CLINICAL CANCER RESEARCH | PRECISION MEDICINE AND IMAGING

CDKN1B Deletions are Associated with Metastasis in African American Men with Clinically Localized, Surgically Treated Prostate Cancer

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ABSTRACT

Purpose: The potential biological determinants of aggressive prostate cancer in African American (AA) men are unknown. Here we characterize prostate cancer genomic alterations in the largest cohort to date of AA men with clinical follow-up for metastasis, with the aim to elucidate the key molecular drivers associated with poor prognosis in this population.

Experimental Design: Targeted sequencing was retrospectively performed on 205 prostate tumors from AA men treated with radical prostatectomy (RP) to examine somatic genomic alterations and percent of the genome with copy-number alterations (PGA). Cox proportional hazards analyses assessed the association of genomic alterations with risk of metastasis.

Results: At RP, 71% (145/205) of patients had grade group ≥3 disease, and 49% (99/202) were non–organ confined. The median PGA was 3.7% (IQR = 0.9%–9.4%) and differed by pathologic

grade ($P < 0.001$) and stage ($P = 0.02$). Median follow-up was 5 years. AA men with the highest quartile of PGA had increased risks of metastasis (multivariable: HR = 13.45; 95% CI, 2.55–70.86; $P = 0.002$). The most common somatic mutations were *SPOP* (11.2%), *FOXA1* (8.3%), and *TP53* (3.9%). The most common loci altered at the copy number level were *CDKN1B* (6.3%), *CHD1* (4.4%), and *PTEN* (3.4%). *TP53* mutations and deep deletions in *CDKN1B* were associated with increased risks of metastasis on multivariable analyses (*TP53*: HR = 9.5; 95% CI, 2.2–40.6; $P = 0.002$; *CDKN1B*: HR = 6.7; 95% CI, 1.3–35.2; $P = 0.026$).

Conclusions: Overall, PGA, somatic *TP53* mutations, and a novel finding of deep deletions in *CDKN1B* were associated with poor prognosis in AA men. These findings require confirmation in additional AA cohorts.

Introduction

African American (AA) men have a 1.8-fold higher incidence of prostate cancer than European American (EA) men and are twice as likely to die from their disease (1). Numerous studies show that AA men still exhibit worse oncologic and mortality outcomes even after controlling for socioeconomic contributors (2, 3). The biological determinants of aggressive prostate cancer in AA men are still unclear and remain an unmet need in cancer health disparities research. There is growing evidence that molecular subtypes of prostate cancer occur at different frequencies in AA and EA men (4–9); yet these differences do not explain the adverse cancer-specific outcomes in AA men. There have been several large-scale genome sequencing studies that have identified unique molecular drivers in prostate cancer (10–12); however these have been largely confined to men of European ancestry. The Cancer Genome Atlas (TCGA) sequencing effort contains only 14% ($n = 43$) AA

men, of whom only 17 have clinically significant cancer [grade group (GG) ≥3]. Although there have been subsequent sequencing studies of AA patients (13–17), many of these have been limited in sample size, number of men with clinically significant cancer, and clinical outcome data necessary to draw prognostic associations. Thus, there remain significant gaps in our understanding of prostate cancer genomics in men of African ancestry. Without representative genomic studies in AA men, we risk worsening the current health care disparities in prostate cancer (18, 19). Using a comprehensive panel designed to target cancer-associated genes, we performed somatic genomic sequencing of the largest cohort of AA men with radical prostatectomy (RP)-treated clinically localized prostate cancer and long-term follow-up for biochemical recurrence (BCR) and metastasis. Our aim is to elucidate the key molecular drivers associated with poor prognosis in this population.

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AACR American Association for Cancer Research®

Materials and Methods

Cohort description

Institutional Review Board approval was obtained from participating institutions [Johns Hopkins Medical Institutions (JHMI) and University of Michigan] in accordance with the U.S. Common Rule, and the study was conducted under a waiver of consent. The AA cohort was selected in two groups, as reported previously (4, 20). First, we identified all consecutive AA men treated by RP at JHMI from 1995 to 2005 with available clinical follow-up. For RP GG1 ($n = 304$ consecutive cases) and RP GG2 ($n = 144$ consecutive cases), 50 AA men were randomly selected from each category but only those with available tissue were included in the study ($n = 28$ GG1, $n = 36$ GG2). All other RP GG categories (GG3-5) had less than 50 AA men treated from 1995 to 2005, thus all consecutive AA men in these GG categories with available tissue were included ($n = 48$ GG3, $n = 26$ GG4, $n = 14$ GG5). For the second group, to increase the cohort size and also enrich for higher GG, all AA RP cases from 2006 to 2010 at JHMI with GG≥3 were additionally included. The final cohort included 220 self-identified AA men: 28 GG1, 36 GG2, 96 GG3, 34 GG4, and 26 GG5. All cases were reviewed by pathologists on this study during cohort selection.

DNA isolation

For all cases, formalin-fixed paraffin embedded (FFPE) RP specimens were obtained, and 5×0.6 mm punches from the dominant tumor nodule (highest GG) were procured for somatic DNA extraction. DNA was extracted from FFPE material using the Qiagen AllPrep FFPE DNA/RNA Kit (Qiagen). DNA concentrations were quantified with the Qubit fluorometer, using a Quant-iT dsDNA High Sensitivity Assay Kit (Invitrogen). Germline DNA from normal tissue was not obtained.

Next-generation sequencing and bioinformatic analysis

We performed targeted, multiplexed PCR-based DNA next-generation sequencing (NGS) as described (21, 22) using a custom designed expanded pan-genitourinary and other cancer (Pan-GU) panel comprised of 3,127 amplicons targeting 135 genes on all autologs and the X chromosome to facilitate genome-wide copy number

profiling (22). The genes included: *ABL1, ALK, APC, AR, ATM, B2M, BAP1, BCL2L1, BCL2L11, BRAF, BRCA1, BRCA2, BRWD1, CCND1, CCNE1, CD274, CDH1, CDH19, CDK12, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CHD1, CTNNA1, DEK, E2F3, EGFR, ERBB2, ERBB3, ERCC2, ERF, ESR1, ETS2, ETV1, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FOXA1, FOXP1, GNAS, JAK1, JAK2, KEAP1, KIF5C, KIT, KLF3, KRAS, LSAMP, MDM2, MDM4, MECOM, MED12, MED12L, MET, MITF, MSH2, MSH6, MTOR, MYC, MYCL, MYCN, NCOR1, NF2, NKX3-1, PBRM1, PDGFRA, PIK3CA, PIK3R1, PPARG, PRKCQ, PTEN, PTPN14, PTPRM, RB1, RET, RYBP, SAV1, SCUBE1, SDHA, SDHB, SDHC, SDHD, SETD2, SMARCB1, SPOPL, STAG2, TCEB1, TFE3, TFEB, TP53, TSC1, TSC2, UBE2G2, WT1, YWHAZ, ZBTB16, ZC3H13, ZFHX3, ZMYM3, ZNF292*. We used 20 to 24 ng of DNA per sample for library construction using the Ion Ampliseq Library Kit 2.0 (Life Technologies) with barcode incorporation and sequencing on the Ion Torrent Proton sequencer as described (21, 22). Data analysis was performed as described to identify high-confidence, prioritized somatic mutations and copy-number alterations (CNA) using validated pipelines based on Torrent Suite 5.0.4.0 (21–24). Samples meeting the following requirements were considered for mutation and CNA calling: ≥1.5M reads, ≥70% reads on target, ≥80% uniformity, and ≥500× mean coverage depth. Samples meeting the following requirements were considered for CNA calling only: ≥1.5M reads, ≥50% reads on target, ≥80% uniformity, and ≥400× mean coverage depth. High-confidence somatic variants occurring at hotspots (>3 observations impacting that residue in COSMIC or cBioPortal) in oncogenes, inframe indels in oncogenes or tumor suppressor genes, or hotspot or deleterious (nonsense/frameshift/splice site altering) variants of tumor suppressor genes were considered driving variants (21, 24). Log2 copy-number ratio cutoffs to define deep deletions, shallow deletions, gains, and amplifications, respectively were < -0.807, 0.415 to -0.807, 0.322 to 0.807, and >0.807. Percent of the genome with copy-number alterations (PGA) was defined as the number of genes with any CNA (as just described)/total number of genes assessed for CNA ($n = 106$). PGA was examined as a continuous variable and by quartiles, similar to previous studies of EA men (25): ≤0.93% (≤25th percentile), 0.94% to 3.74% (25th–50th percentile), 3.75% to 9.35% (50th–75th percentile), and >9.35% (>75th percentile).

Of the 220 cases sequenced, a total of 205 (93%) resulted in interpretable data for CNA/PGA calls; 184 of those 205 cases (90%) had interpretable sequencing data for mutations. All 205 sequenced cases had clinical, pathologic, and long-term follow-up information, including BCR and metastasis. At our institution, men were followed up with PSA assays and digital rectal examinations every 3 months after surgery for the first year, semiannually for the second year, and annually thereafter. A detectable serum PSA level of at least 0.2 ng/mL was evidence of BCR. Time to metastasis was defined as the time from RP to the first detection of distant metastasis on imaging. Distant metastases were diagnosed by radionuclide bone scan, chest radiograph, or other body imaging, which was performed at the time of BCR and annually thereafter.

IHC for p27

p27 IHC was performed manually on standard histologic sections of FFPE prostate tumors using a mouse monoclonal antibody for p27 (SX53G8.5; Cell Signaling Technologies) and an anti-mouse secondary detection kit (Leica). Immunostaining was scored by two pathologists (TLL and DCS), where tumors with any loss (focal or homogeneous) of p27 nuclear staining were considered to have p27 loss, using the prostatic stroma and benign glands as well as inflammatory cells as

internal positive controls. Because p27 immunostaining is sensitive to fixation as reported previously (26), cases without internal control staining were not scored for p27 loss and were considered inevaluable.

Statistical analysis

All statistical analyses were performed using Stata 15.0 (StataCorp). Continuous variables were compared using the rank-sum calculation or Kruskal–Wallis test when appropriate, and proportions were compared with the chi-squared test. BCR-free survival (BFS) and metastasis-free survival (MFS) were analyzed using the Kaplan–Meier method and log-rank statistic. Univariable and multivariable Cox proportional hazards models evaluated the association of genomic alterations with BCR and metastasis; multivariable models were adjusted for age, PSA level, pathologic GG, and pathologic stage. Patients were censored at last follow-up if they did not develop either BCR or metastasis.

Results

Targeted NGS of known cancer-related loci was interpretable for CNA in 205 of 220 AA patients (93%). **Table 1** describes their clinico-pathologic characteristics. Median PSA level was 7.3 ng/mL (IQR = 5.24–13.0 ng/mL), and median age was 58 years (IQR = 53–63 years). Seventy percent of patients had GG3-5 disease (145/205), and 49% of tumors were non–organ confined. Median duration of follow-up was 5 years (IQR = 2–10 years): 39% of patients developed BCR, and 11% developed metastasis. The 10-year BFS and MFS survival rates were 46.8% (95% CI, 36.7%–56.2%) and 81.6% (95% CI, 71.1%–88.6%), respectively.

Median PGA of JHMI AA men was 3.7% (IQR = 0.9%–9.4%). Overall, PGA increased with pathologic grade ($P < 0.001$; **Table 2**) and T-stage ($P = 0.019$). When examined as a continuous variable, PGA was significantly associated with metastasis (**Table 3**; multivariable HR 1.15 per 1% change in PGA; 95% CI, 1.07–1.23; $P < 0.001$) but not with BCR (Supplementary Table S1). In addition, we found that the highest quartile of PGA was significantly associated with metastasis on multivariable analysis (**Fig. 1A**; **Table 3**; HR = 13.45; 95% CI, 2.55–70.86; $P = 0.002$) and with BCR on univariable analysis (HR = 1.91; 95% CI, 1.06–3.45; $P = 0.032$; Supplementary Table S1).

Although it is not possible to compare PGA between different cohorts due to different sequencing methodology, for comparisons of PGA between EA and AA men within a single cohort (**Table 2**), we examined the TCGA dataset which includes 43 self-identified AA men [with race confirmed in 98% (42/43) by evidence of germline African ancestry (27); Supplementary Table S2] with available PGA data. The median PGA for TCGA AA men was 6.5% (IQR = 0.5%–12.3%) and increased with GG ($P < 0.001$). Similarly, median PGA for TCGA EA men was 6.1% (IQR = 1.7%–11.7%) and also increased with GG ($P < 0.001$). There did not appear to be any differences in PGA by ancestry except among GG5 disease (AA 26.2% vs. EA 9.0%, $P = 0.032$), although sample numbers were very small for AA men with GG5 cancer ($n = 4$).

Of the 205 JHMI AA men, 184 had both somatic mutation and CNA data available, and this subset of men was analyzed to identify any associations between genomic alterations (somatic mutations or CNA) and oncologic outcomes in our cohort. Supplementary Table S3 describes the clinico-pathologic characteristics of this subset. Overall, 35% (65/184) of this subset had pathogenic somatic mutations detected using this targeted sequencing panel, consistent with the relatively low mutation burden reported in primary prostate cancer

Table 1. Clinico-pathologic characteristics and PGA for African-American cohort (copy-number alteration data available for $n = 205$ of 220 patients).

Variable	AA ($n = 205$)
Median PSA, ng/mL (IQR)	7.30 (5.24–13.00)
Median age, year (IQR)	58 (53–63)
Clinical T stage, n (%)	
T1c	154 (75.1)
T2a	34 (16.6)
T2b/c	17 (8.3)
Biopsy grade group, n (%)	
1 (GS 6)	72 (35.1)
2 (GS 3+4)	49 (23.9)
3 (GS 4+3)	48 (23.4)
4 (GS 8)	26 (12.7)
5 (GS 9–10)	10 (4.9)
RP grade group, n (%)	
1 (GS 6)	28 (13.7)
2 (GS 3+4)	32 (15.6)
3 (GS 4+3)	91 (44.4)
4 (GS 8)	33 (16.1)
5 (GS 9–10)	21 (10.2)
Pathologic T stage, n (%)	
T2N0	87 (43.1)
T3aN0	71 (35.2)
T3bN0	28 (13.9)
N1	16 (7.9)
Median length of follow-up, year (IQR)	5 (2–10)
BCR, n (%)	74 (39.2)
Median time to BCR, year (IQR)	2 (1–7)
5-Year BCR-free survival (95% CI)	0.603 (0.518–0.678)
10-Year BCR-free survival (95% CI)	0.468 (0.367–0.562)
Metastasis, n (%)	20 (10.6)
Median time to metastasis, year (IQR)	5 (2–9)
5-Year metastasis-free survival (95% CI)	0.918 (0.852–0.956)
10-Year metastasis-free survival (95% CI)	0.816 (0.711–0.886)
Median PGA (IQR)	3.74 (0.93–9.35)
Mean PGA	5.85
Median PGA, high CNV only (IQR)	0 (0,0)
Mean PGA, high CNV only	0.45

($\sim$1 mutation/Mb; ref. 11). The most common somatic mutations and CNA are listed in **Fig. 1B** and Supplementary Tables S4 and S5. The three most commonly mutated genes were *SPOP* (11.2%), *FOXA1* (8.3%), and *TP53* (3.9%). The three loci most commonly altered at the copy-number level were *CDKN1B* (6.3%), *CHD1* (4.4%), and *PTEN* (3.4%).

Because most alterations were present at a low prevalence in this cohort, we selected the six alterations with >3% prevalence (*FOXA1* mutation, *SPOP* mutation, *TP53* mutation, *CDKN1B* deep deletion, *CHD1* deep deletion, and *PTEN* deep deletion) and assessed associations with oncologic outcome. Among the mutated genes, *TP53* mutation was the only one significantly associated with metastasis on univariable analysis and remained significant on multivariable analysis (**Fig. 1C**; **Table 4**; HR = 9.45; 95% CI, 2.20–40.55; $P = 0.002$). *TP53* mutation did not meet the threshold for statistical significance for association with BCR on univariable analysis (Supplementary Table S1; HR = 2.36; 95% CI, 0.95–5.69; $P = 0.065$) but did reach significance on Kaplan–Meier analysis ($P = 0.038$). Among the CNA, deep deletions in *CDKN1B* were associated with increased risk of metastasis (**Fig. 1D**; **Table 4**; multivariable HR = 6.66; 95% CI, 1.26–

Table 2. PGA in JHMI AA cohort ($n = 205$) and TCGA cohort ($n = 313$) by pathologic grade.

	JHMI AA ($n = 205$)		TCGA AA ($n = 43$)		TCGA EA ($n = 270$)	
	PGA, median (IQR)	P value	PGA, median (IQR)	P value	PGA, median (IQR)	P value
Total	3.74 (0.93–9.35)		6.48 (0.45–12.34)	0.877[a]	6.08 (1.74–11.71)	
RP grade group		<0.001[b]		<0.001[b]		<0.001[b]
1	0.94 (0–4.21)		0.61 (0.004–6.39)	0.486[a]	2.11 (0.05–4.99)	
2	1.87 (0–3.27)		3.89 (0.09–7.53)	0.480[a]	4.68 (1.36–7.94)	
3	3.74 (0.94–10.28)		9.57 (6.34–16.73)	0.154[a]	6.94 (2.68–11.38)	
4	8.41 (2.80–11.22)		12.03 (8.86–15.51)	0.780[a]	13.74 (7.40–22.20)	
5	4.67 (0.94–9.35)		26.2 (15.2–34.9)	0.032[b]	8.97 (3.53–17.90)	

[a]P value signifies rank sum calculation to detect differences in PGA between TCGA AA and TCGA EA cohorts within each grade group.
[b]P value signifies Kruskal–Wallis calculation to detect differences in PGA between grade groups within each cohort.

35.22; $P = 0.026$) but were not significantly associated with BCR (Supplementary Table S1). Deep deletions in *PTEN* did not meet the threshold for statistical significance for association with metastasis on multivariable analysis (**Table 4**; HR = 5.16; 95% CI, 0.97–27.48; $P = 0.055$) but were significantly associated with BCR on multivariable analysis (Supplementary Table S1; HR = 2.94; 95% CI, 1.08–8.00; $P = 0.035$). No metastatic events were observed in patients with deep deletions in *CHD1*. Although *NKX3-1* CNA had a prevalence of 3.4% (Supplementary Table S4), these were evenly split between amplifications and deletions, thus association of NKX3-1 with outcomes was not performed.

As deep deletions in *CDKN1B* have not previously been described as a recurrent genomic alteration in AA men associated with prognosis, we aimed to further characterize the patients harboring this genomic lesion. Median PSA for these men was 11.9 ng/mL (IQR = 6.3–21.0 ng/mL), and 60% (6/10) of the lesions occurred in men who were younger than the cohort's median age (58 years). Of the 10 tumors with deep deletions in *CDKN1B*, 50% (5/10) were GG4-5, even though GG4-5 disease only represented about 25% of the entire cohort. In addition, 90% (9/10) of the tumors were non–organ confined at RP. To confirm the presence of *CDKN1B* deep deletions in this subgroup, we performed IHC for p27, the protein encoded by *CDKN1B*. As previously reported, p27 immunostaining is highly sensitive to tissue fixation conditions (26), thus of the 10 cases with *CDKN1B* deep deletions, only 5 had adequate internal control staining in benign glands and stroma to allow evaluation of p27 expression levels in tumor cells. Of those with adequate staining, 60% (3/5) had focal loss of p27 protein in some, but not all tumor cells whereas 40% had homogeneous loss of p27 (**Fig. 2**), confirming the copy-number calls.

Discussion

There is limited understanding of the underlying genetic mechanisms of aggressive disease in AA men with clinically localized prostate tumors. Here we performed targeted NGS in the largest cohort of AA men to date, enriched with intermediate and high-grade disease. We demonstrated that somatic *TP53* mutations, deletions in *CDKN1B*, and extent of PGA were associated with risk of metastasis in AA men in multivariable models controlling for clinical-pathologic parameters, including Gleason grade group. This study is the first to correlate somatic genomic sequencing data with risk of metastasis in AA prostate cancer. Notably, the poor prognosis of patients with deep deletions in *CDKN1B* is a novel finding that has important clinical implications for AA men. These deep deletions were more common in younger men with higher grade and stage disease and correlated with p27 protein loss.

Several large-scale sequencing efforts have greatly contributed to our understanding of the genomic landscape of prostate cancer (11, 12). TCGA identified seven recurrent molecular subtypes of prostate cancer, including four gene fusion events (*ERG, ETV1/4,* and *FLI1*) and three mutational events (*SPOP, FOXA1,* and *IDH1*). However, their cohort of 333 prostate tumors included 43 from AA patients and only 17 AA patients with GG ≥3 disease, limiting the generalizability and clinical relevance of their results. In addition, current genomic risk classifiers used in risk stratification of prostate cancer are derived from primarily EA cohorts, and their use in underrepresented racial groups may exacerbate health disparities (18, 19). Our work in this study contributes to the much needed effort to understand the genomics of AA prostate cancer and reduce the pervasive racial gap in cancer health care.

Table 3. Univariable and multivariable Cox proportional hazards models assessing association of PGA with metastasis.

	Univariable		Multivariable[a]	
Variable	HR (95% CI)	P value	HR (95% CI)	P value
PGA (continuous)[b]	1.11 (1.06–1.17)	<0.001	1.15 (1.07–1.23)	<0.001
PGA (quartile)[c]				
≤25th percentile	1.00 (reference)	—	1.00 (reference)	—
25–50th percentile	0.80 (0.07–8.88)	0.859	0.58 (0.05–6.43)	0.655
50–75th percentile	5.28 (1.02–27.41)	0.048	3.31 (0.60–18.25)	0.170
>75th percentile	14.43 (3.13–66.65)	0.001	13.45 (2.55–70.86)	0.002

[a]Multivariable models were adjusted for age at RP, PSA, RP grade group <4 or ≥4, and pathologic stage. RP grade group was analyzed as dichotomous variable due to low number of metastatic events per grade group.
[b]Per 1% change in PGA.
[c]≤0.93% (≤25th percentile), 0.94%–3.74% (25th–50th percentile), 3.75%–9.35% (50th–75th percentile), and >9.35% (>75th percentile).
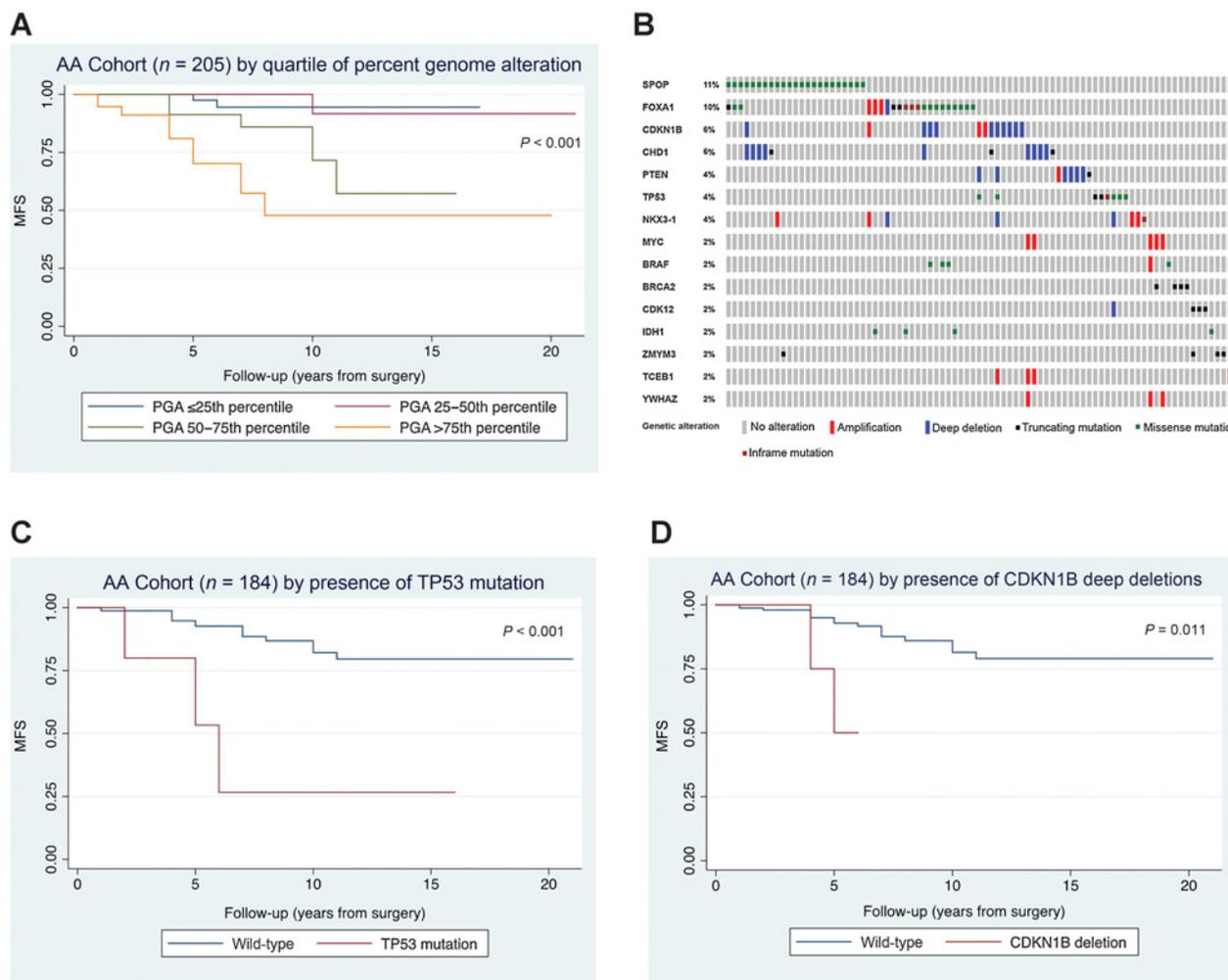
Figure 1.
Correlation of clinical outcomes and genomic alterations in AA cohort. **A,** Kaplan–Meier plot of MFS by quartile of PGA. **B,** Oncoprint showing distribution of somatic mutations and copy-number alterations for AA cohort. **C,** Kaplan–Meier plot of MFS by presence of *TP53* mutation compared with wild type. **D,** Kaplan–Meier plot of MFS by presence of *CDKN1B* deep deletions compared with wild type.

Other groups have included exclusively men of African ancestry in their sequencing studies (13–16), and several unique genomic drivers of carcinogenesis have been identified. However, with the exception of Huang and colleagues (13), these studies have generally had small numbers of AA men with clinically significant disease. Importantly, none have included long-term clinical outcome data for metastasis. In their cohort of 192 AA tumors, Huang and colleagues (13) identified recurrent mutations in *SPOP* (8%), *FOXA1* (6%), and *TP53* (7%) along with *ERF* (5%), a novel *ETS* transcription factor somatic alteration not previously recognized as a recurrent mutation in primary prostate cancer. In our cohort, we found 10 cases with CNA in *ERF* (one deep deletion, six shallow deletions, and three low-level amplifications) but no mutations. Petrovics and colleagues (14) sequenced seven AA prostate tumors and seven EA tumors and discovered novel deletions in the *LSAMP* region enriched in AA patients and associated with BCR. *LSAMP* deletions were seen in three cases of our AA cohort (one deep deletion, two shallow deletions).

In this study we identified two genomic alterations that were associated with increased risk of metastasis among AA men: *TP53* mutations and deletions in *CDKN1B*. The *TP53* tumor suppressor gene is arguably the most frequently mutated gene in human cancer, present in more than 40% of tumors overall (28). However, in prostate cancer surgical cohorts, *TP53* mutations are relatively infrequent: 8% in the TCGA cohort (EA men) and 4% in the AA men of the current study. This prevalence increases up to 50% in metastatic castration-resistant prostate cancer, though again, predominantly EA men were studied (29). Accordingly, the presence of *TP53* mutation is associated with an aggressive prostate cancer phenotype and prostate cancer–specific mortality (30, 31). Recent genome analyses by racial ancestry in pan-cancer cohorts reveal that *TP53* mutations are significantly more common among AA cancer patients than EA patients (27), a finding that does not seem to be replicated in prostate cancer based on the TCGA data described above and additional studies by our group (32). Importantly, although the overall prevalence of *TP53* mutations is generally low

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Table 4. Univariable and multivariable Cox proportional hazards models assessing association of genetic alterations with metastasis.

Variable	Univariable		Multivariable[a]	
	HR (95% CI)	P value	HR (95% CI)	P value
CDKN1B deep deletion	5.71 (1.23–26.61)	**0.026**	6.66 (1.26–35.22)	**0.026**
CHD1 deep deletion[b]	—	—	—	—
PTEN deep deletion	4.19 (0.94–18.62)	0.060	5.16 (0.97–27.48)	0.055
FOXA1 mutation	0.57 (0.08–4.29)	0.586	0.34 (0.04–2.76)	0.310
SPOP mutation	1.05 (0.30–3.62)	0.942	1.34 (0.34–5.32)	0.678
TP53 mutation	7.01 (2.01–24.41)	**0.002**	9.45 (2.20–40.55)	**0.002**

[a]Multivariable models were adjusted for age at RP, PSA, RP grade group <4 or ≥4, and pathologic stage. RP grade group was analyzed as dichotomous variable due to low number of metastatic events per grade group.
[b]No metastatic events were observed for CHD1 genetic alterations, hence HR and P values were not calculated.

in AA men with clinically localized prostate cancer, it remains associated with outcomes as seen in EA cohorts, reminiscent of findings with *PTEN* loss (4).

To our knowledge, no prior studies have identified deletions in *CDKN1B* as a recurrent somatic genomic lesion in AA prostate cancer that is associated with metastasis, although there is some indication of a similar association in previous EA cohorts. *CDKN1B* encodes for p27, a cyclin-dependent kinase inhibitor that negatively regulates cell proliferation and whose loss is associated with poor prognosis in several cancer types. In older studies of predominantly EA cohorts (33–36), prostate tumors that had decreased expression of p27 protein were found to have an increased risk of BCR after surgery. However other studies have shown that the absence or low expression of p27 has no impact on prostate cancer prognosis (36–38), and it often occurs in association with other known prostate cancer molecular subtypes (e.g., *PTEN* loss or *ERG*-negative tumors; refs. 35, 37). Many of these studies are limited in their sample size and also lack a uniform cut-off between high and low p27 protein expression, typically measured by IHC. In addition, prior studies have established that p27 immunostaining is highly sensitive to formalin fixation time, which introduces a significant

preanalytic variable into evaluation of p27 immunostaining, and makes many cases inevaluable (26). In some linkage studies, germline polymorphisms in *CDKN1B* have been associated with hereditary prostate cancer (39–41). In addition, loss of heterozygosity at the *CDKN1B* locus (12p13) has been identified as a recurrent alteration in metastatic prostate cancer, and it is associated with higher GG disease, lymph node metastases, and advanced tumor stage among predominantly EA cohorts (42, 43). In this study, deep deletions in *CDKN1B* occurred in 5% to 6% of AA men and were independently associated with risk of metastasis, findings which have not been previously described in this population. We were able to confirm subclonal (heterogeneous) or clonal (homogeneous) p27 protein loss by IHC in a subset of evaluable samples with *CDKN1B* deep deletions in our cohort, confirming the copy-number calls in this subset of cases and providing additional information on likely clonality. However, we acknowledge that p27 immunostaining is likely not adequate for screening large cohorts due to variability in preanalytic conditions. In addition, we found *CDKN1B* deletions were more common in younger men who presented with higher stage and grade disease, suggesting this cell-cycle pathway may be important in the more aggressive disease state found in AA prostate cancer.

Consistent with an association of *CDKN1B* loss with aggressive prostate cancer, previous sequencing studies have found the prevalence of *CDKN1B* deep deletions is enriched in metastatic compared with primary disease (Supplementary Fig. S1). In most primary tumor cohorts, such as the TCGA, *CDKN1B* deep deletions are reported in only 1% to 2% of cases overall, whereas the prevalence is increased to 2% to 6% of metastatic cohorts. Unfortunately, the low prevalence in the TCGA cohort makes it impossible to determine whether these deletions are more common among AA patients. In the Huang and colleagues (13, 44) study of AA tumors, deep deletions of *CDKN1B* occurred in 2.2% of primary tumors, but there was no matched EA cohort included in this study. As mentioned above, our AA cases were significantly higher grade and stage compared with those included in TCGA and Huang and colleagues, potentially explaining why the rate of *CDKN1B* loss in the current study is somewhat higher.

To better compare deep deletion frequency between AA and EA patients, we interrogated data from the AACR Project GENIE cohort (Supplementary Fig. S1; ref. 45) and found that *CDKN1B* deep deletions occurred in 1.2% (14/1,206) of primary prostate cancer among EA patients and 1.8% (2/109) of primary prostate cancer

Homogeneous loss | Heterogeneous loss
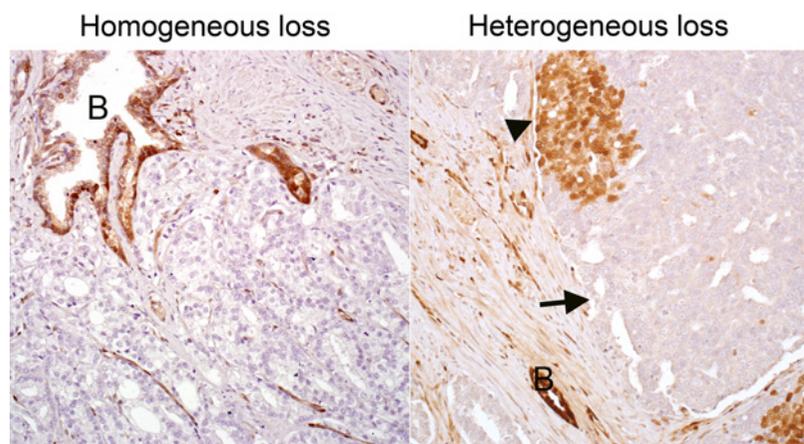
Figure 2.
p27 immunostaining in African-American prostate tumors with *CDKN1B* deep deletions. Both cases showed deep deletions of *CDKN1B* by sequencing, however the tumor on the left demonstrates homogeneous loss of p27 protein, suggestive of a clonal alteration, whereas the tumor on the right shows heterogeneous loss of p27 in some (arrow) but not all (arrowhead) tumor cells, suggestive of a subclonal alteration in *CDKN1B*. In both cases, benign glands (B) provide an internal positive control for p27 staining. All images reduced from 200× magnification.

among AA patients. Consistent with our finding that *CDKN1B* deletion is associated with risk of metastasis, the rate of deletion was higher in both races in metastatic tumors (4.5% or 35/764 in EA patients and 5.7% or 4/70 in AA patients), although this difference between primary and metastatic disease only reached statistical significance in the EA samples ($P < 0.0001$). However, the EA and AA cohorts were not matched in the GENIE samples and may be confounded by selection for specific clinical–pathologic variables (45). Thus, it remains uncertain whether the relatively high rate of *CDKN1B* deletions in our AA primary tumor cohort (6%) is due to the higher grade and stage of these tumors relative to other cohorts, or whether this high rate is a cohort-specific or race-specific finding. Additional AA cohorts will be required to resolve these questions. In addition, future research is warranted to determine if *CDKN1B* deep deletions play a contributory role in outcome disparities between races.

PGA in tumors from men of African ancestry has only been described in a few sequencing studies (13, 15, 16). The extent of CNA, or PGA, was an important predictor of adverse prognosis in the AA men in our cohort. Patients with the highest quartile of PGA had increased risks of metastasis and BCR. Previous work by Hieronymus and colleagues (25) demonstrated in predominantly EA cohorts that CNA burden is a prognostic factor in prostate cancer relapse and metastasis and can also be assessed in biopsy specimens, allowing for improved risk stratification and treatment guidance in the diagnostic setting. Our study further corroborates the association of PGA with high-risk pathology and increased metastasis among AA men with prostate cancer. Similar to our findings, PGA in the TCGA cohort increased with pathologic grade in both AA and EA men. Although the only difference in PGA between the AA and EA men of TCGA was among GG5 disease, this comparison is severely limited due to the small number of men with GG5 tumors (AA $n = 4$, EA $n = 38$). Continued sequencing efforts are necessary to robustly compare the PGA profile between AA and EA men and determine its precise role as a biomarker.

The strengths of our study include the large cohort of intermediate and high-grade AA prostate tumors ($n = 145$ GG≥3) and the long-term clinical outcome data, which allowed for the first correlation of somatic genomic sequencing with risk of metastasis in AA men to our knowledge. The inclusion of men of African ancestry in prostate cancer genomic studies is crucial to understanding the role of race in health disparities. AA race was determined by self-identification in our study, but we did not confirm this with racial ancestry informative genetic markers. A major limitation of our study is the lack of a grade-matched EA cohort that would have allowed comparisons between races. However, it is important to note that the value of studying the AA population is not principally in its comparison to a reference EA cohort; rather, it is critical to fill in the gaps in our knowledge about AA prostate cancer genomics so that we can better tailor risk stratification and precision therapies in the AA population that is disproportionately affected by prostate cancer mortality. Importantly, while *TP53* mutations and deletions in *CDKN1B* were shown to be associated with an increased risk of metastasis in AA men after surgery, overall these genomic alterations remain relatively infrequent in AA men with clinically localized prostate cancer, and the multivariable models are knowingly over-fitted with estimates that could deviate from truth. However, they are presented here for comparison to univariable hazard ratios. Additional AA cohorts are needed to

corroborate our findings. Finally, it is important to note that because this study was designed to look specifically at outcomes in AA patients relative to the most common genomic alterations, we used targeted sequencing and did not perform whole genome or whole-exome sequencing to look for novel low prevalence alterations in AA tumors.

In conclusion, targeted somatic sequencing of AA prostate tumors reveals significant associations of genomic alterations with metastasis in AA men. Notably, our study is the first to describe deletions in *CDKN1B* as a recurrent genomic lesion in AA men that is associated with poor prognosis. Furthermore, the observation that these deletions may occur in younger men with high-grade tumors suggests that this pathway may contribute to a more aggressive disease course. This work begins to fill in the significant gaps in our understanding of the biology of aggressive prostate cancer in AA men. Continued research in AA prostate cancer genomics will guide the advancement of precision medicine in underrepresented populations and help alleviate cancer health disparities.

Disclosure of Potential Conflicts of Interest

D.H. Hovelson is an employee/paid consultant for Strata Oncology. D.E. Spratt is an employee/paid consultant for Janssen, AstraZeneca, and Blue Earth. S.A. Tomlins is an employee/paid consultant for Strata Oncology, Astellas, Janssen, Almac Diagnostics, Sanofi, and Abbvie, reports receiving commercial research grants from Astellas, and holds a patent on ETS gene fusion, licensed to Hologic and Ventana. T.L. Lotan reports receiving commercial research grants from Ventana/Roche and GenomeDx. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F.A. Faisal, S. Murali, H. Kaur, L.B. Guedes, D.C. Salles, J.J. Tosoian, S. Han, D.E. Spratt, T.L. Lotan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F.A. Faisal, T. Vidotto, L.B. Guedes, D.C. Salles, J.J. Tosoian, D.H. Hovelson, K. Hu, A.S. Baras, S.A. Tomlins, T.L. Lotan
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Acknowledgments

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Clinical Cancer Research

CDKN1B Deletions are Associated with Metastasis in African American Men with Clinically Localized, Surgically Treated Prostate Cancer

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