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Evolution of the genomic landscape of circulating tumor DNA (ctDNA) in metastatic prostate cancer over treatment and time

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ABSTRACT

Background: Targeted therapies have shown promise for men with metastatic castration-resistant prostate cancer (mCRPC). Due to the difficulty with obtaining tumor tissue in bony metastases, liquid biopsies are a promising alternative to guide treatment selection. While concurrent tissue next-generation sequencing (tNGS) and liquid biopsy has high concordance, it is unknown whether the genomic landscape of metastatic prostate cancer (mPC) changes over time or treatment. Herein, we hypothesize that the genomic landscape of mPC evolves with new treatments and/or time between tests.

Patients and Methods: Men with mPC from the University of Utah with matched tNGS and liquid biopsy were included. Clinical data was collected retrospectively. Exonic regions from 69 genes covered by both platforms were included for analysis. Paired *t* tests were used to assess number of genomic alterations (GAs) between testing platforms. Number of alterations was assessed by time and number of treatments between testing by multivariate nonparametric trend tests.

Results: 101 men with mPC were eligible and included. In men with no new treatments and $\leq$ 1 year between tests, a similar number of GAs were detected in both tests (2.0 vs. 2.2). In contrast, men with $\geq$ 1 new treatment between tests had significantly more GAs after treatment (5.0 vs. 2.4, $p = 0.005$). Total number of GAs was correlated with number of new treatments between testing ($p = 0.003$) and not time between testing ($p = 0.76$).

Conclusion: The genomic landscape of mPC evolves with subsequent therapies. This finding suggests that contemporary tumor genomic profile upon disease progression may optimize guidance towards subsequent therapy selection.

Introduction

Currently, there are no biomarker-guided therapies approved for the treatment of metastatic prostate cancer (mPC). However, recently, multiple targeted therapies have demonstrated promise in clinical trials for metastatic castration-resistant prostate cancer (mCRPC), including PARP inhibitors for men with DNA-repair gene mutations and PI3K/AKT pathway inhibitors for men with *PTEN* deficient tumors [1,2]. To date, the primary method used to guide targeted treatment selection is next-generation sequencing (NGS) of tumor tissue obtained via biopsy. For men with mCRPC, routine biopsy for tissue NGS (tNGS) is challenging because the majority have metastases located only in bones, which causes significant morbidity and has low yields of tumor DNA [3,4]. Because of these challenges, clinicians frequently use archived primary tumor tissue from prostate biopsy or prostatectomy that is years to decades old. NGS of ctDNA, hereafter referred to as liquid biopsy, circumvents many of the challenges associated with bone biopsies in men with mCRPC. Additionally, the majority of men with mCRPC shed greater than 1% ctDNA, which results in improved yields from liquid biopsies [5,6].

To successfully provide personalized cancer treatment, the majority of a patient's metastatic disease should be driven by a common, targetable genomic alteration (GA). In mCRPC, there does not appear to be extensive heterogeneity between spatially distinct metastases, so personalized treatment may be feasible [7]. Then, for liquid biopsy to be an acceptable alternative to tNGS, a liquid biopsy should accurately reflect

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the driver mutations across a patient's metastases. In a recent study in men with mCRPC, there was high concordance between the GAs detected concurrently in tNGS and liquid biopsy [8]. Now, with these facts established, it's important to understand whether the genomic landscape of mPC significantly changes after new treatments or a set time period. This is important because it could help clinicians determine if NGS testing of a more contemporary liquid biopsy may be preferred over an archived tumor tissue biopsy, and also how frequently liquid biopsy should be performed with subsequent systemic therapies to accurately guide targeted treatment selection.

Herein, we hypothesize that the genomic landscape of mPC evolves with new treatments and/or time between NGS tests. To track the evolution of the genomic landscape, we analyzed the frequency and concordance of GAs in “paired” tNGS and liquid biopsy collected at various time intervals after collecting the initial tumor tissue.

Methods

In this Institution Review Board approved study, men with mPC, defined as metastatic hormone sensitive prostate cancer (mHSPC) or mCRPC, from the University of Utah/Huntsman Cancer Institute were included if they had matched tNGS and liquid biopsy available. tNGS was performed using FoundationOne (Foundation Medicine, Cambridge, MA). Liquid biopsy was performed using Guardant360 (Guardant Health, Inc., Redwood City, CA). All tissue samples for tNGS were collected prior to liquid biopsy. Of the 101 men included, 15 had tNGS performed on a biopsy from metastatic tissue, and the remaining 86 men had tNGS performed on primary prostate cancer. Clinical data was collected through retrospective chart review. Exonic regions from 69 genes covered by both platforms were included for analysis. Specifically, GAs included single nucleotide variants, copy number variations, insertion/deletions, fusions, and rearrangements tested by both platforms.

Paired *t*-tests were used to assess number of genomic alterations (GAs) between testing platforms and were confirmed with nonparametric testing. Number of alterations was assessed by time and number of treatments between tDNA and ctDNA testing by multivariate nonparametric trend tests. Patients without GA detected by liquid biopsy were included in the calculation of concordance.

Results

101 men with mPC who had matched tDNA NGS and liquid biopsy available were included. Baseline characteristics are listed in Table 1. In this cohort, median age at diagnosis was 64 years old, median summed Gleason score was 9, and median PSA at diagnosis was 34.9 ng/ml. In all men, more GAs were detected by liquid biopsy than tNGS (3.8 vs. 2.2, $p < 0.0001$, Table 2). Among all patients included, the median time between tNGS and liquid biopsy was 15.2 months.

In the 21 men with no new treatment and less than 12 months between tNGS and liquid biopsy, there was no significant difference in the number of GAs detected between platforms (2.2 vs. 2, $p = 0.78$, Table 2, Fig. 1). In the same group of men, the concordance between tNGS and liquid biopsy was 96% for all genes tested and 20% for GAs detected (Table 3). In the 23 men with at least 1 new treatment and less than 12 months between tNGS and liquid biopsy, significantly more GAs were detected in the liquid biopsy (5.0 vs. 2.4, $p = 0.0053$, Table 2, Fig. 1). In these men, the concordance between tNGS and liquid biopsy was 91% for all genes tested and 10% for GAs detected (Table 3). In the 5 men with no treatments but greater than 12 months between tests, there was no significant difference in the number of GAs detected by tNGS and liquid biopsy (Fig. 1). In a multivariate analysis, the total number of GAs was associated with number of new treatments between testing ($p = 0.003$) and not time between testing ($p = 0.76$). When analyzing across both NGS platforms, the total number of GAs increased with additional lines of treatment (Fig. 2). In contrast, the total number of GAs did not significantly change with increasing time (Fig. 3).

Table 1
Baseline characteristics.

Median age at diagnosis	64
Median summed Gleason score	9
Median PSA at diagnosis (ng/ml)	34.8
Median time between tests (months)	15.2
Percent with tissue NGS first	97%
Visceral metastases at time of liquid bx	16.8%
Number of new tx between tests	
• 0	25.7%
• 1	17.8%
• 2	24.8%
• 3	15.8%
• 4	7.9%
• 5	7.9%

NGS = next-generation sequencing, bx = biopsy, tx = treatments.

Discussion

In men with mCRPC, a previous study showed that tNGS and liquid biopsy have high concordance when performed concurrently. However, as utilization of both of these tests increases, we need to improve our understanding on how the results from these tests differ across time and treatment. Our study shows that the genomic landscape of mPC significantly evolves with subsequent treatments. Thus, in order to accurately capture the genomic landscape of men with mPC, NGS of either tumor tissue or ctDNA is needed directly prior to initiating any novel targeted therapy. Interestingly, we did not find that the genomic landscape of mPC significantly changes with increasing time but no new treatments. While this finding is surprising, it could be that time without new treatment does not result in sufficient evolution of the genomic landscape to detect a significant difference in our small cohort. These findings should help inform clinicians on how to optimally use tNGS and liquid biopsies in men with mPC.

Currently, targeted therapy is often selected on the basis of tNGS of archived tumor tissue that can be years old. Our findings suggest that archived tissue obtained prior to receipt of systemic treatments may not accurately reflect the current genomic landscape of mPC. Thus, NGS, via either liquid biopsy or tNGS, may preferably be obtained directly prior to initiation of targeted therapy. While targeted therapies are not currently approved for men with mCRPC, PARP inhibitors and PIK3/AKT pathway inhibitors are promising treatments currently under investigation. In a study of 692 men with metastatic prostate cancer, 11.8% had a deleterious germline DNA-repair gene mutation, and there are likely more men with mCRPC who develop somatic mutations in DNA-repair genes [9]. A phase 2 clinical trial, TOPARP-A, showed that men with mCRPC and homozygous deletions and/or deleterious mutations in DNA-repair genes have improved progression-free survival with a PARP inhibitor, olaparib [1]. In men with mCRPC, 40–60% have PTEN loss, which results in hyperactivation of the PI3K-Akt-mTOR pathway [10]. In a phase 2 trial of 253 men with mCRCP, the Akt inhibitor, ipatasertib, plus abiraterone acetate demonstrated increased anti-tumor activity in men with PTEN loss compared to those without PTEN deletion [2]. A phase 3 registration trial is currently ongoing. Beyond these, several other targeted therapies are being investigated in earlier phase trials for men with mPC. For potential targetable alterations, a few large multi-institutional studies have described the genomic landscape of mCRPC as sequenced by tNGS and liquid biopsy [10–12].

Men with mCRPC commonly shed greater than 1% ctDNA, so liquid biopsies are a promising alternative for detection of GAs in this population. To date, one study has evaluated the concordance of GAs detected by matched liquid biopsy and tNGS in mCRPC [8]. In that study of 45 men, 93.6% of the GAs detected by tNGS were also observed by liquid biopsy. However, only 77% of mutations detected by liquid biopsy were also seen in tNGS. In our study, men with less than 1 year

Table 2
Median number of genomic alterations detected by tissue next-generation sequencing and liquid biopsy over treatment and time.

	n	Median GAs in tNGS	Median GAs in liquid biopsy	*p* value
Any time between tests and any number of treatments (all patients)	101	2.2	3.8	<0.0001
≤12 months between tests and 0 new treatments	21	2.2	2	0.78
≤12 months between tests and 1 new treatment	11	1.7	4.7	0.0088
≤12 months between tests and 1–5 new treatments	23	2.4	5	0.0053

GAs = genomic alterations, *n* = number, tNGS = tissue next-generation sequencing.

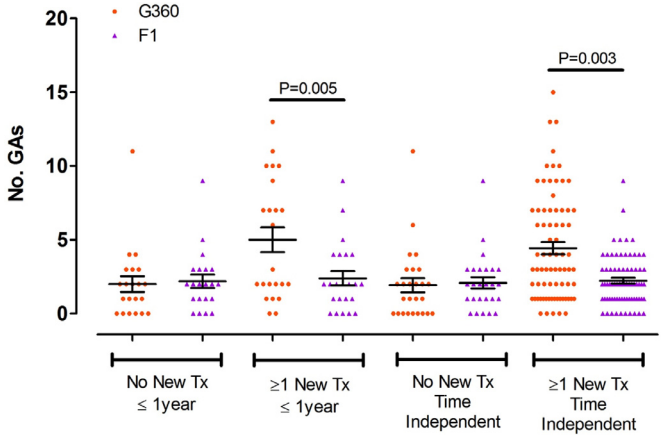

Fig. 1. Genomic alterations detected by tissue next-generation sequencing and liquid biopsy across time and treatment. G360 = liquid biopsy, F1 = tissue next-generation sequencing, GAs = genomic alterations, tx = treatment.

Table 3
Concordance of genomic alterations detected by tissue next-generation sequencing and liquid biopsy.

	n	Concordance (only + GAs)	Concordance (including WT)
Total population	101	13.5%	93%
Time between tests ≤12 months	44	15%	93%
• 0 treatments	21	20%	96%
• 1–5 treatments	23	10%	91%
Time between tests >12 months	57	12%	93%

GAs = genomic alterations, *n* = number, WT = wild type.

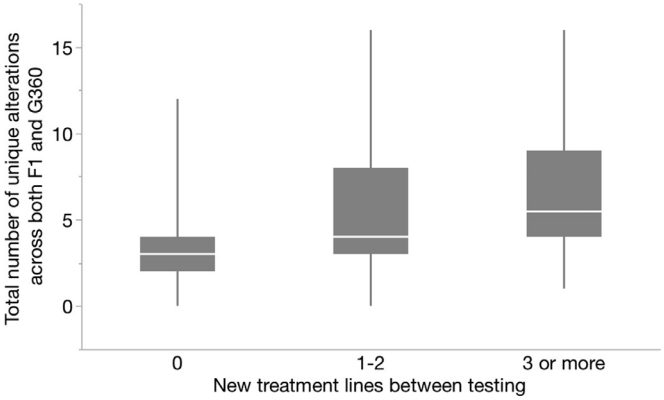

Fig. 2. Genomic alterations across both platforms with additional treatments.

between tests and no new treatments had a significantly lower concordance rate, 20%. There are multiple explanations for the difference in concordance between studies. First, concordance was calculated

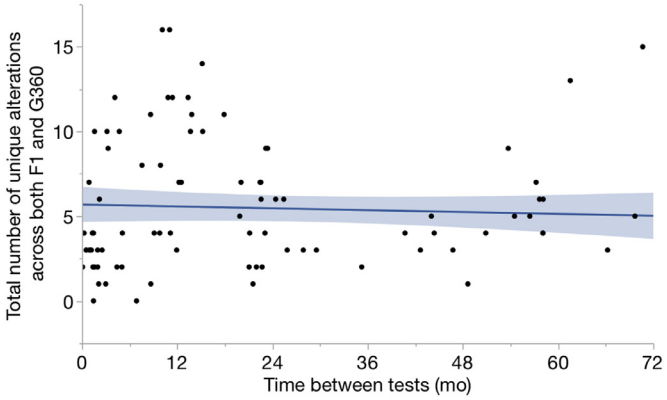

Fig. 3. Genomic alterations across both platforms with increasing time. F1 = tissue next-generation sequencing, G360 = liquid biopsy, mo = months.

differently in the two studies. In the study by Wyatt and colleagues, 11 men without detectable GAs by liquid biopsy were excluded from the concordance analysis, whereas, in our study, we included this cohort. Another potential explanation is that we used two different commercially available NGS platform for the liquid biopsy and tNGS. In contrast, the study by Wyatt and colleagues used the same custom, hot spot NGS platform for the liquid biopsy and tNGS. Finally, we included different stages of mPC in our cohort, while the Wyatt study only evaluated men with mCRPC. In mCRPC, there is limited intratumoral heterogeneity; however, studies suggest that localized prostate cancer has more significant intratumoral heterogeneity [7,13,14]. Considering all of these factors, we believe that our results compliment prior studies of liquid biopsy in mPC since none of them have evaluated how the genomic landscape evolves with time or treatment.

Our study has several limitations. Our study is retrospective and our cohort size is relatively small, which increases the number of potential confounders present. Additionally, we utilized two commercially available platforms for tNGS and the liquid biopsies. While we limited our analysis to genes with identical exonic coverage in both platforms, the pipeline for calling variants may have differed between companies, and to date, studies have noted significant discordance when using commercially available platforms [15,16]. Some common GAs in mCRPC were not tested for by both platforms, such as *RB* loss and *ERG* fusion genes. Moving forward, we are interested in using liquid biopsies to explore how the genomic landscape of mPC evolves after specified treatments, such as novel androgen axis inhibitors or chemotherapy. At this point, further comparisons of tNGS and liquid biopsy are likely unnecessary, but comparisons of somatic alterations detected by tNGS or liquid biopsy with mRNA sequencing are of future interest.

Conclusion

In conclusion, the genomic landscape of mPC evolves after receipt of systemic treatment, and contemporary NGS with either liquid biopsy or metastatic tumor tissue via biopsy may optimize selection of targeted therapy. In contrast, the genomic landscape of mPC does not significantly change with the passing of time but no new treatments. These

findings can help inform clinicians when ordering NGS and starting patients on targeted therapy for mPC.

Clinical practice points

- The genomic landscape of metastatic prostate cancer significantly changes after treatment with systemic therapies; however, it does not significantly change when time passes without new treatments.
- After progression on systemic therapy, contemporary next-generation sequencing with either liquid biopsy or metastatic tumor tissue biopsy may optimize selection of targeted treatment in the clinical trial setting.

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