhanced using ImageJ (NIH). The merged image of immunofluorescence and H&E was generated by removing the background of the fluorescent images in Photoshop CC 2019 (Adobe) and overlaying onto the bright-field image.

Residual cancer burden calculations. Residual tumor was concordantly identified by three board-certified genitourinary pathologists (M.M., R.L., and H.Y.). Residual cancer burden (RCB)³⁰ was measured and calculated by multiplying the number of slices through which each residual tumor extended by the largest cross-sectional width and length and block thickness (0.6 cm). Volume was further corrected by multiplying by 0.4 and the estimated tumor cellularity. Precise length and width measurements were performed on scanned slides using Zen Blue 2012 (Zeiss) with objective/magnification and pixel:distance calibrations recorded within the scanned CZI file. The right-sided lesion extended through four blocks. With a length of 2.09 cm, a width of 1.73 cm, and 10% cellularity, the RCB was 0.35 cc. The left-sided lesion extended through two blocks. With a length of 0.62 cm, a width of 0.35 cm, and 10% cellularity, the RCB was 0.01 cc.

Genomic analysis. Regions of residual tumor identified in whole-mount prostatectomy specimens were registered with post-treatment mpMRI, and co-registered with pre-treatment mpMRI using imaging landmarks for reference. Biopsy paths were superimposed on pre-treatment MR targets to indicate the spatial relationship between biopsy tissue and RP tissue. Serial sections of tumor tissue (and benign regions uninvolved with tumor) were cut onto metal frame PEN-membrane slides (MicroDissect GmbH), stained with Paradise Plus stain (Thermo Fisher), and laser capture microdissected using an ArcturusXT Ti microscope (Thermo Fisher) onto CapSure Macro LCM Caps (Thermo Fisher). Serially stained slides of PIN-4, anti-AR, and anti-ERG immunostaining were used as references.

Using the QIAamp DNA FFPE Tissue Kit (Qiagen), microdissected cells adhered to caps were lysed and DNA was purified according to the manufacturer's instructions. DNA yields were quantified using Picogreen reagent (Thermo Fisher). For exome sequencing, 10–50 ng of DNA were sheared (Covaris), and assembled into

whole-exome libraries using the SureSelect Human All Exon V7 Low Input Exome (Agilent). Samples were sequenced to an average depth of 35× on-target coverage on an Illumina HiSeq4000 at 150 cycles paired-end, eight indexing cycles, and ten molecular barcoding cycles. Pass-filter FASTQ files were trimmed using SureCall Trimmer 4.0.1 (Agilent), aligned with the Burrows Wheeler Aligner BWA-MEM version 0.7.17³¹ to version hg19 of the human genome (b37 with decoy chromosomes), duplicate-marked using LocatIt 4.0.1 (Agilent), and quality score recalibrated using version 4.0.5.2 of the Genome Analysis Toolkit (GATK). Somatic point mutations were called on intervals from the Agilent bait design BED files using MuTect2 (part of the GATK4 package), first by running MuTect2 in tumor-only mode on all BAM files generated by our group using the same Agilent library preparation method from normal (not tumor) FFPE DNA and merging the resultant VCF files using the CreateSomaticPanelOfNormals module of GATK. MuTect2 was run in somatic mode on each tumor BAM paired with the normal BAM from this patient and the somatic panel of normal with af-of-alleles-not-in-resource set to 0.0000025 disable-read-filter set to MateOnSameContigOrNoMappedMateReadFilter. GetPileupSummaries and CalculateContamination were used on each tumor BAM file, and the resultant contamination table was used to filter somatic mutations using FilterMutectCalls. CollectSequencingArtifactMetrics and FilterByOrientationBias were used to further filter mutations for 8-oxoG artifacts using the settings -AM G/T -AM C/T. These pass-filter mutations were then functionally annotated using Oncotator version 1.9.70 (database version April052016). Mutations were then further filtered on a per-sample basis by requiring any locus with a somatic mutation be covered at least half of the mean coverage for the sample and by no fewer than 16 reads and at least 2 unique counts of the alternate allele. Mutations resulting from obvious read bias were removed by running HaplotypeCaller on the reassembled BAM output of MuTect2 in somatic mode and using the directionality of the reads in the strand direction-annotated VCF file. All remaining pass-filter SNVs and indels were then evaluated against the output of MuTect2 from the other samples. For loci where mutations were called confidently in one sample but filtered out, those mutations were backfilled. For loci where no mutations were called, the read depth of the reference allele was backfilled using Pysamstats version 0.24.3 in Python 2.7.

Somatic copy number alterations and aCGH-style plots were generated using Nexus Copy Number version 9 (BioDiscovery). All BAM files generated by our group using the same Agilent library preparation method from normal (not tumor) FFPE DNA were loaded into the Multiscale Reference Builder, specifying the Agilent bait design BED file for selecting regions of the genome to estimate copy number. All default settings were used. After generating the pooled reference, each tumor BAM file was separately loaded with the following analysis settings: systematic correction: quadratic correction; recenter probes: median; analysis: SNP-FASST2 segmentation; significance threshold: 1.0E-6; max contiguous probe spacing (Kbp): 1000.0; percent outliers to remove: 3.0. Because Nexus copy number relies on arbitrary cutoffs for calling gains and losses, we imputed SCNA calls from GATK4 for determining whether deviation from a copy number ratio of 0 was a meaningful alteration. To restrict analysis to only those regions covered by the exome library, the Agilent library design BED file was preprocessed with PreprocessIntervals with bin-length set to 0 and interval-merging-rule set to OVERLAPPING_ONLY. The BED file was also annotated with GC content using AnnotateIntervals. These interval files were used in all GATK4 copy number calling steps. To obtain SCNA calls with GATK4, read counts were first obtained from all BAM files using CollectReadCounts, including from all BAM files generated by our group using the same Agilent library preparation method from normal (not tumor) FFPE DNA. The normal read count files were compiled into a panel of normal using CreateReadCountPanelOfNormals, and the panel of normal was used to smooth read counts across all samples using DenoiseReadCounts. CollectAllelicCounts was also performed on all denoised BAM for identifying regions of potential LOH. ModelSegments used tumor smoothed read counts and the paired normal/tumor allelic counts for generating copy number estimates that were then called using CallCopyRatioSegments.

To determine whether an unbiased assessment of all SNVs and copy number alterations would identify clonal somatic events based on grouped analysis of all six tumor samples, we employed CLONET (version 1.0.0 revision 20171016) with the v2 method for estimating error³². SCNAs jointly called by both Nexus and GATK were reformatted into SEG files, and each tumor BAM file was filtered for informative SNPs using ASEQ version 1.1.1.1. We then parsed the point mutation and SCNA clonality tables for events marked “clonal” or “subclonal” by CLONET, which would indicate that CLONET had identified molecular evidence of a single tumor cell population with expected allelic fractions in interrelated subclones. However, CLONET marked all events as “subclonal.uncertain” or “notanalysed” which occurs when segments and mutations do not fit the assumption of the expected model for identifying subclonal populations³².

Based on pass-filter SNVs and indels identified from exome sequencing, 50 targets were selected randomly from 871 total calls for additional amplicon-based sequencing, with the exception of the TP53 Y236X nonsense alteration, which was subsequently included for verification^{33,34}. Prior to nomination for targeted sequencing, each selected genomic coordinate was inspected in IGV and mutation calls falling in MAPQ < 10 were excluded. Four of the randomly selected mutations were excluded by this vetting process, resulting in 47 total targets. Using parameters to limit the amplicon size to 140 nucleotides, we used the Ion AmpliSeq Designer [<https://www.ampliseq.com>] tool which identified 46 multiplex primer pairs that were compatible with our intended targets in a single pool. All multiplex

primer panel designs were cross-checked using In-Silico PCR [<https://genome.ucsc.edu/cgi-bin/hgPcr>] to prevent production of unintended amplicons for each patient-specific batch of targets. The forward and reverse primer sequences identified above were appended with adaptor sequences³³ at the 5' end, complementary to Illumina Nextera full-length primers (IDT). Dual-barcoded sequencing adaptors were modified³³ to contain longer regions of complementarity and were ordered with NGSO-4 purity from Sigma. Primers were resuspended in 1 × TE buffer (pH 8.0, 100 μM). A designated pre-PCR workstation was used for the first reaction. A mixture of all forward primers was prepared such that each primer was represented at 2 μM concentration. An aliquot of the primer mix (2.5 μL) was combined with uracil N-glycosylase (0.3 U, 2 μL), cDNA (8 μL), 2 × KAPA HiFi HotStart Uracil + ReadyMix (15 μL), and dUTP (3.5 μL, 2.5 μM). Samples were incubated in a thermal cycler at 37 °C for 2 min, 98 °C for 5 min, 65 °C for 2 min, and 72 °C for 7 min. Products from this reaction were cleaned using AMPure XP SPRI beads (54 μL for each 30 μL tagging reaction), following the manufacturer's recommended protocol for mixing, magnetic separation, ethanol washes, and drying. Elution was performed with deionized nuclease-free water (10 μL) to recover the purified tagged product. Purified tagged products (8 μL) were combined with ddATP (2 μL, 0.2 mM), 10 × CoCl₂ (2 μL), 10 × terminal deoxynucleotidyl transferase (Tdt) buffer (2 μL), low TE buffer (4.4 μL), and Tdt enzyme (1.6 μL of 20 U) for a total volume of 20 μL. Samples were incubated for 90 min at 37 °C and 15 min at 75 °C. Samples were held at 4 °C until clean-up, which was performed using the Select-A-Size Clean & Concentrator Kit (Zymo) according to the manufacturer's instructions for a 100 bp one-sided cutoff with the following modifications: the last wash step was centrifuged at maximum speed for 1 min, and elution was performed with 10 μL Zymo elution buffer with a 5 min incubation. 8 μL of purified product were recovered. For the amplification of targets using the reverse primers, a mixture of reverse oligonucleotides was created with 1 μL of each 100 μM primer in a total volume of 100 μL TE, for a final concentration of 1 μM each primer. To 1.25 μL of this primer mix, 3.5 μL of 2.5 μM dUTP, 1 μL of TE, 8 μL of product from the terminal transferase reaction, 1.25 μL of a 2 μM stock of i5 barcoded sequencing adaptor³³, and 15 μL of 2 × KAPA HiFi HotStart Uracil + ReadyMix were added. Samples were incubated in a thermal cycler at 98 °C for 5 min initially and followed by 20 cycles of PCR (98 °C–65 °C–72 °C for 30 s each with 7 min of final extension at 72 °C). Clean-up was performed using the Select-A-Size Clean & Concentrator Kit using the guidelines for a 200 bp one-sided cutoff according to the manufacturer's protocol with the following modifications: the last wash step was centrifuged at maximum speed for 1 min, and elution was performed with 10 μL Zymo elution buffer following a 5 min incubation. In total, 8 μL of purified product were recovered. Final construction of each sequencing library was performed by additional amplification using both the NGSO-4 i5 and i7 sequencing adaptors to prime PCR. All 8 μL of product from the previous step was mixed with 4 μL of 4 μM i7 adaptor stock, 4 μL of 2 μM i5 adaptor stock, 4 μL of TE, and 20 μL of 2 × KAPA HiFi HotStart Uracil + ReadyMix. Samples were incubated in a thermal cycler at 98 °C for 5 min and followed by 10–30 cycles of PCR (98 °C–72 °C for 30 s each with 7 min of final extension at 72 °C). The exact number of cycles of PCR to use were determined empirically by substituting control FFPE genomic DNA in the first step of the library preparation protocol, substituting 4 μL of 6 × SYBR green in the final PCR step above, and performing qPCR on a QuantStudio 3 (Thermo Fisher). The number of cycles was determined by cycle threshold value corresponding to the ½-maximum of amplification saturation on a linear curve. Clean-up was performed using the Select-A-Size Clean & Concentrator Kit with a 200 bp one-sided cutoff as described above, but with a 20 μL elution. Quality control was performed on the completed library using D1000 ScreenTapes on a TapeStation looking for a peak size between 250 bp and 350 bp with primer dimer comprising <10% of the library. The library was then quantified for functional concentration using the KAPA Illumina Quantification Kit for NGS, using a 1:50,000 dilution of the library per the manufacturer's protocol. The library was adjusted to a final concentration of 10 μM.

The amplicon library was sequenced on a MiSeq (Illumina) instrument with the v2 Reagent Kit (500-cycle). 20% Phi-X was added to the final library pool. Sequencing was performed with 195 cycles paired-end and dual 8-cycle indexing reads on the P5 and P7 adaptors. This sequencing strategy intentionally sequenced through the entire insert molecule and into the adaptor on the other side, reading the i5 and i7 indexing adaptor an additional time. Individually barcoded libraries were recovered and filtered for quality using bcl2fastq (Illumina). Pass-filter FASTQ files were trimmed with Trimmomatic version 0.36, preserving the trimmed-off sequences and parsing them for the expected i5 and i7 adaptor based on bcl2fastq binning. Comparing each library to the expected adaptor pair, read pairs corresponding to the incorrect adaptors were discarded. Remaining read pairs in FASTQ files were aligned to b37-decoy using BWA-MEM version 0.7.10. BAM files were sorted and indexed using samtools version 1.9, and unique base calls were identified by running using Pysamstats version 0.24.3 in Python 2.7 over the defined intervals for each target, and all calls were manually inspected using IGV version 2.4.19.

Of the 46 targets, 1 target was in a region of poor complexity and was removed from future consideration. One target failed to amplify in all samples, while five targets preferentially demonstrated bias toward the alternate allele in the normal sample and were also removed. Based on counts of the alternate allele determined a priori from exome sequencing, a sample was determined to harbor the mutation in

question if (1) the frequency of the mutation in the tumor sample was three times greater than in the benign reference; (2) if the alternate absolute allele read count was greater than 50; and (3) if the read count for the base in question was at least three times greater than the average alternate allele for the remainder of the locus (i.e., above the background error rate of library preparation and sequencing).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

For the protection and privacy of the human subject in this study, the raw whole-exome and targeted sequencing data have been deposited in NCBI Database of Genotypes and Phenotypes (dbGaP), a controlled-access database, under the accession ID [phs001938.v1.p1](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150193). All the other data supporting the findings of this study are available within the article and its supplementary information files, and from the corresponding author upon reasonable request. A reporting summary for this article is available as a Supplementary Information file. The source data underlying Fig. 1j, Supplementary Table 3, and Supplementary Table 4 are provided as a Source Data file.

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Author contributions

S.W., S.H., H.Y., B.T., and A.G.S. designed the research study. P.A.P., P.L.C., and W.L.D. designed the clinical study. F.K., P.A.P., R.A.M., D.J.V., G.C., J.L.G., M.J.M., and B.T. were involved in patient care and acquired patient data. S.W., D.J.V., R.T.L., R.L., R.A., J.R.B., N.V.C., S.Y.T., M.J.M., H.Y., and A.G.S. performed histopathological analyses. S.H. and B.T. performed imaging analyses. S.W., N.T.T., S.Y.T., and A.G.S. performed genomics analyses. S.W., S.H., D.J.V., H.Y., R.T.L., B.T., and A.G.S. interpreted results. S.W., S.H., and A.G.S. prepared figures. S.W., S.H., N.V.C., H.Y., and A.G.S. drafted the paper. All authors approved the final paper.

Competing interests

H.Y. and R.T.L. perform consulting in an advisory role for Janssen Pharmaceuticals. The remaining authors declare no competing interests.

Additional information

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Prostate Cancer

Nascent Prostate Cancer Heterogeneity Drives Evolution and Resistance to Intense Hormonal Therapy

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Abstract

Background: Patients diagnosed with high risk localized prostate cancer have variable outcomes following surgery. Trials of intense neoadjuvant androgen deprivation therapy (NADT) have shown lower rates of recurrence among patients with minimal residual disease after treatment. The molecular features that distinguish exceptional responders from poor responders are not known.

Objective: To identify genomic and histologic features associated with treatment resistance at baseline.

Design, setting, and participants: Targeted biopsies were obtained from 37 men with intermediate- to high-risk prostate cancer before receiving 6 mo of ADT plus enzalutamide. Biopsy tissues were used for whole-exome sequencing and immunohistochemistry (IHC).

Outcome measurements and statistical analysis: We assessed the relationship of molecular features with final pathologic response using a cutpoint of 0.05 cm³ for residual cancer burden to compare exceptional responders to incomplete and nonresponders. We assessed intratumoral heterogeneity at the tissue and genomic level, and compared the volume of residual disease to the Shannon diversity index for each tumor. We generated multivariate models of resistance based on three molecular features and one histologic feature, with and without multiparametric magnetic resonance imaging estimates of baseline tumor volume.

Results and limitations: Loss of chromosome 10q (containing *PTEN*) and alterations to *TP53* were predictive of poor response, as were the expression of nuclear ERG on IHC and the presence of intraductal carcinoma of the prostate. Patients with incompletely and nonresponding tumors harbored greater tumor diversity as estimated via phylogenetic

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tree reconstruction from DNA sequencing and analysis of IHC staining. Our four-factor binary model (area under the receiver operating characteristic curve [AUC] 0.89) to predict poor response correlated with greater diversity in our cohort and a validation cohort of 57 Gleason score 8–10 prostate cancers from The Cancer Genome Atlas. When baseline tumor volume was added to the model, it distinguished poor response to NADT with an AUC of 0.98. Prospective use of this model requires further retrospective validation with biopsies from additional trials.

Conclusions: A subset of prostate cancers exhibit greater histologic and genomic diversity at the time of diagnosis, and these localized tumors have greater fitness to resist therapy.

Patient summary: Some prostate cancer tumors do not respond well to a hormonal treatment called androgen deprivation therapy (ADT). We used tumor volume and four other parameters to develop a model to identify tumors that will not respond well to ADT. Treatments other than ADT should be considered for these patients.

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1. Introduction

The vast majority of prostate cancers are detected and treated early while potentially curable by surgery or radiation [1]. By contrast, localized prostate tumors that progress despite definitive therapy are characterized by extensive evolutionary heterogeneity at the time of initial treatment and later at recurrence, and therapy resistance in metastatic castration-resistant prostate cancer (mCRPC) can be associated with multiple subclones that emerged from a common ancestor [2,3]. Numerous studies have identified subsets of human tumors, including prostate cancers, with a higher risk of recurrence because of greater intratumoral heterogeneity, and evidence suggests that active mutational processes are more likely to give rise to a daughter clone with resistance in more evolutionarily complex cancer systems [4–6]. For patients with prostate cancer, who may go for more than a decade between initial therapy and development of radiographically confirmed metastases, intervening chemohormonal treatments and their effects on tumor progression are based on biochemical recurrence with rare opportunities for sampling tumors by biopsy until later in the disease course. Consequently, little is known about the relationship between the evolutionary trajectory of treatment-naïve localized prostate tumors and their propensity to resist direct treatment.

The androgen receptor (AR) remains a molecular target for prostate cancer therapy via inhibition of the release of testicular androgen (androgen deprivation therapy, ADT) and newer-generation inhibitors including abiraterone and enzalutamide. These agents are the standard of care for treatment of mCRPC, but recent clinical trials have demonstrated their efficacy in nonmetastatic CRPC as well as in de novo metastatic hormone-sensitive prostate cancer [7–10]. Although the rationale for applying these drugs earlier in the disease course is that AR activity is critical for progression, it is logical that inhibition of AR activity can ablate organ-confined disease or prevent it from spreading.

Neoadjuvant ADT (NADT) and, more recently, intense NADT (ie, ADT plus one or more AR-targeted agents) has been tested in a series of phase 2 clinical trials in efforts to

decrease intratumoral testosterone levels and diminish the volume of residual disease before surgery [11–13]. Analyses of these cohorts have demonstrated that greater baseline tumor volume is a significant predictor of greater final tumor volume [14]. A recent meta-analysis of several studies also indicated that patients with less residual disease at the end of treatment experience longer durations of biochemical recurrence-free survival [15]. With pathologic complete response rates varying across cohorts, analyses of post-treatment surgical specimens have indicated that molecular alterations driving resistance are subclonal and are present before treatment, pointing to a relationship between divergence from the truncal clone for a given tumor and greater fitness associated with subclonal heterogeneity in a hormone-sensitive state [16,17].

We thus hypothesized that evolutionary principles relating diversity and heterogeneity to clinical outcome could explain in part why a heterogeneous cohort of high-risk tumors at baseline could result in diverse resistance mechanisms after treatment. We present an integrated histologic and genomic analysis of 141 tumor exomes sampled via laser capture microdissection and representing 110 baseline biopsy tissues from 37 patients with intermediate- to high-risk prostate cancer who were enrolled in a single-arm phase 2 clinical trial of NADT intensified with enzalutamide [14]. Using both tissue- and DNA-based analyses, we observed that the volume of residual disease at the end of therapy increases proportionally with tumoral diversity at baseline. Localized prostate cancers with more complex evolutionary trajectories are thus more likely to be resistant to intense systemic hormonal therapy and have a lower chance of relapse-free survival. To predict the likelihood of nonresponse, we demonstrate that a straight-forward four-factor histogenomic model recapitulates the relationship between nonresponse and greater baseline subclonal diversity. Baseline tumor volumes estimated via multiparametric magnetic resonance imaging (mpMRI) are complementary to diversity measures and further improve prediction. Thus, our results indicate that the greater fitness of more diverse tumors contributes, at least in part, to treatment resistance in high-risk localized prostate cancer.

2. Patients and methods

2.1. Biospecimen procurement

All patients provided informed consent to join National Cancer Institute study 15-c-0124 (NCT02430480), approved by the National Institutes of Health Institutional Review Board in accordance with the Declaration of Helsinki [14].

Patients underwent baseline mpMRI, and MRI/ultrasound fusion biopsies were targeted to one or more mpMRI lesions. A 12-core template biopsy was also obtained in a subset of patients. A sample of saliva (Oragene OG-500; DNA Genotek, Ottawa, Canada) or blood, from which genomic DNA was further derived, was also collected before treatment initiation. Following 6 mo of ADT plus enzalutamide, patients underwent a second mpMRI before radical prostatectomy (RP).

2.2. Sample processing

Serial sections (5 μ m) of formalin-fixed, paraffin-embedded biopsy tissues were stained with hematoxylin and eosin, stained with antibodies via immunohistochemistry (IHC), or cut onto polyethylene naphthalate membrane slides (MicroDissect GmbH, Herborn, Germany) for laser capture microdissection (LCM). LCM was performed to sample 141 histologically distinct tumor foci from 110 biopsies (median of four foci per patient), plus regions of benign tissue. DNA was extracted, assembled into libraries for whole-exome sequencing, and analyzed for detection of somatic mutations and copy number alterations. IHC was performed with an IP FLX autostainer (Biocare Medical, Pacheco, CA, USA) using AR, prostate-specific antigen (PSA), glucocorticoid receptor (GR), ERG, PTEN, synaptophysin, PIN-4, Ki-67, NKX-3.1, CAM5.2, p53, and p63 antibodies. The Supplementary material provides complete descriptions of the sampling and processing steps.

2.3. Statistical analysis

Statistical analyses were performed with GraphPad Prism version 8 (GraphPad Software Inc., San Diego, CA, USA), Microsoft Excel for Mac version 16 (Microsoft Corporation, Redmond, WA, USA), and SAS/STAT software version 14.1 (SAS Institute, Cary, NC, USA). Null hypothesis tests of associations (enrichments) between response and individual dichotomous factors were performed using a two-sided Fisher's exact test at a per-patient and a per-lesion level. Estimated odds ratios were calculated with 95% confidence intervals (CIs) derived from inverted one-sided exact tests. Associations between factors were measured using Pearson or Spearman correlations. Comparisons of single factors between exceptional responder (ER) and incomplete and nonresponder (INR) cases were performed using Student or Welch's *t* tests or the Wilcoxon rank sum test. Comparisons of covariates within single factors between ER and INR cases were performed using the Cochran-Armitage test for trend. The four features used in the histogenomic model were selected according to the top performing features from genomic and histologic univariate analyses with odds ratios favoring INR. Logistic regression models including the four factors were fitted to the data. Estimated probabilities were compared to the observed response proportions for the factor combinations. The accuracy of the dichotomized estimated probabilities was examined using receiver operating characteristic curves. Response probabilities were estimated as the same function of the factor values in an independent validation population. Genomic Shannon diversity index (SDI) scores were tested for Pearson correlations with these probabilities. Owing to the small cohort size, corrections for multiple comparisons were not performed.

3. Results

3.1. Genomic alterations consistent with high-risk prostate cancer

We analyzed a spectrum of somatic copy-number alterations and gene mutations frequently reported for human tumors in 141 LCM foci from baseline biopsies using whole-exome sequencing (Supplementary Fig. 1 and Supplementary Table 1). Germline alterations to cancer-related genes were detected via whole-genome sequencing of DNA from saliva or buffy coats (Supplementary material). These 141 foci, based on different histologies or biopsy blocks, were sampled from 48 distinct MRI-contoured lesions (Supplementary Fig. 2 and Supplementary Table 2) from all 37 patients who completed our clinical study. There was variable genomic heterogeneity between foci from the same MRI-defined lesion and between lesions in the same patient (Supplementary Figs. 1 and 2). We curated 28 somatic alterations for deeper analysis on the basis of their frequent reporting in large prostate cancer cohort studies [18–21]. When collapsed to single data points for each patient (Fig. 1A and Supplementary Table 3) on the basis of the index MRI lesion, the landscape of these alterations was not significantly different from that for 188 high-risk, Gleason pattern 4 and 5 tumors from The Cancer Genome Atlas (TCGA) PRAD [19] data set (Supplementary Fig. 3A and Supplementary Table 4). Individually, 27 of these features did not significantly differ (χ^2 test) between our clinical trial and the TCGA cohort (Supplementary Fig. 3B; $p=0.098$, Wilcoxon matched-pairs test), validating the frequency of these alterations overall.

Following treatment, we used RP specimens to assess local responses to therapy by calculating the residual cancer burden (RCB), a validated intermediate endpoint for systemic therapy that has clinical significance for predicting recurrence after surgery [13,15]. On the basis of minimal residual disease rates in prior neoadjuvant studies, we preselected 0.05 cm³ as the cutpoint (Fig. 1B) between ER and INR cases at the patient level, resulting in 15 ER and 22 INR patients. One patient whose tumor expanded during therapy underwent transurethral resection of prostate tumor instead of RP and thus RCB could not be calculated. Within the INR group, registration of pretreatment biopsies and imaging to final MRI and pathology identified 12 additional biopsy-confirmed lesions of which ten exhibited an exceptional pathologic response, for a total of 25 ER and 24 INR MRI-visible lesions (23 INR lesions with genomic data).

We then compared the frequency of 27 recurrent genomic events between the two groups in univariate analysis (Fig. 1C). Arm-level loss (encompassing $\geq 50\%$) for chromosome 10q was exclusive to the INR group and hotspot mutations to *SPOP* were only observed in the ER group. Somatic copy-number losses, frameshifts, and hotspot mutations to *TP53* were significantly enriched in the INR group ($p=0.023$, Fisher's exact test) at baseline (Supplementary material). Similar trends were also observed at the lesion level (Fig. 1D) and loss of chromosome

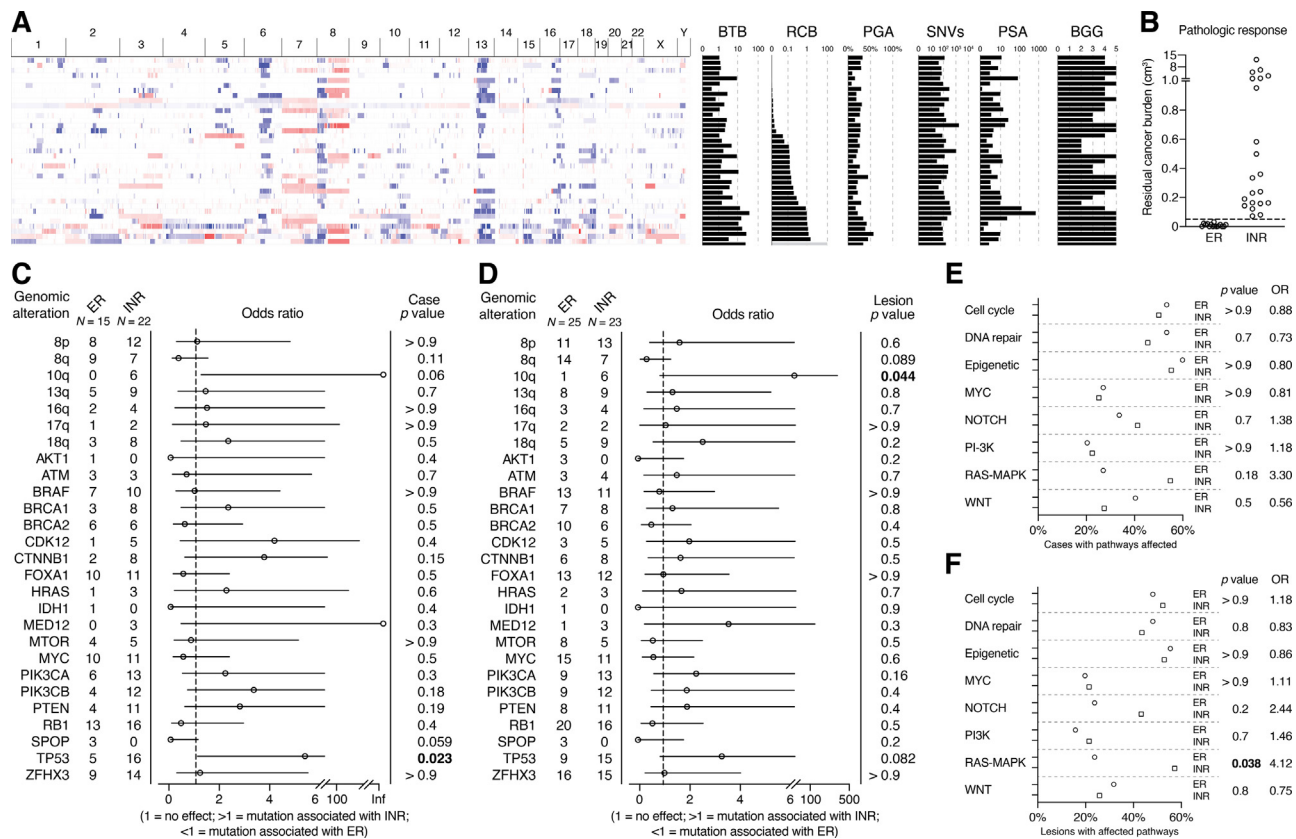


Fig. 1 – Genomic landscape of prostate tumors before treatment with neoadjuvant androgen deprivation therapy plus enzalutamide. (A) Genomic and clinical data per patient ($n=37$). One patient classified clinically as a nonresponder underwent transurethral resection of prostate tumor instead of radical prostatectomy so RCB could not be calculated. BTB=baseline tumor burden (cm^3); RCB=residual cancer burden (cm^3); PGA=percent genome altered; SNVs=small nucleotide variants; PSA=prostate-specific antigen (ng/ml); BGG=highest biopsy Gleason group. **(B)** Distribution of RCB values across 15 exceptional responder (ER) and 21 incomplete and nonresponder (INR) cases with a cutpoint of 0.05 cm^3 . **(C,D)** Frequency and odds ratio for 27 genomic features for comparison of INR and ER **(C)** cases and **(D)** lesions. **(E,F)** Grouping of altered genes into eight pathways, with the proportion of affected **(E)** cases and **(F)** lesions with genes altered per pathway. For C–F, each factor was considered independently in a two-sided Fisher's exact test, and the effect size is given as an odds ratio (OR). ORs >1 favor INR. For C–F, these are exploratory analyses that are underpowered for per-gene analyses, and thus may not be replicable in other cohorts when adjustments for multiple testing are applied.

10q was significantly associated with poor response to NADT ($p = 0.044$). Although the significance levels for these alterations may not be conclusive because of multiple testing, further exploration is warranted for these top genomic features associated with poor response.

After grouping gene alterations into pathways [22], we found that a greater proportion of INR tumors accumulated somatic alterations to RAS/RAF/MAPK pathway genes, which was statistically significant at the lesion level (Fig. 1E,F). Germline frameshift mutations to DNA homologous repair genes (*BRCA1*, *BRCA2*, and *PALB2*) were observed in an ER case and two INR cases (Supplementary Table 3), but neither group was significantly enriched for alterations in DNA damage repair genes, consistent with similar mutation-type proportions and signatures between ER and INR patients (Supplementary Fig. 4A–D). Thus, both ER and INR tumors demonstrated features of high-risk tumors and significantly altered genomes, but there were relatively few differences between the groups.

3.2. Pathologic and histologic features distinguish ER from INR tumors

With a broad range of pathologic responses across the cohort, we explored whether clinicopathologic parameters can explain, at least in part, differences in treatment response. Although INR patient tumor burdens generally were greater at baseline (Table 1, Fig. 2A, and Supplementary Fig. 5A), there was no difference between ER and INR patients in baseline serum PSA levels (Fig. 2B), highest Gleason grade group for pretreatment biopsies per patient (Fig. 2C) and per mpMRI-visible lesion (Supplementary Fig. 5B), or the number of mpMRI-visible lesions (Supplementary Fig. 5C). On univariate analysis, the proportion of cases or lesions harboring cribriform architecture, ductal morphology, or Gleason pattern 4 or 5 did not distinguish ER from INR, but nuclear ERG and intraductal carcinoma (IDC-P) were significantly enriched in INR cases (Fig. 2D,E and Supplementary Fig. 5D; $p=0.0020$ and $p=0.013$, respectively).

Table 1 – Patient characteristics

Parameter	Exceptional responders	Incomplete or nonresponders	p value
Patients (n)	15	22	
Median age, yr (IQR)	63 (58–71)	62 (56–68)	0.20
Race, n (%)			0.60
White	11 (73)	14 (64)	
Black	3 (20)	3 (14)	
Other	1 (7)	5 (23)	
Median baseline PSA, ng/ml (IQR)	9.58 (5.90–21.37)	8.05 (5.37–15.38)	0.55
Median baseline PSA density, ng/ml/ml (IQR)	0.22 (0.16–0.40)	0.21 (0.15–0.36)	0.96
Median prostate volume, ml (IQR)	43.0 (35.0–56.5)	36.5 (31.6–47.9)	0.14
Median MRI tumor burden, cm ³ (IQR)	1.58 (1.27–3.07)	6.99 (3.13–18.53)	0.0002
Pretreatment clinical T stage, n (%)			0.15
cT1	2 (13)	2 (9)	
cT2	5 (33)	1 (5)	
cT3a	5 (33)	10 (45)	
cT3b	1 (7)	6 (27)	
cT4	2 (13)	3 (14)	
Pretreatment cN1 stage, n (%)	3 (20)	8 (36)	0.47
Biopsy ISUP grade group, n (%)			0.52
2	0 (0)	4 (18)	
3	2 (13)	3 (14)	
4	7 (47)	4 (18)	
5	6 (40)	11 (50)	

IQR = interquartile range; ISUP = International Society of Urological Pathology; MRI = magnetic resonance imaging; PSA = prostate-specific antigen.

As suggested by the frequency of *PTEN* genomic alterations (Fig. 1C), reductions in *PTEN* on IHC staining were enriched in INR cases (Fig. 3A; $p=0.0015$, Fisher's exact test). We also observed intratumoral heterogeneity in the co-occurrence of *PTEN*-intact and -reduced, or ERG-positive and -negative, tumors. Most cases (Fig. 3B) or lesions (Fig. 3C) were uniformly positive or negative for both *PTEN* and ERG (score of 0), but the frequency of tumors with either heterogeneous *PTEN* or ERG staining (score of 1) or both *PTEN* and ERG heterogeneous staining (score of 2) was greater in the INR group (Fig. 3D; $p=0.0059$ and $p=0.0068$, respectively, Cochran-Armitage test for trend).

Because *TMPRSS2-ERG* fusion and *PTEN* loss tend to occur early during prostate tumorigenesis [23], our observation of their heterogeneity enriched among INR tumors at baseline raised the possibility of increased treatment resistance being due to a greater number of tumor subclones. As these subclones may display a wider array of phenotypes apparent in situ, we extended our histologic analyses to five additional immunostains of pretreatment biopsy tissues: AR, PSA, GR, synaptophysin (SYP), and Ki-67. Automated staining within the linear range of colorimetric development for each antibody, coupled with machine-learning-based image analysis, resolved up to four subpopulations of tumor cells on the basis of staining intensity, which in turn was used to derive weighted histology intensity (HI) scores and histology heterogeneity measures based on the SDI (Fig. 3E). All stains demonstrated significant associations between HI and SDI (Fig. 3F), indicating staining homogeneity in areas of high (AR, PSA) or low (Ki-67, SYP, GR) intensity. The HIs for PSA were negatively correlated with RCB (Fig. 3G) and SDI (Fig. 3H), but PSA SDI and RCB were positively correlated (Fig. 3I), indicating that INR cases displayed more PSA heterogeneity as HI decreased proportional to RCB (Fig. 3J). Thus, ER cases

were more uniformly strong for PSA staining, with lower SDI at lower RCB. Unlike PSA, the HI for GR was positively correlated with RCB (Fig. 3K) and SDI (Fig. 3L), yet diversity was still correlated with RCB (Fig. 3M). The heterogeneity of these markers in a subset of tumors at baseline may represent subpopulations less similar to the truncal clone, and reflecting future treatment resistance.

3.3. Resistance to neoadjuvant enzalutamide is associated with greater genomic diversity at baseline

We observed greater within-patient similarities when examining the whole-genome somatic copy-number alteration (SCNA) data for individual foci from ER than from INR cases (Supplementary Fig. 1). Across a defined set of 69 amplifications and 111 deletions, correlation matrices revealed greater coefficients for patients with lower residual cancer burdens (Fig. 4A), irrespective of foci number. Across all cases with two or more foci, there was a statistically significant negative correlation ($\rho=-0.43$, 95% CI -0.68 to -0.086 ; $p=0.014$) between the median correlation coefficient for each case and its RCB (Fig. 4B) showing more intratumoral homogeneity in ERs. Although this could reflect variable tumor purity in the INR group, heterogeneity could also reflect increasingly divergent subclones within the tumor systems. We therefore considered all callable, high-confidence small nucleotide variants (SNVs) and boundary-harmonized SCNAs from every LCM focus to identify tumor subpopulations via bioinformatic reconstruction of tumor phylogenies (Fig. 4C). We observed two general types of clone tree structures (Fig. 4D): (1) a linear evolution path with few subclones inferred from nodes; and (2) a branched phylogenetic tree with multiple subclones. A depiction of each tree and supporting SNV/SCNA assignments is provided in Supplementary Figure 6.

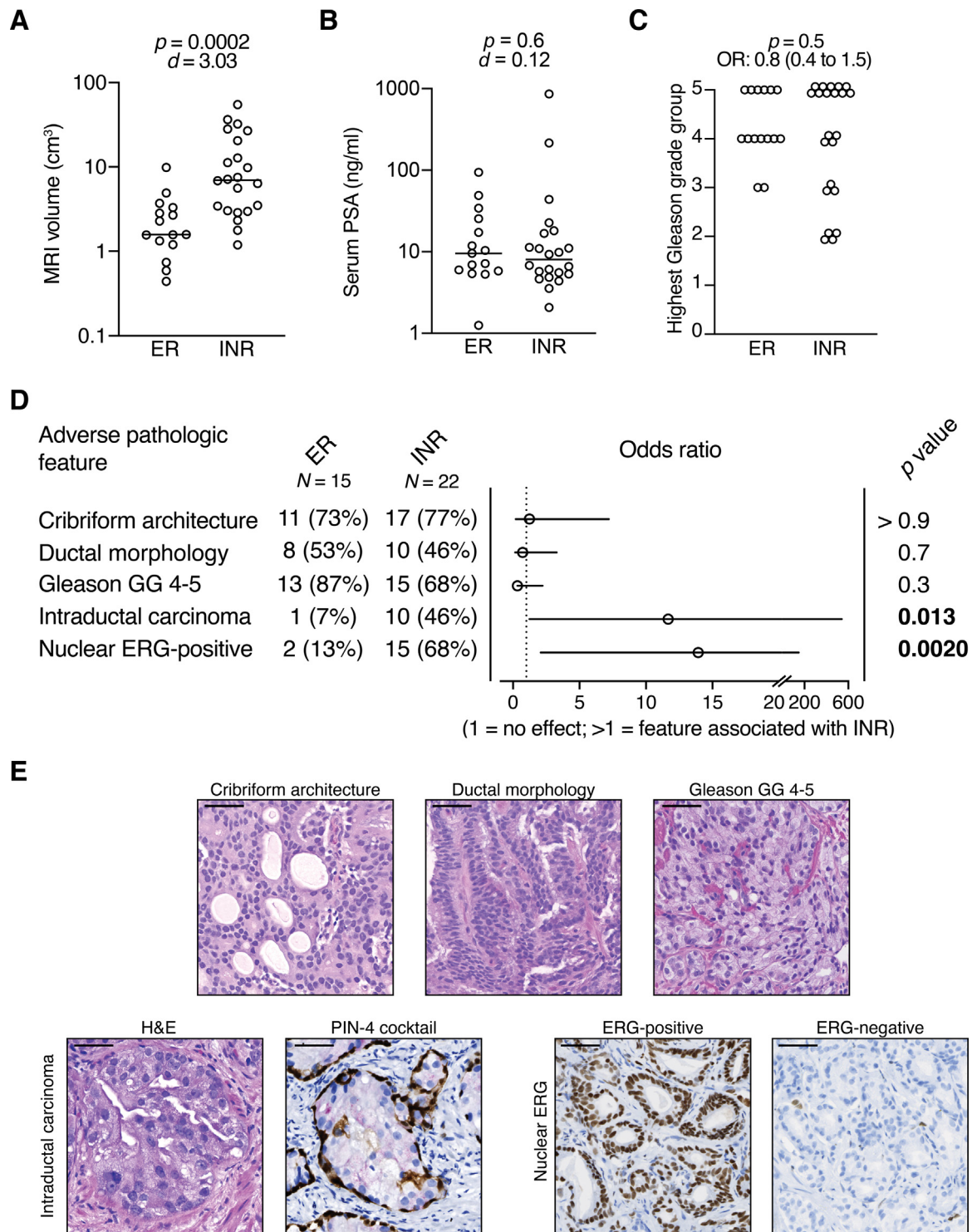


Fig. 2 – Comparison of clinicopathologic features between exceptional responder (ER) and incomplete and nonresponder (INR) cases. (A) Baseline patient-level magnetic resonance imaging (MRI) tumor volumes for ER versus INR cases, plotted on a log₁₀ scale. $p = 0.0002$ by Mann-Whitney *U* test. (B) Baseline serum prostate-specific antigen (PSA) for ER versus INR cases, plotted on a log₁₀ scale. $p = 0.6$ by Mann-Whitney *U* test. For A and B, the effect size *d* is determined as the Hodges-Lehmann median percent difference, based on the log₁₀-transformed value. (C) Highest Gleason grade group (GG) among all pretreatment biopsies for each case; $p = 0.5$ by the Cochran-Armitage test for trend for each GG between ER and INR. The effect size is given by the estimated odds ratio (OR; with 95% confidence interval) for each increment in Gleason grade group. (D) Frequency and odds ratio for five histologic and pathologic features for INR versus ER cases. Odds ratio >1 favors INR. (E) Representative micrographs of the adverse histologic features considered in D. For intraductal carcinoma, PIN-4 cocktail stains basal cells with brown chromogen (antibodies against cytokeratin 5, cytokeratin 14, and p63) and luminal cells with red chromogen (antibody against α -methylacyl-CoA racemase). For nuclear ERG staining, endogenous ERG is observed in endothelial cells for ERG-negative cases. H&E = hematoxylin and eosin. Scale bar: 50 μ m.

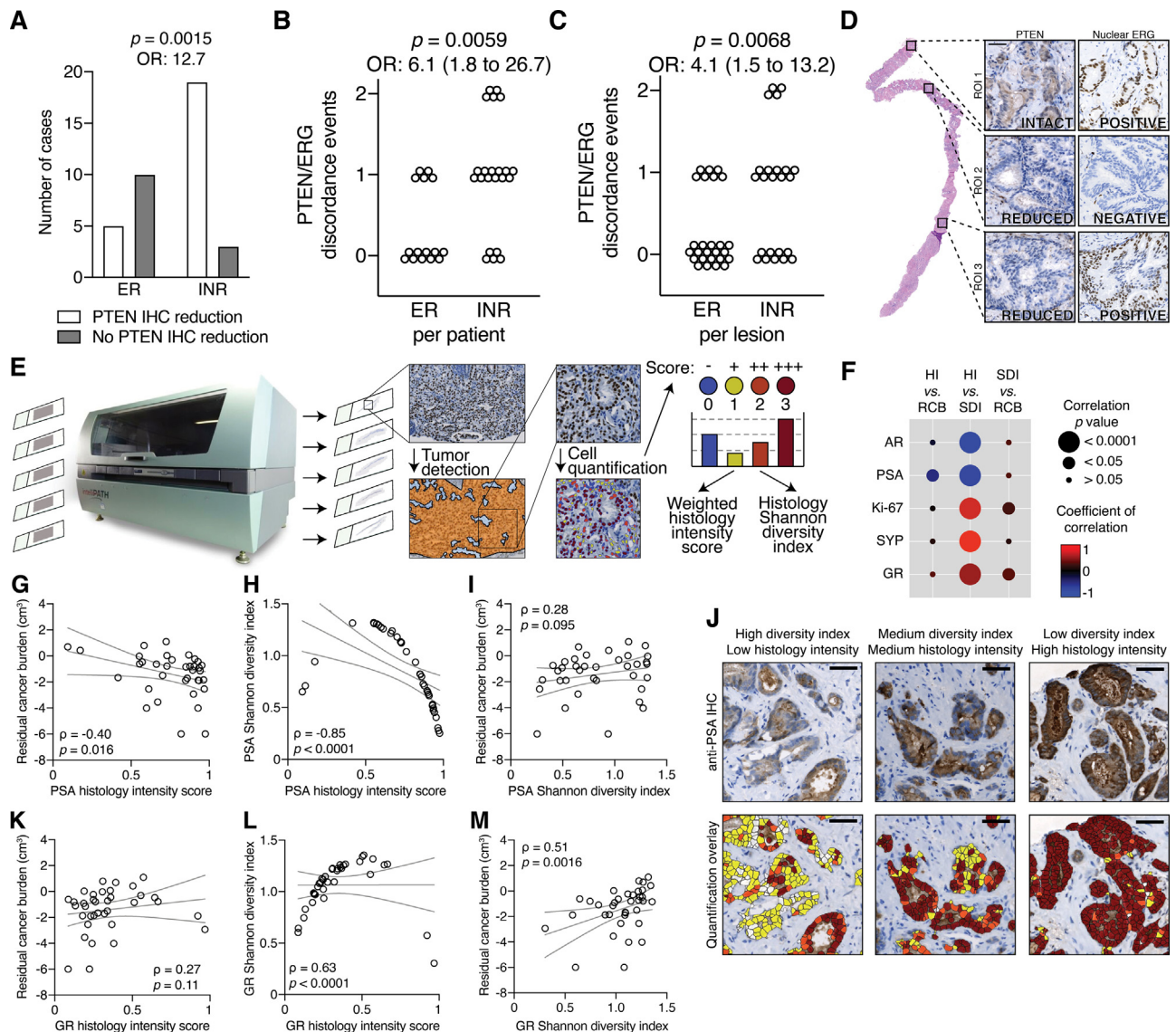


Fig. 3 – Observations of histologic diversity in baseline biopsies. (A) Enumeration of PTEN reduction observed via anti-PTEN immunohistochemistry (IHC) in exceptional responder (ER) and incomplete and nonresponder (INR) cases. The effect size is given as an odds ratio (OR), for which a value >1 favors IHC reduction with INR. (B) Per-patient and (C) per-lesion enumeration when PTEN and/or ERG IHC is positive and negative within the same case. A score of 1 corresponds to either PTEN or ERG showing discordance. A score of 2 indicates both PTEN and ERG show heterogeneity within the same case. Statistical significance was determined using the Cochran-Armitage test for trend. The effect size is given by the estimated OR (95% confidence interval), which is for each increment in heterogeneity observations. (D) A representative case demonstrated PTEN and ERG heterogeneity by IHC (score of 2), showing a region with intact PTEN and positive ERG staining (top), a region with reduced PTEN staining but negative staining for ERG (middle), and a region with reduced PTEN and positive ERG staining (bottom). All three insets are from the same biopsy sample. (E) Workflow for machine-based IHC quantification using an autostainer for reproducible staining conditions, followed by slide scanning and cell/nucleus quantification using the Definiens platform. (F) Bubble plot of IHC correlations between histology intensity (HI) score, residual cancer burden (RCB), and Shannon diversity index (SDI). The bubble size is inversely proportional to the Spearman rank correlation p value, and the color scale is red for more positive coefficients of correlation and blue for negative. (G,K) Correlation of the $\log_{10}$ -transformed RCB for each case plotted against the HI score for each case for (G) anti-prostate-specific antigen (PSA) and (K) anti-glucocorticoid receptor (GR) IHC. (H,L) Shannon diversity index for (H) anti-PSA and (L) anti-GR IHC for each case plotted against the HI score for each case for anti-PSA (H) or anti-GR IHC. (I,M) Correlation of the $\log_{10}$ -transformed RCB for each case plotted against the Shannon diversity index for each case for (I) anti-PSA and (M) or anti-GR IHC. For G–I and K–M, nonparametric Spearman correlation analyses p values are shown with their respective p values. Lines and bands represent linear regression lines and 95% confidence intervals, respectively. Owing to the small number of points, the scatter plots in H and L appear parabolic with no known explanation, although the trend over most of the data suggests a linear relationship. (J) Representative regions of high, medium, and low staining diversity index and low, medium, and high HI, respectively, for anti-PSA IHC. Scale bar: 50 μm .

We observed more branched trees among INR cases (86% vs. 73%); the number of nodes per tree (Fig. 4E) was positively correlated with RCB ($p = 0.35$, 95% CI 0.0095–0.62; $p = 0.039$). Using the prevalence of each tumor subpopulation to calculate a tree SDI, we observed a modest positive correlation ($p = 0.22$, 95% CI -0.13 to 0.52 ; $p = 0.20$) between

baseline SDI and post-treatment RCB (Fig. 4F). A modest inverse correlation was observed between SCNA interfocal homogeneity and genomic SDI ($p = -0.29$, 95% CI -0.59 to 0.067 ; $p = 0.098$) associating genomic heterogeneity with processes of evolutionary diversity (Fig. 4G). Moreover, the percentage genome altered (PGA), a metric associated with

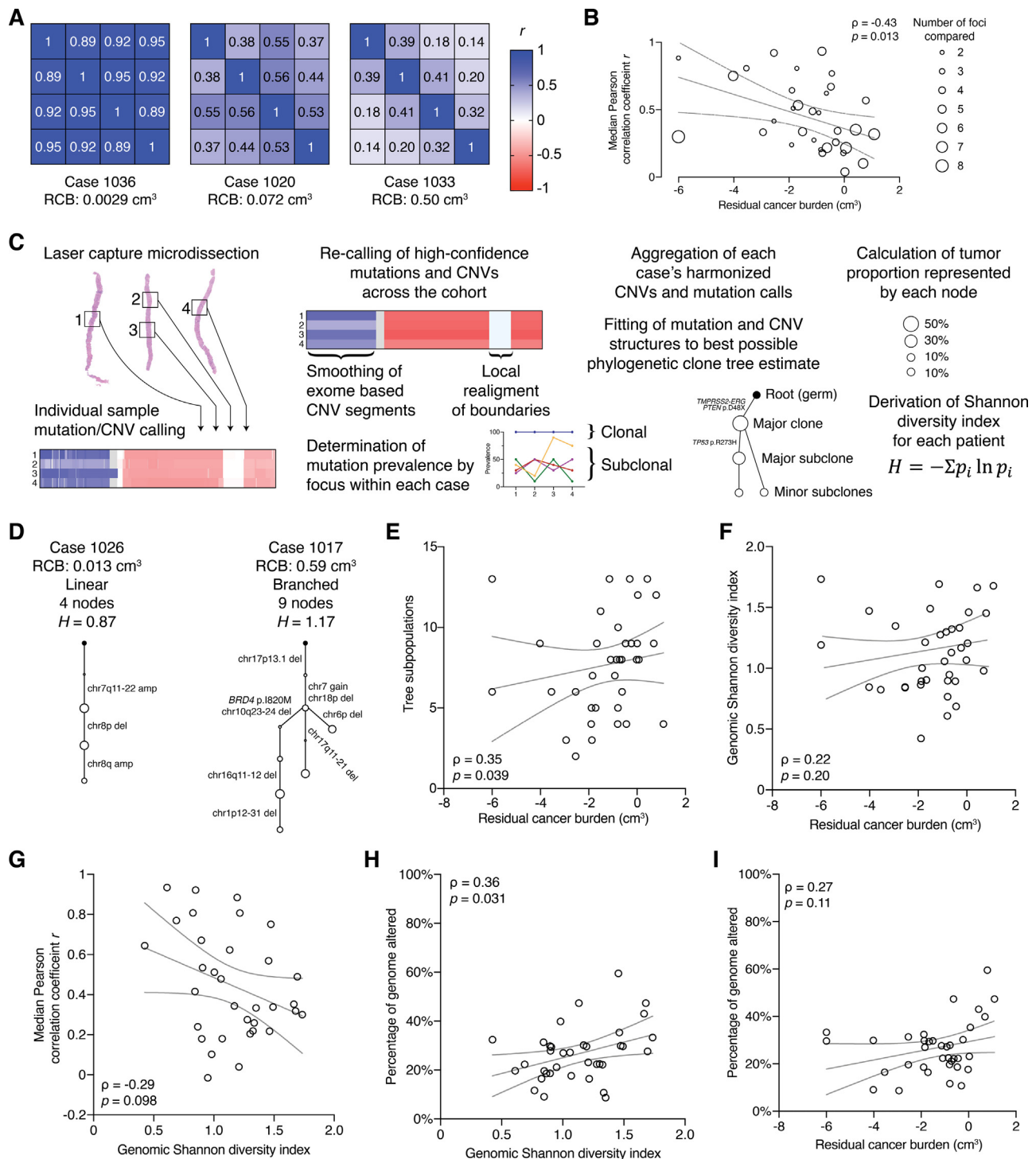


Fig. 4 – Measures of genomic diversity. (A) Correlation matrices for three representative cases with four tumors each, showing the range of similarities among genomic gains and losses across 180 predefined regions. (B) Median Pearson correlation coefficient for each multiregion case as a function of the log₁₀-transformed residual cancer burden (RCB) value. The number of foci dissected for each case is depicted by the size of the circle. (C) Schematic showing the workflow to harmonize multiregion sampling data and processing for phylogenetic clone tree reconstruction. CNV = copy number variation. (D) Representative linear and branched tree structures. (E) Scatter plot of the number of nodes in each phylogenetic tree versus the log₁₀-transformed RCB for each case. (F) Scatter plot of the genomic Shannon diversity index (SDI) for each case versus the corresponding log₁₀-transformed RCB. (G) Median Pearson correlation coefficient for each multiregion case from B versus the genomic SDI for each case. (H) Percentage of the genome altered (PGA) plotted

tumor aggressiveness, was positively correlated with genomic SDI ($p = 0.36$, 95% CI 0.031–0.62; $p = 0.03$) and RCB ($p = 0.27$, 95% CI –0.071 to 0.56; $p = 0.11$; Fig. 4H,I). We therefore conclude that tumors resistant to NADT may harbor a greater number of major preexisting subclones,

and that sensitivity to NADT, even in tumors with driver alterations, is conferred by lower fitness associated with the simpler evolutionary history of these tumors.

To validate these findings, we selected four histogenomic factors according to their performance in univariate

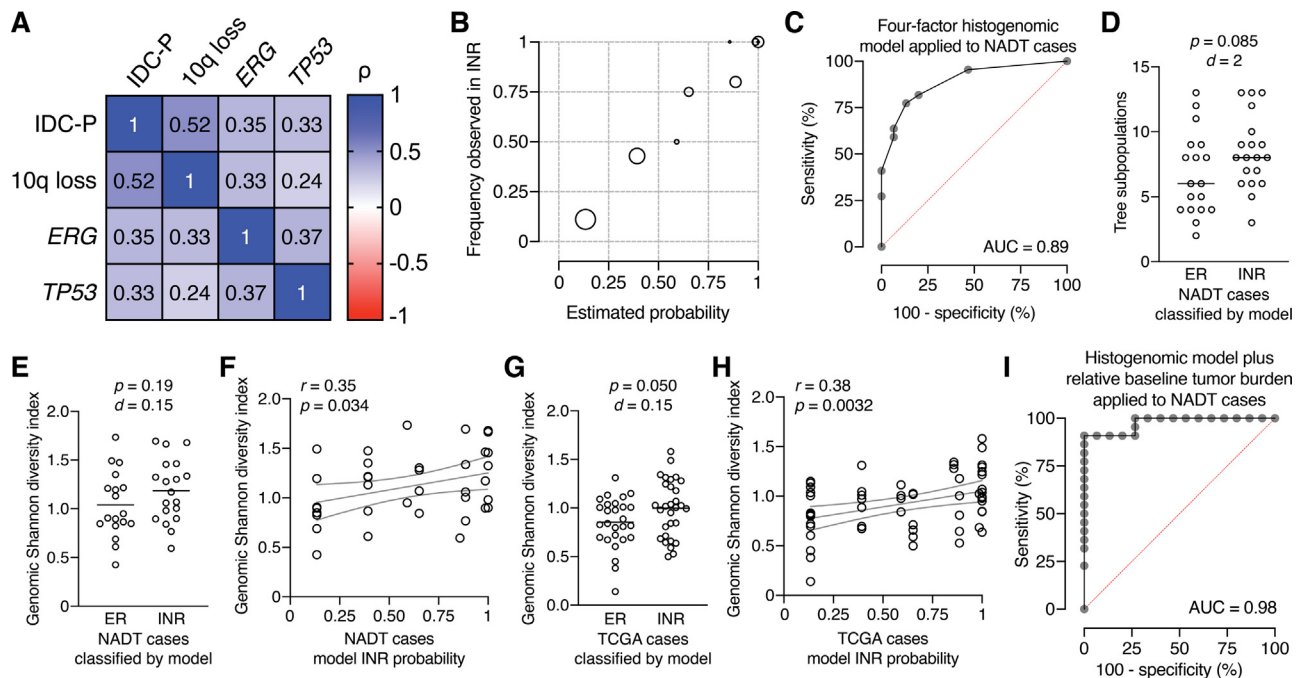


Fig. 5 – A multivariate model of poor response to intense neoadjuvant androgen deprivation therapy (NADT) is associated with genomic diversity. (A) Spearman correlation matrix of each binary factor (1 = presence, 0 = absence) across each of the 37 patients in the study cohort. IDC-P = presence of intraductal carcinoma; 10q loss = at least half of chromosome arm 10q deleted hemizygotously as determined using the GISTIC algorithm; ERG = overexpression of nuclear ERG determined via immunohistochemistry; TP53 = loss-of-function alterations or hotspot mutations to TP53, including copy number loss, as determined via GISTIC. (B) Bubble plot of the frequency of each four-factor combination observed in the trial cohort and the proportion observed in the incomplete and nonresponder (INR) group. (C) Receiver operating characteristic curve for the ability of the INR probability to distinguish exceptional responder (ER) from INR cases in the trial cohort using the four-factor model. (D,E) Reapplication of the four-factor model to trial patients and (D) the number of tumor subpopulations or (E) the genomic Shannon diversity index (SDI) for each group after reclassification. Statistical significance determined using the Mann-Whitney *U* test for the number of tumor populations and Welch's *t* test for genomic SDI. The line represents the (D) median or (E) mean value. The effect size *d* is determined as the Hodges-Lehmann (D) difference of medians or (E) difference of means. (F) Pearson correlation of the genomic SDI and INR probability for the trial cohort. (G) Genomic SDI for The Cancer Genome Atlas (TCGA) validation cohort as classified as ER or INR by the four-factor model. Statistical significance was determined using Welch's *t* test. The line represents the mean value. The effect size *d* was determined as the difference of means. (H) Pearson correlation of the genomic SDI and INR probability for the validation cohort. (I) Receiver operating characteristic curve for the ability of the INR probability to distinguish ER from INR cases in the trial cohort based on the four-factor model plus the baseline relative tumor burden.

analyses that showed imperfect associations with each other (Fig. 5A) to construct a multivariate model for poor response using logistic regression (Supplementary Table 6). These features (*TPRS2:ERG* fusion/nuclear ERG expression, deletion of $\geq 50\%$ of chromosome 10q, loss-of-function or hotspot alterations to TP53, and IDC-P) allow up to 16 possible combinations and probabilities of being INR (Fig. 5B). This model (Fig. 5C) has an area under the receiver operating characteristic curve (AUC) of 0.89 ($p < 0.0001$), and a cutpoint of 60% offered the best discrimination, classifying 30 of 37 NADT cases correctly. This cutpoint also recapitulated the trend towards greater diversity in the number of nodes per tree and greater genomic SDI for each case with higher INR probability (Fig. 5D–F), demonstrating potential utility in our cohort of diverse tumor profiles.

Our validation group for this four-factor histogenomic model consisted of 57 cases of Gleason score 8–10 tumors from the prostate TCGA cohort with evaluable genomic and histologic data. The 60% INR cutpoint resulted in 30 cases classified as INR that harbored a significantly higher genomic SDI than ER ($p = 0.050$, Welch's *t* test; Fig. 5G), and the probability of INR across the group was positively correlated ($r = 0.38$, 95% CI 0.14–0.59; $p = 0.0032$) with

genomic SDI (Fig. 5H), suggesting that greater genomic diversity in a subset of untreated prostate tumors is associated with a higher likelihood of predicted treatment resistance.

As mpMRI estimates of normalized baseline tumor volume were predictive of treatment response at AUC = 0.89 [14], we investigated whether addition of relative volume as a fifth factor would improve the fit of our model in classifying ER versus INR cases. MRI volume measurements were independent of tumor heterogeneity or diversity (Supplementary material), so adding baseline relative tumor burden to the model improved the AUC to 0.98 (Fig. 5I). Thus, genomic diversity and tumor volumes provide complementary data to inform on tumor behavior when predicting outcome following NADT.

4. Discussion

Patients diagnosed with intermediate- to high-risk localized prostate cancer have variable outcomes, and in this clinical trial we found that pathologic responses to intense NADT did not trend with classical clinicopathologic

parameters observed at baseline, such as Gleason score and clinical stage. We used whole-exome sequencing and IHC to identify potential molecular differences between ER and INR cases that could be exploited to further sensitize resistant tumors or select the best trial candidates in future studies. Despite extensive multiregion sampling, we identified relatively few genes that were significantly altered in solely one group; nearly all cases demonstrated hallmarks of aggressive tumors. However, among ER cases, which we defined as harboring RCB of $<0.05\text{ cm}^3$, these somatic alterations were clonal and histologic phenotypes were homogeneous. By contrast, tumors that demonstrated therapeutic resistance had greater subclonal heterogeneity, and phylogenetic reconstruction indicated more complex, branched evolutionary paths, such that both genomic and histologic diversity at baseline positively correlated with residual tumor. At the extreme end of this phenomenon was one patient in our study who harbored two completely independent tumors that exhibited differential responses to therapy [24].

The long-recognized variability and heterogeneity of localized prostate cancer demonstrate that tumor branching occurs at key times during its development, including on transition from high-grade prostatic intraepithelial neoplasia to cancer, from low- to high-grade cancer, and from localized disease to metastasis, with concomitant heterogeneity in IHC marker expression and somatic mutation profiles [3,25–28]. Although some earlier studies demonstrated selection bias in the analysis of larger tumors that were more conducive to multiregion sampling and sequencing, more recent cohort-based molecular analyses have demonstrated strong relationships between tumor evolution and aggressiveness [4,5]. Here, we attempted to subdivide otherwise high-risk tumors on the basis of their reconstructed evolution paths before treatment. While tumors with greater diversity were more likely to resist therapy, they were not necessarily larger, despite larger tumors presumably having undergone more cell divisions since tumor initiation. Indeed, even smaller tumors in our study were sampled comprehensively and exhibited a spectrum of diversity values, such that our bioinformatic reconstruction of tumor phylogenies was not biased by less complex data for ER cases. Meanwhile, more homogeneous tumors were more sensitive, but not necessarily smaller, and still harbored features of advanced cancer such as higher Gleason grade and a cribriform glandular architecture.

The range of residual tumor volumes lends itself to several possible strategies for patient management determined according to prognostic factors at baseline. Combined with MRI, which is increasingly common for staging and guiding biopsies of primary prostate cancer [29], and a series of straightforward immunostains readily available in most hospital laboratories, use of a commercial cancer sequencing panel would provide enough information to determine the probability of response to NADT according to the five-factor model. For patients in the INR group, those with the largest residual tumor volumes often harbored three or four histogenomic factors, and thus may benefit

from either immediate surgery (with or without adjuvant radiotherapy) or systemic chemotherapy before surgery. INR patients with intermediate RCBs (Fig. 1B) may represent incomplete responders and thus may have responded further to androgen annihilation via ADT in conjunction with abiraterone and an AR antagonist.

This study has several limitations. Until future studies with similar analyses of baseline tissue are performed, we will be unable to validate the interaction of these factors with RCB following intense NADT. As many of the factors we identified were based on exploratory studies, they are underpowered and require confirmation in larger studies. In particular, the features selected for generating the four-factor model were not selected on the basis of their likelihood to remain noteworthy after correction for multiple testing, but rather largely because of their emergence as top distinguishing features among those tested and that they did not strongly correlate with each other. In addition, while the selected factors are predicted to correlate with poor response to intense NADT, the presence of these factors needs to be confirmed in post-treatment tissue specimens from this study and other trials to confirm their status as drivers of resistance. Such analyses are ongoing.

The four factors selected to develop a multivariate model of INR probability independently correlated with genomic diversity and offered even stronger discrimination between ER and INR when combined with baseline volume. Prospective assessment of patients lacking these factors despite harboring high-risk prostate cancer may select for a greater proportion of ER cases in future studies.

5. Conclusions

Outcomes for patients with intermediate- to high-risk prostate cancer remain variable, but in the context of prior studies [4,26,27], connecting short-term responses to treatment with extensive histogenomic analyses has revealed that the evolutionary history of a tumor is clearly related to its fitness. Patients with smaller tumors harboring more linear evolution paths exhibited responses to preoperative intense NADT, which is expected to delay or prevent recurrence. By contrast, patients with more diverse tumor systems may harbor oligometastatic disease, for whom sensitive molecular imaging modalities such as PSMA positron emission tomography could identify opportunities for radiation therapy and treatment regimens other than ADT. A study using intense NADT with imaging and serial prostate biopsies to evaluate intraprostatic tumor responses at multiple time points is currently in progress (NCT02430480).

Author contributions: Adam G. Sowalsky 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: Ye, VanderWeele, Dahut, Sowalsky.

Acquisition of data: Wilkinson, Carrabba, Whitlock, Bright, Atway, Trostel, Lotan, Turkbey, Harmon, Walker.

Analysis and interpretation of data: Wilkinson, Ye, Terrigino, Capaldo, Bright, Lis, Sater, Merino, Salles, Kaur, Lotan, Choyke, Pinto, Gulley, Madan, Sowalsky.

Drafting of the manuscript: Wilkinson, Terrigino, Carrabba, Lotan, Venzon, Sowalsky.

Critical revision of the manuscript for important intellectual content: Karzai, VanderWeele.

Statistical analysis: Venzon.

Obtaining funding: Dahut.

Administrative, technical, or material support: Chun.

Supervision: Choyke, Dahut, Sowalsky.

Other: None.

Financial disclosures: Adam G. Sowalsky certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Huihui Ye and Rosina T. Lis perform consulting in an advisory role for Janssen Pharmaceuticals. The remaining authors have nothing to disclose.

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Data sharing statement: All raw sequencing data have been deposited in the Database of Genotypes and Phenotypes (dbGaP) at accession number phs001938.v2.p1. The custom code used for processing data is available at <https://github.com/CBIIT/lgcp>. Slides used for histologic analysis have been deposited in The Cancer Imaging Archive. The complete study protocol and associated forms are available on request to the corresponding author.

CRediT authorship contribution statement

Scott Wilkinson: Methodology, Validation, Investigation, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Huihui Ye:** Conceptualization, Investigation, Data curation. **Fatima Karzai:** Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing. **Stephanie A. Harmon:** Investigation, Data curation, Writing - review & editing. **Nicholas T. Terrigino:** Validation, Formal analysis, Investi-

gation, Data curation, Writing - original draft, Visualization. **David J. VanderWeele:** Conceptualization, Resources, Writing - original draft. **John R. Bright:** Investigation. **Rayann Atway:** Investigation. **Shana Y. Trostel:** Investigation. **Nicole V. Carrabba:** Methodology, Investigation, Writing - original draft. **Nichelle C. Whitlock:** Investigation. **Stephanie M. Walker:** Investigation, Data curation. **Rosina T. Lis:** Validation, Data curation. **Houssein A. Sater:** Investigation, Data curation. **Brian J. Capaldo:** Methodology, Software, Formal analysis. **Ravi A. Madan:** Resources. **James L. Gulley:** Resources. **Guinevere Chun:** Resources. **Maria J. Merino:** Investigation, Resources. **Peter A. Pinto:** Resources. **Daniela C. Salles:** Investigation. **Harsimar B. Kaur:** Investigation. **Tamara L. Lotan:** Investigation, Writing - original draft. **David J. Venzon:** Software, Formal analysis, Data curation, Writing - original draft, Visualization. **Peter L. Choyke:** Resources, Supervision. **Baris Turkbey:** Methodology, Resources, Writing - review & editing, Supervision, Project administration. **William L. Dahut:** Resources, Supervision, Funding acquisition. **Adam G. Sowalsky:** Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.eururo.2021.03.009>.

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Integrated radiologic, genomic and pathologic analysis revealed intratumoral divergent responses to neoadjuvant anti-androgen therapy

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Background:

Localized prostate cancers are uniquely genetically variable and frequently multifocal, comprising both spatially separate regions and multiple independently evolving clones within each region. The multifocality of prostate cancer is a well-acknowledged problem for diagnosis, and the differential aggressiveness of potentially independent clones remains largely unknown. Further, appropriate prostate cancer staging using multiparametric MRI (mpMRI) and biopsy tissue can be confounded by sampling error. To date, there has been no understanding of whether this variability influences management decisions for localized prostate tumors. Here, we sought to identify the sensitivity and genomic profile of distinct localized tumors from a single patient following systemic intense neoadjuvant androgen deprivation therapy.

Methods:

A 66-year-old man with high risk prostate cancer was enrolled onto a Phase 2 study of neoadjuvant androgen deprivation therapy plus enzalutamide. mpMRI was performed at baseline, showing a single semi-contiguous lesion encompassing the right apical-mid peripheral zone also extending into the left distal apical peripheral zone. MR/ultrasound-fusion targeted biopsy was performed before undergoing 6 months of intense neoadjuvant androgen deprivation therapy (goserelin and enzalutamide). Biopsies of the left- and right-sided tumors showed differing histologies. A second mpMRI was performed following therapy before radical prostatectomy. Laser capture microdissection was performed to isolate pure populations of tumor cells from the pre-treatment biopsy and post-treatment surgical specimen. Whole exome sequencing was performed on microdissected tissues to identify somatic mutations and copy number alterations, which were further used to assess tumor clonal architecture and genomic/phenotypic evolution of treatment resistant tumor.

Results:

At baseline, mpMRI identified a large semi-contiguous tumor extending from the right apical-mid peripheral zone to the left distal apical zone, which was further resolved into two independent lesions sampled separately upon targeted biopsy. The biopsies showed remarkably differencing histologies, with one lesion TMPRSS2:ERG fusion positive and PTEN reduced, while the other lesion lacked the fusion and had heterogeneous PTEN staining. After six months of systemic therapy and radical prostatectomy, mpMRI and pathology showed near complete response of one tumor and substantial resistance of the other, which exhibited a large intraductal component. The resistant focus exhibited strong AR expression, reduced but present TMPRSS2:ERG expression, PTEN reduction, and low expression of potential bypass mechanisms including AR-V7 and GR.

Using point mutations as definitive clonal markers, we found that two clonally-independent tumors exhibited intrinsic heterogeneity at baseline which correlated with response or resistance. Whole exome data highlighted a divergence in the status of tumor suppressor genes implicated in prostate cancer progression both pre- and post-treatment. The pre-treatment resistant focus contained a one copy arm-level deletion of *PTEN* and a focal deletion in *TP53*, both of which were not present in the responding focus. The post-treatment intraductal resistant tumor contained an additional *PTEN* and *TP53* copy loss, rendering the intraductal resistant tumor both negative for both PTEN and p53. The responding tumor focus lacked any genomic hits to *PTEN* but showed reduced PTEN expression, suggesting alternative mechanisms of PTEN expression reduction.

Conclusions:

These data highlight a patient containing a polytumor, defined by two completely independent primary tumor systems in the same organ. Resistant intraductal tumor containing AR overexpression and reduced bypass mechanisms and dedifferentiation markers highlight a castration-resistant adenocarcinoma. Evolutionary trajectories that resulted in tumor heterogeneity in this case likely contributed to our observation that two independent prostate tumor nodules with distinct genetic alterations responded differently to neoadjuvant intense ADT.

Multiple primary prostate tumors with differential drug sensitivity

Scott Wilkinson¹, Huihui Ye², Nicholas T. Terrigino¹, Nicole Carrabba¹, Rayann Atway¹, Shana Y. Trostel¹, John R. Bright¹, S. Thomas Hennigan¹, Rosina T. Lis³, Ross Lake¹, Stephanie Harmon¹, Baris Turkbey¹, Peter A. Pinto¹, Peter L. Choyke¹, Fatima Karzai¹, David J. VanderWeele¹, Kathleen Kelly¹, William L. Dahut¹, and Adam G. Sowalsky¹

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Background:

The differential aggressiveness of potentially independent prostate cancer clones remains largely unknown. Appropriate prostate cancer staging using multiparametric MRI (mpMRI) and biopsy tissue can be confounded by sampling error. To date, there has been no understanding of whether clonal variability influences management decisions for localized prostate tumors. We sought to identify the sensitivity and genomic profile of distinct localized tumors from a patient following systemic intense neoadjuvant androgen deprivation therapy (ADT).

Methods:

A 66-year-old man with high risk prostate cancer enrolled in a Phase 2 study of intense neoadjuvant ADT (goserelin + enzalutamide; inADT). Baseline mpMRI showing a single semi-contiguous lesion encompassing the right apical-mid PZ extending into the left distal apical PZ. MR/US-fusion targeted biopsy was performed before 6 months of inADT. A second mpMRI was performed before radical prostatectomy. Whole exome sequencing on microdissected tumor foci identified somatic mutations and copy number alterations, which were further used with immunohistochemistry to assess tumor clonal architecture and genomic/phenotypic evolution of treatment resistant tumor.

Results:

We found that two clonally-independent tumors exhibited intrinsic heterogeneity at baseline which correlated with response or resistance. Biopsies of distinct left- and right-sided tumors showed differing histologies. mpMRI and pathology showed near complete response of the left-sided tumor and substantial resistance of the right-sided tumor, which exhibited a large intraductal component. Histology and whole exome data highlighted a divergence in the status of PTEN and TP53, tumor suppressor genes implicated in prostate cancer progression.

Conclusions:

These data highlight that even nascent prostate cancer is heterogenous and neoadjuvant therapeutic strategies will need to consider this for clinical optimization. Evolutionary trajectories that resulted in tumor heterogeneity in this case likely contributed to our observation that two independent prostate tumor nodules with distinct genetic alterations responded differently to neoadjuvant intense ADT.

Integrated radiologic, genomic and pathologic analysis revealed intratumoral divergent responses to neoadjuvant anti-androgen therapy

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Background:

Localized prostate cancers are uniquely genetically variable, comprising both spatially separate regions and multiple independently evolving clones within each region. The multifocality of prostate cancer is a well-acknowledged problem for diagnosis, and the differential aggressiveness of potentially independent clones remains largely unknown. Further, appropriate prostate cancer staging using multiparametric MRI (mpMRI) and biopsy tissue can be confounded by sampling error. To date, there has been no understanding of whether this variability influences management decisions for localized prostate tumors. Here, we sought to identify the sensitivity and genomic profile of distinct localized tumors from a single patient following systemic intense neoadjuvant androgen deprivation therapy.

Methods:

A 66-year-old man with high risk prostate cancer was enrolled onto a Phase 2 study of neoadjuvant androgen deprivation therapy plus enzalutamide. mpMRI was performed at baseline, showing a single semi-contiguous lesion encompassing the right apical-mid peripheral zone also extending into the left distal apical peripheral zone. MR/ultrasound-fusion targeted biopsy was performed before undergoing 6 months of intense neoadjuvant androgen deprivation therapy (goserelin and enzalutamide). Biopsies of the left- and right-sided tumors showed differing histologies. A second mpMRI was performed following therapy before radical prostatectomy. Laser capture microdissection was performed to isolate pure populations of tumor cells from the pre-treatment biopsy and post-treatment surgical specimen. Whole exome sequencing was performed on microdissected tissues to identify somatic mutations and copy number alterations, which were further used to assess tumor clonal architecture and genomic/phenotypic evolution of treatment resistant tumor.

Results:

After six months of systemic therapy and radical prostatectomy, mpMRI and pathology showed near complete response of one tumor and substantial resistance of the other tumor, which exhibited a large intraductal component. Histopathological staining and whole exome data highlighted a divergence in the status of tumor suppressor genes implicated in prostate cancer progression, with the non-responding tumor at baseline exhibiting additional alterations absent from the pre-treatment tumor that responded to therapy. Using point mutations as definitive clonal markers, we found that two clonally-independent tumors exhibited intrinsic heterogeneity at baseline which correlated with response or resistance.

Conclusions:

These data highlight that even nascent prostate cancer is heterogenous and neoadjuvant therapeutic strategies will need to consider this for clinical optimization. Evolutionary trajectories that resulted in tumor heterogeneity in this case likely contributed to our observation that two independent prostate tumor nodules with distinct genetic alterations responded differently to neoadjuvant intense ADT.

Conflicts of Interest:

Huihui Ye: Janssen (consulting)

Funding Acknowledgments:

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EDUCATION

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CRTA Postdoctoral Fellow

October 2016 – Present

Emory University, Atlanta, GA
PhD in Biological and Biomedical Sciences, Cancer Biology

June 2011 – August 2016

Berry College, Mt Berry, GA
BS in Biology

December 2009

RESEARCH SUPPORT

- **Early Investigator Research Award** 2019-2021
W81XWH-19-1-0712, Department of Defense,
- **Young Investigator Award** 2018-2021
18YOUN28, Prostate Cancer Foundation, * (NCI is an ineligible recipient of PCF funds)
- **Ruth L. Kirschstein National Research Service Award** 2015-2016
1F31CA200383, National Institutes of Health,
- **Graduate Research Fellowship Program Recipient** 2011-2014
National Science Foundation,

RESEARCH EXPERIENCE

Postdoctoral Research, National Institutes of Health, Bethesda, MD

October 2016 – Present

CTRA Fellow, Dr. Adam Sowalsky lab

My postdoctoral work investigates the genetic features underlying response to anti-androgen therapy in patients with localized aggressive prostate cancer. Although androgen deprivation therapies (ADT) have been utilized in the clinic for metastatic prostate cancer, the genetic features that would predict response or non-response to this therapy is as of yet not understood. We were successful in identifying genetic features within baseline tumors of exceptional response to ADT, as well as features that would predict intrinsic resistance to this therapy. I was able to utilize my graduate training in cancer cell biology to initiate functional and mechanistic studies of these genetic predictors of response to therapy, with the ultimate goal of identifying novel targets to sensitize aggressive prostate cancers to hormone therapy.

- I served as a major contributor to three successfully funded Department of Defense Prostate Cancer Research Program awards to study ctDNA in pre-surgical and post-surgical/pre-biochemical recurrence patients and to examine intrinsic resistance to neoadjuvant ADT. I conducted specific aims under the research plan, and supervised/mentored 3 postbaccalaureate fellows and 1 summer student under the project.
- I applied for, and received, research grants from the Prostate Cancer Foundation's Young Investigator Award and the DOD Early Investigator Research Award based on my preliminary work and proposed future studies in this lab.
- Served as a reviewer for Scientific Reports, as well as peer-reviewed Prostate Cancer Foundation Young Investigator Awards, NCI Graduate Student Research Program applications, and ASCB microsymposia abstracts.
- I presented my work at several national research conferences, including the American Society for Cell Biology, American Association for Cancer Research, and AACR Advances in Prostate Cancer Research.

Thesis Research, Emory University, Atlanta, GA

March 2012 – August 2016

PhD Candidate, Dr. Adam Marcus lab

The tumor suppressor LKB1 is the third most commonly mutated gene in non-small cell lung adenocarcinoma. Identifying the consequences of LKB1 mutation during lung cancer invasion and metastasis is critical to understanding the mechanism of localized tumor invasion. My graduate work focused on identifying functional consequences of domain mutation in LKB1. I identified LKB1 farnesylation as critical for driving cellular polarization and directional persistence in 2D and 3D invasion, as well as promoting its actin association during cell motility. I also highlighted the LKB1 kinase activity in regulating cell membrane dynamics and leading-edge focal adhesion deposition and maturation during cell motility.

- I applied for and received an NSF predoctoral Graduate Research Fellowship to cover my first three years of my degree, focusing on the cell biology of lung cancer during cell motility.
- Received an NIH F31 pre-doctoral fellowship for my final two years describing the role of LKB1 in regulating cell polarization and invasion in a 3D microenvironment.

- Presented my research at several local and national conferences, including presentations at ASCB, AACR Tumor Metastasis, the Winship Cancer Institute Research Symposium, and Emory's graduate student symposium.
- Served as a mentor to 2 graduate students in the lab, while also developing and teaching courses on cancer cell biology to undergraduate and high school students.

Rickettsial Zoonoses Branch, CDC, Atlanta, GA

February 2010 – June 2011

Biologist (ORISE Fellow)

- I created an ecological niche model of Rickettsial pathogens throughout the Congo basin in sub-Saharan Africa to track previous Rickettsial outbreaks and predict future outbreaks based on climate and environmental conditions.
- I traveled to Arizona three times to participate in community awareness and Epi-Aid efforts to treat for and prevent *R. rickettsii* infection in epidemic regions of rural eastern Arizona.

Rickettsial Zoonoses Branch, CDC, Atlanta, GA

June 2009 – February 2010

Guest Researcher

- I developed an ELISA to detect antibodies to *R. rickettsii* in canines, which has been deployed to eastern Arizona for an *R. rickettsii* epidemic where canines are serving as reservoir hosts for ticks harboring rickettsial disease.

Berry College, Mount Berry, GA

August 2008 – December 2009

Undergraduate Research Assistant, Dr. Chris Mowry lab

- My undergraduate research examined dietary habits of coyotes (*Canis latrans*) in Yellowstone National Park to identify the impact of reintroduction of the grey wolf (*C. lupus*) on the local ecosystem.

PUBLICATIONS

S. Wilkinson, H. Ye, F. Karzai, S. Harmon, N. T. Terrigino, D. VanderWeele, J. Bright, R. Atway, S. Trostel, N. Carrabba, N. Whitlock, S. Walker, R. Lis, H. Sater, B. Capaldo, R. Madan, J. Gulley, G. Chun, M. Merino, P. Pinto, D. Salles, H. Kaur, T. Lotan, D. Venson, P. Choyke, B. Turkbey, W. L. Dahut, A. G. Sowalsky. (2020). Nascent prostate cancer heterogeneity drives evolution and resistance to intense hormonal therapy. *European Urology*. S0302-2838(21)00207-4. doi: 10.1016/j.eururo.2021.03.009.

S. Wilkinson*, S. Harmon*, N. T. Terrigino, K. Karzai, P. A. Pinto, R. A. Madan, D. J. VanderWeele, R. Lake, R. Atway, J. R. Bright, N. V. Carrabba, S. Y. Trostel, R. T. Lis, G. Chun, J. L. Gulley, M. J. Merino, P. L. Choyke, H. Ye, W. L. Dahut, B. Turkbey, A. G. Sowalsky. (2020). A case report of multiple primary prostate tumors with differential drug sensitivity. *Nature Communications*. 11, 837. DOI: 10.1038/s41467-020-14657-7.

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N.C. Whitlock, S.Y. Trostel, **S. Wilkinson**, N.T. Terrigino, S.T. Hennigan, R. Lake, N.V. Carrabba, R. Atway, E.D. Walton, B.E. Gryder, B.J. Capaldo, H. Ye, A.G. Sowalsky. (2020). *MEIS1* down-regulation by MYC mediates prostate cancer development through elevated *HOXB13* expression and AR activity. *Oncogene*. <https://doi.org/10.1038/s41388-020-01389-7>

F. Karzai*, S. M. Walker*, **S. Wilkinson**, R. A. Madan, J. H. Shih, M. J. Merino, S. Harmon, D. VanderWeele, L. M. Cordes, N. V. Carrabba, J. R. Bright, N. T. Terrigino, G. Chun, M. Bilusic, A. Couvillon, A. Hankin, M. N. Williams, R. T. Lis, H. Ye, P. L. Choyke, J. L. Gulley, A. G. Sowalsky, B. Turkbey, P. A. Pinto, W. L. Dahut. (2020). Sequential prostate MRI imaging in newly diagnosed high-risk prostate cancer treated with neoadjuvant enzalutamide prior to surgery. *Clinical Cancer Research*. DOI: 10.1158/1078-0432.CCR-20-2344.

C. S. Jansen, N. Prokhnevskaya, V. A. Master, M. Sanda, J. W. Carlisle, M. A. Bilen, M. A. Cardenas, **S. Wilkinson**, R. Lake, A. G. Sowalsky, R. Valanparambil, W. Hudson, D. McGuire, K. R. Melnick, A. Khan, K. S. Kim, Y. M. Chang, A. Kim, C. Filson, M. Alemozaffar, A. Osunkoya, P. Mullane, C. Ellis, R. Akondy, S. J. Im, A. O. Kamphorst, A. Reyes, Y. Liu, H. Kissick. (2019). TCF1+ stem-like CD8 T cells in human tumors are required to generate anti-tumor effector T cells. *Nature*. 576: 465-470.

S. T. Hennigan*, S. Y. Trostel*, N. T. Terrigino, O. S. Voznesensky, R. J. Schaeffer, N. C. Whitlock, **S. Wilkinson**, N. V. Carrabba, R. Atway, S. Shema, R. Lake, A. R. Sweet, D. J. Einstein, F. Karzai, J. L. Gulley, P. Chang, G. L. Bubley, S. P. Balk, H. Ye, A. G. Sowalsky. (2019). Low abundance of circulating tumor DNA in localized prostate cancer. *Journal of Clinical Oncology Precision Oncology*. DOI: 10.1200/PO.19.00176.

A. Sheahan, K. Morel, D. Burkhart, S. Baca, D. Labbe, K. Roehle, M. Heckler, C. Calagua, H. Ye, P. Galbo, S. Panja, A. Mitrofanova, **S. Wilkinson**, N. Whitlock, S. Y. Trostel, A. Hamid, A. Kibel, A. Choudhury, M. Pomeranta, M. Freedman, S. Sweeny, S. Dougan, A. Sowalsky, M. Loda, B. Olson, L. Ellis. Targeting EZH2 increases therapeutic efficacy of checkpoint blockade in models of prostate cancer. In Revision at *Nature Cancer*.

S. Wilkinson, Y. Hou, J. T. Zoine, J. Saltz, L. A. D. Cooper, A. I. Marcus. (2017). Coordinated cell motility is regulated by a combination of LKB1 farnesylation and kinase activity. *Scientific Reports*. 7:40929.

J. Konen*, **S. Wilkinson***, B. Lee, W. Zhou, Y. Jiang, A. I. Marcus. LKB1 kinase-dependent and -independent defects disrupts polarity and adhesion signaling to drive collagen remodeling during invasion. *Molecular Biology of the Cell*. 2016. 27(7): 1069-84.

**Selected for Highlights Article, Cover Image for Journal

B. Lee, J. Konen, **S. Wilkinson**, A. I. Marcus, Y. Jiang. (2016). Local alignment vectors reveal cancer cell-induced ECM fiber remodeling dynamics. *Scientific Reports*. 7:39498.

F. Liu, R. Jin, X. Liu, H. Huang, **S. Wilkinson**, D. Zhong, F. R. Khuri, H. Fu, A. I. Marcus, Y. He and W. Zhou. LKB1 promotes cell survival by modulating TIF-IA-mediated pre-ribosomal RNA synthesis under uridine depleted conditions. *Oncotarget*. 2016. 7(3): 2519-31.

SCIENTIFIC SERVICE

• Member: Honorific Awards Taskforce, ASCB	2020-2021
• Member: Nominating Committee, ASCB	2020
• Member: Governance Committee, ASCB	2018-2020
• Moderator: Microtubule Motors & Transport Microsymposium, ASCB	December 2019
• Moderator: New Perspectives: Frontiers of Microscopy Microsymposium, ASCB	December 2019
• Reviewer, Prostate Cancer Foundation Young Investigator Awards	April 2019
• Reviewer, NCI Graduate Student Recruiting Program	February 2019
• Member, ASCB Governance Committee	2019-2021
• Reviewer, Scientific Reports	January 2018 – Present
• Moderator: Cell Cycle, Cell Division, Cell Death Microsymposium, ASCB	December 2018
• Moderator: Cytoskeleton, Motility, and Cell Mechanics Microsymposium, ASCB	December 2018
• Moderator: Cancer Cell Biology Microsymposium, ASCB	December 2017
• Moderator: Cell Adhesion and Motility Microsymposium, ASCB	December 2017
• Moderator: Bioengineering and Signaling Microsymposium, ASCB	December 2017
• Moderator: Cytoskeletal Molecular Dynamics Microsymposium, ASCB	December 2016
• Moderator: Development and Invasion Microsymposium, ASCB	December 2016
• Moderator: Signaling and Bioengineering Microsymposium, ASCB	December 2016
• Moderator: Cell Motility and Migration Microsymposium, ASCB	December 2015
• Moderator: Actin Cytoskeleton Dynamics Microsymposium, ASCB	December 2015

AWARDS & HONORS

• Fellows Award for Research Excellence (FARE), NIH	2020
• Early Investigator Research Award, Department of Defense	2019-2021
• Young Investigator Award, Prostate Cancer Foundation	2018-2021
• Cover Image, Molecular Biology of the Cell	April 2016
• First Author <i>Highlights Article</i>, Molecular Biology of the Cell	April 2016
• Cancer Close-Up Imaging Award, National Cancer Institute	April 2016
• Runner-Up, DSAC Image Competition, Emory University	January 2016
• Graduate Student Travel Award, American Society for Cell Biology	December 2015
• Graduate Career Teaching Award, Emory University	September 2015
• William and Catherine Rice Endowed Research Award, Emory University	September 2015
• Honorable Mention, Ford Foundation Dissertation Fellowship	April 2015
• 2nd Place, Division Student Activity Council Symposium, Emory University	January 2015
• Center for Faculty Development and Excellence Mini-Grant, Emory University	November 2014
• Runner-Up in Winship Scientific Research Symposium, Emory University	October 2014
• Emory-HHMI Curriculum Development Fellowship, Emory University	June-August 2014
• COMPASS Outreach Award, American Society for Cell Biology	May 2014
• 1st Place in Division Student Activity Council Research Symposium	January 2014
• GDBBS Annual Award for Excellence in Student Teaching, Emory University	September 2013
• ORISE Fellow, Centers for Disease Control and Prevention	2010-2011

NATIONAL MEMBERSHIPS

Society for Basic Urologic Research	2018-Present
American Association for Cancer Research	2015-Present
American Society for Cell Biology	2013-Present
American Society for Rickettsiology	2010-2011

COMMUNITY OUTREACH AND LEADERSHIP

• Co-Chair: COMPASS leadership committee, ASCB	January 2019-December 2020
• Panelist: Politicians Don't Bite, ASCB Annual Meeting	December 2016
• Member: Public Policy Committee, ASCB	November 2016-2019
• USA Science and Engineering Festival, ASCB Exhibitor	April 2016, April 2018
• COMPASS Hill Day, ASCB	April 2016
• Co-Chair: Outreach Subcommittee, COMPASS, ASCB	January 2016-December 2018
• AACR Early Career Hill Day, AACR	February 2016
• Member: Education Committee, ASCB	April 2015-November 2016
• Member: Committee for Postdocs and Students (COMPASS), ASCB	March 2015-Present
• Imagining the Future: Atlanta Science Festival	March 2015
• Hill Day, American Society for Biochemistry and Molecular Biology	April 2014
• Students for Science, Educator	2012-2016

FORMAL TEACHING, AND MENTORING

• NIH Postbaccalaureate Mentor Program, Mentor to Jack Bright	February 2019-Present
• NIH Postbaccalaureate Mentor Program, Mentor to Nicholas Terrigino	June 2018-Present
• NIH Postbaccalaureate Mentor Program, Mentor to Nicole Carrabba	October 2017-March 2019
• NIH Summer Internship in Biomedical Research, Mentor to Rayann Atway	2017, 2019
• Winship Summer Scholars Program, Mentor to Stuti Rao	2015
• Bio 285: The Non-Fiction of Cancer, Instructor, Emory University	Spring 2015
• Mentor to Emily Summerbell, Jaquelyn Zoine, PhD Rotation Students	2014-2016
• Cancer: The Biology Behind the Disease, Instructor, Emory Pre-College Program	2014-2016
• Cancer: The Biology Behind the Disease, Course Developer	Spring 2014
• Emory Pipeline Program, Senior Curriculum Instructor	2013-2014
• Infectious Disease Institute, Mentor to Lucy Baker, Emory Pre-College Program	2016
• Bio 250: Cell Biology, Teaching Assistant, Emory University	Fall 2012

OUTREACH WRITING

- **Promote Public Awareness of Science with a COMPASS Outreach Grant**
[ASCB Post](#), January 20, 2017
- **How to Inspire Future Generations of Scientists Without Quitting Our Day Jobs**
[ASCB Compass Points](#), August 7, 2015
- **Exploring "Alternative" Careers: Are universities doing enough to help?**
[ASCB Compass Points](#), June 26, 2015
- **The Importance of Elections in Scientific Societies (including ASCB)**
[ASCB Compass Points](#), April 10, 2015

ABSTRACTS

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2020). Multiple primary prostate tumors exhibit divergent responses to intense neoadjuvant androgen deprivation therapy. 2020 AACR Prostate Cancer meeting. Denver, CO.

*Conference was canceled due to COVID-19 pandemic.

C. S. Jansen, N. Prokhnevskaya, M. Cardenas, V. Master, J. W. Carlisle, M. A. Bilen, **S. Wilkinson**, R. Lake, A. G. Sowalsky, A. O. Osunkoya, P. Mullane, C. Ellis, A. Reyes, Y. Liu, H. Kissick. (2020). CD8 T cell infiltration predicts progression in renal cell carcinoma and stratifies low and high-risk patients with stage III disease. 2020 SESAUA Meeting, New Orleans, LA.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2020). Multiple primary tumors with differential drug sensitivity. 2020 GU ASCO Symposium. San Francisco, CA.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2019). Multiple primary tumors with differential drug sensitivity. 2019 ASCB Annual Meeting. Washington, DC.

C. S. Jansen, N. Prokhnevskaya, V. Master, J. W. Carlisle, M. A. Bilen, M. Cardenas, **S. Wilkinson**, R. Lake, A. G. Sowalsky, A. O. Osunkoya, P. Mullane, C. Ellis, A. Reyes, Y. Liu, H. Kissick. (2019). CD8 T cell infiltration into renal tumors required a supportive antigen presenting niche and is independent of PD-L1 status. Society for Immunotherapy in Cancer Annual Meeting. National Harbor, MD.

S. Wilkinson, H. Ye, N. T. Terrigino, N. Carrabba, R. Atway, S. Y. Trostel, J. R. Bright, S. T. Hennigan, R. T. Lis, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2019). Integrated radiologic, genomic and pathologic analysis revealed intratumoral divergent responses to neoadjuvant anti-androgen therapy. 26th Annual Prostate Cancer Foundation Scientific Retreat. Carlsbad, CA.

S. Harmon, **S. Wilkinson**, H. Ye, F. Karzai, N. V. Carrabba, R. Atway, N. Terrigino, J. Bright, R. Lake, D. VanderWeele, P. Choyke, P. Pinto, W. L. Dahut, A. G. Sowalsky, B. Turkbey. (2019). Baseline multiparametric MRI characteristics of exceptional pathologic response to neoadjuvant enzalutamide for high-risk, localized prostate cancer. 2019 RSNA Annual Meeting. Chicago, IL.

S. Wilkinson, H. Ye, N. Carrabba, R. Atway, S. Y. Trostel, T. Hennigan, R. Lake, S. Harmon, G. Chun, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2019). Combining genetic and histopathologic features to predict response to anti-androgen therapy in aggressive prostate cancer. 2019 AACR Annual Meeting. Atlanta, GA.

N. T. Terrigino, S. T. Hennigan, S. Trostel, **S. Wilkinson**, H. Ye, A. G. Sowalsky. (2019). Development of an allele-specific assay for detecting circulating tumor DNA prostate cancer. 2019 AACR Annual Meeting. Atlanta, GA.

F. Karzai, R. A. Madan, D. J. VanderWeele, G. Chun, M. Bilusic, L. M. Cordes, N. Carrabba, **S. Wilkinson**, H. Ye, S. Harmon, A. G. Sowalsky, B. Turkbey, P. A. Pinto, P. L. Choyke, J. L. Gulley, W. L. Dahut. (2019). A study of intense neoadjuvant testosterone lowering therapy with goserelin and enzalutamide (Enza) in high-risk prostate cancer (PC) with multiparametric MRI (mpMRI). 2019 GU ASCO Annual Meeting, San Francisco, CA.

A. G. Sowalsky, **S. Wilkinson**, H. Ye, S. Harmon, B. Turkbey, N. Carrabba, R. Atway, G. Chun, D. J. VanderWeele, F. Karzai, R. A. Madan, P. A. Pinto, P. L. Choyke, W. L. Dahut. (2019). Molecular and imaging correlates of exceptional pathologic response to neoadjuvant ADT plus enzalutamide. 2019 GU ASCO Annual Meeting. San Francisco, CA.

S. Wilkinson, H. Ye, N. Carrabba, R. Atway, S. Y. Trostel, S. T. Hennigan, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, W. L. Dahut, K. Kelly, D. J. VanderWeele, A. G. Sowalsky. (2018). Genetic features predictive of response to anti-androgen therapies in aggressive prostate cancer. 2018 ASCB Annual Meeting. San Diego, CA.

S. Wilkinson, H. Ye, N. Carrabba, R. Atway, S. Y. Trostel, S. T. Hennigan, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2018). Genetic features predictive of response to anti-androgen therapies in aggressive prostate cancer. 2018 SBUR Annual Meeting. Palm Springs, CA.

A. Sheahan, K. Morel, D. Burkhart, N. Boufaied, S. Panja, C. Calagua, Y. Huang, H. Ye, S. Trostel, N. Whitlock, **S. Wilkinson**, A. G. Sowalsky, A. Kibel, M. Loda, C. Sweeney, A. Mitrofanova, S. Dougan, D. Labbe, B. Olson, L. Ellis. (2018). Therapeutic targeting of Ezh2 enhances PD-1 blockage by induction of interferon gamma response. 2018 SBUR Annual Meeting. Palm Springs, CA.

S. Wilkinson, H. Ye, N. Carrabba, R. Atway, S. Y. Trostel, S. T. Hennigan, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, D. J. VanderWeele, K. Kelly, W. L. Dahut, A. G. Sowalsky. (2018). Genetic features predictive of response to anti-androgen therapies in aggressive prostate cancer. 25th Annual Prostate Cancer Foundation Scientific Retreat. Carlsbad, CA.

D. J. VanderWeele, B. Turkbey, F. Karzai, S. Harmon, A. G. Sowalsky, H. Ye, **S. Wilkinson**, G. Chun, S. Gold, P. A. Pinto, P. L. Choyke, W. L. Dahut. (2018). Neoadjuvant androgen deprivation therapy and enzalutamide: imaging and pathological responses. 2018 ASCO Annual Meeting. Chicago, IL.

N. Carrabba, **S. Wilkinson**, H. Ye, S. Harmon, B. Turkbey, P. A. Pinto, F. Karzai, W. L. Dahut, D. J. VanderWeele, P. L. Choyke, A. G. Sowalsky. (2018). Genetic and molecular mechanisms in high risk prostate cancer. 18th Annual CCR-FYI Colloquium. Bethesda, MD.

A. G. Sowalsky, **S. Wilkinson**, H. Ye, D. J. VanderWeele, B. Turkbey, S. T. Hennigan, R. Atway, S. Y. Trostel, R. Lake, F. Karzai, S. Harmon, G. Chun, K. Kelly, P. A. Pinto, P. L. Choyke, W. L. Dahut. (2018). Molecular and histopathologic correlates of imaging and biological responses to neoadjuvant GnRH agonist plus enzalutamide for high risk prostate cancer. 2018 GU ASCO Meeting. San Francisco, CA.

D. J. VanderWeele, B. Turkbey, F. Karzai, S. Harmon, A. G. Sowalsky, H. Ye, **S. Wilkinson**, P. A. Pinto, P. L. Choyke, W. L. Dahut. (2018). Neoadjuvant enzalutamide and androgen deprivation therapy for high risk prostate cancer: early results from a feasibility trial. 2018 GU ASCO Meeting. San Francisco, CA.

S. Wilkinson, H. Ye, R. Atway, S. Y. Trostel, N. C. Whitlock, S. T. Hennigan, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, W. L. Dahut, K. Kelly, D. J. VanderWeele, A. G. Sowalsky. (2017). Genomic correlates of imaging response in men receiving intense neoadjuvant androgen deprivation therapy. 2017 ASCB Annual Meeting. Philadelphia, PA.

S. Wilkinson, H. Ye, R. Atway, S. Y. Trostel, N. C. Whitlock, S. T. Hennigan, R. Lake, S. Harmon, B. Turkbey, P. A. Pinto, P. L. Choyke, F. Karzai, W. L. Dahut, K. Kelly, D. J. VanderWeele, A. G. Sowalsky. (2017). Genomic correlates of imaging response in men receiving intense neoadjuvant androgen deprivation therapy. AACR's Prostate Cancer: Advances in Basic, Translational, and Clinical Research. Dec 2017. Orlando, FL.

N. C. Whitlock, R. Atway, **S. Wilkinson**, Q. Yang, H. Ye, A. G. Sowalsky. (2017). Genetic contribution of MYC to the development of primary prostate cancer. AACR's Prostate Cancer: Advances in Basic, Translational, and Clinical Research. Dec 2017. Orlando, FL.

S. Wilkinson, A. I. Marcus. (2016). Cellular polarization and motility during invasion is driven by a combination of LKB1 farnesylation and kinase activity. *Mol. Bio. Cell* 27, pg(311).

R. Commander, Y. Hou, J. Konen, **S. Wilkinson**, M. Gilbert-Ross, L. Cooper, A. I. Marcus. (2016). Defining the invasive biology of clinically relevant LKB1 mutations in lung cancer. *Mol. Bio. Cell* 27, pg (312).

R. Jin, F. Liu, X. Liu, H. Huang, **S. Wilkinson**, D. Zhong, F. Khuri, H. Fu, A. I. Marcus, Y. He, W. Zhou. (2016). The regulation of pre-ribosomal RNA synthesis by LKB1. Proceedings: AACR 107th Annual Meeting. New Orleans, LA.

S. Wilkinson, J. Konen, A. I. Marcus. (2016). LKB1 kinase-dependent and -independent defects disrupt polarity and adhesion signaling to create a uniquely invasive amoeboid cancer cell. 13th Annual Division Student Advisory Council Student Research Symposium. 14 Jan 2016. Atlanta, GA.

E. Summerbell, **S. Wilkinson**, A. I. Marcus. (2016). Farnesylated LKB1 associates with actin to promote stress fiber formation in mesenchymal cells. 13th Annual Division Student Advisory Council Student Research Symposium. 14 Jan 2016. Atlanta, GA.

S. Wilkinson, J. Konen, A. I. Marcus. (2015). LKB1 kinase-dependent and -independent defects disrupt polarity and adhesion signaling to create a uniquely invasive amoeboid cancer cell. *Mol. Bio. Cell* 26, pg(1116).

E. Summerbell, **S. Wilkinson**, A. I. Marcus. (2015). Farnesylated LKB1 associates with actin to promote stress fiber formation in mesenchymal cells. *Mol. Bio. Cell* 26, pg(1515).

S. Wilkinson, J. Konen, A. I. Marcus. (2015). LKB1 kinase-dependent and -independent defects disrupt polarity and adhesion signaling to drive collagen remodeling during invasion. AACR Tumor Metastasis Conference. Austin, TX.

Y. Hou, **S. Wilkinson**, D. Brat, A. I. Marcus, L. Cooper. (2015). Spatiotemporal Visualization of Cell Membrane Dynamics and Protein Colocalization Reveals Correlation between Membrane Dynamics and Metastatic Invasion. The 6th ACM Conference on Bioinformatics, Computational Biology, and Health Informatics. 9 Sept 2015. Atlanta, GA.

J. Konen*, **S. Wilkinson***, A. I. Marcus. (2015). Better together: A dual function of LKB1 in navigating the 3-D microenvironment. 12th Annual Division Student Advisory Council Student Research Symposium. 15 Jan 2015. Atlanta, GA.

*These authors contributed equally to this work.

S. Wilkinson, A. I. Marcus. (2015). Farnesylation regulates a molecular switch between distinct tumor suppressive functions of LKB1. 12th Annual Division Student Advisory Council Student Research Symposium. 15 Jan 2015. Atlanta, GA.

S. Wilkinson, A. I. Marcus. (2014). Farnesylated LKB1 regulates cell polarization and invasion through a 3D collagen microenvironment in a kinase-independent manner. *Mol. Bio. Cell* 25, pg(2055).

S. Wilkinson*, J. Konen*, A. I. Marcus. (2014). Twice as nice: the dual tumor suppressive function of LKB1 in regulating cell polarization and invasion. Winship Scientific Research and Academic Development Symposium. 23 Oct 2014. Atlanta, GA.

* These authors contributed equally to this work.

S. Wilkinson, A. I. Marcus. Farnesylated LKB1 associates with actin to regulate cell polarity and motility. Emory University STEM Research and Career Symposium. 27 March 2014. Atlanta, GA.

S. Wilkinson, A. I. Marcus. LKB1 regulates cell polarity and motility in a farnesylation-dependent manner. 11th Annual Division Student Advisory Council Student Research Symposium. 10 Jan 2014. Atlanta, GA.

S. Wilkinson, A. I. Marcus (2013). Post-translational modification of LKB1 regulates its actin association in a kinase-independent manner. *Mol Bio Cell* 24(suppl), Abstract No. 1167.

R. Ryan Lash, **Scott Wilkinson**, Marguerite Madden, William L. Nicholson. Transforming analog rickettsial disease data into a digital geospatial database. 2011 ESRI Health GIS Conference. 9 Sept 2011. Arlington, VA.

Scott C Wilkinson, R Ryan Lash, William L Nicholson. Creation of a legacy database on Rickettsial pathogens and diseases found in Africa. 2010. 24th Annual Meeting of the American Society for Rickettsiology. 2010 August 1-4. Stevenson, WA.

S. Wilkinson, A. J. Lepore, J. Isenhower, C. Mowry. (2009). Determination of prey eaten by coyotes in Yellowstone National Park. Symposium on Student Scholarship. Berry College. Mount Berry, GA.

PRESENTATIONS

Scott Wilkinson. LKB1: Controlling the clutch that drives invasion. Elkin Seminar Series. Emory University. 16 Nov 2015. Atlanta, GA.

Scott Wilkinson. Twice as nice: the dual tumor suppressive functions of LKB1 in regulating cell polarization and invasion. Tuesday Morning Seminar Series. Berry College. 7 April 2015. Mount Berry, GA.

Scott Wilkinson. Dual functions of LKB1 in regulating cell polarity during invasion. Integrated Cellular Imaging Data Club. Emory University. 2 April 2015. Atlanta, GA.

Scott Wilkinson. Dual functions of LKB1 in regulating cell polarization and invasion. Elkin Seminar Series. Emory University. 16 Feb 2015. Atlanta, GA.

Scott Wilkinson. Farnesylated LKB1 associates with actin to regulate cell polarity. Elkin Seminar Series. Emory University. 31 Jan 2014. Atlanta, GA.

Scott Wilkinson. LKB1 Farnesylation Regulates its Actin Association. Elkin Seminar Series. Emory University. 22 Feb 2013. Atlanta, GA.

Scott Wilkinson. Possible Interactions Between Bcl-2 Family Proteins. Elkin Seminar Series. Emory University. 2 March 2012. Atlanta, GA.

Scott Wilkinson. Targeting Vimentin Overexpression in Metastatic Cancer. Elkin Seminar Series. Emory University. 2 Dec 2011. Atlanta, GA.

Scott Wilkinson. Characterization of the BAI1 Promoter Region and Regulatory Elements in Mouse and Human DNA. Elkin Seminar Series. Emory University. 2 Sept 2011. Atlanta, GA.

Scott Wilkinson. Recent CDC Investigations into Rocky Mountain Spotted Fever in Arizona. Tuesday Morning Seminar Series. Berry College. 19 Oct 2010. Mount Berry, GA