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SHORT COMMUNICATION

Targeted Next-Generation Sequencing in Men with Metastatic Prostate Cancer: a Pilot Study

Pedro C. Barata[1] · Prateek Mendiratta[1] · Brandie Heald[2] · Stefan Klek[1] · Petros Grivas[1] · Davendra P. S. Sohal[1] · Jorge A. Garcia[1,3]

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Abstract

Introduction Tumor profiling by targeted next-generation sequencing (tNGS) and personalized treatment based on these results is becoming increasingly common in patients with metastatic solid tumors, but it remains unclear whether this strategy results in benefit to patients with metastatic prostate cancer (mPCa).

Objective To assess the clinical utility of tNGS in treatment decision-making for patients with mPCa.

Patients and Methods Patients with available genomic profiling using tumor tissue (FoundationOne, F1) or cell-free DNA (FoundationACT, Guardant360) were included. Targetable genomic alterations (tGA) included a change in the copy number or mutations in DNA repair genes, mismatch repair genes, *PTEN*, cyclin-dependent kinases, *ERBB2*, *BRAF*, *TSC*, and the PIK3/mTOR pathway.

Results The study included 66 patients, 86% of which had metastatic castration-resistant prostate cancer (mCRPC), and who had received a median of 3 (range 0–7) treatments prior to tNGS. The most frequent alterations were found in *TP53* (42%), *PTEN* (35%), androgen receptor (AR) (30%), DNA repair (30%), PIK3CA signaling pathway (21%), cyclin-dependent kinases (15%), BRAF (9%), and MMR/MSI (6%) genes. Among the 45 (68%) tGA+ patients, tNGS influenced treatment in 13 (29%) [PARP inhibitor ($n = 7$), mTOR inhibitor ($n = 4$), anti-PD-1 ($n = 2$), anti-HER2 ($n = 1$)]. The median progression-free survival (PFS) was 4.1 months [95% confidence interval (CI), 2.8–5.4]. Among tGA+ patients who did not receive tNGS-based therapy, systemic treatment ($n = 17$) included chemotherapy (71%), new generation anti-androgen therapy (24%), and cabozantinib (6%); the median PFS was 4.3 months (95% CI, 2.6–6.0; $p = 0.7$ for tGA+ with personalized therapy vs. tGA+ without personalized therapy).

Conclusion In this cohort, the use of tNGS was feasible, detected frequent genomic alterations, and was used late in the disease course. Further studies and larger portfolios of targeted therapy trials are needed to maximize the benefit of tNGS in this population.

Key-points

The clinical utility of targeted next-generation sequencing (tNGS) in treatment-decision making for patients with metastatic prostate cancer (mPCa) is undetermined.

In this pilot study of 66 patients with mPCa, the use of tNGS was feasible, detected frequent genomic alterations and was used late in the course of the disease.

Further studies and larger portfolios of targeted therapy trials are needed to optimize benefit of tNGS in this setting.

✉ Jorge A. Garcia
garciaj4@ccf.org

[1] Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

[2] Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH, USA

[3] Case Comprehensive Cancer Center, Cleveland Clinic—Taussig Cancer Institute, 9500 Euclid Ave/CA60, Cleveland, OH 44195, USA

1 Introduction

Metastatic prostate cancer (mPCa) was the most common cancer and the third leading cause of death from cancer among men in the United States in 2017 [1]. In general, when mPCa becomes resistant to androgen deprivation therapy, the management of castration-resistant prostate cancer (mCRPC) involves the sequential use of different therapies, with the goal of improving quality of life, minimizing complications, and prolonging progression-free and overall survival (PFS and OS, respectively) [2–7].

Acknowledging the molecular heterogeneity among primary and metastatic prostate tumors [8, 9], there has been a growing emphasis on precision medicine by using genomic data to help stratify patients based on prognostic estimates; however, treatment, to date, has not been molecularly tailored in routine practice.

A number of targeted next-generation sequencing (tNGS) panels analyzing tumor tissue or cell-free circulating tumor DNA (cfDNA) are commercially available for clinical testing, facilitating the genomic sequencing of prostate tumors in clinical practice. Therapeutic implications include targeting putative key tumor drivers and pathways in genomically defined subsets of patients [10, 11], but it is unclear whether this strategy translates into clinical benefit to mCRPC patients. We hypothesized that the "actionability" rate (proportion of NGS-based therapy selection among patients with NGS testing) is low and the median PFS is not significantly longer than expected in patients with NGS-based selected therapies.

2 Methods

This retrospective study was approved by the Cleveland Clinic IRB (research protocol #14-1322) and included consecutive patients with mPCa and available tNGS treated at the Cleveland Clinic. Patient data were collected in compliance with guidelines and informed consent was waived for this study.

The FoundationOne (F1; Foundation Medicine, Cambridge, MA) test uses formalin-fixed, paraffin-embedded (FFPE) tumors to sequence exons from 315 cancer-associated genes and introns from 28 genes involved in rearrangements, as well as microsatellite instability and tumor mutational burden (low < 5, intermediate 6–19, high ≥ 20 mutations/Mb) [12, 13]. The FoundationACT (FACT; Foundation Medicine) test uses cfDNA from blood to sequence 62 different genes and the Guardant360 (G360; Guardant Health, Redwood City, CA) test uses cfDNA from blood to sequence 73 genes [10, 11]. Targetable genomic alterations (tGA) were defined by a change in the copy number (amplification/duplication) or a mutation (deletion/rearrangement/truncation/fusions) in DNA repair genes, mismatch repair genes, *PTEN*, cyclin-dependent kinases, *ERBB2*, *BRAF*, *TSC*, and the PIK3/mTOR pathway; gene selection was based on consensus among investigators.

OS was defined as the time from the diagnosis of metastatic disease until the date of last follow-up or death, whichever came first. PFS was defined as the time from treatment initiation at the time of tNGS to radiologic or clinical progression or death. Patients were censored at the time of last follow-up. The clinical benefit of targeted therapy was defined by a ratio of PFS of specific therapy/PFS on prior therapy of ≥ 1.3, per Von Hoff et al. [14]. The cut-off date for analysis was January 2nd, 2018. Descriptive statistics were used to tabulate patient and treatment characteristics, as well as treatment outcomes. Outcome data were analyzed using Fisher's exact test, Chi-square test, analysis of

Table 1 Baseline patient and disease characteristics

Characteristics	*N* = 66 (%)
Median age, years (range)	68 (49–85)
ECOG Performance Status	
0	20 (30)
1	37 (56)
2	9 (14)
Gleason score	
6	3 (5)
7	11 (17)
8–10	40 (61)
Unknown	12 (18)
Neuroendocrine features	9 (14)
Prior local therapy	
None	23 (35)
Surgery	14 (21)
Radiation	10 (15)
Surgery plus radiation	17 (26)
Unknown	2 (3)
Site of metastasis at stage IV	
Lymph nodes	38 (58)
Bones	58 (88)
Visceral	31 (47)
Median number of lines of treatment for CRPC prior to tNGS	3 (0–7)
Prior therapies for stage IV*	
Androgen deprivation therapy	66 (100)
Abiraterone acetate	48 (73)
Enzalutamide	46 (70)
Sipuleucel-T	10 (15)
Docetaxel	46 (70)
Cabazitaxel	28 (42)
Radium-223	24 (36)
Platinum-based chemotherapy	15 (23)
Mitoxantrone	9 (14)

*≥ 10% of the patients

variance (ANOVA) test, linear regression, log rank test, and proportional hazard models using IBM SPSS Statistics V23. A p-value < 0.05 was considered significant.

3 Results

Table 1 shows the baseline characteristics of the 66 mPCa patients included in this study. All patients had at least one tNGS with available results, but, in one case, FACT did not detect a genomic aberration in the cfDNA collected, while the patient was responding to systemic therapy. The same patient underwent a second cfDNA (G360) test at the time of progression and a *TP53* mutation was detected.

The most common alterations included the genes *TP53* (42%), *PTEN* (35%), androgen receptor (AR) (30%), DNA repair (30%), the mTOR/PIK3CA signaling pathway (21%), cyclin-dependent kinases (15%), BRAF (9%), and MMR/MSI genes (6%) (Fig. 1). Forty-five tumors had at least one targetable genomic alteration (tGA+). The sequencing results influenced treatment in 29% of the CRPC patients: PARP inhibitors ($n = 7$), mTOR inhibitors ($n = 4$), immune checkpoint inhibitors ($n = 2$), and an anti-HER2 antibody ($n = 1$) (Table 2). Only two patients received a targeted therapy on a clinical study (NCT02091141, NCT03248570). All seven patients who received PARP inhibitors were treated off-label, one of them (14%) after prior platinum-based regimen. The median PFS for tGA+ patients receiving specific therapy was 4.1 months [95% confidence interval (CI), 2.8–5.4], with 9/13 patients (69%) progressing on therapy. Four patients had a PFS ratio of ≥ 1.3, including two patients treated with everolimus, one patient treated with trastuzumab, and another patient treated with olaparib (Table 3).

Among tGA+ patients not treated with genomic alteration-guided therapy, the first subsequent treatment ($n = 17$) included chemotherapy (71%), new generation anti-androgen therapy (24%), and cabozantinib (6%). The median PFS was 4.3 months (95% CI, 2.6–6.0) and 12/17 patients (71%) progressed on therapy. No difference in the median PFS between the tGA+ and tGA− groups was noted (Table 2).

Patients with tGA+ did not receive NGS-guided treatment due to physician or patient's choice (44%), being on observation (19%), having ongoing response to treatment at the time of tNGS testing (16%), best supportive care or hospice (9%), or other reasons (13%).

For the six patients with repeated tNGS, a second test was ordered after a median of 15.8 months (range 4.8–26.1). The

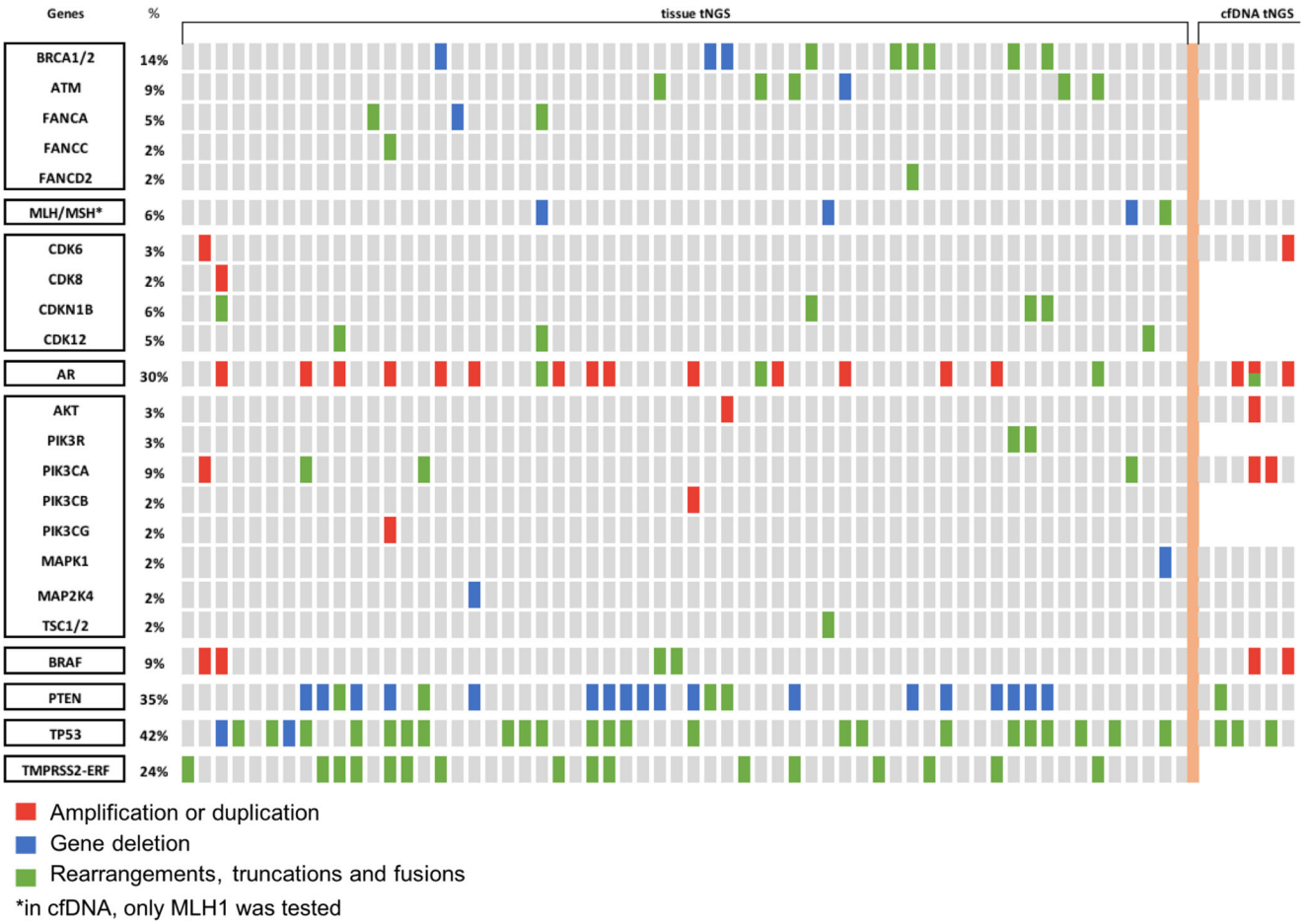

Fig. 1 Genomic alterations detected by targeted next-generation sequencing (tNGS) in the study cohort

Table 2 Targeted next-generation sequencing test results and clinical outcomes

Characteristics	*N* (%)			
First tNGS ordered				
Tissue	60 (91)			
cfDNA (G360/FACT)	6 (9)			
Median time from tissue collection to tNGS testing, months (range)	11.1 (2.9–187.9)			
Tissue sample				
Primary tumor	27 (41)			
Retroperitoneal lymph nodes	3 (5)			
Pelvic lymph nodes	5 (8)			
Liver	8 (12)			
Bone	6 (9)			
Soft tissue	5 (8)			
Other	6 (9)			
Patients with targetable genomic alterations	45 (68)			
Reasons for not receiving tNGS-based therapy	32 (100)			
Ongoing response to prior therapy	5 (16)			
Observation	6 (19)			
Physician/patient's choice	14 (44)			
Cytopenia	1 (3)			
Best supportive care/hospice	3 (9)			
Lost to follow-up	3 (9)			
	tGA+		tGA−	*p*-Value
	Specific therapy, *N* = 13	No specific therapy, *N* = 32	*N* = 21	
Subsequent lines of therapy, median number	1 (0–7)	1 (0–3)	1 (0–2)	
First subsequent therapy	13 (100)	17 (100)	9 (100)	
Olaparib/niraparib	6/1 (54)	–	–	
Everolimus/temsirolimus	3/1 (31)	–	–	
Pembrolizumab	2 (15)	–	–	
Trastuzumab	1 (8)	–	–	
Oral hormonal therapy	–	3 (18)	2 (22)	
Docetaxel/cabazitaxel	–	3/6 (53)	2 (22)	
Platinum-based chemotherapy	–	2 (12)	2 (22)	
Cabozantinib	–	1 (6)	–	
Radium-223	–	–	2 (22)	
Sipuleucel-T	–	–	1 (11)	
PFS, median (95% CI)	4.1 (2.8–5.4)	4.3 (2.6–6.0)	4.6 (3.5–5.7)	0.397
OS for stage IV, median (95% CI)	60.4 (54.6–66.1)	107 (37.7–177.1)	79.9 (19.9–139.9)	0.305
OS after tNGS test, median (95% CI)	12.7 (11.5–13.9)	17.0 (NE)	11.2 (6.0–16.4)	0.525

NE, not estimated; OS, overall survival; PFS, progression-free survival; tNGS, targeted next-generation sequencing; cfDNA, cell-free circulating tumor DNA

F1/G360 was the sequence of tNGS panels ordered in all except one patient who had FACT followed by G360 testing. In one patient, tNGS (G360) re-tested after 26 months identified one tGA (*PIK3CA*) not previously detected (F1).

In the tNGS cohort, the median OS for stage IV disease was 64.5 months (95% CI, 55.4–73.4) and 13 months (95% CI, 6.6–19.4) after the tNGS test was ordered. No difference in the median OS between groups was found (Table 2).

4 Discussion

This is one of the first feasibility reports of advanced NGS-based treatment analysis in clinical practice assessing the impact of the results in the management of mCRPC.

The frequency of genomic aberrations detected in this heavily pretreated population was very similar to other published cohorts and represents relevant drivers in prostate

Table 3 CRPC patients with tGA+ treated with specific therapy ($n = 13$)

Lines for CRPC before tNGS	Prior therapy	PFS prior therapy (months)	Specific therapy	PFS specific therapy (months)	Ratio of PFS specific/ prior therapy
3	Cabazitaxel	10	Trastuzumab	14.2	1.42
1	Carboplatin/etoposide	4	Olaparib	4.2	1.05
4	Enzalutamide	3.5	Everolimus	2.4	0.69
4	Cabazitaxel	5.1	Everolimus	10.8	2.12
3	Docetaxel	5.2	Pembrolizumab	4.5	0.87
3	Enzalutamide/radium-223	3.9	Olaparib/pembrolizumab	3.7	0.95
2	Cabazitaxel	6.2	Temsirolimus	3.1	0.5
2	Enzalutamide	20.8	Olaparib	1.0	0.05
3	Sipuleucel-T	2.9	Olaparib	1.5	0.52
4	Enzalutamide	11.7	Olaparib	0.3	0.03
2	Enzalutamide	8.3	Olaparib	11.0	1.33
3	Abiraterone/radium-223	4.8	Everolimus	9.2	1.92
4	Cabazitaxel	4	Niraparib	1.8	0.45

CRPC, castration-resistant prostate cancer; tGA+, targetable genomic alterations; PFS, progression-free survival; tNGS, targeted next-generation sequencing

cancer development and progression, while alterations in MMR/MSI genes were slightly higher than expected [15]. In the era of precision oncology, the concept of "actionable" genomic alterations is vague and the list of "targetable" genes is continuously changing. The existing data from personalized trials in advanced solid tumors were used for the purpose of this study and included inhibitors of PARP, immune checkpoints, mTOR, and HER2 based on consensus among investigators.

The proportion of patients with "targetable" alterations who received tNGS-based therapy was relatively low, mainly due to physician/patient decision and/or patient treatment status. Among other factors, the lack of level I evidence for clinical use, available clinical trials, and insurance coverage of these therapies may help explain these findings. While only a small fraction of patients received specific targeted therapies enrolled in biomarker-driven studies available at our institution during the study period, the portfolio of genomic-based clinical trials differs significantly among centers with implications in the "actionability" rate of NGS results and the success rate of personalized treatment approaches.

In general, responses to targeted therapies were modest without notably prolonged median PFS, and less than one third of patients who received tNGS-based therapy had a PFS ratio ≥ 1.3. This preliminary evaluation of the efficacy of biomarker-based therapy was underpowered to draw definitive conclusions. In addition to the heterogenous composition of this sample size, other known prognostic factors and response to prior therapies, such as prior platinum-based regimen for patients treated with PARP inhibitors, may have impacted the results. Nonetheless, this dataset raises the question whether the best timing for personalized therapy attempt might be earlier in the disease course, when tumors had been exposed to fewer lines of treatment and may be less resistant to subsequent therapies. Several ongoing phase III trials investigating novel agents targeting DNA repair or the mTOR/Akt pathway (NCT02952534, NCT03072238) are enrolling patients with a limited number of prior therapies and may help to answer this question. The number of "umbrella" and "basket" trials with several tNGS-based treatment modules is increasing, e.g., NCI-MATCH (NCT02465060), and can also provide additional therapeutic options.

As previously reported [16], the number of cfDNA correlates with (lack of) response to systemic therapies in mCRPC; thus, the timing of cfDNA collection may impact the successful detection of genomic aberrations. For tumor tissue, the optimal collection timing is undefined. In general, clinicians favor tumor re-biopsy—when feasible—since the archival tissue may not reflect the tumor evolution process that may occur after the exposure to multiple interim therapies. Whether the use of primary tumor and/or metastatic tissue impacts the quality and "actionability" of the genomic data gathered is unknown and needs further research. In addition, published data have shown very low concordance in tumor-specific alterations for patient-paired samples from tissue and cfDNA in this and other cancers [17, 18]. Insufficient genomic profiling concordance could jeopardize the clinical benefit of personalized medicine, and this aspect was not assessed in this dataset [18]. Lastly, consideration of germline findings is very important and genetic counselors should review all tNGS results; however, tNGS panels are not considered adequate for full screening for germline mutations.

Limitations of this study include the small size of the cohort, its retrospective nature, limited genomic-based clinical

trial options, variability in time points of tumor tissue and cfDNA collection for analysis, as well as heterogeneity in physician treatment, surveillance practices, and follow-up. The presence of several selection and confounding biases could not be excluded. The analysis was underpowered to draw a conclusion regarding any individual biomarker and the genomic alterations reported may have included both somatic and germline aberrations, while no comparison between metastatic and primary prostate tumors was conducted.

5 Conclusions

In this cohort, genomic analysis of mCRPC using commercially available tNGS panels was feasible and detected frequent genomic defects, e.g., in the androgen receptor, DNA repair, and the PIK3CA/mTOR pathway. As the paradigm of personalized medicine continues to evolve, the optimal tNGS test, type and timing of tumor sample collection, as well as reproducibility and validation of putative biomarkers with clinical utility need to be elucidated. The role of prospective biomarker-driven clinical trials remains critical. Further studies and larger portfolios of targeted therapy trials are needed to optimize the benefit of tNGS in this setting.

Compliance with Ethical Standards

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Conflict of Interest Brandie Heald has disclosed to be on an advisory board for Invitae and the speakers' bureau for Myriad Genetics Laboratory. Dr. Petros Grivas has disclosed to be a consultant or advisor for Foundation Medicine, Genentech, Dendreon, Bayer, Driver Inc., Exelixis, Merck & Co., Bristol-Myers Squibb, AstraZeneca, Biocept, Clovis Oncology, EMD Serono, and Seattle Genetics; has received research funding from Mirati Therapeutics, Genentech/Roche, Merck, Oncogenex, Bayer, Pfizer, and AstraZeneca. Dr. Davendra Sohal has disclosed to be a consultant or advisor for Perthera and Foundation Medicine, and received research funding from Novartis, Celgene, OncoMed, Bayer, and Genentech/Roche. Dr. Jorge Garcia has disclosed to be a consultant or advisor for Sanofi, Pfizer, Bayer, Eisai, Exelexis, Medivation/Astellas, and Genentech/Roche; has received research funding from Pfizer, Astellas Pharma, Orion Pharma GmbH, Bayer, Janssen Oncology, Genentech/Roche, and Lilly. Pedro C. Barata, Prateek Mendiratta, and Stefan Klek declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

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