original reports abstract

Genomic Biomarkers and Genome-Wide Loss-of-Heterozygosity Scores in Metastatic Prostate Cancer Following Progression on Androgen-Targeting Therapies

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PURPOSE To study the impact of standard-of-care hormonal therapies on metastatic prostate cancer (mPC) clinical genomic profiles in real-world practice, with a focus on homologous recombination-repair (HRR) genes.

PATIENTS AND METHODS Targeted next-generation sequencing of 1,302 patients with mPC was pursued using the FoundationOne or FoundationOne CDx assays. Longitudinal clinical data for correlative analysis were curated via technology-enabled abstraction of electronic health records. Genomic biomarkers, including individual gene aberrations and genome-wide loss-of-heterozygosity (gLOH) scores, were compared according to biopsy location and time of sample acquisition (androgen deprivation therapy [ADT]-naïve, ADT-progression and post-ADT, and novel hormonal therapies [NHT]-progression), using chi-square and Wilcoxon rank-sum tests. Multivariable analysis used linear regression. False-discovery rate of 0.05 was applied to account for multiple comparisons.

RESULTS Eight hundred forty (65%), 132 (10%), and 330 (25%) biopsies were ADT-naïve, ADT-progression, and NHT-progression, respectively. Later-stage samples were enriched for *AR*, *MYC*, *TP53*, *PTEN*, and *RB1* aberrations (all adjusted *P* values $< .05$), but prevalence of HRR-related *BRCA2*, *ATM*, and *CDK12* aberrations remained stable. Primary and metastatic ADT-naïve biopsies presented similar prevalence of *TP53* (36% *v* 31%) and *BRCA2* (8% *v* 7%) aberrations; 81% of ADT-naïve *BRCA2*-mutated samples presented *BRCA2* biallelic loss. Higher gLOH scores were independently associated with HRR genes (*BRCA2*, *PALB2*, and *FANCA*), *TP53*, and *RB1* aberrations, and with prior exposure to hormonal therapies in multivariable analysis.

CONCLUSION Prevalence of HRR-gene aberrations remains stable along mPC progression, supporting the use of diagnostic biopsies to guide poly (ADP-ribose) polymerase inhibitor treatment in metastatic castration-resistant prostate cancer. gLOH scores increase with emerging resistance to hormonal therapies, independently of individual HRR gene mutations.

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INTRODUCTION

Metastatic prostate cancer (mPC) is a lethal disease with pronounced genomic heterogeneity between patients.[1-4] The recent approval of poly (ADP-ribose) polymerase (PARP) inhibitors[5-7] for mPC with mutations in homologous-recombination repair (HRR) genes, together with other genomic aberrations showing potential value to guide treatment decisions,[8-11] has accelerated the incorporation of genomic testing into clinical practice, and guidelines now recommend offering tumor genomic profiling to patients with mPC.[12-14]

The inclusion of genomic biomarkers into mPC clinical management is still challenged by inequalities in access to testing and unresolved questions about optimal sources of tumor material. Also, the impact of tumor evolution and therapy-induced selective pressure on clinically relevant biomarkers is not well established.[11,15-17] A key question is whether biopsies collected at the time of diagnosis can inform biomarker-driven clinical decisions at later stages, after resistance to androgen deprivation (ADT) and novel hormonal therapies (NHT). Obtaining repeated metastatic biopsies to capture the evolving genomic landscape is often unfeasible, especially outside academic centers. Indeed, most of the evidence endorsing the adoption of molecular profiling has been generated from rigorously selected patient populations

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To study how prostate cancer genomic profiles change upon drug resistance, and particularly to study the impact of genomic evolution in the identification of candidates for poly (ADP-ribose) polymerase inhibitor treatment using a clinical next-generation sequencing assay.

Knowledge Generated

The prevalence of homologous recombination-repair (HRR) gene alterations is stable across different disease states, before/after hormonal therapy. Contrarily, biomarkers such as *AR* and *MYC* amplifications, or *TP53* and *RB1* loss are enriched after hormone therapy resistance. We observed that genome-wide loss-of-heterozygosity, a marker of genomic instability, independently associates with the presence of HRR gene alterations, *TP53*/*RB1* loss and resistance to hormonal therapies.

Relevance

Our data support the use of archival, untreated diagnostic prostate cancer samples for next-generation sequencing testing in clinical practice toward identification of patients with HRR defects for poly (ADP-ribose) polymerase inhibitor therapy indication. Other precision medicine strategies driven by biomarkers such as *AR*, *TP53*, or *RB1* may be in need of contemporaneous samples for accurate patient stratification.

from a relatively small number of academic institutions. Data from more diverse, real-world, patient sets would facilitate the delivery of precision medicine to patients with prostate cancer.

The use of PARP inhibitors for mPC with selected HRR gene mutations such as *BRCA1/2* represents a prime example of molecularly guided mPC management. However, clinical outcomes of patients with different tumor HRR-associated gene mutations, and even among those with mutations in the same gene, are heterogeneous[6,7,18]; thus, there is a need to refine biomarkers of HRR deficiency beyond individual gene mutations. One candidate biomarker is the fraction of the genome affected by loss-of-heterozygosity (gLOH), a measure of how much of the genome is affected by irreversible loss-of-allele events, reflective of genomic instability.[19] *BRCA1/2* mutations exhibit higher gLOH scores in different cancer types,[20] and high gLOH scores associate with PARP inhibitor sensitivity in ovarian cancer.[21] Yet, gLOH levels are lower overall in prostate cancer compared with ovarian cancer, even in *BRCA1/2* defective tumors.[20,22,23] Hence, independent evaluation of gLOH in prostate cancer should be pursued.

In this study, we leverage correlative clinicogenomic data from a large cohort of patients with mPC who underwent tumor genomic profiling as part of routine clinical care, mostly in community clinics. We hypothesized that the prevalence of *BRCA2* and other HRR-gene aberrations would be similar, independently of prior androgen-targeted treatment exposure or sampled disease site (primary or metastatic). We also aimed to analyze clinicogenomic variables in relation to gLOH scores in early versus later stages of lethal prostate cancer progression.

PATIENTS AND METHODS

Study Population

All patients with confirmed metastatic (de novo or recurrent) and/or castration-resistant prostate cancer presentation included in the US-wide Flatiron Health-Foundation Medicine deidentified real-world clinicogenomic database between January 2011 and April 2021, with available genomic profiling data on primary or metastatic tumor tissue specimens. All tissue samples were obtained between March 2002 and December 2020. Specimens were sequenced from November 2013 to December 2020. Follow-up clinical data were collected until April 2021.

Next-Generation Sequencing

A hybrid capture-based next-generation sequencing (NGS) assay was performed on tumor tissue biopsies in a Clinical Laboratory Improvement Amendments–certified laboratory and College of American Pathologists–accredited laboratory (Foundation Medicine, Cambridge, MA). FoundationOne or FoundationOne CDx assays interrogated exons from 315 to 324 cancer-related genes (depending on panel version), plus select introns from at least 28 genes frequently rearranged in cancer. At least 50 ng of DNA per specimen was isolated and sequenced to high, uniform coverage (mean, $>$ 600×) as previously described.[24] Samples were evaluated for aberrations including single-nucleotide substitutions, indels (short variants, denoted as sv), copy-number alterations (amplifications or homozygous deletions, denoted as amp or as del), and other select gene fusions/rearrangements. Only deep deletions, those modeled to translate homozygous deletions taking into consideration sample purity and ploidy, were reported; shallow deletions, predicted to translate single copy losses,

were only considered toward calling zygosity of mutations. Amplifications were defined as ≥ 6 copies. For tumor suppressor genes, all mutations predicted to result in truncation (frameshift mutations, canonical splice-site alterations, and nonsense mutations), rearrangements, or deep deletions were considered pathogenic, together with selected known pathogenic missense mutations. For HRR-related tumor suppressor genes, prediction of zygosity status and biallelic gene loss was calculated as described in prior studies.[21]

Genomic aberrations per gene, and per pathway, were annotated for each patient (Data Supplement). Tumor mutational burden was determined on 1.1 Mb of sequenced DNA.[25] gLOH was defined as the percentage of the genome demonstrating loss-of-heterozygosity using previously validated pipelines and excluding whole-arm and whole-chromosome events.[26] gLOH assessment was pursued in those samples passing copy-number alteration–based quality-control metrics (signal to noise ratio) and tumor purity ≤ 30%.

Clinical Data, Patient Eligibility, and Classification

Deidentified baseline and longitudinal follow-up clinical data from patients treated at approximately 280 cancer clinics (approximately 800 sites of care) in the United States were retrospectively captured from electronic health records, comprising patient-level structured and unstructured data, using technology-enabled abstraction of clinical notes and radiology/pathology reports. Clinical data captured included demographics, disease extent, therapy exposure (with start and stop dates for each therapy line), and survival outcomes. Clinical data were linked to genomics data by deidentified, deterministic matching.

Cases were classified into one of three categories, on the basis of the clinical state at the time when the tumor biopsy used for genomic profiling was acquired: (1) ADT-naïve: tumor biopsy or radical prostatectomy specimens collected before ADT exposure; (2) ADT-progression/CRPC: tumor specimen obtained within 90 days before or 30 days after diagnosis of CRPC per clinical notes, with no prior exposure to NHT; and (3) NHT-progression (NHT-CRPC): tumor specimen collected after progression to treatment with NHT in the CRPC setting. To minimize confounders, we discarded any sample obtained within 30 days of initiating NHT (Data Supplement).

Objectives and Statistical Analysis Plan

The primary objective was to compare the prevalence of clinically relevant genomic biomarkers in primary and metastatic tissue biopsies across the mPC spectrum and therapy exposure, with emphasis on HRR-associated genes. The secondary objective was to examine clinical and genomic variables associated with gLOH scores. Overall survival was calculated from start of systemic treatment to death from any cause. gLOH was assessed as a continuous variable (percentage of the genome affected), as prior studies demonstrated that the distribution of gLOH scores in prostate cancer differs significantly from other tumor types in which dichotomic thresholds were defined.[20]

Chi-square tests and Wilcoxon rank sum tests were used to assess differences between groups of categorical and continuous variables, respectively. Adjustment for multiple testing with a false-discovery rate at 0.05 (Benjamini and Hochberg method) was performed. For the secondary objective related to gLOH scores, we first pursued a univariable linear regression. Next, we pursued a multivariable analysis for those biomarkers with > 5 events in the study population using a least absolute shrinkage and selection operator (LASSO) regression[27] to exclude those gene alterations with a null impact in the model. Time to event end points was assessed using the Kaplan-Meier method and the log-rank test. Cox proportional-hazard models were used to obtain hazard ratios with 95% CIs. We relaxed the linearity assumption for gLOH using restricted cubic splines using the *rms* R package. The threshold for significance was set at 0.05 (two-sided) after adjustment. R v3.6.3 software program was used for all analyses.

This study was conducted according to the ethical principles for medical research described in the Declaration of Helsinki. Institutional review board approval of the study protocol and this analysis was obtained before study conduct and included a waiver of informed consent.

RESULTS

Sample and Patient Characteristics

We identified 1,847 cases with confirmed mPC who had been entered in the clinicogenomics integrated database between 2011 and 2021. After clinical data curation, 1,302 cases with follow-up clinical and treatment data available were retained for this analysis. Of those, genomic profiling had been pursued in tissue samples acquired either before any systemic therapy (ADT-naïve group, n = 840; 65%), after development of castration-resistance but before any NHT exposure (ADT-progression or CRPC group, n = 132; 10%), or after exposure to ≥ 1 NHT (NHT-CRPC group, n = 330; 25%; Fig 1). Five hundred seventeen (40%) cases were sequenced using the FoundationOne panel, and 785 (60%) using the later FoundationOne CDx panel. The median sequencing depth was 794× (interquartile range [IQR], 648-895), with no significant differences among samples in each of the three groups (median depth of 787×, 807×, and 805× for the ADT-naïve, ADT-progression, and NHT-exposed, respectively; see the Data Supplement for tumor content details).

The majority of samples in the ADT-naïve group were prostate primary tumor biopsies or radical prostatectomy specimens (674/840, 80%, *v* 20% of lymph nodes or other metastatic site biopsies), whereas in the post-NHT groups, biopsies were predominantly from lymph nodes (28%), liver (25%), or bone (16%) metastases, representing usual patterns of biopsy acquisition in real-world clinical practice. Patient and sample characteristics, as well as prior history

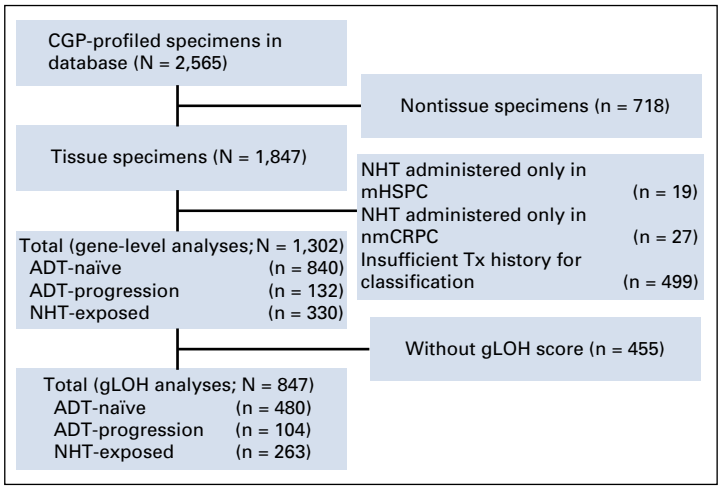

FIG 1. CONSORT diagram of the study population and specimens included in the analysis by clinical state of acquisition. ADT, androgen deprivation therapy; CGP, comprehensive genomic profiling; gLOH, genome-wide loss-of-heterozygosity; mHSPC, metastatic hormone sensitive prostate cancer; NHT, novel hormonal therapy; nmCRPC, non-metastatic castration resistant prostate cancer; Tx, treatment.

of treatment exposure before the specimen acquisition are detailed in Table 1.

Figure 2 depicts the genomic landscape of the study cohort. Overall, 33% cases presented *TMPRSS2* rearrangements. The prevalence of *TP53* and *RB1* aberrations was 41% and 5%, respectively. 33% cases showed *PTEN* loss-of-function alterations (truncating mutations or homozygous deletions) and 6% presented amplifications of activating mutations in *PIK3CA*. Among HRR-associated genes, 9% patients had deleterious *BRCA2* aberrations, compared with 1.4% for *BRCA1*, 1% for *PALB2*, and 6% for *ATM*. Pathogenic mutations in *CDK12* were detected in 7% of the overall population. Aberrations in MMR genes were infrequent (1.8% *MSH2*; 1.4% *MSH6*).

Associations Between Genomic Aberrations and Disease States

A significant enrichment in more advanced disease states (from ADT-naïve to CRPC and to NHT-CRPC) was observed for aberrations in *AR* (0.1% *v* 28% *v* 40%), *MYC* (5.5% *v* 18.2% *v* 20%), *TP53* (35.4% *v* 47.7% *v* 51.5%), *PTEN* (27.6% *v* 38.6% *v* 36.4%), and *RB1* (3.8% *v* 5.3% *v* 7.9%; all adjusted *P* values $< .05$; Fig 3A and 3B, Data Supplement). Combined inactivation of at least two among *TP53, PTEN*, and *RB1* was also identified more frequently in later disease states: 12% ADT-naïve, 21% CRPC, and 22% NHT-CRPC ($P < .001$).

Conversely, the prevalence of pathogenic events in *BRCA2*, *ATM*, and *CDK12* were similar among disease states (all adjusted *P* values $> .3$), and overall prevalence of HRR pathway aberrations was 17% ADT-naïve versus 17% CRPC and 21% NHT-CRPC cases. We next looked at whether second *BRCA2* allele loss could be detected in archival diagnostic samples. Biallelic loss was predicted in 58/72 (81%) of the ADT-naïve samples with *BRCA2* deleterious mutations; in 5/72 (7%), there was no evidence of biallelic loss by NGS, whereas in 9/72 (12%), the bioinformatics algorithm could not confidently call zygosity. Similar trends were observed in the CRPC and NHT-CRPC groups, with 10/12 (83%) and 19/28 (68%) of *BRCA2* mutated cases, respectively, showing evidence of biallelic loss by targeted NGS (Data Supplement). As observed in other series, the percentage of *BRCA1*-mutated cases predicted to harbor biallelic loss was lower (3/18; 17%).

The percentage of cases with tumor mutational burden $\geq$ 10 mutations/Mb was higher in the post-NHT-CRPC setting compared with the earlier settings (8.5% *v* 4.5% CRPC *v* 2.3% ADT-naïve; $P < .001$).

Genomic Landscape of ADT-Naïve, Primary Versus Metastatic Biopsies

Next, we focused on the ADT-naïve biopsy cohort (n = 840) to compare the genomic profile of primary prostate tumor (n = 674) versus metastatic (n = 166) biopsies. No significant differences were observed in primary versus metastasis in the prevalence of aberrations in *TP53* (36.4% *v* 31.3%), *PTEN* (26.3% *v* 33.1%), *BRCA2* (8.2% *v* 6.6%), or *MYC* (5% *v* 7.2%; all adjusted *P* values $> .3$; Fig 3C, Data Supplement). Considering pathway analyses, HRR-associated gene aberrations were also evenly distributed in primary versus metastatic ADT-naïve specimens (16% *v* 18%).

gLOH Score in Metastatic Prostate Cancer

A total of 847 specimens were evaluable for gLOH (65% of the overall study population; Data Supplement). The median gLOH score was 8.2% (IQR, 5.6%-11.5%). Later disease states were enriched for higher gLOH values: median 7.24% ADT-naïve (IQR, 4.92-10.1), 8.92% CRPC (5.99-12.5), and 9.94% NHT-CRPC (7.14-13.6; $P < .001$).

Higher gLOH score was associated with poor prognosis in the ADT-naïve group. Risks of CRPC progression or death were incrementally greater with increases in gLOH scores up to 10%, then reaching a plateau, whereas the association with the risk of death followed a near-linear increase (Data Supplement). If gLOH values were dichotomized on the basis of the median, higher gLOH values related to higher risk of progression to CRPC (hazard ratio = 1.26; 95% CI, 1.01 to 1.57; $P = .03$).

Next, we studied associations between gLOH scores and clinical and genomic features. In univariable analysis, aberrations in *BRCA2* significantly associated with higher gLOH values (adjusted $P < .001$). Higher gLOH scores by means of more advanced disease states were observed for both *BRCA2*-aberrant and wild-type patient subgroups (Fig 4).

Beyond DDR genes, we also identified significant positive associations between gLOH score and aberrations in *TP53*

TABLE 1. Clinical Characteristics of the Study Population

Variable	ADT-Naïve (n = 840)	ADT-Progression (n = 132)	NHT-Exposed (n = 330)	Total (N = 1,302)	*P*
Specimen site					< .001
Bladder	13 (1.5)	16 (12.1)	18 (5.5)	47 (3.6)	
Bone	52 (6.2)	17 (12.9)	53 (16.1)	122 (9.4)	
Liver	9 (1.1)	27 (20.5)	83 (25.2)	119 (9.1)	
Lymph node	57 (6.8)	25 (18.9)	91 (27.6)	173 (13.3)	
Other	35 (4.2)	26 (19.7)	67 (20.3)	128 (9.8)	
Prostate	674 (80.2)	21 (15.9)	18 (5.5)	713 (54.8)	
Specimen collection to NGS, months					< .001
Median (Q1, Q3)	18.8 (3.8, 40.2)	2.1 (1.0, 10.2)	1.1 (0.8, 1.9)	6.9 (1.4, 27.1)	
Practice type					< .001
Academic	260 (31.0)	16 (12.1)	36 (10.9)	312 (24.0)	
Community	580 (69.0)	116 (87.9)	294 (89.1)	990 (76.0)	
Race					.026
AA	66 (7.9)	4 (3.0)	32 (9.7)	102 (7.8)	
Asian	13 (1.5)	2 (1.5)	5 (1.5)	20 (1.5)	
Other	108 (12.9)	22 (16.7)	61 (18.5)	191 (14.7)	
Unknown	51 (6.1)	3 (2.3)	12 (3.6)	66 (5.1)	
White	602 (71.7)	101 (76.5)	220 (66.7)	923 (70.9)	
PSA at specimen collection					< .001
Median (Q1, Q3)	74.8 (20.3, 308.9)	14.6 (4.2, 85.8)	51.5 (10.4, 196.3)	60.0 (15.6, 246.2)	
Missing	92	9	82	183	
Histology					< .001
Adenocarcinoma	811 (96.5)	124 (93.9)	291 (88.2)	1,226 (94.2)	
Other, mixed histology or not specified	29 (3.5)	8 (6.1)	39 (11.8)	76 (5.8)	
Therapy exposure before specimen acquisition					
Prior ADT exposure	0	132 (100)	330 (100)		
Time to CRPC, months					
Median (Q1, Q3)	NA	41.6 (13.1, 101.1)	43.3 (15.8, 99.1)		
Prior NHT exposure	0	0	330 (100)		
Time on NHT, months					
Median (Q1, Q3)	NA	NA	13.6 (7.4, 26.5)		
Prior taxane exposure	0	40 (30.3)	168 (50.9)		
Prior platinum exposure	0	6 (4.5)	14 (4.2)		
Prior Radium 223 exposure	0	3 (2.3)	44 (13.3)		

Abbreviations: AA, African American; ADT, androgen deprivation therapy; CRPC, castration-resistant prostate cancer; NA, not available; NGS, next-generation sequencing; NHT, novel hormonal therapy; PSA, prostate-specific antigen.

($P < .001$), *RB1* ($P < .001$), *AR* ($P < .001$), and *PTEN* ($P = .01$) in univariate analysis. Conversely, significant inverse associations were identified between gLOH scores and mutations in *CDK12*, *CTNNB1*, and *MSH2* (Data Supplement). We explored the features of those samples with higher gLOH scores; a threshold of gLOH = 13.6% defined the top quartile in our study population. Among those patients, and after excluding patients with canonical *BRCA1/2* alterations, *TP53* and *MYC* amplifications were significantly enriched (*P*-adjusted .001 for both).

To confirm these findings, we performed a multivariable analysis (MVA) including the clinical and genomic variables of interest (from the total gene list, we excluded those with ≤ 5 events and those showing high multicollinearity or null

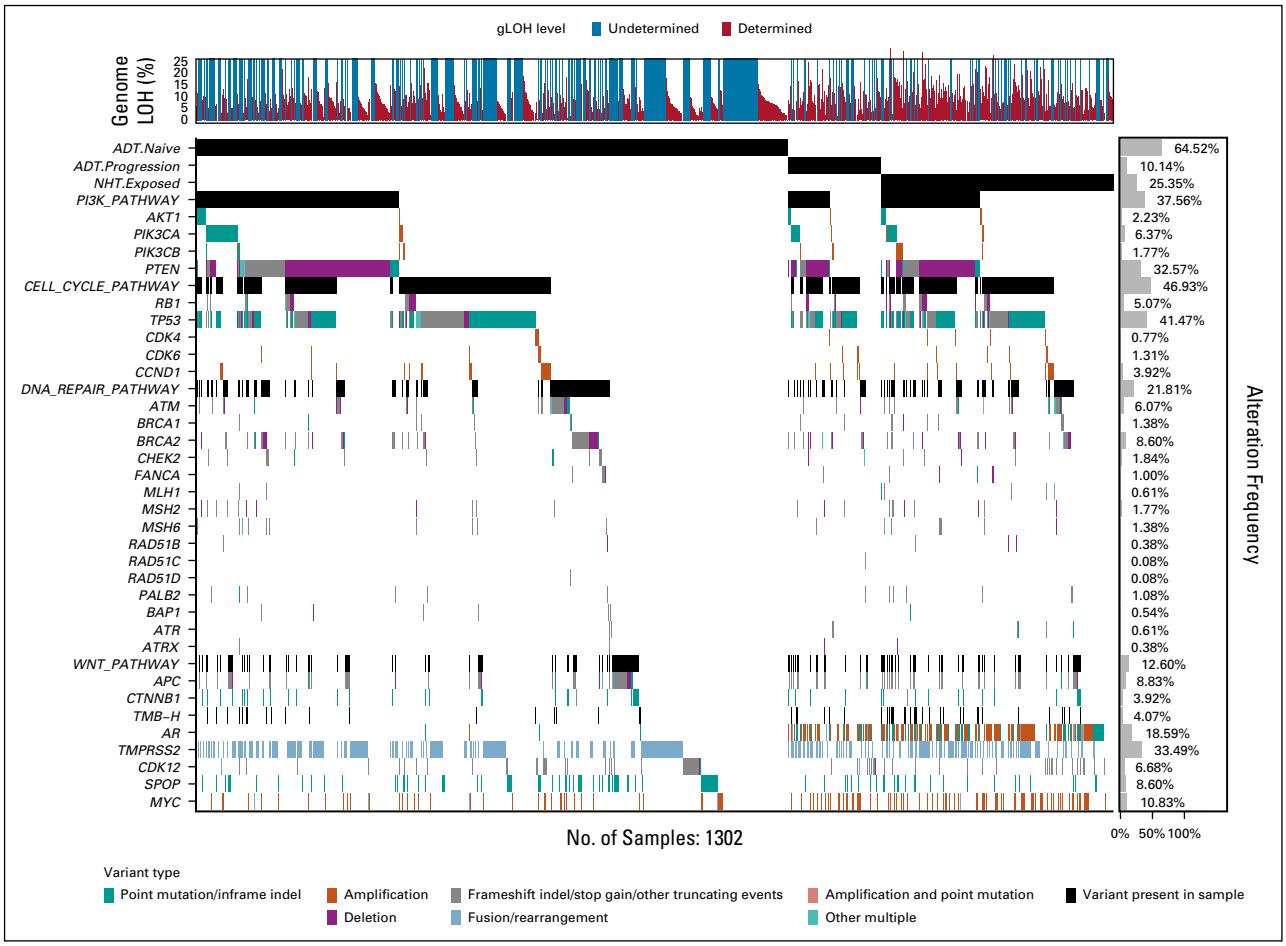

FIG 2. OncoPrint summarizing the genomic landscape of the study cohort. Cases are sorted by disease states. The legend depicts the color code for different alteration types. For copy-number alterations, only deep/homozygous deletions and amplifications (≥ six copies) are included in the OncoPrint; shallow/single copy deletions or mild copy gains (< six copies) events are not represented. The top row shows gLOH scores (as the percentage of genome affected by LOH) per patient. gLOH, genome-wide loss-of-heterozygosity; indel, insertion/deletion.

association with gLOH scores on a LASSO regression analysis). In the MVA, prior hormonal therapy and, particularly, having previously received and progressed to both ADT and NHT were independently associated with higher gLOH scores. By contrast, prior taxane exposure, or the primary versus metastatic origin of the tumor specimen, did not affect gLOH scores. Among the genes included in the MVA, aberrations in *BRCA2*, *FANCA*, *PALB2*, *TP53,* and *RB1* also showed significant independent associations with increased gLOH scores. *CDK12*, *CTNNB1*, and *MSH2* mutations, and *TMPRSS2-ERG* fusions, independently associated with lower gLOH scores (Fig 5). AR aberrations no longer associated with gLOH when considering prior treatment exposure in the MVA.

A sensitivity analysis was performed independently in the subsets of primary and metastatic samples, obtaining similar results in each subgroup (Data Supplement), further suggesting that it is treatment-driven pressure, rather than differences between anatomical locations, which drives the enrichment for higher gLOH scores.

DISCUSSION

Integrating genomics and clinical data is central to the development and clinical implementation of precision cancer medicine strategies.[28] In this study, we leveraged a large 'real-world' cohort of clinically and genomically annotated mPC cases to assess how disease progression and androgen-targeted therapy–induced selective pressure may affect the evolving genomic landscape of lethal prostate cancer.

Importantly, all patients in our study including those who were ADT-naïve at the time of biopsy acquisition had confirmed progression to late-stage mPC. Prior comparisons of the genomic landscape studies of primary versus metastatic prostate tumors could be confounded by the inclusion of patients with localized prostate cancer who never developed recurrence.[4] We identified no significant differences in the prevalence of key gene aberrations between primary and metastasis in ADT-naïve samples, suggesting that both might be similarly

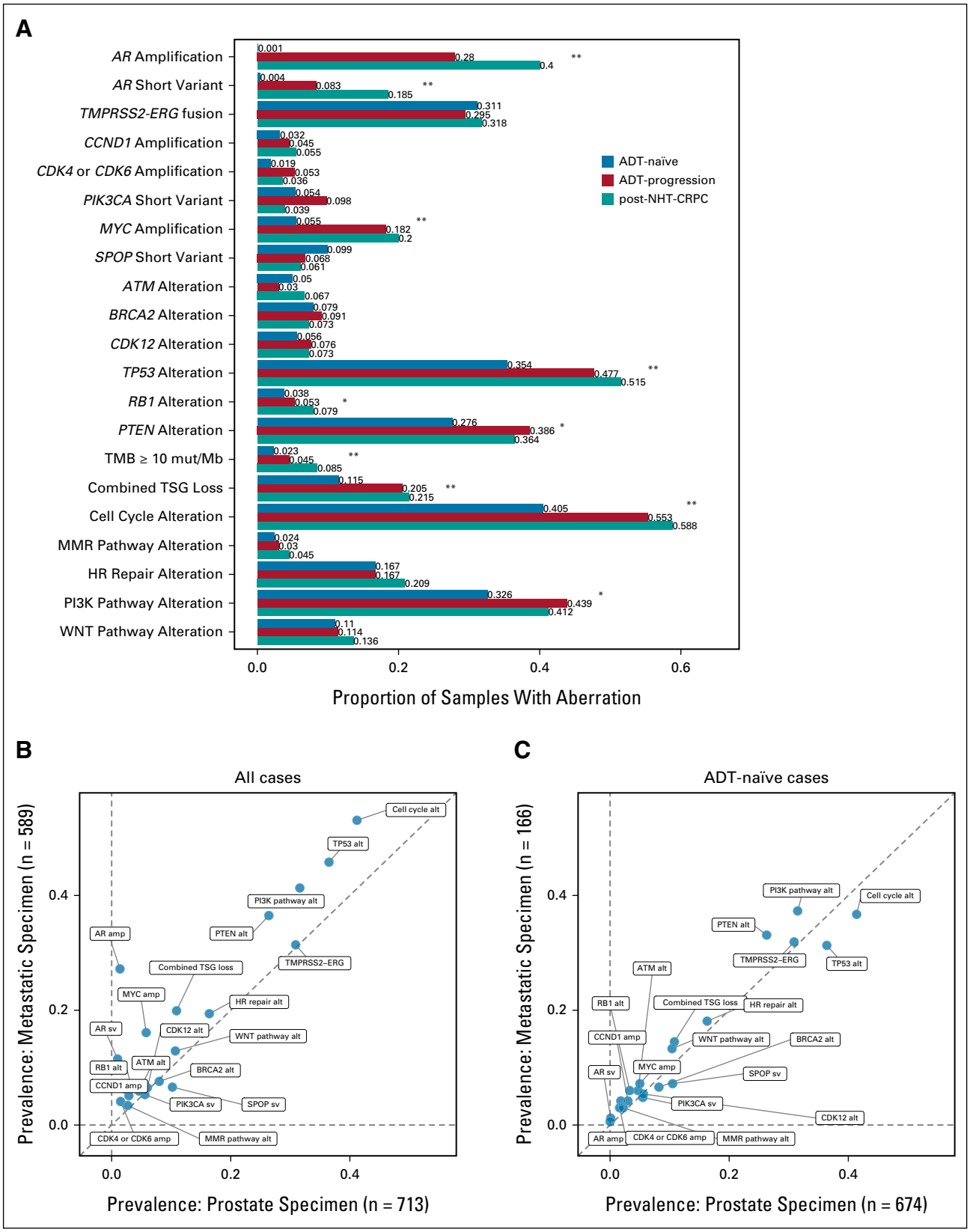

FIG 3. (A) Prevalence of clinically relevant genomic aberrations in the study population by clinical state at the time of sample acquisition (blue: ADT-naïve, n = 840; red: ADT-progression, n = 132; teal: post-NHT-CRPC, n = 330). Prevalence figures by site of biopsy (prostate *v* metastasis) in (B) all cases and in (C) ADT-naïve cases. $*P < .05$; $**P < .001$. ADT, androgen deprivation therapy; CRPC, castration-resistant prostate cancer; NHT, novel hormonal therapy.

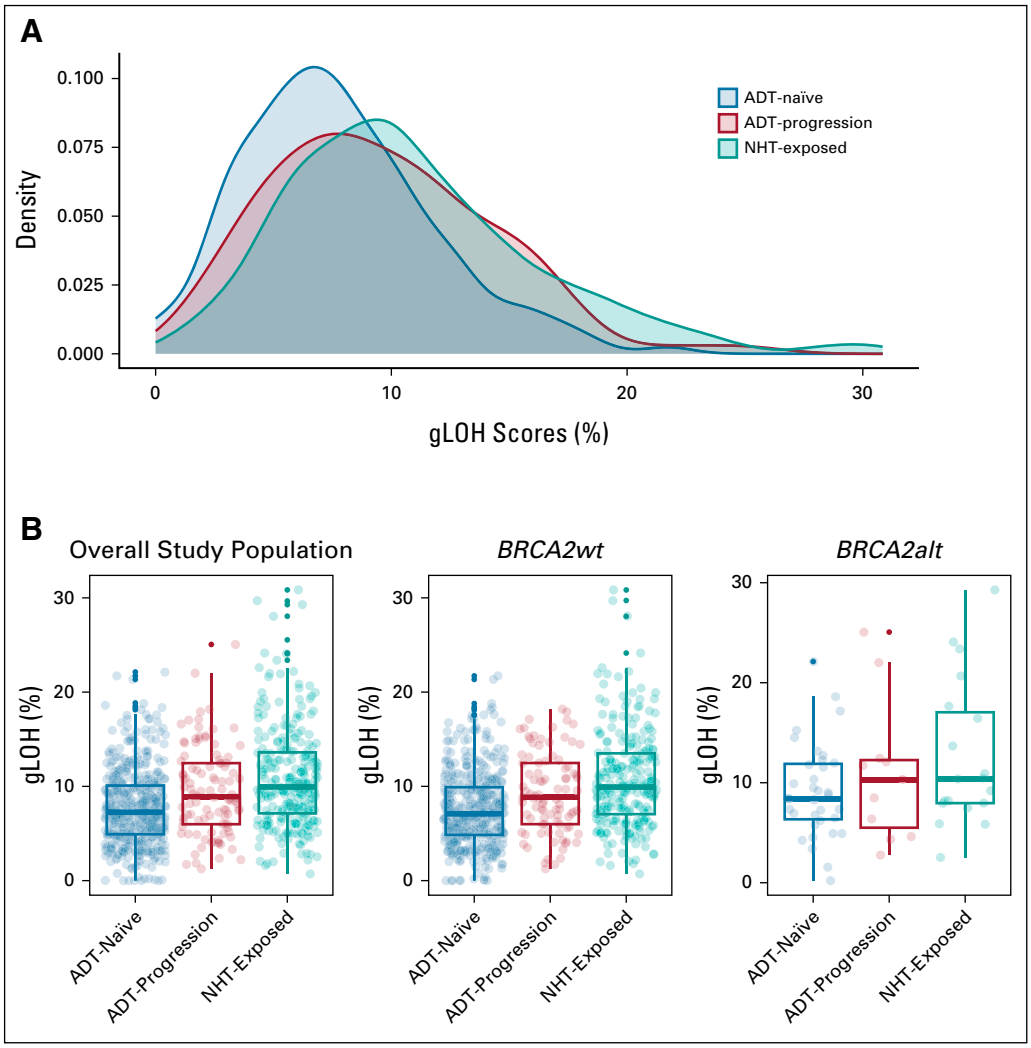

FIG 4. gLOH distribution by clinical state: (A) histogram of gLOH scores in the overall study population, by disease states at the time of sample acquisition, and (B) boxplots comparing gLOH scores. ADT, androgen deprivation therapy; gLOH, genome-wide loss-of-heterozygosity; NHT, novel hormonal therapy.

informative in newly diagnosed mPC patients, a population for whom genomic testing is now recommended by clinical guidelines.[12,29]

We found enrichment of aberrations in *AR*, *MYC*, tumor suppressor genes such as *TP53* and *RB1*, and of cell cycle and PI3K/PTEN pathway genes, from ADT-naïve to CRPC and NHT-CRPC disease, highlighting their relevance to lethal prostate cancer progression and drug-resistance.[30-32] Conversely, the prevalence of aberrations in *BRCA2* and other HRR genes remained stable along disease progression. Even if some of these HRR gene mutations were of germline origin, we also observed that loss of the second allele in the tumor was detected similarly in pretreatment and postresistance biopsies.

Our study lacks longitudinal same-patient biopsies collected over time, as repeated genomic profiling is not commonly obtained in routine clinical practice; we compared samples from different patients. We and others have recently reported small series of patient-matched tumor tissue biopsies[15,33] or correlative tumor-plasma paired samples[34] that also suggest that HRR mutations are present in early stages of mPC progression. Our study complements this evidence with a much larger cohort, albeit with indirect comparisons. Together, these data support the use of diagnostic, archival tissue biopsies to stratify patients with metastatic castration-resistant prostate cancer for PARP inhibitor treatment on the basis of HRR gene status, regardless of later exposures to subsequent lines of therapy.[6,7,35] Contrarily, assessment of *AR* and cell-cycle–related genomic biomarkers may require contemporaneous specimens.

Patterns of genome-wide aberrations such as gLOH may help toward further clinical qualification of less common

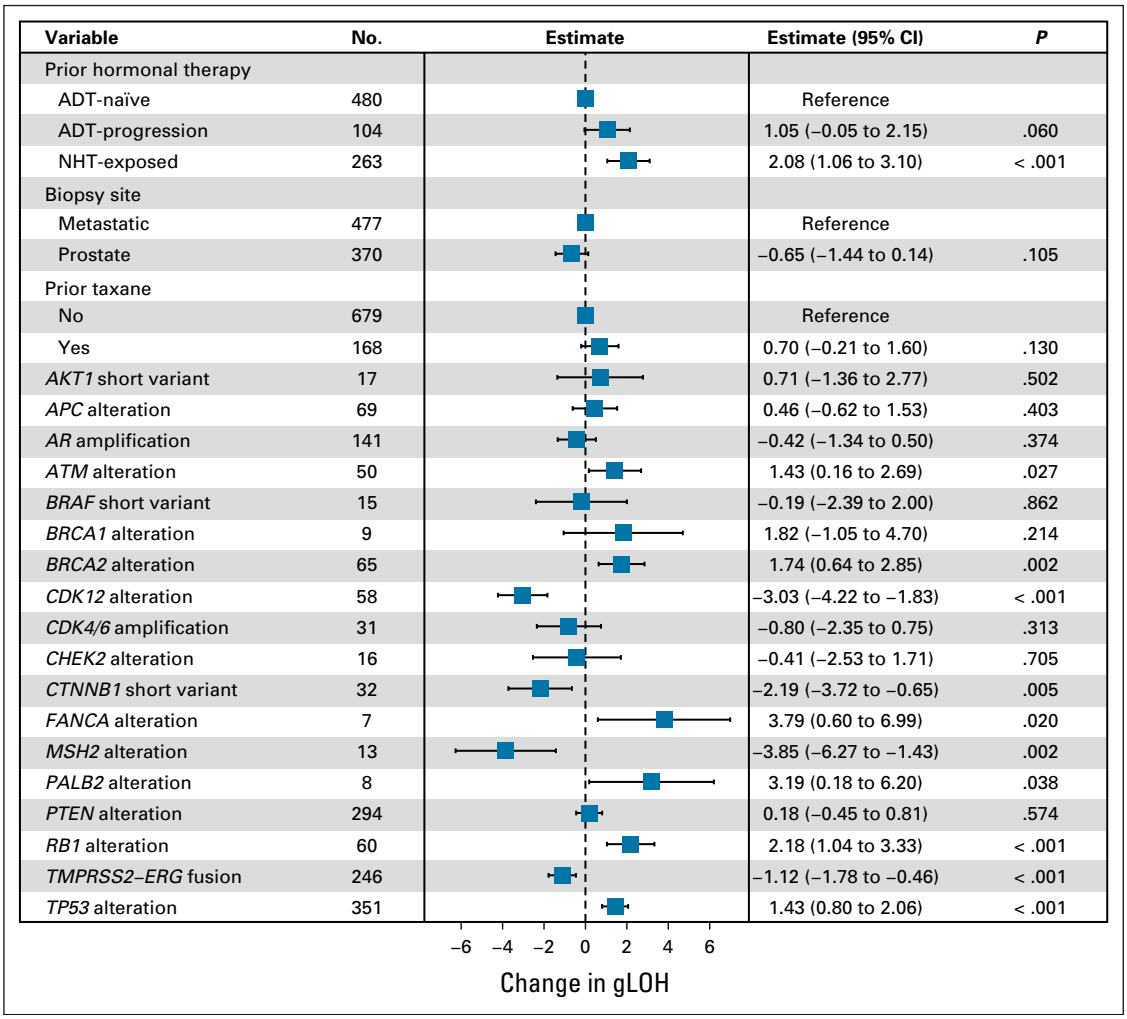

FIG 5. Clinical features and genomic alterations associated with gLOH. A multivariable model was constructed to evaluate the associations of clinical and genomic variables with gLOH. Point estimates and confidence intervals indicate the average difference in gLOH independently associated with each feature. For genomic aberrations, the reference is wild-type. The threshold for significance was established at adjusted $P < .05$ (two-sided). The dashed line represents a null association; squares (and CI) to the right represent a direct association of the variable with higher gLOH, whereas squares (and CI) to the left indicate an inverse association between the variable and gLOH levels. ADT, androgen deprivation therapy; gLOH, genome-wide loss-of-heterozygosity; NHT, novel hormonal therapy.

HRR gene aberrations beyond *BRCA2* mutations. We found that progression to hormonal therapy, but not to taxanes, associates with increasing gLOH scores independent of specific HRR gene aberrations or subtypes; and that *TP53* or *RB1* loss, which is also more prevalent in later disease stages, independently contributes to increased gLOH scores. This increase with mPC hormonal resistance possibly reflects positive selection of genomically unstable subclones, in line with the increased level of large-scale transition (LST) events, another candidate marker of genomic instability, shown in liquid biopsies in later stages of mPC.[36,37] On the basis of these findings, clinical trials evaluating PARP inhibitor as monotherapy or in combination in prostate cancer should take into consideration the time of biopsy acquisition and the *TP53* and *RB1* mutational status when analyzing the predictive value of gLOH scores.

We acknowledge that the retrospective nature of our study prevented us from controlling for potential confounding factors, although access to correlative clinical annotations allowed us to adjust gene-specific analyses to distinct clinical states. Moreover, different from previous studies, our cohort was formed by patients receiving treatment mostly in community practices and not in academic centers, including a wide range of institutions not involved in clinical trials. As genomic testing becomes part of the standard prostate cancer patient journey, real-world data sets including patients under-represented in research studies (ie, those with comorbidities, poor performance status, elderly, or minorities[38] on the basis of geographical,

ethnicity, or socioeconomic factors) will become critical to inform strategies for delivering precision medicine in diverse clinical settings.

In conclusion, prevalence of mutations in *BRCA2* and other HRR-associated genes is stable along mPC progression, supporting the use of diagnostic tumor biopsies for mPC patient stratification for PARP inhibitor treatment in clinical practice after progression to NHT. Progression to androgen-targeted therapies is linked to enrichment in *AR*, *MYC*, *TP53*, *PTEN*, *RB1*, and PI3K/PTEN pathway aberrations, as well as in genomic instability as per increasing gLOH scores, independent of *BRCA1/2* status.

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