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Elevated serum CEA is associated with liver metastasis and distinctive circulating tumor DNA alterations in patients with castration-resistant prostate cancer

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Abstract

Background: Elevated serum carcinoembryonic antigen (CEA) is used to identify "treatment emergent" forms of castration-resistant prostate cancer (CRPC) such as aggressive variant prostate cancer (AVPC). However, its individual utility as a prognostic marker and the genetic alterations associated with its expression have not been extensively studied in CRPC.

Methods: This study retrospectively analyzed clinical outcomes and circulating tumor DNA profiles in 163 patients with CRPC and elevated or normal serum CEA. These same patients were then classified as AVPC or non-AVPC and compared to determine the uniqueness of CEA-associated gene alterations.

Results: Patients with elevated CEA demonstrated higher rates of liver metastasis (37.5% vs. 19.1%, $p = 0.02$) and decreased median overall survival from CRPC diagnosis (28.7 vs. 73.2 mo, $p < 0.0001$). In addition, patients with elevated CEA were more likely to harbor copy number amplifications (CNAs) in *AR*, *PIK3CA*, *MYC*, *BRAF*, *CDK6*, *MET*, *CCNE1*, *KIT*, *RAF1*, and *KRAS*. Based on variant allele frequency we also defined "clonal" single-nucleotide variants (SNVs) thought to be driving disease progression in each patient and found that CEA expression was negatively correlated with clonal *AR* SNVs and positively correlated with clonal *TP53* SNVs. Of these genetic associations, only the increases in clonal *TP53* SNVs and *KRAS* amplifications were recapitulated among patients with AVPC when compared to patients without AVPC.

Conclusions: Together these findings suggest that CEA expression in CRPC is associated with aggressive clinical behavior and gene alterations distinct from those in AVPC.

KEYWORDS
aggressive variant prostate cancer, carcinoembryonic antigen, castration resistant prostate cancer, circulating tumor DNA, gene amplifications, liver metastasis

1 | INTRODUCTION

Prostate cancer is the second most frequently diagnosed malignancy in men worldwide.[1] Locally recurrent and metastatic prostate cancers typically exhibit an exceptional initial response to androgen deprivation therapy (ADT), but over time invariably progress on this first line of hormone therapy and become castration resistant. The onset of castration-resistant prostate cancer (CRPC) carries a grim prognosis and has been attributed to the evolution of persistent intratumoral androgen receptor (AR) signaling in the absence of circulating testosterone.[2] Recent studies have described a distinct subtype of CRPC, termed aggressive variant prostate cancer (AVPC), characterized by histologic and clinical characteristics atypical of prostatic adenocarcinoma.[3–5] These cancers have features of primary small cell and neuroendocrine prostate cancers, and they appear to be inherently nonreliant on AR signaling.[6] In addition, their incidence is increasing alongside use of novel highly potent antiandrogens such as enzalutamide and abiraterone, raising concern that they represent treatment emergent entities.[6]

Carcinoembryonic antigen (CEA) is used to identify AVPC when present alongside other markers of neuroendocrine cancer, and its expression has been associated with decreased overall survival in this patient population.[3] However, its utility as an independent marker of disease behavior and prognosis in CRPC has not been extensively studied. Moreover, only a subset of AVPCs express CEA, and it is unclear whether this is indicative of underlying genetic heterogeneity within this subtype of CRPC.[3] In this study, we retrospectively compared the clinical and genetic characteristics of CRPCs in patients with elevated and normal serum CEA. We also attempted to determine whether the genetic alterations identified in CEA expressing cancers were distinct from the genetic differences which distinguish AVPCs and non-AVPCs. While genetic profiling of individual cancers is conventionally performed via solid tissue biopsy, this technique is subject to sampling bias and may fail to capture the predominant driver mutations in clonally diverse cancers.[7] To circumvent this issue, we utilized circulating tumor DNA (ctDNA) analysis that is an acceptable alternative to solid tissue-based techniques per the National Comprehensive Cancer Network (NCCN) guidelines for CRPC.[8]

2 | MATERIALS AND METHODS

2.1 | Patient selection

Patients with CRPC who underwent ctDNA profiling via the Guardant360 platform (Guardant Health Inc.) at the Medical University of South Carolina (MUSC) between August 2014 and June 2020 were eligible for analysis. To be included, patients were required to have undergone CEA and ctDNA analysis at least once at the time of, or following, CRPC diagnosis. Patients with a second active malignancy were excluded. CRPC was defined as radiologic or laboratory evidence of disease progression despite a castrate level

testosterone. Laboratory progression was defined as a rise in prostate-specific antigen (PSA) of >2 ng/ml and >25% from treatment nadir with a second subsequent PSA confirming a positive trend, or alternatively as a change in therapy due to rising PSA if this occurred before the above. Patients with histology-proven small cell carcinoma of the prostate were classified as having CRPC regardless of androgen deprivation history. Nonsmoking patients with CEA > 5 ng/ml (institutional upper limit of normal) were defined as having elevated CEA. A cutoff of CEA > 10 ng/ml was used for current smokers.

Previously described Aparicio criteria were used to identify patients with AVPC.[3] These criteria include: histologic evidence of small-cell carcinoma, exclusively visceral metastases, predominantly lytic bone metastases, bulky lymphadenopathy or bulky high-grade tumor mass in prostate/pelvis, low PSA despite high volume bony disease, neuroendocrine markers in serum or histology coupled with hypercalcemia and/or elevated CEA/LDH, and short interval to androgen-independent progression following initiation of androgen deprivation therapy.[3] Average PSA was calculated from values obtained at time of ctDNA analysis. Average LDH and CEA were calculated from the maximum value observed following prostate cancer diagnosis. The individual clinical and laboratory characteristics of patients eligible for retrospective analysis are listed in Supporting Information: Table S1. This study was approved by the Institutional Review Board at MUSC.

2.2 | ctDNA sequencing

Blood for ctDNA profiling was obtained during clinic visits at time of diagnosis, significant disease progression, or establishment of care. ctDNA sequencing and analysis were performed by Guardant Health Inc., a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists-accredited, New York State Department of Health-approved laboratory. The technical aspects of the Guardant360 platform have been previously discussed.[7,9] Briefly, the platform uses digital sequencing to characterize single-nucleotide variants (SNVs), insertions/deletions (indels), copy number amplifications (CNAs), and fusions in predefined exons and genes in ctDNA from patient plasma. Only Guardant360 results obtained at the time of or after CRPC diagnosis in each patient were examined. For patients who underwent multiple ctDNA tests at the time of or after CRPC diagnosis, a single "consensus" ctDNA profile was used which contained all identified amplifications, SNVs, and clonal SNVs. SNVs deemed "synonymous" were excluded from final analysis. A complete list of the ctDNA alterations identified in each individual patient are available in Supporting Information: Table S2. Over the course of the study, six sequential iterations of the assay were used (54-, 68-, 70-, 73-, 74-, and 83 gene panels). The first iteration of the assay was incapable of detecting CNAs, but no patient included in the retrospective analysis underwent ctDNA characterization with iteration 1 of the assay alone. Gene alterations not detected by all 5 subsequent assay iterations were excluded from comparative

analyses. The variant allele frequencies (VAFs) of these excluded SNVs, as well as synonymous SNVs, were still used to identify clonal non-synonymous SNVs and maximum VAFs in each patient.

2.3 | Statistical analysis

All analyses were performed using SAS version 9.4. No corrections for multiple comparisons were made and statistical significance was based on $p < 0.05$. Baseline clinical and demographic variables were summarized using frequencies and percentages for categorical variables and mean, median, standard deviation, and range for continuous variables. Associations between CEA elevation status (elevated vs. normal) and incidence of metastasis (yes or no) were evaluated using Fisher's exact test. Likewise, associations of CEA status with: CNA, SNV, and clonal SNV incidence, and AVPC status (yes or no) were evaluated using Fisher's exact test. Among patients with at least one CNA, SNV, or clonal SNV, a Wilcoxon rank-sum test was used to compare the average number of these alterations in CEA elevated and CEA normal patients. Serum CEA, LDH, and PSA concentrations, as well as maximum VAF, were also compared using a Wilcoxon rank-sum test. Additionally, the associations between AVPC status and CNA, SNV, and clonal SNV incidence were evaluated using Fisher's exact test.

Survival times were constructed as the time interval from CRPC diagnosis to death from any cause. Survival times for patients still alive at the time of last follow-up or the data cutoff date (August 31, 2021) were censored. Survival curves were constructed using Kaplan–Meier methods and median survival times reported for CEA elevated and normal patients. Corresponding 95% confidence intervals (95% CI) were constructed using Greenwood's variance estimator. Differences in survival were evaluated using a two-sided log-rank test. Hazard ratio (HR) estimation was performed using univariate and multivariable hazard regression models to obtain unadjusted and adjusted estimates, respectively. Although the univariate model was fitted using Cox proportional hazards regression, the multivariable model was fitted using a stratified Cox model because of an observed violation of proportional hazards

3 | RESULTS

3.1 | Characteristics of patients undergoing retrospective analysis

A total of 302 patients underwent ctDNA profiling in the 6 years before the initiation of this retrospective research study. Fifty-four of these 302 patients carried a diagnosis of castration-sensitive prostate cancer (CSPC) at the time of ctDNA analysis and were excluded from the study. Of the remaining 248 patients, 174 had a documented serum CEA level obtained following diagnosis of CRPC. Eleven patients were further excluded due to the presence of a coexisting active malignancy. The remaining 163 patients with CRPC underwent

TABLE 1 Clinical and demographic characteristics of CRPC patients selected for retrospective analysis (N = 163)

Characteristic	Statistic	Result
Age at CRPC diagnosis (year)	Mean (SD)	68.1 (8.7)
	Median (range)	69 (46–95)
PSA (ng/ml)	Mean (SD)	274.8 (734.1)
	Median (range)	53.9 (0.1–6000)
Race	No. (%)	
Non-Black		107 (65.6)
Black		56 (34.4)
Therapy received	No. (%)	
Docetaxel		117 (71.8)
Enzalutamide		113 (69.3)
Abiraterone		109 (66.9)
Cabazitaxel		67 (41.1)
Radium-223		35 (21.5)
Sipuleucel-T		26 (16.0)
Metastatic site	No. (%)	
Bone		141 (86.5)
Lymph node		123 (75.5)
Viscera		53 (32.5)
Liver		40 (24.5)
Lung		18 (11.0)
Other		13 (8.0)
AVPC	No. (%)	63 (38.7)
Elevated CEA	No. (%)	48 (29.5)

Note: PSA obtained at time of ctDNA analysis. Other visceral metastatic sites include adrenal gland, brain, dura, and peritoneum.

Abbreviations: AVPC, aggressive variant prostate cancer; CAE, carcinoembryonic antigen; ctDNA, circulating tumor DNA; CRPC, castration-resistant prostate cancer; PSA, prostate-specific antigen.

retrospective clinical and genetic analysis. Baseline clinical and laboratory characteristics of these 163 patients are shown in Table 1. Fifty-three patients (32.5%) had visceral metastasis to either the lungs, liver, brain, adrenal glands, dura, or peritoneum. After androgen deprivation therapy, docetaxel was the most common therapy received before or during the study period (71.8% of patients). Enzalutamide (69.3%) and abiraterone (66.9%) were also frequently received. Forty-eight patients (29.4%) were classified as having elevated serum CEA. Sixty-three patients (38.7%) were defined as having aggressive variant prostate cancer (AVPC) based on the presence of one or more previously described Aparicio criteria, which include: histologic evidence of small-cell carcinoma, exclusively visceral metastases, predominantly lytic bone metastases, bulky lymphadenopathy or bulky high-grade tumor mass in prostate/

pelvis, low PSA despite high volume bony disease, neuroendocrine markers in serum or histology coupled with hypercalcemia and/or elevated CEA/LDH, and short interval to androgen-independent progression following initiation of androgen deprivation therapy.[3]

3.2 | Metastasis and overall survival in patients with elevated CEA

Rates of metastasis in patients with elevated CEA were examined to assess whether this serum marker was associated with clinical outcomes in our study population. Patients with elevated CEA exhibited a higher rate of visceral metastasis (45.8% vs. 27.0%, p = 0.03) (Table 2). This appeared to be entirely due to differences in rates of liver metastasis (37.5% vs. 19.1%, p = 0.02) as there were no significant differences in the rates of metastasis to the lungs or other visceral sites. There was also no significant increase in the prevalence of lymph node or bone metastasis in patients with elevated serum CEA.

The relationship between CEA status and overall survival (OS) was also examined. Of the 163 patients in our study, nine were excluded from this analysis due to missing date of diagnosis. For instances in which day of diagnosis was missing (n = 31) but month and year were present, we imputed the 15th of the month. To facilitate comparability between univariate and multivariable analyses, the data were further limited to patients with complete covariate data for: age, race (Black and non-Black), AVPC status, average PSA, maximum LDH, incidence of liver metastasis, incidence of nonliver visceral metastases (lung, brain, adrenal gland, peritoneum, and dura), incidence of bone metastasis, incidence of lymph node metastasis, history of antiandrogen therapy (abiraterone or enzalutamide), and history of taxane chemotherapy (docetaxel or cabazitaxel), which further reduced the available sample size for analysis of OS to n = 147. Overall survival of patients with elevated CEA was significantly lower than that of patients with normal CEA (HR = 4.27, 95% CI = 2.48–7.36; log-rank p < 0.0001, Figure 1). Specifically, median OS estimates for patients with elevated and normal CEA were 28.7 months (95% CI = 17.9–52.9 months) and 73.2 months (95% CI = 64.0–88.0 months), respectively. A multivariable model

FIGURE 1 Kaplan–Meier curves of overall survival (OS) from onset of castrate-resistant disease in patients with elevated and normal CEA. Median OS for patients with elevated and normal CEA was 28.7 months (95% CI = 17.9, 52.9) and 73.2 months (95% CI = 64.0, 88.0), respectively. Unadjusted hazard ratio (HR) was estimated using Cox proportional hazards regression (n = 147). Models were fitted to data from the subset of patients with complete covariate data to ensure comparability of HR estimates between unadjusted and adjusted analyses. CEA, carcinoembryonic antigen; CI, confidence interval.

including the previously stated covariates was fitted to estimate an adjusted HR. A significant violation of proportional hazards was detected for the liver metastasis variable (Kolmogorov-type supremum test p = 0.002).[10] We, therefore, fitted a stratified Cox model stratified by liver metastasis status, an approach that remedies the proportional hazards violation by assuming different baseline hazards for patients across levels of the stratification variable but equal covariate effects on the hazard of death. The adjusted analysis indicated elevated CEA remained strongly associated with the hazard of death in patients with CRPC (adjusted HR = 3.19, 95% CI = 1.72–5.91).

3.3 | Copy number amplifications in patients with elevated CEA

The ctDNA profiling platform utilized in this study can detect copy number alterations in the form of copy number amplifications (CNAs). Of the 48 patients with CRPC and elevated CEA, 39 (81.3%) demonstrated a CNA in at least one gene examined by the ctDNA assay. This frequency was higher than the rate of 59.1% observed among CRPC patients with normal CEA (p = 0.007). Among patients with at least one CNA, elevated CEA was also associated with a

TABLE 2 Frequency (%) of patients with metastasis

Metastatic site	Elevated CEA (n = 48)	Normal CEA (n = 115)	p value
Lymph node	38 (79.2)	84 (73.9)	0.55
Bone	45 (93.8)	96 (83.5)	0.13
Viscera	22 (45.8)	31 (27.0)	0.03
Liver	18 (37.5)	22 (19.1)	0.02
Lung	8 (16.7)	10 (8.7)	0.17
Other	3 (6.3)	10 (8.7)	0.76

Note: Other visceral metastatic sites include adrenal gland, brain, dura, and peritoneum. Fisher's exact test used to calculate p value.
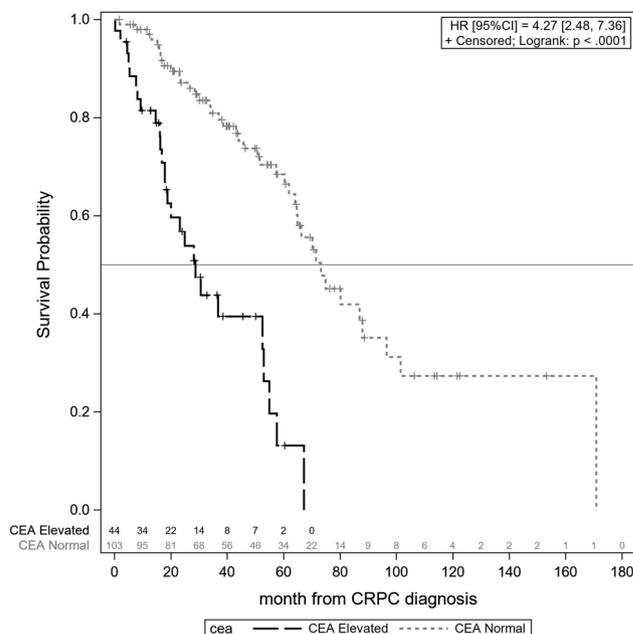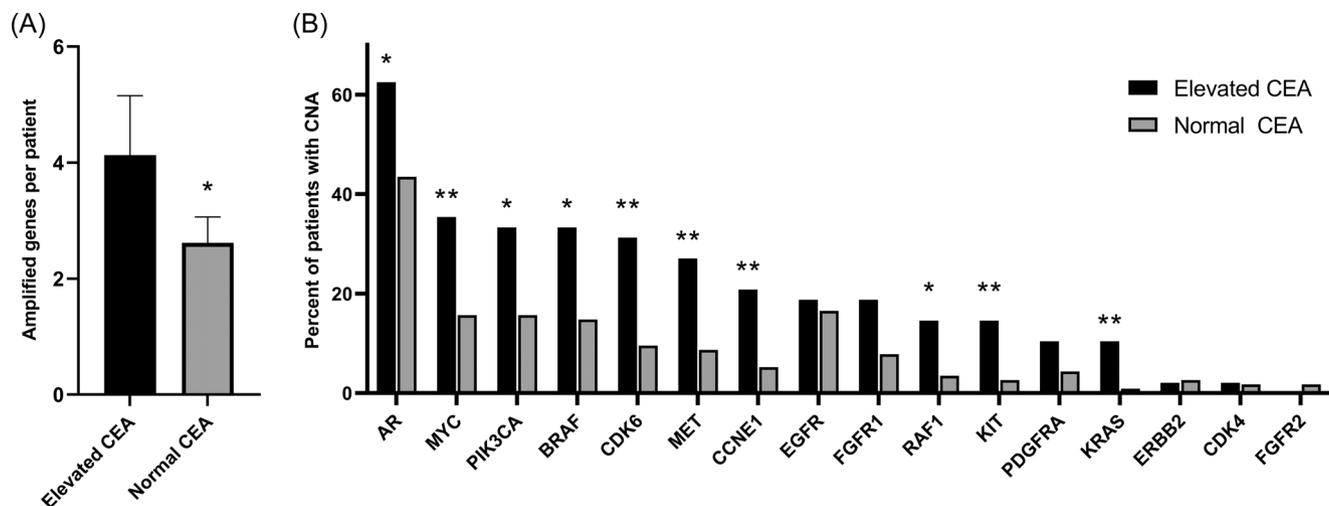Abbreviation: CAE, carcinoembryonic antigen.

FIGURE 2 Copy number amplifications detected in ctDNA of CRPC patients with elevated and normal CEA. (A) Average number of amplified genes per patient among patients with at least one CNA. (B) Percent of patients with CNAs in individual genes targeted by ctDNA analysis. *$p < 0.05$; **$p < 0.01$. Error bars denote 95% CI. CEA, carcinoembryonic antigen; CI, confidence interval; ctDNA, circulating tumor DNA; CRPC, castration-resistant prostate cancer.
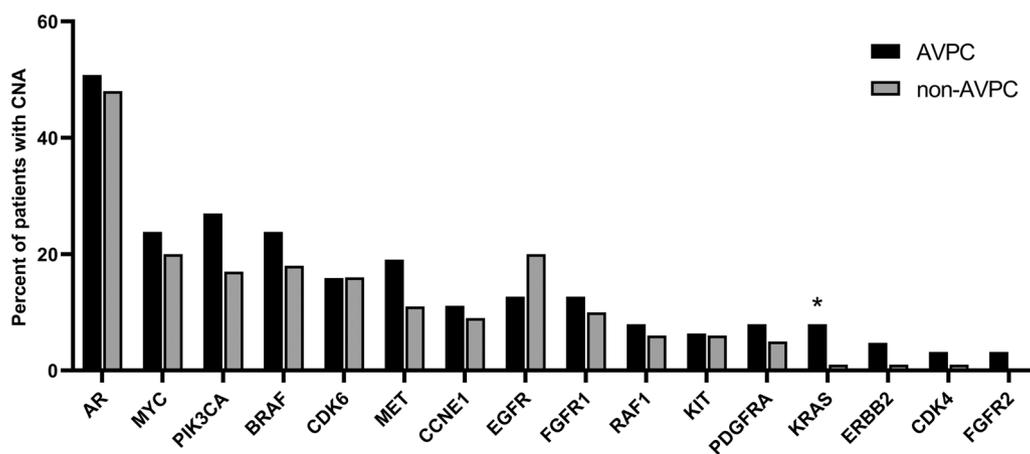

FIGURE 3 Percentage of AVPC and non-AVPC patients with copy number amplifications in individual genes detected via ctDNA analysis. AVPC, aggressive variant prostate cancer; ctDNA, circulating tumor DNA.

higher number of amplified genes per patient (4.1 genes vs. 2.6 genes, $p = 0.01$) as illustrated in Figure 2A. With regard to specific genes, patients with elevated serum CEA were significantly more likely to harbor CNAs in *AR*, *PIK3CA*, *MYC*, *BRAF*, *CDK6*, *MET*, *CCNE1*, *KIT*, *RAF1*, and *KRAS* (Figure 2B).

3.4 | CEA-associated CNAs are not replicated in patients with AVPC

Elevated serum CEA is a component of the Aparicio criteria used to identify AVPC, and 27 of 48 (56.3%) patients with elevated CEA also met the criteria for AVPC. Fewer patients with normal CEA (31.3%) met the same AVPC criteria ($p = 0.005$), raising concerns that the

ctDNA alterations we observed in association with elevated CEA could be attributed to a higher prevalence of AVPC in this population. To determine the uniqueness of CEA-associated genetic findings, the ctDNA profiles of patients with and without AVPC were compared. In accordance with previous studies, AVPC patients in our study possessed significantly higher median serum levels of LDH (482.0 vs. 242.0 U/L, $p < 0.0001$) and CEA (4.5 vs. 2.6 ng/ml, $p = 0.001$), as well as lower median serum PSA (34.6 vs. 66.0 ng/ml, $p = 0.055$).[3] In contrast to patients with elevated CEA, patients with AVPC did not demonstrate an increased prevalence of CNAs compared to non-AVPC patients (73.0% vs. 61.0%, $p = 0.13$). Similarly, AVPC displayed a higher frequency of CNAs in *KRAS* (7.9% vs. 1.0%, $p = 0.03$) but not in *AR*, *PIK3CA*, *MYC*, *BRAF*, *CDK6*, *MET*, *CCNE1*, *KIT*, or *RAF1* (Figure 3).

3.5 | Single nucleotide alterations in patients with elevated CEA and AVPC

Among the 163 total patients examined, 148 (90.08%) possessed at least one nonsynonymous SNV detectable by ctDNA analysis. Mutations in our patient population were most frequently observed in *TP53* (58.9% of patients), *AR* (31.9% of patients), and *APC* (16.0% of patients). Unlike copy number alterations, the total SNV rate did not vary between patients based on circulating CEA level (95.8% vs. 98.7%, *p* = 0.23). In addition, amongst patients with at least one SNV, the average number of SNVs per patient did not differ between those with elevated and normal CEA (3.0 vs. 3.6, *p* = 0.76), nor did the prevalence of SNVs in any individual genes detected by the ctDNA assay (Figure 4A). When patients with and without AVPC were compared there were similarly no differences in total SNV prevalence or frequency of individual gene mutations (data not shown). Of note, both patients with elevated CEA and patients with AVPC demonstrated maximum VAFs which were elevated relative to their controls at 23.5% versus 12.7% (*p* = 0.007) and 20.8% versus

12.8% (*p* = 0.007), respectively, indicative of greater "shedding" of tumor DNA among these cancers.

To better characterize mutations driving the predominant tumor cell subclones in each patient we identified "clonal" SNVs in each ctDNA profile. Clonal SNVs were defined as SNVs occurring at a frequency of ≥50% of the maximum VAF in each patient. As with total SNVs, the average number of clonal SNVs per patient did not differ between patients with elevated and normal CEA (1.7 vs. 1.8, *p* = 0.74). Clonal SNVs in our patient population were most frequently observed in *TP53* (47.2%%), *AR* (17.2%), *APC* (9.2%), *PIK3CA* (8.6%), *ATM* (7.4%), and *CTNNB1* (5.5%). All other clonal SNVs identified occurred in less than 5% of patients. As illustrated in Figure 4B, patients with elevated CEA were more likely to harbor clonal SNVs in*TP53* (60.4% vs. 41.7%, *p* = 0.04) and less likely to possess clonal SNVs in *AR* (6.3% vs. 21.7%, *p* = 0.021). AVPCs were analogously associated with an increased frequency of clonal SNVs in *TP53* (58.7% vs 40.0%, *p* = 0.02) when compared with non-AVPCs, but no difference in the rate of clonal *AR* SNVs was observed (Figure 4C).
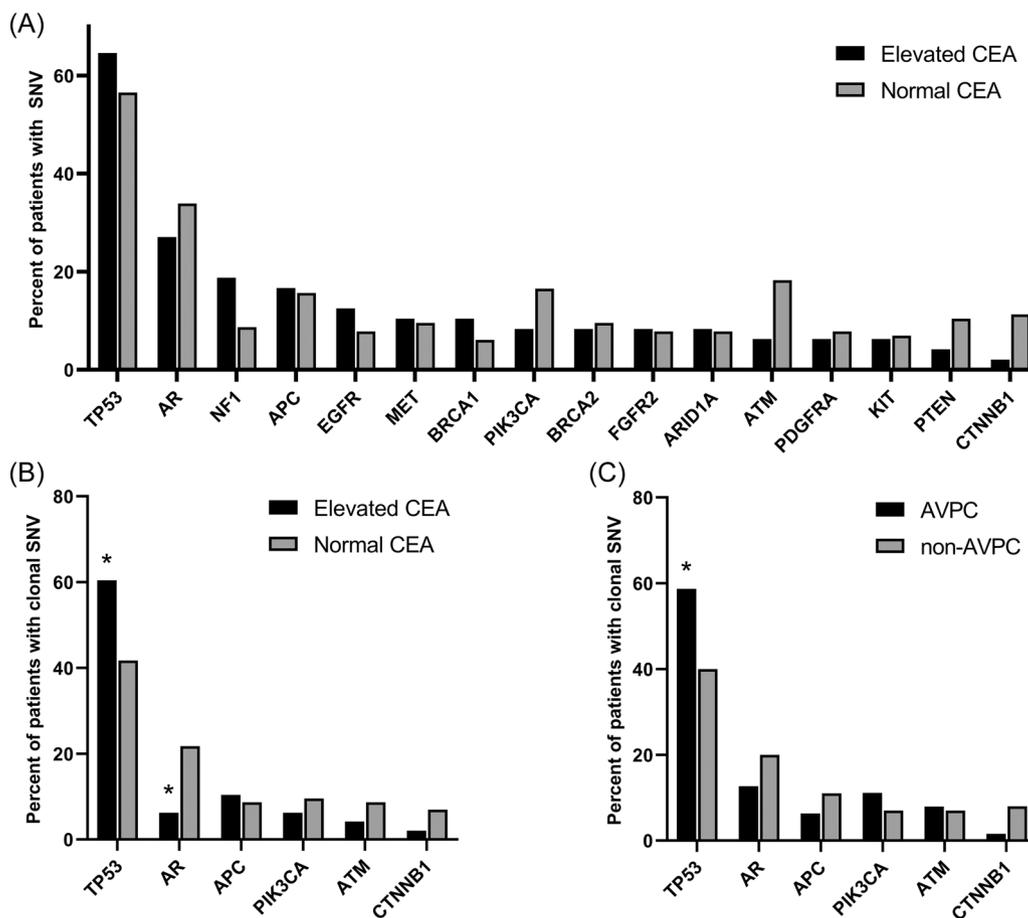

FIGURE 4 Prevalence of single-nucleotide variants (SNVs) as detected by ctDNA analysis. (A) Percent of patients with elevated and normal CEA harboring SNVs in the study population's 16 most frequently mutated genes. (B) Prevalence of clonal SNVs in patients with elevated and normal serum CEA. (C) Prevalence of clonal SNVs in AVPC and non-AVPC patients. Listed are the 6 genes with the highest frequency of clonal SNVs. *p < 0.05. AVPC, aggressive variant prostate cancer; CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA.

4 | DISCUSSION

Elevated serum CEA is a known marker of AVPC, but studies examining its utility as a prognostic indicator in CRPC have yielded inconsistent results.[3,11–13] The current study provides evidence that elevated serum CEA is associated with liver metastasis and decreased overall survival. Previous data which failed to establish a link between CEA and clinical outcomes involved patients enrolled in clinical trials at the National Cancer Institute before the introduction of advanced androgen axis blockers such as abiraterone and enzalutamide.[11] It is worth considering that the potential prognostic value of CEA observed by both our lab and Aparicio et al. is due to treatment-induced differences in prostate cancer biology which have emerged since the earlier NCI study. The specificity of CEA for liver metastases is also intriguing and mirrors previous studies in colorectal cancer which demonstrated that CEA binds a receptor on Kupffer cells leading to inflammatory cytokine release and the upregulation of adhesions molecules on hepatic endothelium that in turn promote metastatic colonization.[14–16]

This study also examined circulating tumor DNA alterations associated with CEA expression in CRPC. CEA expression was not correlated with an increased or decreased frequency of any SNVs during initial ctDNA analysis. However, when variant allele frequency was used to identify certain SNVs driving the dominant tumor cell subclone in each patient, CEA expression was found to be negatively correlated with such "clonal" SNVs in the *AR* gene, and positively correlated with clonal SNVs in *TP53*. CEA-positive cancers were also found to exhibit copy number gains (CNAs) in multiple genes including: *AR*, *MYC*, *PIK3CA*, *BRAF*, *CDK6*, *MET*, *CCNE1*, *KIT*, *RAF1*, and *KRAS*. These genes span chromosomes X, 3, 4, 7, 8, 12, and 19, and *CCNE1* is the only gene that co-localizes with CEA, which itself is encoded by a family of genes at 19q13. Previous genome-wide association studies on patients with prostate cancer have mapped a susceptibility locus for aggressive tumor behavior to this same genomic region.[17] The responsible genetic elements at 19q remain the subject of debate, but it is possible that increased expression of CEA may reflect cis-acting mutations in this chromosomal region.

To the best of our knowledge, this is the first study to illustrate a possible correlation between elevated serum CEA and certain ctDNA alterations in CRPC. Amplifications of *AR*, *MYC*, *CDK6*, *RAF1*, *PIK3CA*, *BRAF*, and *MET* have been observed in patients with prostate cancer, and amplification of *KRAS* has been documented to drive malignant potential in prostate cells in vitro.[9,18–25] CNAs in *KIT* and *CCNE1* have not been reported in prostate cancer, but they have been associated with poor prognosis and aggressive behavior in other malignancies.[26,27] Moreover, increased activity of each of the proteins encoded by genes exhibiting CNA in this study has been implicated in prostate cancer progression.[2,28–36] The findings surrounding *AR* SNVs mirror earlier reports which demonstrated that treatment-related neuroendocrine prostate cancers are characterized by a diminished reliance on androgen receptor signaling.[4] Interestingly, decreased androgen receptor transcriptional activity has been observed alongside *AR* gene amplifications in a subset of these patients, and CEA expressing tumors were similarly associated with *AR* gene amplifications in our study.[18] This seeming contradiction has been attributed to altered epigenetic regulation within neuroendocrine prostate cancers.[18]

Of the CEA-associated ctDNA alterations observed in this study, only clonal SNVs in *TP53* and CNAs in *KRAS* were similarly overrepresented in patients with AVPC relative to patients without APVC. This finding suggests that AVPC, as it is currently defined, represents a genetically heterogenous entity and that CEA expression may define a distinct subpopulation of neuroendocrine cancers. Of note, the observed prevalence of AVPC in our study population (~40%) is somewhat higher than the documented estimates of ~20% which appear in the literature.[6] This is potentially explained by the requirement that patients have undergone CEA testing to be eligible for study enrollment, as this test is frequently ordered out of suspicion for AVPC at our institution. In accordance with prior reports, patients with AVPC in this study exhibited higher serum levels of CEA and LDH compared to patients without APVC.[3]

Aparicio et al. first developed their AVPC criteria to clinically identify patients with treatment-related neuroendocrine prostate cancer responsive to platinum chemotherapy without the need for biopsy.[3] These criteria have since been used in several phase 2 clinical trials and based on the results of these studies and others, NCCN guidelines now recommend platinum chemotherapy for biopsy-proven treatment-related neuroendocrine or small cell prostate cancer.[8] Molecular studies have suggested that clinically defined AVPC and biopsy-proven neuroendocrine and small cell prostate cancer share the same underlying genetic alterations.[5] However, the majority of medical societies, including the NCCN, have yet to incorporate the clinical AVPC criteria into their guidelines, and the Advanced Prostate Cancer Consensus Conference (APCCC) 2017 voted in favor of standard mCRPC treatment for patients with AVPC.[6]

It has been proposed that AVPCs are genetically unified by copy number losses in *RB1* and PTEN along with alterations in *TP53*.[5] The present study made use of liquid ctDNA-based biopsy techniques developed as an alternative to traditional solid tissue-based techniques due to concerns of sampling bias and a rising demand for serial biopsies.[7,37] A limitation of our presented assay is its inability to detect copy number losses. However, the assay was able to identify an association between clonal *TP53* SNVs and AVPCs, thus aligning with previous results produced with tissue-based techniques. Liquid biopsy-based assays have already gained acceptance within the NCCN for genetic characterization of CRPC.[8] However, these results lend additional credence to the use of these techniques in AVPC and CEA-positive CRPC patient populations.

In conclusion, patients with CRPC and elevated serum CEA in this retrospective study demonstrated more aggressive disease and unique genetic alterations identifiable via ctDNA analysis. These CEA-associated gene alterations were not replicated among patients with AVPC. Together these findings suggest that CEA expressing prostate cancers represent a distinct molecular subtype of CRPC and that CEA may serve as a valuable biomarker of prognosis. However,

these results must be interpreted with several caveats in mind. The retrospective nature of this study renders it incapable of establishing any independent prognostic value for serum CEA in patients with CRPC. In addition, the clinical and genetic associations observed were not validated in any additional clinical datasets or patient populations, the time of ctDNA collection was not uniform, and all patients came from a single center. As a result, additional studies will be required to conclusively demonstrate an independent prognostic role for serum CEA level in CRPC, to elucidate the molecular mechanisms which underlie CEA-associated genetic alterations, and to determine whether CEA expression carries any therapeutic implications.

CONFLICT OF INTEREST

LMD is an employee of Guardant Health with stock and ownership interests in the company. TSG is on the advisory board for Exelixis Pharmaceuticals and receives institutional support from Ferring Pharmaceuticals. The other authors report no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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