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Clinical and pathological features associated with circulating tumor DNA content in real-world patients with metastatic prostate cancer

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Abstract

Background: Liquid biopsy is a powerful tool that can enable treatment decisions for metastatic prostate cancer patients with difficult-to-biopsy tumors. However, the detection of genomic alterations via liquid biopsy is limited by the fraction (tumor fraction [TF]) of circulating tumor DNA (ctDNA) within the total cell-free DNA content. While prior work has preliminarily correlated TF with clinical features of prostate cancer, we sought to validate and provide additional resolution, such that a clinical practitioner might anticipate the probability of successful liquid biopsy profiling leveraging commonly assessed clinical and laboratory features.

Methods: A total of 813 liquid biopsy specimens were assessable, with 545 associated with a PSA prostate specific antigen measurement, collected in standard-of-care settings across approximately 280 US academic or community-based cancer clinics from September 2018 to July 2021. Deidentified data were captured into a real-world clinico-genomic database (CGDB). Comprehensive genomic profiling (CGP) was performed on extracted cell-free DNA from liquid biopsy samples.

Results: In multivariable models, higher PSA level, lower hemoglobin, lower albumin, higher alkaline phosphatase (all $p < 0.001$), and collection of liquid biopsy blood draw within 60 days of new treatment initiation ($p = 0.002$) were the most strongly associated features with higher TF. At PSA levels of <5 ng/ml, 43% of patients had a TF of <1% indicating an increased likelihood of unevaluable results. Conversely, at PSA levels of >5 ng/ml, 78% of patients had a TF of at least 1% and 46% had a TF of ≥10%, suggesting improved sensitivity for detection of targetable alterations.

Conclusions: Universal genomic profiling of prostate cancers will require complementary use of liquid biopsy and tumor tissue profiling for suitable patients. The likelihood of adequate ctDNA shedding into plasma is one consideration when

deciding whether to pursue CGP via liquid biopsy versus tumor profiling. Our real-world data suggest that PSA < 5 ng/ml is associated with lower ctDNA yield on liquid biopsy, potentially increasing the incidence of negative results or a need for confirmation with tissue testing.

KEYWORDS
ctDNA, genomic profiling, liquid biopsy, mCRPC, tumor fraction

1 | INTRODUCTION

Molecular evaluation of prostate cancer tissue and liquid specimens via comprehensive genomic profiling (CGP) is becoming standard practice for metastatic prostate cancer care. There are three main approaches to assess CGP: archival primary tissue, metastatic tissue, or circulating tumor DNA (ctDNA) from a liquid biopsy (contemporaneous). Many patients with metastatic castration-resistant prostate cancer (mCRPC) may have a banked archival tissue sample from their original diagnostic specimen. Recent work has helped to elucidate the concordance of genomic findings between archival and metastatic tissue in this disease. While the prevalence of alterations in genes related to homologous recombination DNA repair (enabling PARP poly(ADP-ribose) polymerase inhibitor use) appears consistent across prostate cancer disease states,[1] some alterations, such as those in*AR*, *MYC*, and *RB1* can change in prevalence with increasing systemic therapy exposures.[2] Depending on the clinical question being asked and the availability of archival tissue, it may be necessary to consider CGP using a new metastatic tissue or liquid biopsy.

Contemporaneous solid tissue metastatic biopsies can be difficult, as many patients with mCRPC have metastatic deposits confined to bones. The last few years have seen a rapid improvement and availability of liquid biopsy CGP assays, some having gained Food and Drug Administration (FDA) approval,[3] where cell-free DNA is obtained from a blood draw, amplified, and profiled.[4,5] However, not all patients are able to have successful CGP from a liquid biopsy, the resolution of which is limited by the fraction of tumor DNA that is present in the overall cell-free DNA content of the sample, known as the tumor fraction (TF).[5] Higher levels of TF enable more comprehensive genomic profiling; thus, being able to anticipate the level of TF may be valuable for decisions about ordering contemporaneous tissue versus liquid biopsy profiling.

Prior small studies of prostate cancer patients' TF assessments with clinical and pathological features reported tumor burden and proxies thereof (e.g., PSA prostate specific antigen level) to be associated with TF levels.[6–8] Assessing commonly available clinical and pathologic features in a larger, real-world treatment population across many clinical sites, we sought to add resolution to the ability to anticipate TF level, to guide decisions for optimal use of contemporaneous metastatic liquid versus tissue profiling in advanced prostate cancer patients.

2 | METHODS

2.1 | Study design and patient selection

Our cohort comprised patients with a confirmed diagnosis of metastatic prostate cancer included in the Flatiron Health (FH)-Foundation Medicine (FMI) deidentified clinico-genomic database (CGDB) between September 2018 and July 2021. All patients underwent genomic testing using Foundation Medicine CGP liquid biopsy assays (FoundationOne®Liquid or FoundationOne®Liquid CDx).

Deidentified clinical data originated from approximately 280 US cancer clinics (~800 sites). Retrospective longitudinal clinical data were derived from electronic health records (EHRs), comprising patient-level structured and unstructured data, curated via technology-enabled abstraction of clinical notes and radiology/pathology reports, which were linked to CGP data by deidentified, deterministic matching.[9] Clinical data included demographics, clinical-pathologic and laboratory features, and timing of treatment relative to liquid biopsy blood draw.

Patients were included in this study if they received liquid biopsy profiling ordered by their clinical provider at any point in their patient journey, but only patients with metastatic prostate cancer are reported here. Only the earlier timepoint was chosen and was linked to the Figure 1 depicts the study flowchart. Institutional review board approval of the study protocol was obtained before study conduct and included a waiver of informed consent.

2.2 | Comprehensive genomic profiling and TF estimation

Hybrid capture-based next-generation sequencing (NGS) assays were performed on blood specimens in Clinical Laboratory Improvement Amendments (CLIA)–certified, College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine, Cambridge, MA). FoundationOne®Liquid (interrogating select regions of 70 genes) or FoundationOne®Liquid CDx assays (interrogating 324 cancer-related genes) were utilized. Samples were evaluated for genomic alterations as previously described.[3] Circulating TF was determined via the composite tumor fraction (cTF) technique. cTF leverages two complementary methods: a proprietary tumor fraction estimator

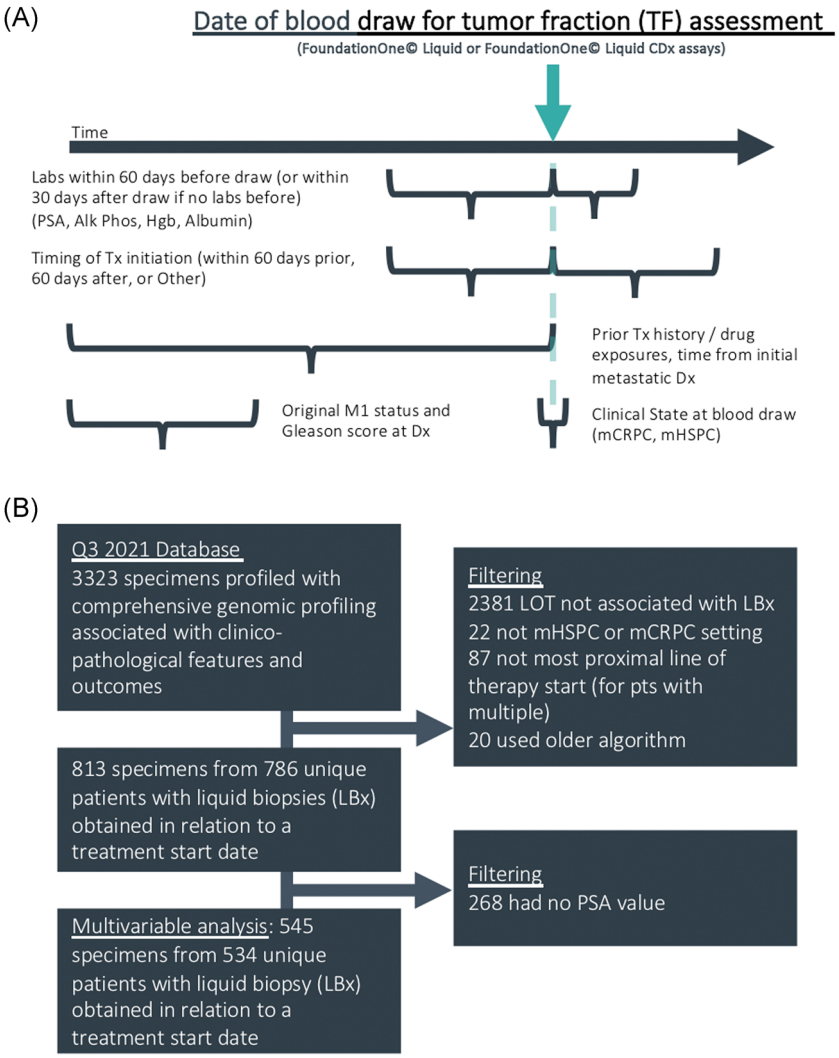


FIGURE 1 Cohort selection schema. (A) Graphical representation of clinical and pathological features evaluated in relation to blood draw for liquid biopsy. (B) Cohort selection diagram [Color figure can be viewed at wileyonlinelibrary.com]

(TFE) based on a measure of tumor aneuploidy, and the maximum somatic allele frequency (MSAF) method. cTF defaults to TFE's value when available. When tumor aneuploidy does not generate an informative estimate (approximately 10% TF threshold), then MSAF is used. MSAF is determined using the allele fraction for all somatic short variants and rearrangements detected at >2000X median unique coverage by non-PCR duplicate read pairs. cTF excludes a select list of variants associated with clonal hematopoiesis (CH) for MSAF determination.[4]

2.3 | Statistical analysis

Pearson χ^2 tests and Wilcoxon rank-sum tests were used to assess differences between groups for categorical and continuous variables, respectively. Multiple comparison adjustments were not performed since this was a hypothesis-generating study; *p* values are reported to quantify the strength of association for biomarker and each outcome, not for hypothesis significance testing. Multivariable models made use of linear regression with continuous variables being log2 transformed as needed for model assumptions. TF was log2(x + 1) transformed to avoid log2(0). Missing values were not imputed for statistical analyses; samples without PSA values were excluded for statistical analyses, and missingness in categorical values was included as a categorical variable. Patients who were not metastatic at time of treatment were excluded, as were rare patients with exceptionally high PSA values due to statistical model fit considerations.

3 | RESULTS

3.1 | Characteristics of analysis cohort

A total of 3323 specimens were included in the database at the time of analysis (November 25, 2021). For descriptive analyses, 813 liquid biopsies from 786 unique patients tied to treatment information were eligible (Figure 1B). For statistical analyses (Figures 3 and 4), 545 patients were eligible, after excluding those without available PSA measurements. Circulating TF was associated with M stage at

FIGURE 2 Clinical and pathological features at the time of liquid biopsy tumor fraction (TF) assessment. The clinical and pathological features present at time of blood draw for liquid biopsy are shown associated with the the resulting TF level. Numbers at right side of graphs indicate the number of specimens contributing to that bar. Specimens with unavailable or unknown values for each assessment were excluded [Color figure can be viewed at wileyonlinelibrary.com]

diagnosis, Gleason sum at diagnosis, clinical state (mHSPC, mCRPC) at time of liquid biopsy collection, number of prior metastatic treatment lines, time from treatment start date, and laboratory values (PSA, albumin, alkaline phosphatase, and hemoglobin) (all $p < 0.001$). Of the evaluated features, only ECOG was not strongly associated with TF ($p = 0.17$). See Table 1 and Figure 2, which descriptively summarize the relationships between these clinical factors and TF levels in unadjusted analyses.

3.2 | Additive and independent features associated with TF

We further sought to evaluate the additive and independent clinical, laboratory, and pathological features associated with TF (Figure 3). Evaluating TF as a continuous variable, all features included in Table 1 were incorporated into the multivariable model. Specimens without available PSA values were not included in the analysis. The model estimate reflects the log2-transformed values for TF. Every doubling of PSA resulted in an estimate increase of 0.40 (95% confidence interval [CI]: 0.21–0.58), $p < 0.001$, the units being TF as a percent that has been log2(x + 1) transformed (see Section 2). Transformed back to linear estimates, and adjusted for all other features in the model, the model estimated that two hypothetical patients, with all features identical except for one having a 10-fold higher PSA value at treatment start, would on average have 0.32% higher TF (95% CI: 0.16–0.51). Other features additive and independent to PSA level with respect to predicting TF level were: having alkaline phosphatase levels above the upper limit of normal (1.75%, 95% CI: 1.20%–2.43%, $p < 0.001$), having albumin levels below the lower limit of normal (0.57%, 95% CI: 0.14%–1.16%, $p < 0.001$), having hemoglobin levels below the lower limit of normal (0.43%, 95% CI: 0.13%–0.83%, $p < 0.001$), and whether the timing of the liquid biopsy draw was within 60 days before treatment start (0.40%, 95% CI: 0.13%–0.72%, $p = 0.002$) compared with patients whose clinical provider drew blood outside of time range proximal to a treatment decision.

3.3 | Anticipating TF by PSA level

We then attempted to delve deeper into the correlation between PSA level (the most actionable clinical parameter in real-world practice) and TF. This relationship is captured in Figure 4, in which PSA levels were categorized into five strata (PSA < 5, 5–20, 20–50, 50-200, and >200 ng/ml) and TF was considered as a continuous variable (Figure 4A) or was categorized into four groups (TF ≥ 1%,

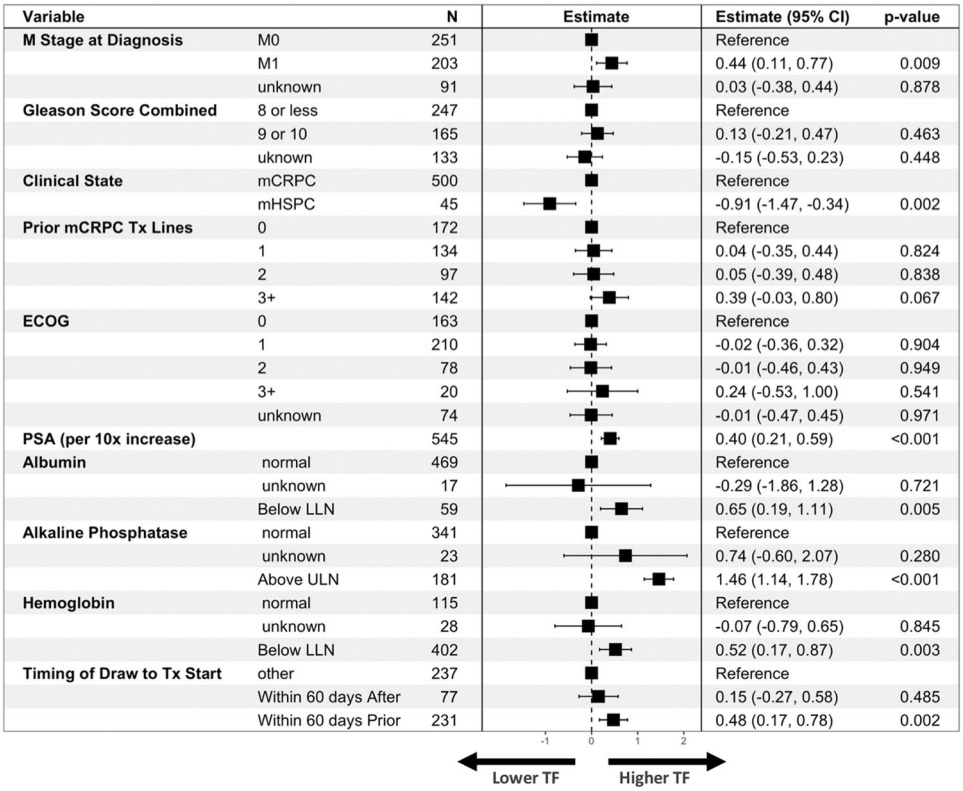


FIGURE 3 Clinical features associated with TF. The clinical and pathological features present at time of blood draw for liquid biopsy are shown associated with TF in a multivariable model adjusting for the features indicated. Point estimates and confidence intervals are shown relative to average in the cohort, with estimates to the right of center indicating higher than average TF values, left of center indicating TF values lower than average. TF, tumor fraction

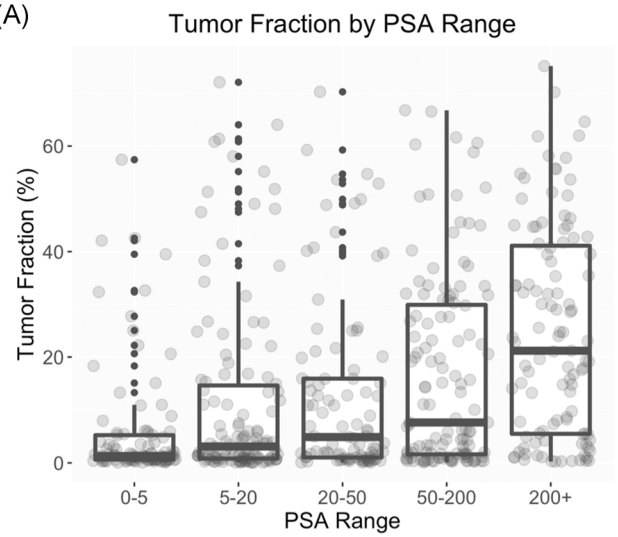
TF ≥ 10%, TF ≥ 30%) (Figure 4B). As shown, patients with high or low TF can be present in any of the PSA strata, but the overall trend is such that patients with higher PSA in aggregate have higher TF levels. Viewed categorically it can be demonstrated, for instance, that patients with a PSA range of 5–20 ng/ml had ≥1% TF 70.5% of the time. Alternatively, in patients with a PSA level <5 ng/ml, 56.7% of cases would have a TF ≥ 1% and only 15.5% of cases would have a TF ≥ 10%. As expected, at higher PSA ranges, the prevalence of highly resolvable TF levels increases. For example, when considering the entire group of patients with PSA levels of ≥5 ng/ml (PSA 5–200+ ng/ml), 77.7% would have a TF of ≥1%, and 46.0% would have a TF of ≥10% (Figure S1).

4 | DISCUSSION

Using a large real-world cohort of prostate cancer patients representing diverse clinical practices, we expanded upon prior work[6–8] preliminarily evaluating the association of circulating TF with various clinical, laboratory, and pathological features. Consistent with prior work, we found that proxies for disease burden, most notably PSA level, strongly associated with TF in prostate cancer (Figures 2 and 3). Prior work has suggested that tumor burden might be the most predictive feature associated with TF.[7] However, while tumor burden is arguably more causally valid than PSA, methods to quantify tumor burden in prostate cancer are not as analytically valid as PSA, for which quantification is well established by many diagnostics with standard units and criteria for quantification. We further resolved the association of PSA with TF (Figure 4) such that one might anticipate the estimated probability of successful CGP using liquid biopsy given a patient's PSA level at a particular point along the treatment trajectory.

When deciding whether to use liquid biopsy or tissue tumor profiling for a given cancer patient, one consideration is the likelihood of adequate ctDNA shed at the time of testing. Quantifying TF is one way to assess the chance of reduced ctDNA content and reduced assay sensitivity.[10] In the absence of adequate ctDNA content, a liquid biopsy is more likely to return a negative result, which per FDA label should be confirmed with tissue testing.[11] Furthermore, higher TF can increase the reliability of ctDNA-based variant detection, particularly copy number alterations (i.e., BRCA2 homozygous loss or AR amplification) which require higher TF for detection.[4,11] Our data suggest that a PSA < 5 ng/ml is associated with a greater chance of low ctDNA yield, with TF > 1% in only about one in two patients (Figure 4). But if PSA is >5 ng/ml there is a three in four chance of having TF > 1%, as well as a higher incidence of elevated TF > 10% where detection of, for example, BRCA2 loss or AR amplification would be more robust.

(A)



(B)

PSA Range	Patients	Prop TF 1%+	Prop TF 10%+	Prop TF 30%+
PSA 0-5	n=97	0.567	0.155	0.062
PSA 5-20	n=122	0.705	0.311	0.131
PSA 20-50	n=95	0.726	0.400	0.147
PSA 50-200	n=124	0.782	0.476	0.250
PSA >200	n=107	0.897	0.664	0.374

FIGURE 4 Association between PSA strata and TF. The association of TF by PSA range is shown graphically (A) with dots indicating individual specimens, boxes indicating interquartile range, and whiskers showing 95% confidence intervals. Numerical values are depicted in (B), again according to the five PSA strata. TF, tumor fraction

If liquid biopsy is less favorable for a given clinical decision, then it will be important to consider whether an archival prostate specimen might be valid, or if a contemporaneous tissue biopsy might be necessary. A key consideration will be the probability that an alteration of interest might develop or be lost over time. Data suggest that HRD alterations associated with PARP inhibitor sensitivity appear to be predominantly truncal events such that archival tissue is an acceptable option when available.[1,12] In contrast, alterations associated with drug resistance such as AR alterations can increase in prevalence over time.[5,6,12,13] As these markers continue to gain clinical utility with additional data, the relevance of their profiling will likely increase moving forward, as will the need for optimal assessment of contemporaneous tissue and/or liquid biopsies.

While the lack of detectable ctDNA could indicate reduced sensitivity for targetable alterations, lower (or undetectable) TF ranges themselves have also been associated with improved patient prognosis in prospective clinical trials.[6] If clinically stable, such patients may be well suited for confirmatory tissue testing to look for targetable alterations missed in the ctDNA. Additionally, ongoing clinical trials, such as the PROTRACT study (NCT04015622), are prospectively evaluating whether risk stratification based on TF levels can be used to direct patients to chemotherapy versus second-generation hormonal therapy. Such risk-stratification approaches are increasingly relevant as the

TABLE 1 Cohort overview, and by tumor fraction (TF) strata

TF strata	Below 1% (*N* = 202)	1%–10% (*N* = 280)	10%–30% (*N* = 171)	Above 30% (*N* = 160)	Total (*N* = 813)	*p* value
M stage at diagnosis						< 0.001
M0	99 (49.0%)	146 (52.1%)	58 (33.9%)	61 (38.1%)	364 (44.8%)	
M1	66 (32.7%)	94 (33.6%)	75 (43.9%)	75 (46.9%)	310 (38.1%)	
unknown	37 (18.3%)	40 (14.3%)	38 (22.2%)	24 (15.0%)	139 (17.1%)	
Gleason Sum						0.001
6	13 (6.4%)	30 (10.7%)	17 (9.9%)	11 (6.9%)	71 (8.7%)	
7	55 (27.2%)	60 (21.4%)	39 (22.8%)	25 (15.6%)	179 (22.0%)	
8	31 (15.3%)	47 (16.8%)	25 (14.6%)	21 (13.1%)	124 (15.3%)	
9	50 (24.8%)	74 (26.4%)	41 (24.0%)	46 (28.8%)	211 (26.0%)	
10	5 (2.5%)	8 (2.9%)	13 (7.6%)	22 (13.8%)	48 (5.9%)	
unknown	48 (23.8%)	61 (21.8%)	36 (21.1%)	35 (21.9%)	180 (22.1%)	
Clinical State at time of LBx						< 0.001
mCRPC	167 (82.7%)	247 (88.2%)	156 (91.2%)	154 (96.2%)	724 (89.1%)	
mHSPC	35 (17.3%)	33 (11.8%)	15 (8.8%)	6 (3.8%)	89 (10.9%)	
Prior mCRPC Tx Lines						0.001
0	93 (46.0%)	110 (39.3%)	59 (34.5%)	49 (30.6%)	311 (38.3%)	
1	53 (26.2%)	69 (24.6%)	43 (25.1%)	34 (21.2%)	199 (24.5%)	
2	29 (14.4%)	50 (17.9%)	23 (13.5%)	25 (15.6%)	127 (15.6%)	
3+	27 (13.4%)	51 (18.2%)	46 (26.9%)	52 (32.5%)	176 (21.6%)	

TABLE 1 (Continued)

TF strata	Below 1% (*N* = 202)	1%–10% (*N* = 280)	10%–30% (*N* = 171)	Above 30% (*N* = 160)	Total (*N* = 813)	*p* value
ECOG performance status						0.166
0	71 (35.1%)	85 (30.4%)	50 (29.2%)	37 (23.1%)	243 (29.9%)	
1	63 (31.2%)	99 (35.4%)	60 (35.1%)	57 (35.6%)	279 (34.3%)	
2	19 (9.4%)	35 (12.5%)	21 (12.3%)	30 (18.8%)	105 (12.9%)	
3+	5 (2.5%)	5 (1.8%)	9 (5.3%)	5 (3.1%)	24 (3.0%)	
unknown	44 (21.8%)	56 (20.0%)	31 (18.1%)	31 (19.4%)	162 (19.9%)	
PSA (ng/ml)						< 0.001
Median (Q1, Q3)	16.2 (4.2, 61.4)	23.7 (6.3, 88.2)	56.8 (19.8, 261.5)	113.0 (33.0, 449.3)	34.0 (8.6, 140.4)	
Missing observations	58	97	55	53	263	
Albumin (g/L)						< 0.001
Median (Q1, Q3)	41.0 (38.0, 43.0)	40.0 (38.0, 43.0)	40.0 (37.0, 43.0)	38.0 (35.0, 41.0)	40.0 (37.0, 42.0)	
Missing Observations	50	84	47	39	220	
Alkaline Phosphatase (IU/L)						< 0.001
Median (Q1, Q3)	76.0 (60.0, 103.0)	83.5 (66.2, 120.5)	127.0 (86.0, 233.0)	174.5 (92.2, 345.0)	96.0 (68.0, 164.5)	
Missing Observations	51	86	48	42	227	
Hemoglobin (g/dl)						< 0.001
Median (Q1, Q3)	12.5 (11.4, 13.5)	12.0 (11.0, 13.0)	11.8 (10.1, 12.8)	10.7 (9.4, 11.8)	11.8 (10.6, 13.0)	
Missing Observations	54	84	49	42	229	
Time from Tx Start to LBx						0.003
other	118 (58.4%)	166 (59.3%)	83 (48.5%)	75 (46.9%)	442 (54.4%)	
Within 60 days After	31 (15.3%)	27 (9.6%)	16 (9.4%)	17 (10.6%)	91 (11.2%)	
Within 60 days Prior	53 (26.2%)	87 (31.1%)	72 (42.1%)	68 (42.5%)	280 (34.4%)	

Note: Numerical representation of cohort overview according to each clinical characteristic, separated by the four TF strata and overall.
Abbreviation: ECOG, eastern cooperative oncology group; mCRPC, metastatic castration-resistant prostate cancer; mHSPC metastatic hormone sensitive prostate cancer.

number of therapeutic options grows for patients with advanced prostate cancer.

It is important to note that while PSA may be a reasonable proxy to anticipate TF levels, we did observe considerable heterogeneity between the two measures. As an example: some patients with low PSA had very high TF measurements (Figure 4A) and vice versa. The phenomena surrounding these discordances are still being resolved, such as neuroendocrine differentiation and lineage plasticity, which is characterized by rapid progression, visceral metastatic spread, and often low PSA levels.[14–17] It has been noted that both PSA and TF levels are additive and independent prognostic features,[6] suggesting an added benefit from evaluation of both in tandem beyond genomic profiling.

4.1 | Limitations

There are several shortcomings with this analysis. This was not a prospectively enrolled study. The patients included reflect those for whom liquid biopsies have been ordered in the time period reflected. It has been previously established that tumor burden and PSA burden correlate with TF. However, our database does not contain quantified measures of metastatic tumor burden, which is often difficult to assess given frequent bone-dominant metastases in mCRPC. Also, PSA levels are influenced by castration status, and this analysis did not interpret PSA levels separately in the hormone-sensitive and castration-resistant metastatic settings. Finally, the estimation of TF performed here relied upon genomic features associated with cancer biology, yet which also could at times be due to other somatic signals such as CH. We hope that the increasing emergence of tumor-informed monitoring assays for reliable quantification of tumor content will allow further validation of the clinical associations that we identified here.

5 | CONCLUSIONS

These data suggest that the utility of liquid biopsy could be optimized for the right patients at the right time using heuristics from routine clinical practice. Liquid biopsy has more reliable sensitivity in the

setting of elevated circulating TF, which is positively correlated with PSA level, alkaline phosphatase level, albumin level, hemoglobin level, Gleason score, M stage at diagnosis, castration state, and timing of blood draw relative to initiation of systemic therapy. Patients with PSA level of <5 ng/ml have a reduced probability of successful liquid biopsy, especially for resolution of copy number alterations, due to low ctDNA levels. Our proposed threshold for clinical utility of liquid biopsy assessment is a PSA of >5 ng/ml, at which level 78% of patients would be expected to have a circulating TF of at least 1%, and 23% would have a TF of at least 30%. Conversely, at PSA concentrations of <5 ng/ml in the metastatic prostate cancer setting, a tumor biopsy would be expected to yield more robust CGP results than a liquid biopsy. Liquid and tissue CGP are fundamentally two complementary diagnostics and must be used in parallel to optimize diagnostic yields and to aid treatment decisions for cancer patients.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Emmanuel S. Antonarakis, Marni Tierno, Ryon P. Graf. *Administrative support*: Geoffrey R. Oxnard. *Provision of study materials or patients*: N/A. *Collection and assembly of data*: N/A. *Data analysis and interpretation*: Ryon P. Graf, Emmanuel S. Antonarakis. *Manuscript writing*: Ryon P. Graf, Emmanuel S. Antonarakis, Geoffrey R. Oxnard. *Final approval of manuscript*: All authors. *Accountable for all aspects of the work*: All authors.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study originated from Flatiron Health, Inc. and Foundation Medicine, Inc. These deidentified data may be made available upon request, and are subject to a license agreement with Flatiron Health and Foundation Medicine; interested researchers should contact <cgdb-fmi@flatiron. com> and <dataaccess@flatiron. com> to determine licensing terms.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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