original report abstract

Prospective Comprehensive Genomic Profiling of Primary and Metastatic Prostate Tumors

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PURPOSE Comprehensive genomic profiling (CGP) is increasingly used for routine clinical management of prostate cancer. To inform targeted treatment strategies, 3,476 clinically advanced prostate tumors were analyzed by CGP for genomic alterations (GAs) and signatures of genomic instability.

METHODS Prostate cancer samples (1,660 primary site and 1,816 metastatic site tumors from unmatched patients) were prospectively analyzed by CGP (FoundationOne Assay; Foundation Medicine, Cambridge, MA) for GAs and genomic signatures (genome-wide loss of heterozygosity [gLOH], microsatellite instability [MSI] status, tumor mutational burden [TMB]).

RESULTS Frequently altered genes were *TP53* (44%), *PTEN* (32%), *TMPRSS2-ERG* (31%), and *AR* (23%). Potentially targetable GAs were frequently identified in DNA repair, phosphatidylinositol 3-kinase, and RAS/RAF/MEK pathways. DNA repair pathway GAs included homologous recombination repair (23%), Fanconi anemia (5%), *CDK12* (6%), and mismatch repair (4%) GAs. *BRCA1/2*, *ATR*, and *FANCA* GAs were associated with high gLOH, whereas *CDK12*-altered tumors were infrequently gLOH high. Median TMB was low (2.6 mutations/Mb). A subset of cases (3%) had high TMB, of which 71% also had high MSI. Metastatic site tumors were enriched for the 11q13 amplicon (*CCND1/FGF19/FGF4/FGF3*) and GAs in *AR*, *LYN*, *MYC*, *NCOR1*, *PIK3CB*, and *RB1* compared with primary tumors.

CONCLUSION Routine clinical CGP in the real-world setting identified GAs that are investigational biomarkers for targeted therapies in 57% of cases. gLOH and MSI/TMB signatures could further inform selection of poly (ADP-ribose) polymerase inhibitors and immunotherapies, respectively. Correlation of DNA repair GAs with gLOH identified genes associated with homologous recombination repair deficiency. GAs enriched in metastatic site tumors suggest therapeutic strategies for metastatic prostate cancer. Lack of clinical outcome correlation was a limitation of this study.

INTRODUCTION

Genomic alterations (GAs) that drive prostate cancer have been elucidated by profiling primary tumors.[1,2] Comprehensive genomic profiling (CGP) is increasingly used for routine clinical management of patients with prostate cancer,[3] with accumulating evidence associating GAs with responses to therapy. In clinical trials, candidate genomic biomarkers include *BRCA1/BRCA2/ATM* for poly (ADP-ribose) polymerase (PARP) inhibitors,[4] *PTEN/AKT* for AKT inhibitors,[5,6] and *PIK3CB* for phosphatidylinositol 3-kinase (PI3K)-β inhibitors.[7] Responses to immunotherapy have been associated with *CDK12* GAs,[8] *POLE* GAs,[9] and tumor mutational burden-high (TMB-H)[9] or microsatellite instability-high (MSI-H) genomic signatures.[10]

GAs that are associated with castration resistance and metastatic progression have been identified by comparing primary versus metastatic tumors,[2,11-13] including an analysis of 1,013 prostate tumors from seven independent whole-exome sequencing studies[2] and longitudinal genomic profiling.[14,15] To date, the largest study of primary versus metastatic tumors using a single targeted sequencing assay included 200 primary and 304 metastatic tumors.[3] To refine further the genomics of prostate cancer in the real-world setting and to inform rational therapy selection and drug development, we assessed GAs and genomic signatures from routine prospective CGP on 1,660 primary and 1,816 metastatic site tumors from unmatched patients.

METHODS

Consecutive CGP results were reported for 3,476 unique patients with prostate cancer by prospective sequencing (median coverage, 743×) of tissue

ASSOCIATED CONTENT

Appendix

Data Supplement

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

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CONTEXT

Key Objective

In this study, we use real-world data from routine prospective clinical genomic profiling to evaluate genomic alterations (GAs) and genomic signatures in primary and metastatic prostate cancer.

Knowledge Generated

We evaluated the landscape of GAs in prostate cancer and identified those that are enriched in metastatic site tumors. Approximately 3% of cases had high microsatellite instability and/or high tumor mutational burden status. Specific DNA damage response alterations were associated with genome-wide loss of heterozygosity.

Relevance

Fifty-seven percent of cases harbored a GA associated with targeted therapy approaches. GAs enriched in metastatic site tumors suggest therapeutic strategies for metastatic prostate cancer. Genomic signatures, including microsatellite instability, tumor mutational burden, and genome-wide loss of heterozygosity, may further refine biomarker development for poly (ADP-ribose) polymerase inhibitors and immunotherapies.

samples using a validated assay[16] (FoundationOne; Foundation Medicine, Cambridge, MA; Appendix Table A1). For patients with multiple samples, the sample with the highest sequencing quality metrics was included. Age and site of specimen collection were abstracted from accompanying pathology reports, clinical notes, and requisition forms. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin–stained slides. Results were analyzed for GAs and gene signatures (TMB, MSI, genome-wide loss of heterozygosity [gLOH]). Germline/somatic mutation calls were predicted without a matched normal; in validation testing of 480 tumor-only sequencing calls against matched normal samples, accuracy was 95% for somatic and 99% for germline calls.[17] Enrichment was defined as the difference in GA frequency between metastatic and primary sites. Potentially targetable GAs were defined by European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets criteria.[18] The Appendix provides additional details on the methods used in this study.

RESULTS

Patient Characteristics

CGP was performed in the course of routine clinical care on tissue samples from 3,476 unique patients with prostate cancer (median age, 66 years; range, 34 to 94 years), including 1,660 samples from the prostate primary site and 1,816 samples from metastatic sites of unmatched patients (Appendix Fig A1).

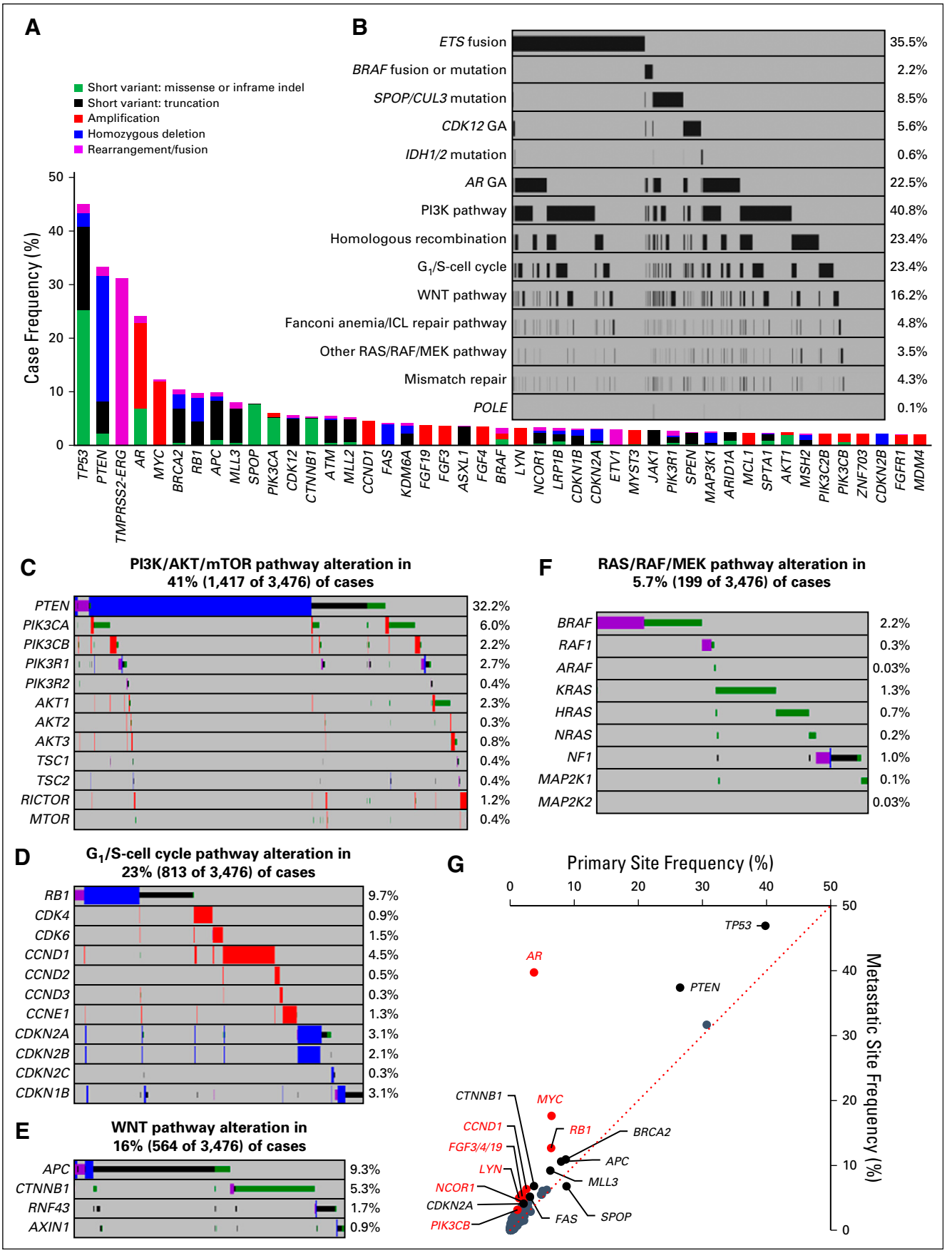
GAs in Primary and Metastatic Site Tumors

Overall, there was an average of 4.5 GAs per tumor (primary, 3.5 GAs; metastatic, 5.5 GAs). Frequently altered genes were *TP53* (43.5%), *PTEN* (32.2%), *TMPRSS2-ERG* (31.2%), *AR* (22.5%), *MYC* (12.3%), *BRCA2* (9.8%), *RB1* (9.7%), *APC* (9.3%), *MLL3/KMT2C* (7.8%), *SPOP* (7.7%), *PIK3CA* (6.0%), and *CDK12* (5.6%; Fig 1A). *ETS* fusions were observed in 35.5% of cases. Activating *BRAF* or *RAF1* fusions/rearrangements were observed in 1.2% of cases (35 *BRAF* and seven *RAF1*; Appendix Fig A2).

The PI3K/AKT/mammalian target of rapamycin (mTOR) pathway was frequently altered (40.8%; Figs 1B and 1C). As expected, *PTEN* GAs were frequent, and we observed activating mutations and amplifications in *PIK3CA*, *PIK3CB*, *AKT1/2/3*, *MTOR*, and *RICTOR* and loss-of-function alterations in *PIK3R1/2* and *TSC1/2*. The G_1/S-cell cycle pathway was altered in 23.4% of cases, most frequently *RB1* loss-of-function alterations (9.7%), as well as copy number alterations (CNAs) in *CDK4/6*, *CCND1/2/3*, *CCNE1*, *CDKN2A/B/C*, and *CDKN1B* (Figs 1B and 1D). The WNT pathway was altered in 16.2% of cases (Figs 1B and 1E). RAS/RAF/MEK pathway alterations (5.7%; Figs 1B and 1F) included oncogenic *BRAF* mutations (Appendix Fig A2), *BRAF*/*RAF1* rearrangements, activating mutations in *RAF1/ARAF*, *KRAS*/*NRAS*/*HRAS* and *MAP2K1/2*, and *NF1* loss-of-function alterations. Homologous recombination repair (HRR)–related pathway alterations were observed in 23.4% of cases (Fig 1B), and other DNA repair pathway alterations included Fanconi anemia/interstrand crosslink repair (FA/ICL) genes (4.8%), *CDK12* (5.6%), mismatch repair (MMR) genes (4.3%), and the DNA polymerase gene *POLE* (0.1%; Fig 1B).

Overall, 57% of cases harbored GAs that are investigational biomarkers for targeted therapies with varying levels of supporting clinical evidence[18] (Appendix Table A2), including candidate biomarkers for targeted therapies in advanced phases of development in prostate cancer (eg, *PTEN*, *AKT1*, *BRCA1/2*, *ATM*) and those that have been successfully targeted in other tumor types, such as activating *BRAF* and *ERBB2* GAs.

Distinct genomic subsets of prostate cancer have been described, including those defined by *ETS* fusions, *SPOP* mutations, *IDH1* mutations,[1] and *CDK12* GAs.[8] Consistent with distinct subsets, *ETS* fusions were mutually exclusive with *SPOP*/*CUL3* GAs ($P < .001$; Fisher's exact test, two-tailed),


A
Short variant: missense or inframe indel
Short variant: truncation
Amplification
Homozygous deletion
Rearrangement/fusion
Case Frequency (%)
50
40
30
20
10
0
TP53
PTEN
TMPRSS2-ERG
AR
MYC
BRCA2
RB1
APC
MLL3
SPOP
PIK3CA
CDK12
CTNNB1
ATM
MLL2
CCND1
FAS
KDM6A
FGF19
FGF3
ASXL1
FGF4
BRAF
LYN
NCOR1
LRP1B
CDKN1B
CDKN2A
ETV1
MYST3
JAK1
PIK3R1
SPEN
MAP3K1
ARID1A
MCL1
SPTA1
AKT1
MSH2
PIK3C2B
PIK3CB
ZNF703
CDKN2B
FGFR1
MDM4
B
ETS fusion 35.5%
BRAF fusion or mutation 2.2%
SPOP/CUL3 mutation 8.5%
CDK12 GA 5.6%
IDH1/2 mutation 0.6%
AR GA 22.5%
PI3K pathway 40.8%
Homologous recombination 23.4%
G1/S-cell cycle 23.4%
WNT pathway 16.2%
Fanconi anemia/ICL repair pathway 4.8%
Other RAS/RAF/MEK pathway 3.5%
Mismatch repair 4.3%
POLE 0.1%
C
PI3K/AKT/mTOR pathway alteration in 41% (1,417 of 3,476) of cases
PTEN 32.2%
PIK3CA 6.0%
PIK3CB 2.2%
PIK3R1 2.7%
PIK3R2 0.4%
AKT1 2.3%
AKT2 0.3%
AKT3 0.8%
TSC1 0.4%
TSC2 0.4%
RICTOR 1.2%
MTOR 0.4%
F
RAS/RAF/MEK pathway alteration in 5.7% (199 of 3,476) of cases
BRAF 2.2%
RAF1 0.3%
ARAF 0.03%
KRAS 1.3%
HRAS 0.7%
NRAS 0.2%
NF1 1.0%
MAP2K1 0.1%
MAP2K2 0.03%
D
G1/S-cell cycle pathway alteration in 23% (813 of 3,476) of cases
RB1 9.7%
CDK4 0.9%
CDK6 1.5%
CCND1 4.5%
CCND2 0.5%
CCND3 0.3%
CCNE1 1.3%
CDKN2A 3.1%
CDKN2B 2.1%
CDKN2C 0.3%
CDKN1B 3.1%
E
WNT pathway alteration in 16% (564 of 3,476) of cases
APC 9.3%
CTNNB1 5.3%
RNF43 1.7%
AXIN1 0.9%
G
Primary Site Frequency (%)
0
10
20
30
40
50
Metastatic Site Frequency (%)
TP53
AR
PTEN
CTNNB1
MYC
CCND1
RB1
BRCA2
FGF3/4/19
APC
LYN
MLL3
NCOR1
CDKN2A
FAS
SPOP
PIK3CB

CDK12 GA ($P < .001$), and *BRAF* rearrangements/mutations ($P < .001$; Fig 1B).

To compare primary and metastatic site tumors, we assessed relative enrichment in GAs (Fig 1G; Appendix Table A3). GAs enriched by 2% or more in metastatic site tumors included *AR* (36.0% enrichment), *MYC* (11.2%), *PTEN* (10.9%), *TP53* (7.1%), *RB1* (6.3%), the 11q13 amplicon (*CCND1* [3.8%], *FGF19* [3.7%], *FGF3* [3.3%], *FGF4* [3.3%]), *LYN* (3.6%), *CTNNB1* (3.0%), *MLL3* (2.9%), *APC* (2.6%), *NCOR1* (2.3%), *BRCA2* (2.2%), *FAS* (2.1%), *PIK3CB* (2.0%), and *CDKN2A* (2.0%; all $P < .05$). Of these, 10 were enriched in metastatic site tumors by twofold or more (all $P < .001$), including *AR* (10.6-fold), *LYN* (3.6-fold), 11q13 (*CCND1* [2.5-fold], *FGF19* [3.0-fold], *FGF3* [2.8-fold], *FGF4* [2.9-fold]), *MYC* (2.7-fold), *NCOR1* (2.1-fold), *PIK3CB* (2.7-fold), and *RB1* (2.0-fold overall, 1.8-fold for homozygous deletions, 2.5-fold for mutations). The fraction of tumor suppressor gene mutations predicted to result in biallelic inactivation was not higher in primary site tumors, which suggests that metastatic enrichments were functionally relevant (Appendix Fig A3A). Collectively, G_1/S-cell cycle genes were altered in 30.7% of metastatic site versus 15.4% of primary site tumors (Appendix Fig A3B). The only gene enriched in primary site tumors was *SPOP* (2.0%; 1.3-fold enrichment; $P = .03$).

DNA Repair Pathway GAs

HRR and FA/ICL pathway GAs have been associated with responses to PARP inhibitors in prostate cancer,[4] and *CDK12* has been implicated in HRR in preclinical studies but also has distinct functions associated with DNA replication-related repair.[19,20] Collectively, these genes were altered in 31.0% of cases (Figs 1B and 2A), and genes altered in more than 1% of cases included *BRCA2* (9.8%), *CDK12* (5.6%), *ATM* (5.2%), *CHEK2* (1.8%), *BRCA1* (1.4%), *FANCA* (1.3%), and *ATR* (1.1%). MMR pathway GAs[10] or *POLE* V411L,[9] which have been associated with increased TMB and responses to immunotherapy, were observed in 4.3% and 0.1% of cases, respectively (Fig 2B).

Germline/somatic status predictions[17] were made to estimate the prevalence of germline DNA repair mutations; 933 of 1,261 of DNA repair mutations (74%) yielded an available germline/somatic call of which 35.7% were germline (Fig 2C). Predicted germline mutations were identified in 57.8% of *BRCA2*-, 25.0% of *BRCA1*-, 35.8% of *ATM*-, 80.0% of *CHEK2*-, 52.2% of *FANCA*-, 42.3% of *MSH2*-, 20.0% of *MSH6*-, 25.0% of *MLH1*-, and 44.4% of *PMS2*-mutated cases; only 9.2% of *CDK12*-mutated cases harbored a predicted germline mutation (Fig 2C).

Genomic Signatures: gLOH

In addition to GAs in individual DNA repair genes, genomic signatures represent the phenotypic readout of deleterious DNA repair and are potential predictive biomarkers. For example, gLOH is a measure of homologous recombination deficiency (HRD) and is associated with PARP inhibitor clinical benefit in *BRCA1/2* wild-type ovarian cancer.[21,22] We assessed the association between genomic signatures and DNA repair GAs.

Percent gLOH was assessable for 2,624 cases, and the median gLOH score was 8.5% (interquartile range, 5.8%-12.2%). Overall, 447 of 2,624 cases (17%) were gLOH high (gLOH-H; Fig 2D), of which 35.9% harbored an HRR or FA/ICL pathway GA (Appendix Fig A4A). We evaluated the association between gLOH and DNA repair GAs (Fig 2D). Consistent with the established relationship between *BRCA1/2* and HRD,[21] *BRCA1/2*-altered cases (both predicted germline and somatic mutations) were more frequently gLOH-H compared with the overall data set (Fig 2D).

To evaluate the potential relevance of non-*BRCA1/2* DNA repair genes as biomarkers for PARP inhibition, we assessed their association with gLOH. gLOH-H frequency was comparable between the overall data set and cases with non-*BRCA1/2* DNA repair GAs, although cases with homozygous deletions in non-*BRCA1/2* HRR pathway or FA/ICL pathway genes were more frequently gLOH-H (Appendix Fig A4B). While considering each gene individually, cases with *ATR* or *FANCA* GA (particularly *FANCA* homozygous deletion) were more frequently gLOH-H, whereas *CDK12*- or *NBN*-altered cases were significantly less frequently gLOH-H (Fig 2D).

Genomic Signatures: TMB and MSI

MSI-H and TMB-H genomic signatures are biomarkers of sensitivity to immunotherapies[23,24]; therefore, we evaluated the association between MSI/TMB and MMR/polymerase

FIG 1. Genomic characterization of primary and metastatic site prostate tumors. (A) The frequency of genomic alterations (GAs) identified in tumors from 3,476 patients with prostate cancer. Genes altered in 2% or more of cases are shown. (B) Frequency of major pathway alterations, including *ETS* fusions, *BRAF* rearrangements/mutations, *SPOP/CUL3* mutations, *CDK12* GAs, *IDH1/2* mutations, *AR* GAs, phosphatidylinositol 3-kinase (PI3K) pathway GAs, homologous recombination GAs, G_1/S-cell cycle GAs, WNT pathway GAs, Fanconi anemia/interstrand crosslink repair (FA/ICL) repair pathway GAs, RAS/RAF/MEK pathway GAs (other than *BRAF*), mismatch repair GAs, and *POLE* mutations (see Figs 1C to 1F for details). Each column represents a single patient sample, and samples that harbored an alteration in each pathway are indicated in black. (C to F) GAs identified in each pathway, including the (C) PI3K/AKT/mammalian target of rapamycin (mTOR) pathway, (D) G_1/S-cell cycle pathway, (E) WNT pathway, and (F) RAS/RAF/MEK pathway (*NF1* mutation, rearrangement, or copy number loss; *BRAF* or *RAF1* mutations or rearrangements; *ARAF, K/N/H-RAS*, or *MAP2K1/2* mutations were included); percent of altered cases is indicated. (G) Comparison of alteration frequencies for each gene in primary site samples versus metastatic site samples. The dotted line represents a 1:1 relationship. Genes that were enriched (difference between primary site *v* metastatic site frequency) by at least 2% are indicated (all $P < .05$ by Fisher's exact test [two-tailed]), with genes enriched at least twofold indicated in red (all $P < .001$ by Fisher's exact test [two-tailed]). Details are listed in Appendix Table A3. indel, insertion/deletion.

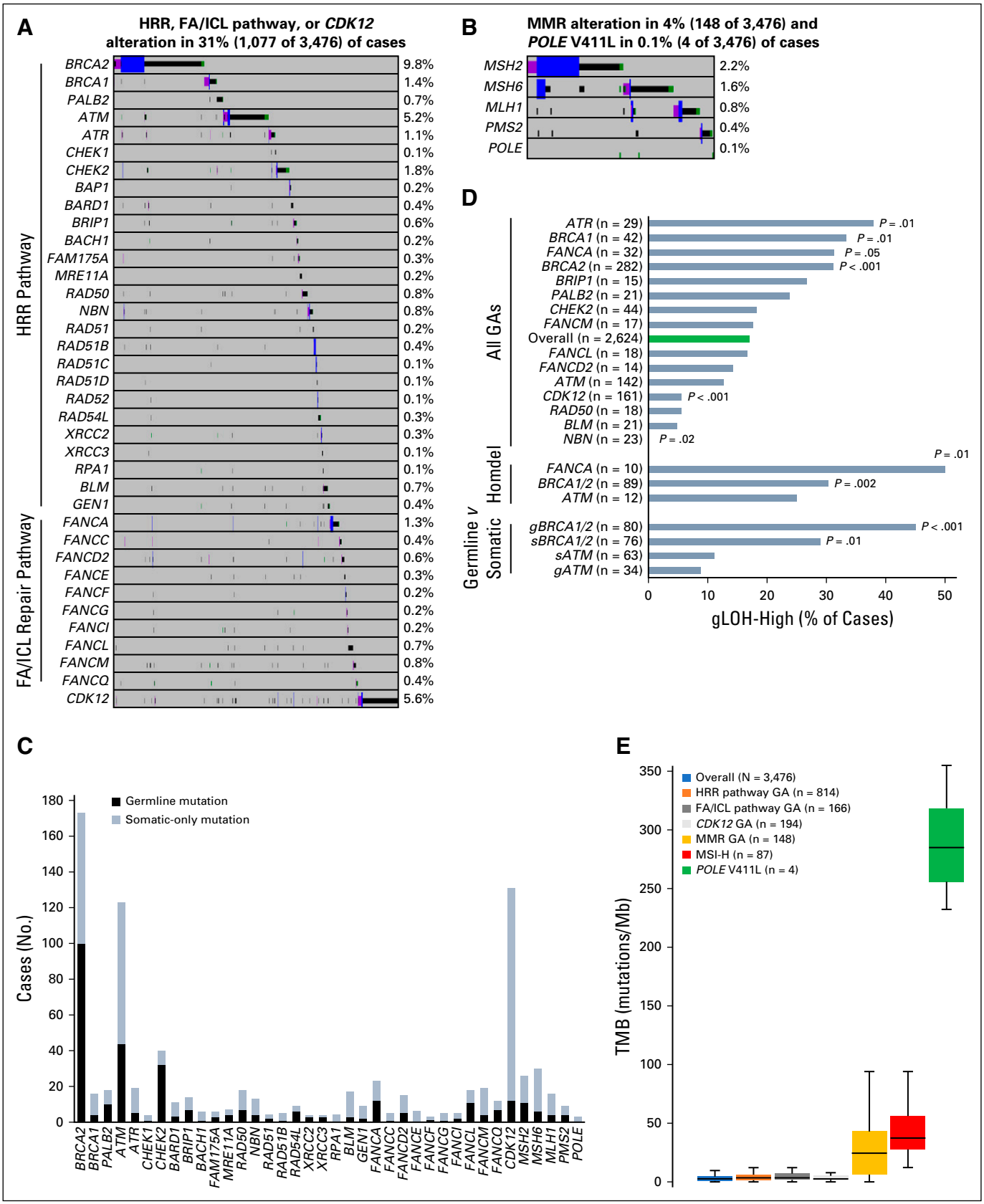


FIG 2. DNA repair genomic alterations (GAs) and association with genomic signatures. GAs identified in the (A) homologous recombination repair (HRR) pathway, Fanconi anemia/interstrand crosslink repair (FA/ICL) repair pathway, and *CDK12* or (B) mismatch repair (MMR; *MSH2*, *MSH6*, *MLH1*, *PMS2*) or

pathway GAs. MSI status was assessable for 3,326 cases, and overall, 87 of 3,326 of cases (2.6%) were MSI-H (2.0% primary site, 3.1% metastatic site tumors). MSI-H status significantly co-occurred with MMR GA, with 78.2% MSI-H cases also harboring a GA in the MMR pathway. For cases with an MMR GA, 48.6% were MSI-H; for the subset of MMR mutations resulting in biallelic inactivation, 69.5% were MSI-H (Appendix Fig A5A).

Deleterious MMR or *POLE* GAs can cause an accumulation of mutations that can be quantitatively measured by TMB. Median TMB was low overall (2.6 mutations/Mb) and low for primary site and metastatic site tumors; a subset of cases (3.3%) was TMB-H (≥ 20 mutations/Mb), including 2.5% of primary site and 4.0% of metastatic site tumors (Appendix Fig A5B). At a lower TMB threshold,[25] 5.1% of cases overall had 10 mutations/Mb or more (Appendix Fig A5B). TMB was significantly increased for cases with MMR GA (median, 24.4 mutations/Mb) and MSI-H (median, 37.4 mutations/Mb); median TMB for *POLE* V411L–mutated cases was 285 mutations/Mb (Fig 2E). As expected, median TMB was low for cases with GA in other DNA repair pathways (Fig 2E). For cases with an HRR GA, the frequency of TMB-H was higher (9.3%) than the overall data set (3.3%); however, homozygous deletions in HRR pathway genes and gLOH-H score were not associated with TMB-H, which suggests that HRD is likely not causal for the TMB-H phenotype.

For 111 TMB-H cases with assessable MSI status, the TMB-H phenotype was explained by concurrent MSI-H status for 71.2% of cases and by *POLE* V411L mutation for 3.6% of cases (Appendix Fig A5C). For the remaining cases, TMB-H phenotype was not attributed to MSI-H or *POLE*.

Landscape of *ETS* Fusions and *AR* GAs

In total, 1,236 *ETS* fusions were detected (one sample harbored two *ETS* fusions; Fig 1B). *TMPRSS2-ERG* comprised the majority (87.7%) of *ETS* fusions (Fig 3A), with the remaining consisting of *ETV1* (8.5%), *ETV4* (2.6%), and *ETV5* (1.2%) with diverse fusion partners, including several not previously described (Fig 3B). Consistent with previous studies, breakpoints were most frequently in *ERG* intron 3[26] (Fig 3C). We also identified breakpoints that juxtaposed *TMPRSS2* with the intergenic region upstream of *ERG* (0.1 to 75 kb upstream; Fig 3C); similar upstream intergenic breakpoints were observed for *TMPRSS2* fusions with *ETV4* (10 of 32 cases) and *ETV5* (two of 15 cases).

AR GAs are associated with castration-resistant disease and were most enriched in metastatic site tumors (39.7% of metastatic site and 3.7% of primary site tumors; Fig 1G). In total, 905 *AR* GAs were observed in 783 cases (557 CNAs, 303 mutations, and 45 rearrangements; Fig 4A). CNAs were observed in 16.0% of cases (2.4% primary site and 28.5% metastatic site). Five hundred fifty-five of 557 CNAs were amplifications (median copy number, 24; range, six to 366); two homozygous deletions that encompass exons 5 to 7 or exons 5 to 8 are likely activating.[27] *AR* missense mutations were observed in 6.9% of cases (1.3% primary site and 11.9% metastatic site), and most were ligand-binding domain antiandrogen resistance mutations (Fig 4B).[28] Finally, *AR* rearrangements were observed in 1.3% of cases (0.4% primary site and 2.2% metastatic site); however, because the sequencing strategy was not explicitly designed to detect *AR* rearrangements and does not fully cover *AR* intronic regions, the true frequency of *AR* rearrangements is difficult to establish. The 45 rearrangements included 18 duplications, 17 deletions, six inversions, and four translocations that were predicted to result in a truncated *AR* gene retaining exons 1 to 3 that encode the DNA binding domain (Fig 4C); such alterations are likely activating.[27,29]

DISCUSSION

CGP of 3,476 prostate tumors identified frequent GAs in the PI3K, cell cycle, HRR, and WNT pathways and diverse GAs that are investigational biomarkers for targeted therapies in 57% of cases (Appendix Table A2). GAs that co-occur with targetable GAs (Fig 1B) and their impact on response to therapy warrant consideration in biomarker-driven trials. MSI-H and TMB-H have been associated with immunotherapy benefit,[10,24,25] and gLOH-H has been associated with PARP inhibitor benefit[21]; therefore, assessment of signatures of genomic instability potentially expands the population of patients addressable with immunotherapy or targeted therapy.

We identified a subset of *TMPRSS2* rearrangements fused to upstream intergenic regions of *ERG*, *ETV4*, or *ETV5* as well as novel *ETS* fusion partners. However, the assay is

FIG 2. (Continued). polymerase (*POLE*) V411L genes. (C) Mutations in DNA repair genes were assessed for germline or somatic status. For each gene, the number of cases with a germline mutation or somatic-only mutation is shown. (D) Genome-wide loss of heterozygosity (gLOH) score was evaluated for the overall data set (blue) and for association with each DNA repair gene altered at 0.5% or greater frequency. The frequency of cases with a gLOH-high score for each subset is shown. The term "all GAs" represents the association with any reportable GA (short variant mutation, homozygous deletion or rearrangement) in the specified gene. Homozygous deletions (homdel) were individually assessed for *BRCA1/2*, *ATM*, and *FANCA*. Germline (g) and somatic (s) mutations were individually assessed for *BRCA1/2* and *ATM*. Each subset was individually compared with the overall data set, and unadjusted *P* values are shown (Fisher's exact test [two-tailed]). (E) Tumor mutational burden (TMB) was evaluated for the overall data set and compared with various genomic subsets, including those harboring an HRR pathway GA, an FA/ICL pathway GA, a *CDK12* GA, an MMR GA, a microsatellite instability-high (MSI-H) genomic signature, or a *POLE* V411L mutation. Box and whisker plots: Boxes span first and third quartiles, the median is denoted by the horizontal line in the box, and whiskers indicate maximum and minimum values within 1.5× the interquartile range.

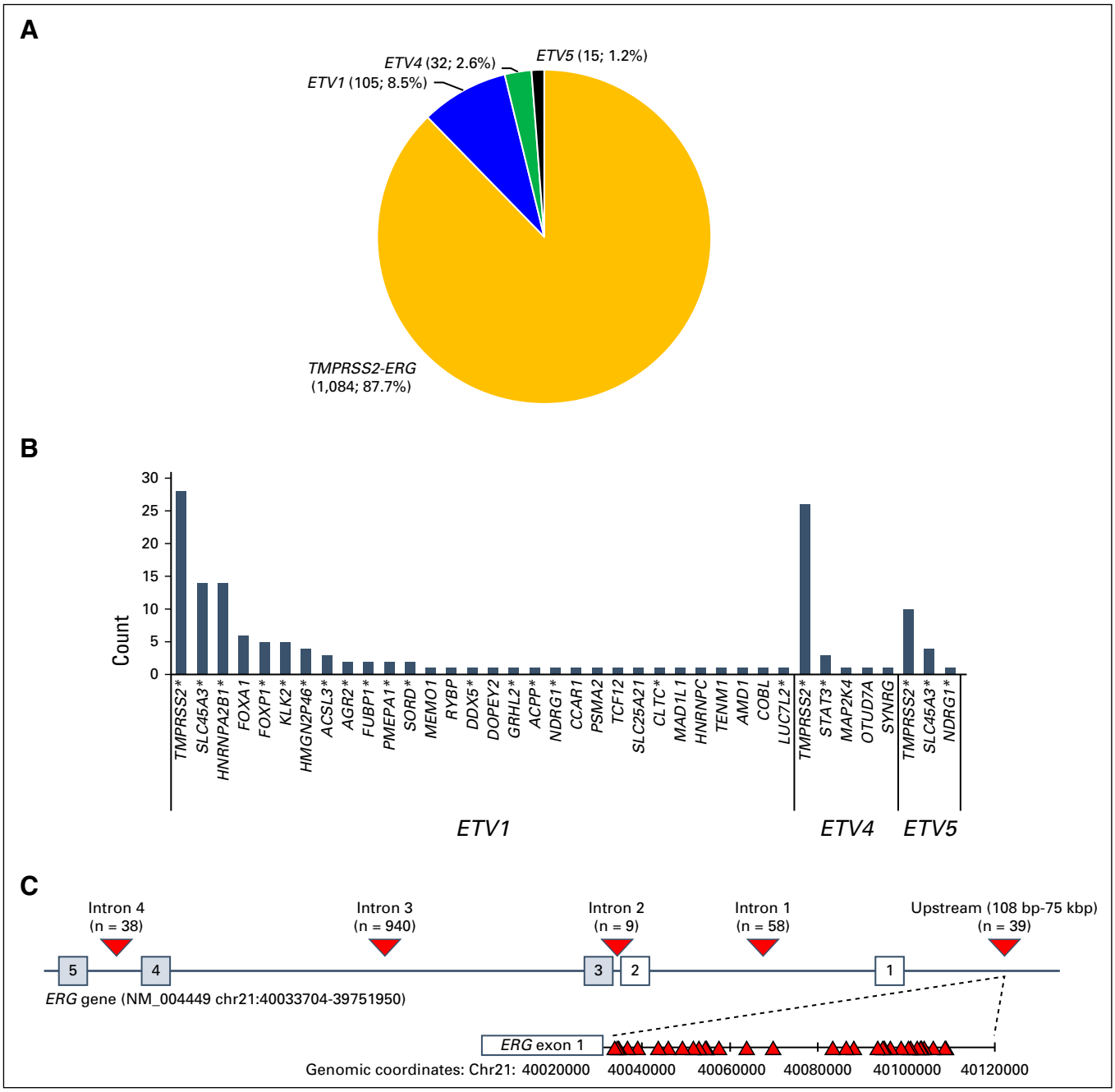


FIG 3. *TMPRSS2-ERG* and other *ETS* fusions. (A) Distribution of 1,236 *ETS* fusions in this study. (B) The 5′ partner genes are identified. (C) Distribution of breakpoints identified in the *ERG* gene. Numbered boxes represent exons. Triangles represent observed breakpoints. (*) Fusion partners that have been previously described. bp, base pair; kbp, kilobase pair.

limited to common breakpoints and may incompletely identify rare *ETS* fusions with novel breakpoints. Consistent with distinct molecular subsets of prostate cancer,[1,8] *ETS* fusions were mutually exclusive with *SPOP*/*CUL3*, *CDK12* mutations, or *BRAF* rearrangements/mutations that may potentially comprise a clinically relevant genomic subset.[30,31]

Diverse *AR* GAs can mediate androgen axis inhibitor resistance[28,29] and were strongly enriched in metastatic site tumors. *AR* rearrangements that disrupted the ligand-binding domain can mediate resistance to current AR inhibitors.[29] Development of novel AR inhibitors that target the diverse spectrum of *AR* alterations is needed. *AR* GAs commonly co-occurred with alterations in other targetable pathways (Fig 1B); such concurrent alterations may present an opportunity for targeted therapy in androgen axis inhibitor–resistant tumors.

In addition to metastatic enrichment in *AR* GAs, we identified first that *CCND1*/*FGF3*/*FGF4*/*FGF19* (11q13) amplifications

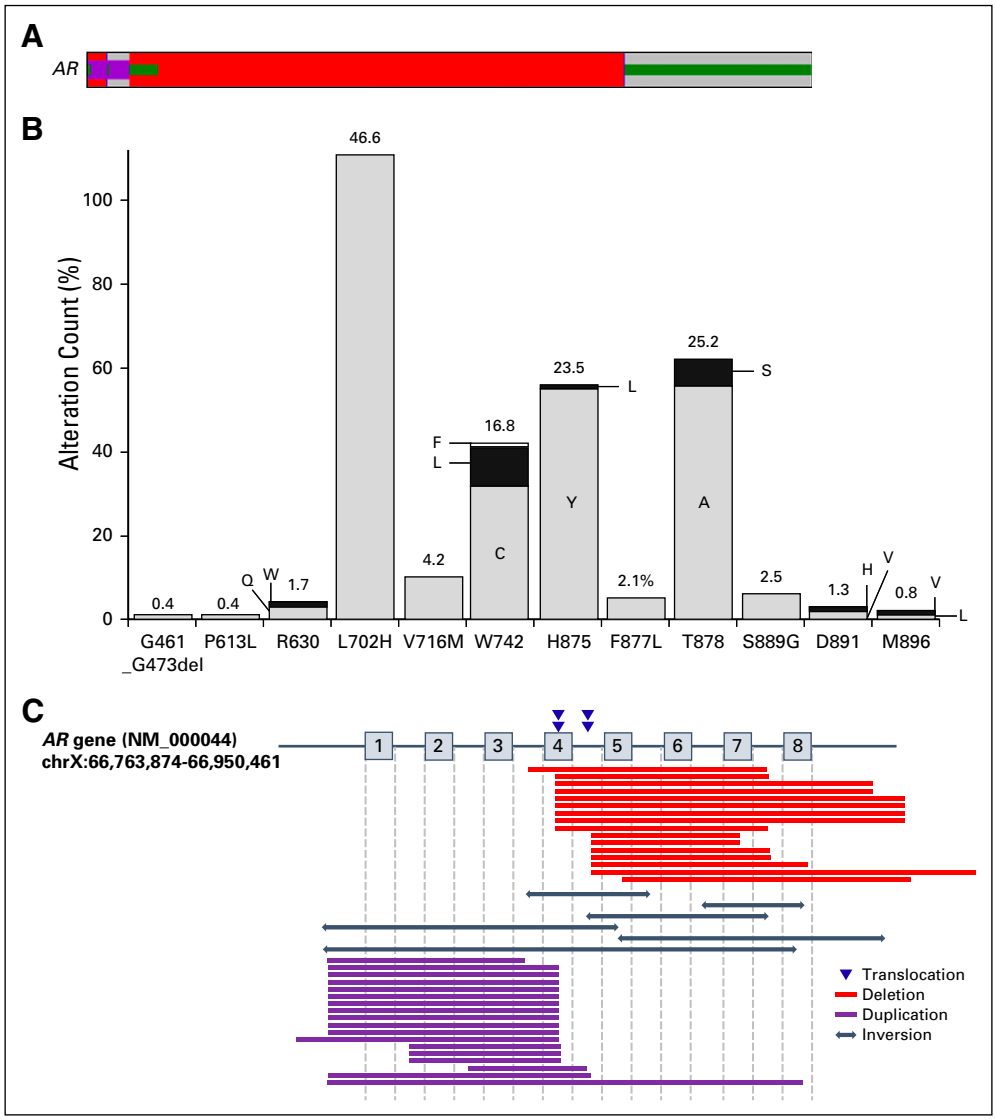


FIG 4. Landscape of *AR* alterations. (A) *AR* genomic alterations (GAs) were identified in 721 cases, including amplification (red), mutation (green), and rearrangements (purple). (B) Graph that represents the number of cases with each *AR* GA. The percentages indicate the frequency of *AR* mutations at each codon as a fraction of all *AR* mutations. Letters denote amino acid abbreviations as recommended by the International Union of Pure and Applied Chemistry. (C) Map of *AR* rearrangements that describe breakpoints for translocations and deleted, duplicated, or inverted regions.

were enriched in metastatic sites. Of note, 11q13 amplification is associated with endocrine resistance in breast cancer and potentially targetable by fibroblast growth factor receptor inhibitors[32-34] and, therefore, warrants investigation in prostate cancer. Second, *CDKN2A* GAs were enriched in metastatic site tumors (1.9-fold), and although not previously described in primary versus metastatic studies,[1-3] acquired *CDKN2A* alterations were described in enzalutamide-resistant tumors.[15] Metastatic enrichment of cell cycle GAs suggests that CDK4/6 inhibitors could be explored. Third, *NCOR1* GAs were enriched in metastatic site tumors. Downregulation of *NCOR1*, which encodes a negative regulator of AR, is associated with androgen axis inhibitor resistance.[35] Finally, we independently confirmed[1-3] metastatic site enrichment of *MYC*, *PTEN*, *TP53*, *RB1*, *CTNNB1*, *MLL3*, *APC*, *BRCA2*, and *PIK3CB* and primary site enrichment of *SPOP*.

DNA repair pathway GAs are associated with responses to PARP inhibitors or immunotherapy in many solid tumor types.[4,10] Collectively, HRR, FA/ICL, *CDK12*, or MMR/DNA polymerase genes were altered in 32.6% of cases. On the basis of a germline/somatic prediction algorithm,[17] we estimate that 35.7% of DNA repair gene mutations were germline. Current guidelines (National Comprehensive Cancer Network version 4.2018)[36] recommend testing for

certain HRR genes, MMR genes, or MSI status; identification of such DNA repair GA by tumor sequencing warrants follow-up germline testing and genetic counseling. A limitation of tumor-only sequencing is that germline/somatic calls are not definitive and require confirmation by dedicated germline testing. However, compared with a recent study,[3] we identify similar relative proportions of germline/somatic mutations for frequently mutated HRR genes (*BRCA2*, *ATM*, *CHEK2*). Furthermore, another study of prostate cancer similarly identified germline mutations for many of the DNA repair genes evaluated here.[37] We also describe potential *CDK12* germline mutations (four of 12 were rs138292741). Although not identified in one recent study of *CDK12* in prostate cancer,[8] germline truncating *CDK12* mutations were described in other studies, including prostate cancer,[38-40] and in germline databases.[40]

Although preclinical studies have identified non-*BRCA1/2* HRR genes, their association with HRD phenotype in clinical samples is unclear. As expected, *BRCA1/2* was associated with gLOH-H. Beyond *BRCA1/2*, associations with gLOH-H were observed for *ATR* and *FANCA*. *CDK12* is a candidate biomarker for PARP inhibition[4] on the basis of preclinical studies[19,20]; however, gLOH-H was significantly less frequent for *CDK12*-altered cases; this finding is consistent with recent studies suggesting that *CDK12* GAs are associated with a focal tandem duplication phenotype that is distinct from HRD.[8,41,42] In trials for metastatic prostate cancer, germline or somatic *BRCA1/2* alterations were associated with response to PARP inhibitor monotherapy.[4,43] In contrast, non-*BRCA* HRR genes have been less consistent in predicting response[4,43,44] and require further refinement in clinical trials.

The evaluation of both individual GAs and genomic signatures together may be important when exploring predictive biomarkers. Identification of gLOH-H cases lacking DNA repair GAs (Appendix Fig A4A) may have clinical value, but further investigation in PARP inhibitor trials is required to assess the clinical utility of gLOH as a biomarker in prostate cancer. Similarly, DNA repair GAs were associated with MSI-H or TMB-H genomic signatures that are associated with benefit from immunotherapy (Fig 2; Appendix Fig A5). A subset of MSI/TMB-H cases do not harbor corresponding DNA repair GAs potentially because GAs may occur in genes involved in DNA repair that have not yet been discovered, complex rearrangements with intronic breakpoints may be challenging to detect, or genomic instability can occur through mechanisms such as gene silencing or extrinsic DNA damage.

Limitations of this study should be acknowledged. First, limited access to patient-level clinical data prevented further subclassification of primary and metastatic samples by castration resistance status. Furthermore, because samples were collected for routine clinical testing, primary samples in this cohort may be biased toward patients who have received prior treatment or developed metastatic disease that resulted in a distinct genomic landscape from untreated primary tumors. In contrast, metastatic samples may not all represent castration-resistant disease. Therefore, sensitivity to identify genomic associations with primary/aggressive disease may be reduced. However, many findings in this study recapitulated those described in previous studies of smaller cohorts with better clinical annotation that compared metastatic castration-resistant tumors with noncastrate primary site tumors.[2,3,13] Particularly, the low *AR* GA frequency in primary site samples is consistent with mostly noncastrate disease, and the high *AR* GA frequency in metastatic site samples is consistent with mostly castration-resistant disease.[2] Second, in contrast to whole-exome sequencing studies of prostate cancer, this study uses an assay that is used in routine clinical practice and was therefore limited to the 395 genes assessed.

In this study, routine CGP for prostate cancer identified frequent alterations in genes and pathways as well as in genomic signatures. These findings may suggest routes to targeted therapy or immunotherapy for patient's refractory to current therapies.

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APPENDIX

Methods

Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol 20152817). Comprehensive genomic profiling (CGP) results were reported clinically by prospective sequencing of tissue samples from 3,476 unique patients with prostate cancer (August 2014 to February 2018) using a validated hybrid capture-based CGP assay (FoundationOne [baitset version T7 was used during this period])[16] in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State–approved laboratory (Foundation Medicine, Cambridge, MA). For patients with multiple submitted samples, only a single sample was included, and the sample with the highest sequencing quality metrics was included. Age and site of specimen collection were abstracted from the accompanying pathology reports, clinical notes, and requisition forms submitted by the treating physician. Specimens included 1,660 primary site tumors and 1,816 metastatic site tumors (Appendix Fig A1). The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin–stained slides, and all samples forwarded for DNA extraction contained a minimum of 20% tumor nuclei. CGP was performed on hybridization-captured, adaptor ligation-based libraries to a median coverage depth of 743× for 395 cancer-related genes plus select introns from 31 genes frequently rearranged in cancer (Appendix Table A1). For *ETS* fusions, targeted regions were *TMPRSS2* (introns 1 to 3), *ERG* (all exons), *ETV1* (introns 3 to 4), *ETV4* (intron 8), and *ETV5* (introns 6 to 7).

Results were analyzed for base substitutions, short insertions/deletions, rearrangements, and copy number alterations (amplification and homozygous deletion). Custom filtering was applied to remove benign germline events as previously described (Hartmaier et al: Cancer Res 77:2464-2475, 2017). Genomic alterations (GAs) were called as reportable if the specific variant was present in the Catalog of Somatic Mutations in Cancer database (Forbes et al: Nucleic Acids Res 45:D777-D783, 2017), if the variant has been characterized as pathogenic, or if the variant had likely functional status (disruptive alterations in tumor suppressor genes); all other variants were classified as variants of unknown significance.

To compare relative enrichments in primary site and metastatic site tumors, genes altered at a 2% or greater frequency were assessed for enrichment. Enrichment was defined as the difference in frequency of gene alteration between metastatic site and primary site samples.

To determine microsatellite instability status, 114 intronic homopolymer repeat loci on the FoundationOne panel were analyzed for length variability and compiled into an overall microsatellite instability score through principal components analysis (Chalmers et al: Genome Med 9:34, 2017). Tumor mutational burden was calculated as the number of somatic base substitutions or insertions/deletions per megabase of the coding region target territory of the test (1.1 Mb) after filtering to remove known somatic and deleterious mutations and extrapolating that value to the exome or genome as a whole (Chalmers et al: Genome Med 9:34, 2017). Tumor mutational burden was categorized as low (fewer than six mutations/Mb), intermediate (six to 20 mutations/Mb), or high (20 mutations/Mb or more; Chalmers et al: Genome Med 9:34, 2017). Germline, somatic, and zygosity statuses for mutations were determined without matched normal tissue as previously described[17]; in validation testing of 480 germline/somatic calls from tumor-only sequencing with matched normal reference samples, accuracy was 95% for somatic calls and 99% for germline calls. Biallelic inactivation was defined as mutations under loss of heterozygosity (LOH) as determined by zygosity status.[17] Percent genome-wide LOH (gLOH) was used as a marker of homologous recombination deficiency and calculated as described, and a gLOH score of 14% or greater was defined as gLOH-high.[21] Potentially targetable GAs were defined as those that have been associated with response to targeted therapy in prostate cancer, homologous recombination repair GAs that have been associated with responses to poly (ADP-ribose) polymerase inhibitors, or GAs associated with response to targeted therapy in multiple other tumor types and were ranked according to modified European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets criteria.[18]

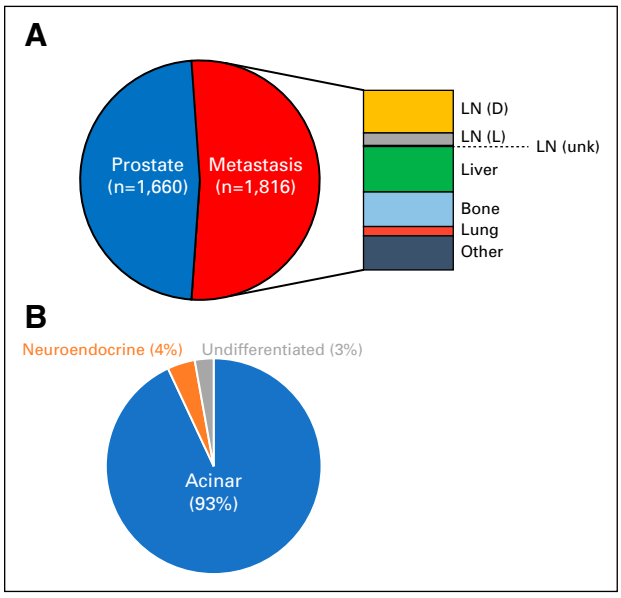


FIG A1. (A) Distribution of sites of sample collection (prostate, 47.8%; distant lymph node [LN (D)], 12.4%; regional LN [LN (R)], 3.7%; unspecified LN [LN (unk)], 0.3%; liver, 13.2%; bone, 10.0%; lung, 2.7%; other metastatic, 9.9%). (B) Distribution of prostate histologic types.

TABLE A1. Genes Sequenced Using the FoundationOne Assay (Version T7)

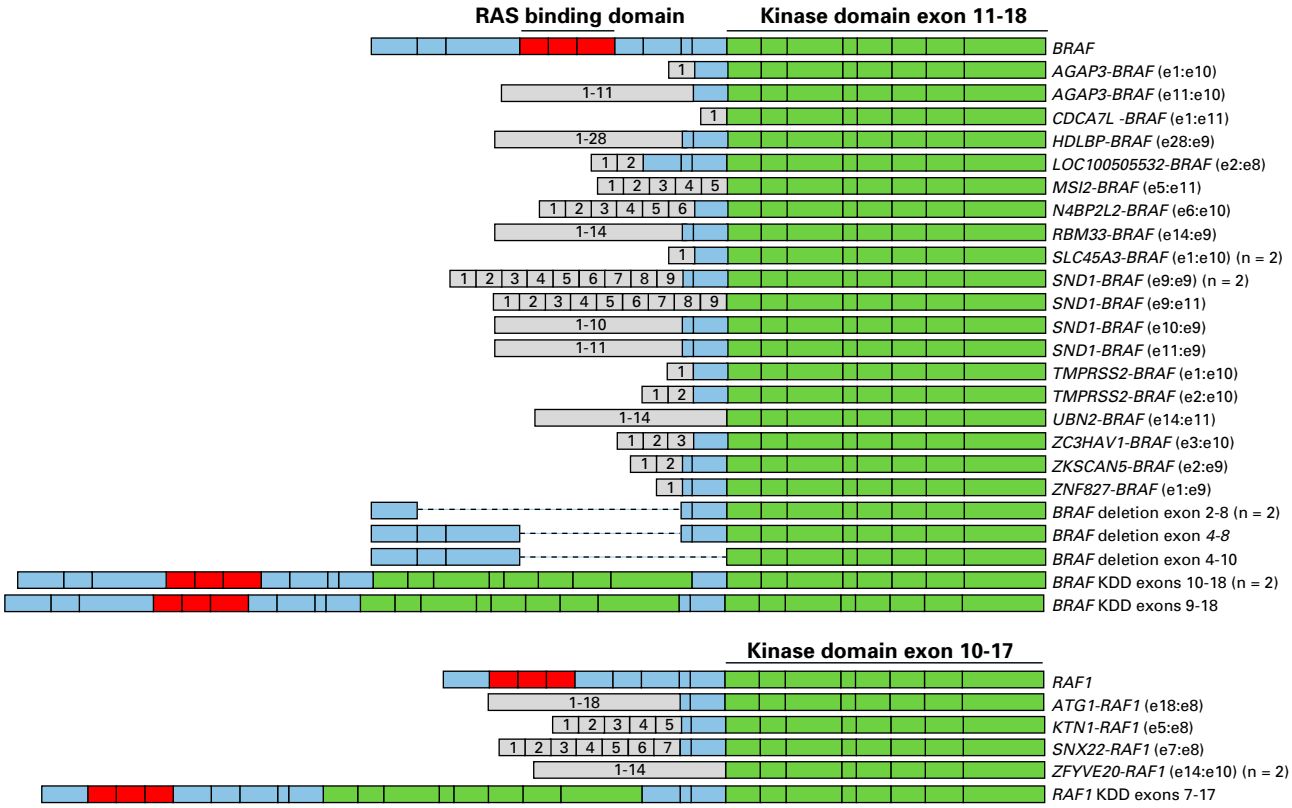
Exonic Capture (395 genes), HUGO Symbol									
ABL1	*BRD4*	*CRLF2*	*FANCF*	*GLI1*	*KDM5A*	*MST1R*	*PHLPP2*	*RB1*	*SYK*
ABL2	*BRIP1*	*CSF1R*	*FANCG*	*GNA11*	*KDM5C*	*MTOR*	*PIK3C2B*	*RBM10*	*TAF1*
ACVR1B	*BTG1*	*CTCF*	*FANCI*	*GNA13*	*KDM6A*	*MUTYH*	*PIK3C2G*	*REL*	*TBX3*
AKT1	*BTK*	*CTNNA1*	*FANCL*	*GNAQ*	*KDR*	*MYC*	*PIK3C3*	*RET*	*TEK*
AKT2	*C11orf30 (EMSY)*	*CTNNB1*	*FANCM*	*GNAS*	*KEAP1*	*MYCL (MYCL1)*	*PIK3CA*	*RICTOR*	*TERC*
AKT3	*CARD11*	*CUL3*	*FAS*	*GPR124*	*KEL*	*MYCN*	*PIK3CB*	*RNF43*	*TERT (promoter only)*
ALK	*CASP8*	*CUL4A*	*FAT1*	*GREM1*	*KIT*	*MYD88*	*PIK3CG*	*ROS1*	*TET2*
ALOX12B	*CBFB*	*CUL4B*	*FAT3*	*GRIN2A*	*KLHL6*	*NBN*	*PIK3R1*	*RPA1*	*TGFBR2*
AMER1 (FAM123B)	*CBL*	*CYLD*	*FBXW7*	*GRM3*	*KMT2A (MLL)*	*NCOR1*	*PIK3R2*	*RPTOR*	*TIPARP*
APC	*CCND1*	*CYP17A1*	*FGF10*	*GSK3B*	*KMT2C (MLL3)*	*NF1*	*PLCG2*	*RUNX1*	*TNF*
APCDD1	*CCND2*	*DAXX*	*FGF12*	*H3F3A*	*KMT2D (MLL2)*	*NF2*	*PMS2*	*RUNX1T1*	*TNFAIP3*
AR	*CCND3*	*DDR1*	*FGF14*	*HGF*	*KRAS*	*NFE2L2*	*PNRC1*	*SDHA*	*TNFRSF14*
ARAF	*CCNE1*	*DDR2*	*FGF19*	*HLA-A*	*LMO1*	*NFKBIA*	*POLD1*	*SDHB*	*TNKS*
ARFRP1	*CD274*	*DICER1*	*FGF23*	*HLA-B*	*LRP1B*	*NKX2-1*	*POLE*	*SDHC*	*TNKS2*
ARID1A	*CD79A*	*DIS3*	*FGF3*	*HLA-C*	*LRP6*	*NOTCH1*	*PPARG*	*SDHD*	*TOP1*
ARID1B	*CD79B*	*DNMT3A*	*FGF4*	*HNF1A*	*LTK*	*NOTCH2*	*PPP2R1A*	*SETD2*	*TOP2A*
ARID2	*CDC73*	*DOT1L*	*FGF6*	*HOXB13*	*LYN*	*NOTCH3*	*PRDM1*	*SF3B1*	*TP53*
ASXL1	*CDH1*	*EGFR*	*FGF7*	*HRAS*	*LZTR1*	*NOTCH4*	*PREX2*	*SH2B3*	*TP53BP1*
ATM	*CDH2*	*EP300*	*FGFR1*	*HSD3B1*	*MAGI2*	*NPM1*	*PRKAR1A*	*SLIT2*	*TRRAP*
ATR	*CDH20*	*EPHA3*	*FGFR2*	*HSP90AA1*	*MAP2K1*	*NRAS*	*PRKCI*	*SMAD2*	*TSC1*
ATRX	*CDH5*	*EPHA5*	*FGFR3*	*IDH1*	*MAP2K2*	*NSD1*	*PRKDC*	*SMAD3*	*TSC2*
AURKA	*CDK12*	*EPHA6*	*FGFR4*	*IDH2*	*MAP2K4*	*NTRK1*	*PRSS1*	*SMAD4*	*TSHR*
AURKB	*CDK4*	*EPHA7*	*FH*	*IGF1*	*MAP3K1*	*NTRK2*	*PRSS8*	*SMARCA4*	*TYRO3*
AXIN1	*CDK6*	*EPHB1*	*FLCN*	*IGF1R*	*MAP3K13*	*NTRK3*	*PTCH1*	*SMARCB1*	*U2AF1*
AXL	*CDK8*	*EPHB4*	*FLT1*	*IGF2*	*MCL1*	*NUDT1*	*PTCH2*	*SMARCD1*	*VEGFA*
BACH1	*CDKN1A*	*EPHB6*	*FLT3*	*IGF2R*	*MDM2*	*NUP93*	*PTEN*	*SMO*	*VHL*
BAP1	*CDKN1B*	*ERBB2*	*FLT4*	*IKBKE*	*MDM4*	*PAK3*	*PTPN11*	*SNCAIP*	*WISP3*
BARD1	*CDKN2A*	*ERBB3*	*FOXL2*	*IKZF1*	*MED12*	*PAK7*	*PTPRD*	*SOCS1*	*WT1*
BCL2	*CDKN2B*	*ERBB4*	*FOXP1*	*IL7R*	*MEF2B*	*PALB2*	*QKI*	*SOX10*	*XPO1*
BCL2A1	*CDKN2C*	*ERCC4*	*FRS2*	*INHBA*	*MEN1*	*PARK2*	*RAC1*	*SOX2*	*XRCC2*
BCL2L1	*CEBPA*	*ERG*	*FUBP1*	*INPP4B*	*MERTK*	*PARP1*	*RAD50*	*SOX9*	*XRCC3*
BCL2L2	*CHD2*	*ERRFI1*	*GABRA6*	*INSR*	*MET*	*PARP2*	*RAD51*	*SPEN*	*ZBTB2*
BCL6	*CHD4*	*ESR1*	*GALNT12*	*IRF2*	*MITF*	*PARP3*	*RAD51B (RAD51L1)*	*SPOP*	*ZNF217*
BCOR	*CHEK1*	*EZH2*	*GATA1*	*IRF4*	*MKNK1*	*PARP4*	*RAD51C*	*SPTA1*	*ZNF703*
BCORL1	*CHEK2*	*FAM175A*	*GATA2*	*IRS2*	*MKNK2*	*PAX5*	*RAD51D (RAD51L3)*	*SRC*	*ZNRF3*
BLM	*CHUK*	*FAM46C*	*GATA3*	*JAK1*	*MLH1*	*PBRM1*	*RAD52*	*STAG2*	
BMPR1A	*CIC*	*FANCA*	*GATA4*	*JAK2*	*MPL*	*PDCD1LG2*	*RAD54L*	*STAT3*	
BRAF	*CRBN*	*FANCC*	*GATA6*	*JAK3*	*MRE11A*	*PDGFRA*	*RAF1*	*STAT4*	
BRCA1	*CREBBP*	*FANCD2*	*GEN1*	*JUN*	*MSH2*	*PDGFRB*	*RANBP2*	*STK11*	

(Continued on following page)

TABLE A1. Genes Sequenced Using the FoundationOne Assay (Version T7) (Continued)

Exonic Capture (395 genes), HUGO Symbol									
BRCA2	*CRKL*	*FANCE*	*GID4 (C17orf39)*	*KAT6A (MYST3)*	*MSH6*	*PDK1*	*RARA*	*SUFU*	
Select Intronic Capture for Rearrangement Analysis (31 genes), HUGO Symbol									
ALK	*BRCA1*	*EGFR*	*ETV5*	*FGFR1*	*KIT*	*MYB*	*NTRK1*	*RAF1*	*ROS1*
BCL2	*BRCA2*	*ETV1*	*ETV6*	*FGFR2*	*KMT2A (MLL)*	*MYC*	*NTRK2*	*RARA*	*RSPO2*
BCR	*BRD4*	*ETV4*	*EWSR1*	*FGFR3*	*MSH2*	*NOTCH2*	*PDGFRA*	*RET*	*TMPRSS2*
BRAF									

Abbreviation: HUGO, Human Genome Organisation.



BRAF Mutation	No.	Functional Characterization
K601E	21	Activating
G469A	3	Activating
A489_Q493del	2	Activating
T488_P492del	1	
V487_P492>G	1	
D594G	2	Kinase activity impaired
L597R	2	Activating
R444Q	1	Unknown
S465_G469del	1	Unknown
N581I	1	Kinase activity impaired
E586K	1	Activating
D594H	1	Kinase activity impaired
D594A	1	Kinase activity impaired
G596R	1	Kinase activity impaired
T599_V600insDFGLAT	1	Oncogenic, kinase activity uncharacterized
V600_W604del	1	Uncharacterized
V600E	1	Activating
K601Q	1	Activating
Total	**43**	

FIG A2. Details of the *BRAF* rearrangements, *RAF1* rearrangements, and *BRAF* short variant mutations. The diagram illustrates *BRAF* (top) and *RAF1* (bottom) rearrangements, including fusions, N-terminal deletions, and kinase domain duplications. Exons are numbered. For fusion, exons (e) are annotated with the last exon included in the 5′ partner and the first exon included in the 3′ partner. In addition, *BRAF* rearrangements at intron 9 (n = 6) and intron 7 (n = 1) and *RAF1* rearrangement at intron 7 with no clear fusion partner were identified (data not shown). The table lists the 43 *BRAF* mutations identified.

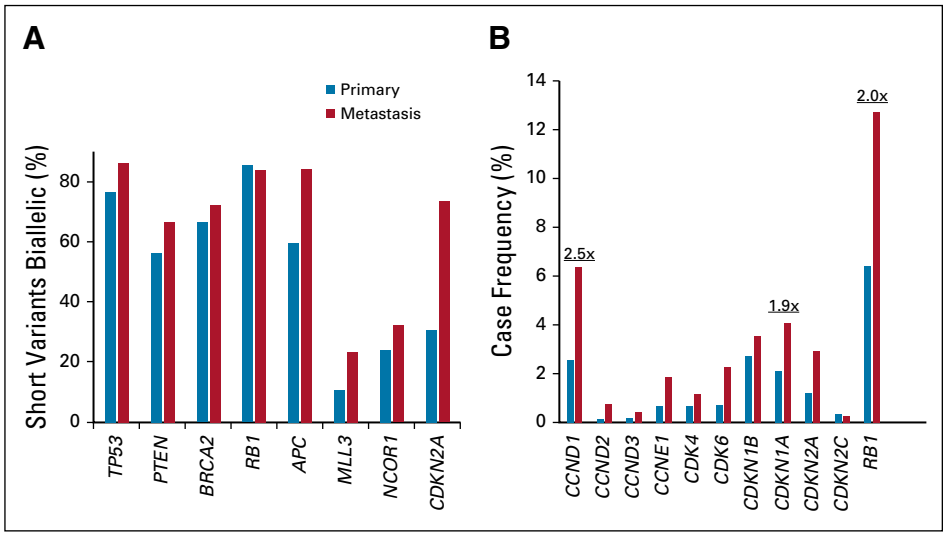


FIG A3. (A) For metastasis-enriched tumor suppressor genes (see Fig 1G), short variant mutations were assessed for loss of heterozygosity. Frequency of short variant mutations under loss of heterozygosity (biallelic) is shown. (B) Frequency of genomic alterations for individual genes in G_1/S-cell cycle pathway in primary versus metastatic site samples. Fold change is indicated.

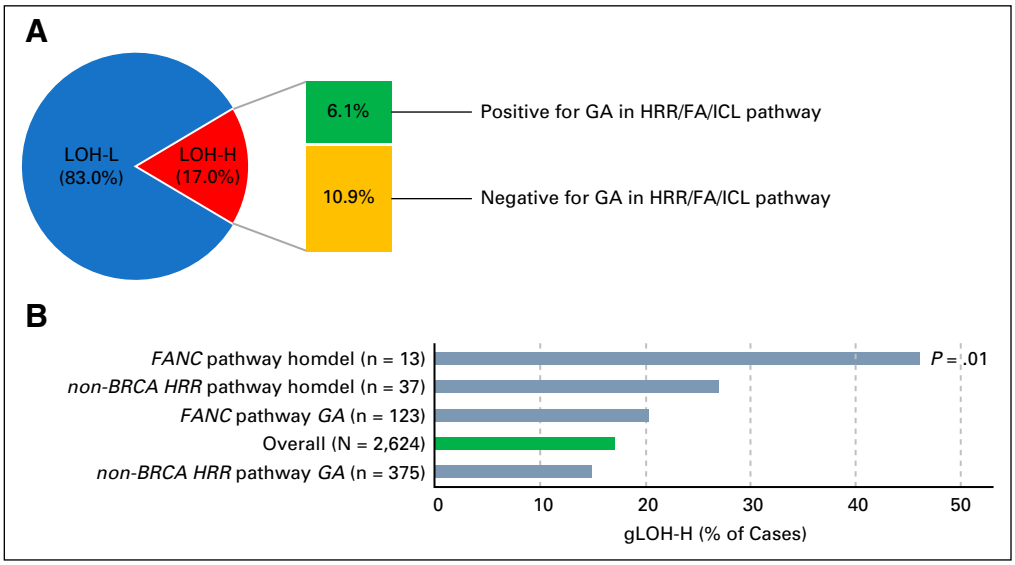
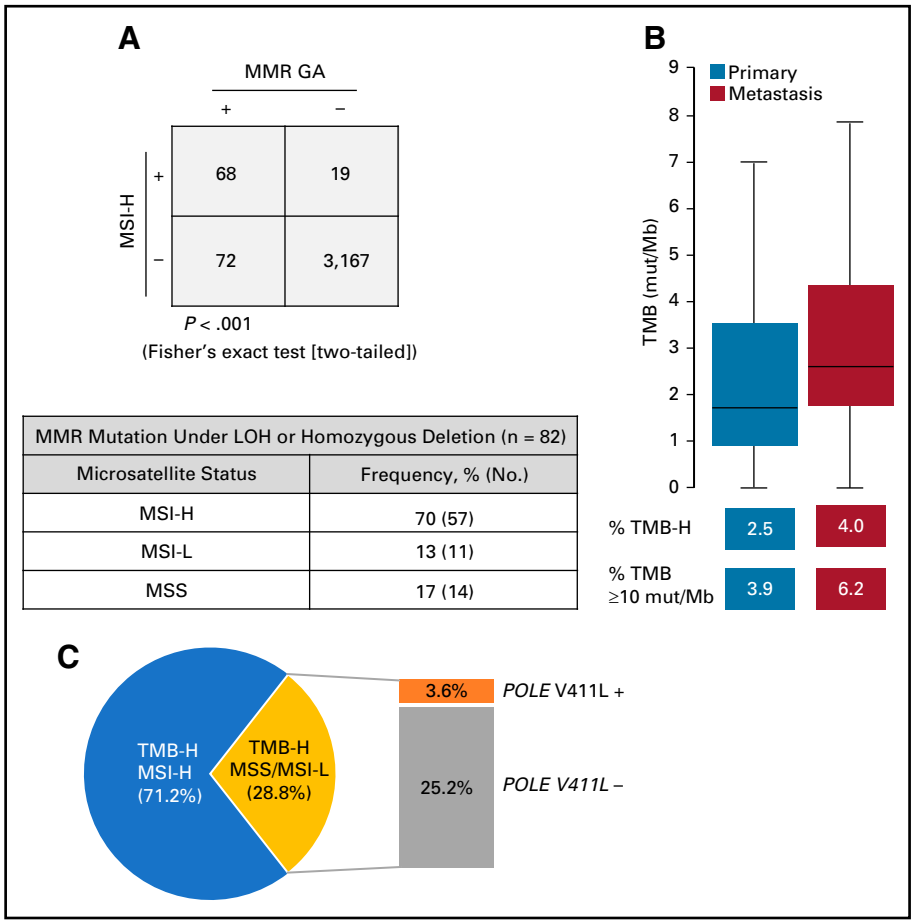


FIG A4. (A) Genome-wide loss of heterozygosity (gLOH) score was assessable for 2,624 cases, and cases with 14% or more gLOH were designated LOH-high (LOH-H). All other cases were LOH-low (LOH-L). LOH-H cases were assessed for the presence (green) or absence (yellow) of a genomic alteration (GA) in the homologous recombination repair (HRR) or Fanconi anemia/interstrand crosslink repair (FA/ICL) pathway. (B) Genes were grouped together into HRR pathway (excluding non-*BRCA1/2*) or FA/ICL pathway (*FANC*); genes were categorized as in Figure 2A. All GAs or only homozygous deletions (homdel) were assessed for LOH-H and compared with the overall data set (green). Each subset was individually compared with the overall data set, and unadjusted *P* values are shown (Fisher's exact test [two-tailed]).



MMR Mutation Under LOH or Homozygous Deletion (n = 82)	
Microsatellite Status	Frequency, % (No.)
MSI-H	70 (57)
MSI-L	13 (11)
MSS	17 (14)

FIG A5. (A) Microsatellite instability (MSI) status was assessable for 3,326 cases, and each case was designated as MSI-high (MSI-H), MSI-low (MSI-L), or microsatellite stable (MSS). MSI-H cases were assessed for the presence or absence of mismatch repair (MMR) genomic alterations (GAs). MSI-H or MMR GA–positive samples are indicated with a plus sign, and negative samples are indicated with a minus sign. Of the cases where both MSI status and MMR GA zygosity status could be determined, 82 cases had MMR GA that resulted in loss of function of both alleles (microsatellite status in this subset listed in table). (B) Tumor mutational burden (TMB) was evaluated for primary site and metastatic site cases. Box and whisker plots: Boxes span first and third quartiles, the median is denoted by the horizontal line in the box, and whiskers indicate maximum and minimum values with 1.5× the interquartile range. The percent TMB-high (TMB-H) or TMB of 10 or more mutations (mut)/Mb cases in primary site and metastatic site tumors is indicated. (C) TMB-H samples were assessed for whether they were MSI-H or MSS/MSI-L. TMB-H cases that were not MSI-H also were assessed for *POLE* V411L pathogenic mutations.

TABLE A2. Recurrent GAs/Genomic Signatures Identified in Prostate Cancer That Have Been Associated With Responses to Targeted Therapy in Prostate and Other Types of Cancer

Gene	Type	Frequency, % (No.)	Targeted Therapy	Cancer Type With Clinical Evidence for Response	First Author	Level of Evidence*
PTEN	Homozygous deletion	32.2 (1,119)	AKT inhibitor	Prostate	de Bono[5]	2A
	Mutation					
	Rearrangement					
BRCA2	Homozygous deletion	9.8 (342)	PARP inhibitor	Prostate	Mateo[4]	2B
	Mutation					
	Rearrangement					
CDK12	Homozygous deletion	5.6 (194)	Immune checkpoint inhibitor	Prostate	Wu[8]	4A
	Mutation					
	Rearrangement					
ATM	Homozygous deletion	5.2 (182)	PARP inhibitor	Prostate	Mateo[4]	2B
	Mutation					
	Rearrangement					
PIK3CB	Amplification	2.2 (76 [amplifications, 53; mutations, 23])	PI3K-β inhibitor	Prostate	de bono JS: AACR Annual Meeting, Philadelphia, PA, April 18-22, 2015 (abstr CT328)	4A
	Activating mutation					
AKT1	Mutation (E17K)	1.5 (51)	AKT inhibitor	Prostate	Hyman[6]	2B
BRCA1	Homozygous deletion	1.4 (50)	PARP inhibitor	Prostate	Mateo[4]	2B
	Mutation					
	Rearrangement					
PALB2	Homozygous deletion	0.7 (25)	PARP inhibitor	Prostate	Mateo[4]	3B
	Mutation					
	Rearrangement					
POLE	Exonuclease domain mutation	0.1 (4)	Immune checkpoint inhibitor	Prostate	Lee[9]	4A
BRIP1	Homozygous deletion	0.6 (20)	PARP inhibitor	Ovarian	Swisher[21]	3B
	Mutation					
	Rearrangement					
RAD51C	Homozygous deletion	0.1 (5)	PARP inhibitor	Ovarian	Swisher[21]	3B
	Mutation					
	Rearrangement				Kondrashova O: Cancer Discov 7: 984-998, 2017	

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TABLE A2. Recurrent GAs/Genomic Signatures Identified in Prostate Cancer That Have Been Associated With Responses to Targeted Therapy in Prostate and Other Types of Cancer (Continued)

Gene	Type	Frequency, % (No.)	Targeted Therapy	Cancer Type With Clinical Evidence for Response	First Author	Level of Evidence*
RAD51D	Homozygous deletion	0.1 (3)	PARP inhibitor	Ovarian	Swisher[21]	3B
	Mutation					
	Rearrangement				Kondrashova O: Cancer Discov 7: 984-998, 2017	
PIK3CA	Activating mutation	4.9 (171)	PI3K inhibitor	Breast, lung	Juric D: Cancer Discov 7:704-715, 2017	3A
					Baselga J: Lancet Oncol 18: 904-916, 2017	
BRAF	Fusion	2.0 (68 [fusion, 35; mutation, 33])	MEK inhibitor	Melanoma, glioma	Ross[32]	3A
					Fangusaro JR: J Clin Oncol 35, 2017 (suppl; abstr 10504)	
	Mutation (V600E and non-V600 kinase activating)				Richtig[31]	
FGFR1	Amplification	1.8 (62)	FGFR inhibitor	Lung, endometrial, breast	Voss MH: J Clin Oncol 35,2017 (suppl; abstr 2500)	4A
					Nogova L: J Clin Oncol 35: 157-165, 2017	
					Pearson A: Cancer Discov 6: 838-851, 2016	
CDK6	Amplification	1.5 (53)	CDK4/6 inhibitor	Unknown primary, ovarian	Peguero JA: J Clin Oncol 34, 2016 (suppl; abstr 2528)	4A
					Konecny GE: J Clin Oncol 34, 2016 (suppl; abstr 5557)	
CDK4	Amplification	0.9 (31)	CDK4/6 inhibitor	Sarcoma, liposarcoma	Peguero JA: J Clin Oncol 34, 2016 (suppl; abstr 2528)	4A
					Dickson MA: J Clin Oncol 31: 2024-2028, 2013	
ERBB2	Activating mutation	0.6 (21 [amplification, 17; mutation, 4])	HER2 inhibitor	Breast, colorectal, bladder, lung, biliary, and salivary gland	Hainsworth JD: J Clin Oncol 36: 536-542, 2018	3A
					Hyman DM: Nature 554:189-194, 2018	
	Amplification				Choudhury NJ: J Clin Oncol 34: 2164-2171, 2016	

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TABLE A2. Recurrent GAs/Genomic Signatures Identified in Prostate Cancer That Have Been Associated With Responses to Targeted Therapy in Prostate and Other Types of Cancer (Continued)

Gene	Type	Frequency, % (No.)	Targeted Therapy	Cancer Type With Clinical Evidence for Response	First Author	Level of Evidence*
PTCH1	Homozygous deletion Mutation Rearrangement	0.5 (16)	SMO inhibitor	Unknown primary, skin, salivary gland, medulloblastoma, and urothelial	Hainsworth JD: J Clin Oncol 36: 536-542, 2018 Robinson GW: J Clin Oncol 33: 2646-2654, 2015	4A
TSC1	Homozygous deletion Mutation Rearrangement	0.4 (12)	mTOR inhibitor	Bladder, kidney	Iyer G: Science 338:221, 2012 Voss MH: Clin Cancer Res 20: 1955-1964, 2014 Ali SM: Eur Urol 68:341-343, 2015	4A
TSC2	Homozygous deletion Mutation Rearrangement	0.4 (14)	mTOR inhibitor	Thyroid	Wagle N: N Engl J Med 371: 1426-1433, 2014 Kwiatkowski DJ: Clin Cancer Res 22:2445-2452, 2016	4A
MET	Amplification	0.4 (13)	MET inhibitor	Lung, kidney	Choueiri TK: J Clin Oncol 35: 2993-3001, 2017 Schrock AB: J Thorac Oncol 11: 1493-1502, 2016	3A
FGFR2	Amplification Activating mutation	0.2 (7 [amplification, 5; mutation, 2])	FGFR inhibitor	Endometrial, cholangiocarcinoma, colorectal, lung, and gastric	Pearson A: Cancer Discov 6: 838-851, 2016 Liao RG: Cancer Res 73: 5195-5205, 2013 Voss MH: J Clin Oncol 35,2017 (suppl; abstr 2500)	4A
CD274/PDCD1LG2	Amplification	0.2 (6)	Immune checkpoint inhibitor	Glioblastoma, HNSCC, basal cell carcinoma, and urothelial	Goodman AM: JAMA Oncol 4: 1237-1344, 2018	4A
MAP2K1	Activating mutation	0.2 (6)	MEK inhibitor	Ovarian, histiocytosis	Grisham RN: J Clin Oncol 33: 4099-4105, 2015 Papapanagiotou M: JCO Precision Oncology 10.1200/PO.16.00070	4A
EGFR	Activating mutation	0.1 (4)	EGFR inhibitor	Lung, breast, urethral	Ali SM: Clin Breast Cancer 14: e14-e16, 2014 Hainsworth JD: J Clin Oncol 36: 536-542, 2018	3A

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TABLE A2. Recurrent GAs/Genomic Signatures Identified in Prostate Cancer That Have Been Associated With Responses to Targeted Therapy in Prostate and Other Types of Cancer (Continued)

Gene	Type	Frequency, % (No.)	Targeted Therapy	Cancer Type With Clinical Evidence for Response	First Author	Level of Evidence*
ERBB3	Activating mutation	< 0.1 (2)	HER2 inhibitor	Urothelial, breast	Choudhury NJ: J Clin Oncol 34: 2165-2171, 2016 Bidard FC: Ann Oncol 26: 1704-1709, 2015	4A
FGFR3	Fusion	< 0.1 (2)	FGFR inhibitor	Glioma, bladder	Di Stefano AL: Clin Cancer Res 21: 3307-3317, 2015 Voss MH: J Clin Oncol 35,2017 (suppl; abstr 2500)	4A
KIT	Activating mutation	< 0.1 (2)	KIT inhibitors	GIST, melanoma	Blay JY: Lancet Oncol. 16: 550-560, 2015; Caravajal RD: JAMA 305: 2327-2334, 2011	3A
Total unique cases with actionable GA		57.1 (1,986)				

NOTE. Targetable GAs included in the analysis were those associated with response to targeted therapy in prostate cancer, homologous recombination repair GAs that have been associated with responses to PARP inhibitors, and GAs associated with response to targeted therapy in multiple tumor types.

Abbreviations: EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GA, genomic alteration; GIST, GI stromal tumor; HER2, human epidermal growth factor receptor 2; HNSSC, head and neck squamous cell carcinoma; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase.

*Levels of evidence were ranked according to the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets criteria[18] with one modification that level 3B/4A alterations required reported clinical responses in addition to the criteria outlined in the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets.

TABLE A3. Relative Enrichment of Genomic Alterations Between Primary Site and Metastatic Site Tumors

	Case Frequency, %										
	Short Variant Mutations		Copy Number Alteration		Rearrangements		All Genomic Alterations				
Gene	Primary	Metastasis	Primary	Metastasis	Primary	Metastasis	Primary	Metastasis	Enrichment	Fold Change	*P**
TP53	36.7	42.2	1.9	3.3	1.6	1.6	39.8	46.9	7.1	1.2	< .001
PTEN	7.6	8.1	17.7	28.6	1.7	1.5	26.5	37.4	10.9	1.4	< .001
AR	1.3	11.9	2.4	28.5	0.4	2.1	3.7	39.7	36.0	10.6	< .001
MYC	0.1	0.0	6.1	17.3	0.2	0.3	6.4	17.6	11.2	2.7	< .001
BRCA2	6.4	7.1	2.0	3.2	0.6	1.0	8.7	10.9	2.2	1.3	.0302
RB1	2.5	6.1	3.1	5.7	0.8	0.9	6.4	12.7	6.3	2.0	< .001
APC	7.0	9.3	0.5	0.9	0.6	0.8	8.0	10.6	2.6	1.3	.0085
MLL3	5.2	8.2	0.0	0.0	1.1	1.1	6.3	9.2	2.9	1.5	.0015
SPOP	8.8	6.8	0.0	0.0	0.0	0.0	8.8	6.8	–2.0	1.3	.026
CTNNB1	3.7	6.4	0.0	0.0	0.1	0.4	3.7	6.8	3.0	1.8	< .001
CCND1	0.1	0.0	2.5	6.3	0.0	0.0	2.5	6.3	3.8	2.5	< .001
FAS	0.2	0.2	2.7	5.0	0.2	0.1	3.1	5.2	2.1	1.7	.0021
FGF19	0.0	0.0	1.9	5.6	0.0	0.0	1.9	5.6	3.7	3.0	< .001
FGF3	0.1	0.1	1.7	5.1	0.0	0.0	1.9	5.2	3.3	2.8	< .001
FGF4	0.0	0.0	1.7	5.1	0.0	0.0	1.7	5.1	3.3	2.9	< .001
NCOR1	1.6	3.1	0.1	0.6	0.4	0.9	2.0	4.4	2.3	2.1	< .001
LYN	0.0	0.0	1.4	5.0	0.0	0.0	1.4	5.0	3.6	3.6	< .001
CDKN2A	0.9	0.9	1.1	3.1	0.1	0.1	2.1	4.1	2.0	1.9	< .001
PIK3CB	0.6	0.7	0.5	2.4	0.0	0.0	1.1	3.1	2.0	2.7	< .001

*All primary site versus metastatic site genomic alterations by Fisher's exact test (two-tailed).