1 **Review**

2

3 # An update of aberrant methylation detection on circulating cell-free DNA as a tool to improve prostate cancer diagnosis and prognosis

4

5 **Nicola Pavan[1], Gabriele Grassi[2], Bruna Scaggiante[2]**

6

7 [1]Department of Medical, Surgical and Health Science, Urology Clinic, University of Trieste, Trieste 34127, Italy.

8 [2]Department of Life Sciences, University of Trieste, Trieste 34127, Italy.

9

10 **Correspondence to:** Prof. Bruna Scaggiante, Department of Life Sciences, University of Trieste, via Valerio 28/1 Trieste 34127, Italy. E-mail:

11 bscaggiante@units.it

12

13 **How to cite this article:** Pavan N, Grassi G, Scaggiante B. An update of aberrant methylation detection on circulating cell-free DNA as a tool to

14 improve prostate cancer diagnosis and prognosis. *J Transl Genet Genom* 2021;5:[Accept]. http://dx.doi.org/10.20517/jtgg.2021.18

15

16 **Received:** 31 Mar 2021 **Accepted:** 28 May 2021 **First online:** 2 Jun 2021

17

© The Author(s) 2018. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or

format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

18

19 **Abstract**

20 Prostate cancer (PCa) remains a disease of high incidence, but orphan of a specific screening program. For this reason, non-invasive techniques
21 capable to predict PCa in patients with high specificity and sensitivity are still an urgent need. One of the major goals is to improve the PCa
22 diagnosis and the identification of patients who benefit from tissue biopsies. Another need is the necessity to have novel biomarkers to better
23 stratify the risk of patients with PCa to predict the aggressiveness of the tumor and the overall survival. Liquid biopsy can be an important
24 non-invasive tool to stratify PCa at the molecular level to improve diagnosis and prognosis, and, possibly, to develop screening programs and
25 follow-up. With this review, we are reporting the lastest update of aberrant methylation detection on circulating tumor DNA as a tool to improve
26 prostate cancer diagnosis and prognosis.

27

28 **Keywords:** Liquid biopsy, DNA, Methylation, Prostate cancer, cfDNA, ctDNA, Biomarker

29

30

31 **INTRODUCTION**

32 Prostate cancer (PCa) is the main cause of cancer-related death in men in Western countries [1].Screening programs to identify adenocarcinoma
33 by the prostate-specific antigen (PSA) have a specificity too low to be largely proposed as the first-line test. Moreover, PSA is elevated as well in
34 benign prostate pathologies, and about 15% of asymptomatic PCa patients do not present elevated PSA levels. Nowadays, prostate biopsy
35 remains the procedure necessary for the diagnosis of PCa despite the invasiveness, possible side effects and cost of the procedure.

36

37 More specific prostate cancer biomarkers are urgently needed not only to anticipate cancer diagnosis, especially the identification of the more

38 aggressive forms, but also for the best management of therapeutic interventions and the surveillance of cancer progression. In particular, for
39 patients in active surveillance (AS), a more specific test and less invasive procedure are needed to improve the adherence to the protocols and
40 maintain a good quality of life for patients.
41
42 At the genomic level, tumor onset and progression are importantly modulated at the epigenetic level by the DNA methylation changes in specific
43 regions, mainly at gene promoter sites. The epigenetic modulation of DNA is a field of intense research to find novel biomarkers for
44 diagnosis/prognosis or targets for innovative therapeutic strategies. Methylation of the CpG islands is a very frequent aberration in cancer that
45 occurs also in prostate cancer [2]. Thus, a part of the scientific interest is directed to the "methylome" analysis of the DNA to better define the
46 onset and the phenotype evolution of cancer.
47
48 To analyze tumor DNA, liquid biopsy can offer a non-invasive tool to monitor specific PCa biomarkers in different biological fluids. Nowadays,
49 it is possible to detect, with high sensitivity and specificity, circulating tumor nucleic acids (DNA and RNA) derived from cancer cells that have
50 died. For example, the search of DNA aberrations of the androgen receptor gene has been found useful at a prognostic and predictive level and
51 they are strongly correlated with patients ' outcomes [3].
52
53 This mini-review summarizes the major and recent discoveries of aberration in the methylation pattern of circulating tumor DNA. We mainly
54 focused on the biomarkers that have been demonstrated to be clinically useful or promising.
55
56 A literature review was performed using PubMed and three main key terms (DNA methylation AND prostate cancer AND circulating cell-free
57 DNA AND liquid biopsy) We selected research articles from 2015 to 2021. Articles included in this review are summarized in **Table 1**.

58

59 **METHYLATED BIOMARKERS IN CIRCULATING CELL-FREE DNA (cfDNA) FROM URINE**

60 **The cfDNA containing ctDNA has been collected after digital rectal examen (DRE) or at first void (FV)**

61 One of the most interesting translational research comes from Brikun et al. [4, 5]. Using cfDNA from urine after DRE or at FV, they
62 demonstrated, using a panel of 19 targets that the number of methylated markers was statistically higher in PCa cases compared to controls, 10 of
63 19 versus 3 of 19, respectively. Six of nineteen methylated markers (6of19) were shown to be the threshold to predict PCa with a negative
64 predictive value (NPV) ≥90% for both DRE and FV urine cfDNA. In addition, authors proved a significative association between the number of
65 methylated markers and the PCa diagnosis with the tissue biopsies. Finally, in post-DRE urine samples, a higher rate of biomarkers was reported
66 when compared to urine at FV. In particular, AOX1, coding an aldehyde oxidase that regulates reactive oxygen species homeostasis, GRFA2,
67 coding a neurotrophic factor involved in cell survival and differentiation, and NEUROG3, coding a transcriptional regulator, cannot be found in
68 FV samples [5].

69

70 Furthermore, in a subsequent study the authors proved that other 13 markers can be used to predict early PCa or to stratify the disease. Using the
71 same sample of cfDNA from urine after DRE or at FV, a panel of 32 markers was proposed and tested [4]. They found that in both groups, the
72 median number of the methylated markers was higher in PCa cases than controls, 16 of 32 versus 5 of 32, respectively. The 10 of 32 positive
73 methylated markers cutoff was found to be the threshold to recommend a patient for prostate biopsy. The positive predictive value did not
74 significantly improve from the previous study with 19 targets, being 71% and 77%, for DRE and FV, respectively. On the contrary, the negative
75 predictive value was confirmed to have high performance being 85% and 93%, for DRE and FV, respectively. Both studies showed a significant
76 increase in the AUC values of the Receiver Operating Characteristic (ROC) curves when compared to PSA. This finding demonstrates the higher
77 specificity and sensitivity of the number of methylated markers in urinary cfDNA compared to PSA level. Notably, HOXD3 and HOXA7, both

78 encoding members of the family of transcription factors, GPR62, coding a signaling factor of the phosphoinositol pathway, and KLK10, coding a
79 serine protease implicated in carcinogenesis, were found in all PCa samples; however, HOXD8rc, encoding a member of the family of
80 transcription factors, CXCL14, encoding a protein involved in inflammatory and immunomodulatory functions, SLC16A5rc, encoding a member
81 of a family of carrier, and GRASP , encoding a scaffold protein involved in phosphoinositide pathway, were more frequently present in highly
82 aggressive PCa, thus suggesting for the last ones a prognostic value for these markers
83
84 Moreover, both studies evaluated the correlation between the number of methylated markers or the average of methylation with the risk score
85 *University of California San Francisco Cancer of the Prostate Risk Assessment* (UCSF-CAPRA). Overall, the results suggested a high
86 performance of the methylation test to identify patients at risk of PCa and the possibility to use these markers and their global status of
87 methylation to stratify patients for PCa aggressiveness. The 32-panel of biomarkers has improved the precision for patient stratification by giving
88 the indication for biopsy in those patients who did not reach the threshold in the 19-panel. Notably, the possibility to determine the risk
89 stratification was assigned to urine cfDNA after DRE, because of the possibility to recover more cancer cells and to avoid dilution and
90 degradation of DNA derived from urine at FV specimens that may cause a higher sampling error. However, the authors underline that in 58
91 patients, both DRE and FV samples were equivalent in the analysis results, thus suggesting that FV remains a useful and simple source for
92 cfDNA. These markers matched with the age of patients and others anamnestic parameters could improve the sensitivity/specificity of the test. A
93 future dedicated clinical trial will be able to find the clinical correlations necessary for the validations of these markers.
94
95 Nekrasov et al. [6] collected 31 urine samples from PCa patients and 33 samples in healthy patients as disease-free control. The methylation
96 status of 17 cancer-associated genes was analyzed using a methylation-specific polymerase chain reaction. They reported 13 genes with increased
97 methylation frequency in patients with PCa compared with the control group. In conclusion, the authors reported a 6-gene panel (APC2, CDH1,

FOXP1, LRRC3B, WNT7A, and ZIC4) able to identify PCa with 78% sensitivity and 100% specificity.

Connel et al. [7], reported a multivariable risk model integrating urinary cell DNA methylation and cfRNA data able to detect significant PCa. In their analysis, 207 post-digital rectal examination urine samples were collected within a Movember cohort (GAP1 urine biomarker). ExoMeth was the name of the model created for this study. Clinical variables (age and PSA) were integrated with methylation and transcript targets. The model was subsequently tested and applied to a final cohort of 197 with available data. With an odds ratio (OR) of 2.04 (95% CI = 1.78-2.35) per 0.1 ExoMeth increase, they were able to increase the likelihood high-grade of the disease being detected on prostate biopsy. In the future, this can potentially avoid unnecessary biopsies in patients on active surveillance (AS) or to guide the necessity of mpMRI in patients with a clinically suspected PCa.

Similarly, Zhao et al. [8] combined the urinary DNA methylation with cf-mRNA biomarkers in a series of 103 CaP patients on AS. The aim of the study was the identification of patients at risk of reclassification. Three marker panels (miR-24, miR-30c and CRIP3 methylation) were identified in the post-DRE urinary sediment using a qPCR-based MethyLight assay. With a negative predictive value of 90% and an OR of 2.17 (95%CI = 1.22-3.85), the authors were able to identify patients with a PCa progression. CRIP3 methylation was found to be a significant predictor of AS reclassification (OR = 1.079, 95% CI = 1.013−1.15).

Silva et al [9], designed a prospective study to investigate the role of blood and urine in capturing the PCa methylome. They selected a cohort of 4 patients with *de novo* metastatic PCa (mPCa) and a post-DRE and FV sample of urine were analyzed. Detection of tumor DNA methylation probes in urine ranged from 6.98% to 39.40%. Authors demonstrated, through a DNA methylation analysis, highly correlated patterns between the different liquid types ($\rho = 0.93$, $P < 0.0001$), with large contributions from non-tumor sources.

118

119 Finally, a promising Danish study [10] investigated the role of novel aberrant promoter hypermethylation of specific genes to improve the
120 diagnosis and prognosis of prostate cancer. The methylation at the promoter region of ST6GALNAC3, encoding a member of the
121 sialyltransferases playing important role in modification of glycoproteins, and ZNF660, encoding a transcriptional regulator, or of T6GALNAC3,
122 encoding a protein identified in neutrophil granules, ZNF660, encoding a transcription factor, CCDC181, encoding a microtubule binding protein,
123 and HAPLN3, encoding a membrane protein, were analyzed in prostate tissues liquid biopsies. 815 samples (705 prostate cancer and 110
124 non-cancer) were processed by methylation-specific qPCR or methylation array. The area under the curve (AUC) of the ROC analysis
125 demonstrated the role of hypermethylation of ST6GALNAC3 and ZNF660 in the diagnosis of prostate cancer (0.917–0.995 vs 0.846–0.903 in
126 cancer vs non-cancer samples, respectively). Moreover, ZNF660 hypermethylation was tested in two radical prostatectomy cohorts of 158 and
127 392 patients.

128

129 ZNF660 hypermethylation was also significantly associated with poor overall and PCa-specific survival in a different cohort of radical
130 prostatectomy (n = 158) with long clinical follow-up available showing a potential prognostic role. In the same study, a panel of hypermethylated
131 circulating tumor DNA (ctDNA) for ST6GALNAC3, ZNF660, HAPLN3, and CCDC181 was proposed for liquid biopsy. A final ctDNA
132 hypermethylation model of 3 genes (ST6GAL- NAC3/CCDC181/HAPLN3) was developed with 100% of specificity and 67% of sensitivity in
133 the detection of PCa.

134

135 **METHYLATED BIOMARKERS IN CIRCULATING CELL-FREE DNA (cfDNA) FROM BLOOD**

136 **The cfDNA, containing ctDNA, has been collected from plasma or from serum.**
137 In 2015, Reis et al.[11] studied, in serum, cfDNA the methylation of GADD45a gene, which they previously found to be methylated at different
138 sites in tissue PCa tissue. The authors found a statistically significant difference between the methylation of GADD45a in Pca with respect to
139 Benign Prostatic Hyperplasia (BHP) patients' serum. The PCa samples were more methylated than BPH controls, although in PCa patients a
140 higher methylation variability than BPH controls was found. No correlation between GADD45a methylation and Gleason score was evidenced.
141 Interestingly, the methylation status of GADD45a and the PSA level better define PCa versus BPH patients than GADD45a methylation alone.
142
143 The role of aberrant DNA promoter methylation was also studied as a possible tool for simultaneous detection of several types of cancers [12]. In
144 a large multicenter study, this hypothesis was tested for lung, prostate, and colorectal cancers. More deeply, cfDNA was extracted from 121 PCa
145 patients and the level of methylation of different promoters was assessed. The authors proposed a "Pan- Cancer" panel (FOXA1me, RARβ2me
146 and RASSF1Ame) able to simultaneously detect PCa and lung cancer (SP 70% and SS 64%). The panel was also able to discriminate between
147 intermediate and high-risk PCa with a sensitivity of 71% and a specificity of 65%. These results can be interesting when future studies will apply
148 this panel in an active surveillance setting or in the decision-making process for a diagnostic biopsy in the suspected cases of PCa.
149
150 As previously described, the study published by Silva et al. [9] analyzed the role of blood in capturing DNA methylation. Utilizing the Infinium®
151 MethylationEPIC BeadChip (Illumina) they were able to detect DNA methylation probes from 7.19% to 64.14% in plasma. Matching liquid and
152 prostate biopsies controls authors prevented the effect of unwanted variables and reduced the inter-individual variability. Despite the small
153 number of patients, the authors have shown that both plasma and urine serve as excellent surrogates for detecting tumoral epigenomic alterations.
154
155 Menschikowski et al. [13] developed a novel amplification system based on digital-droplet PCR (ddPCR), named optimized bias-based

156 pre-amplification ddPCR (OBBPA-ddPCR), for early detection of rare DNA methylation targets. They demonstrated that this technique can
157 specifically detect PLA2R1 gene methylation in serum of PCa patients with a very high sensitivity. PLA2R1 encodes a phospholipase A2
158 receptor. If this novel assay could be usable for the early identification of PCa patients remains to be demonstrated in a large sample cohort.
159
160 Bjerre et al. [14] proved from a panel of 24 candidate biomarkers that three of them, DOCKK2, HAPLN3, encoding an important protein that
161 binds hyaluronic acid involved in many cellular function and cell adhesion, and FBXO30, encoding a member of F-box protein family involved
162 in protein degradation, were strongly related to the progression of hormone-naïve mPCa to castration-resistant mPCa. They used plasma samples
163 that were analyzed by MS-ddPCR. Interestingly, these markers did not result in methylation in healthy controls, BPH, or localized PCa patients.
164 The authors noted that plasma cfDNA quantity did not differ between healthy donors, BPH, localized PCa, or *de novo* mPCa patients. However, a
165 higher level of cfDNA was found to be related to cases in a more advanced clinical stage. It is important to underline that in BPH or localized
166 PCa samples, the cfDNA methylation in the biomarkers was rarely found; on the contrary, in corresponding tissue samples, the methylation of all
167 markers was present. The markers were highly sensitive and specific to identify high tumor volume, *de novo* mPCa. From the clinical point of
168 view, the methylation of any of the three biomarkers was related with shorter OS in these patients, as an independent predictor.
169
170 Beltran et al. [15] studied cfDNA in castration-resistant neuroendocrine prostate cancer (CRPC-NE). A significant proportion of PCa with this
171 phenotype are linked with a poor prognosis. They performed whole-exome sequencing in cfDNA from plasma to identify any aberration in the
172 expression of important tumor suppressor genes such as TP53 and RB1. The same analysis was applied also in genes involved in DNA repair
173 such as BRCA1, BRCA2, FANCA, or an important checkpoint signaling regulators such as Ataxia-telangiectasia mutated gene (ATM) gene. The
174 authors also performed whole-exome genome bisulfite sequencing in a small sample of patients harboring CRPC-adeno or CRPC-NE and
175 compared the results with the methylation pattern in the tissues. A concordance of the methylation status of the targets between cfDNA and tissue

176 biopsy was found. In CRPC-NE samples hypo or hypermethylation status of 20 different sites marked this tumor phenotype in cfDNA.

177

178 **Table 1. Summary of studies.**

First Author	Year of Publication	Number of patients	Cases	Control group	Sample	Methodology	Biomarker	Diagnostic value	Prognostic value	Tissue concordance	Specificity	Sensitivity
I M Reis [11]	2015	82	34 PCa	48 Healthy control	Serum	Ms-SNuPE and Pyrosequencing	GADD45a	Yes	Yes	No	87%	94%
Christa Haldrup [10]	2018	815	705 PCa/ZNF 660 tested in different cohorts	110 Healthy control	Serum	MS-qPCR	ST6GALNAC3, ZNF660; HAPLN3	Yes	Yes	Yes	100%	67%
Mario Menschikowski [13]	2018	2	2 PCa	NA	Serum	OBBPA-ddPCR	PLA2R1	Yes	No	No	NA	NA
Igor Brikun [5]	2018	94	42 PCa	52 Healthy control	Urine (after DRE and at FV)	MS-qPCR by binary presence (>0) or absence (<0) of methylation	ADCY4,AOX1rc,APC,CXCL14, EPHX3,GFRA2,GSTP1,HEMK1 rc,KIFC2,MOXD1,HOXA7,HOX B5,HOXD3,HOXD9,HOXD10,H OXD3a,HOXD3b,NEUROG3,N ODAL,RASSF5	Yes	No	No	71% after DRE and FV	89% after DRE and 94% from FV
Igor Brikun [4]	2019	94	42 PCa	52	Urine (85	MS-qPCR by	PANEL ABOVE PLUS 13	Yes	No	No	76% from	81% from

				Healthy control	after DRE and 65 at FV)	binary presence (>0) or absence (<0) of methylation	additional markers: HOXA11as,KLK10,GPR147,GPR62,HOXD4rc,HOXD3c,FRZB, GRASPrc,HOXBAS3,HOXD8rc, RASSF1, SLC16A5rc				DRE-77% after FV	DRE-93% after FV
Kostyantyn A. Nekrasov[6]	2019	64	31 PCa	33 Healthy control	Urine	qMSP	APC2, CDH1, FOXP1, FOXP2, FOXP3, FOXP4, H1C1, HOXA9, LRRC3B, MGMT, NDRG4, PLCL2, PTEN, UBE2E2, VHL, WNT7A, ZIC4	Yes	Yes	No	100%	78%
Eve O'Reilly [16]	2019	463	209 PCa/31 +178 tissue samples	254 Healthy control	Urine	HumanMethylation 450 BeadChip (HM450k)	Methylation array chip	Yes	Yes	Yes	76%	73%
Shea P. Connell[7]	2019	207	207 PCa	NA	Urine	MS-qPCR	GSTP1, SRFP2, IGFBP3, IGFBP7, APC, PTSG2	Yes	Yes	No	NA	NA
Fang Zhao[8]	2019	103	103 PCa on AS	NA	Urine	Multiplex MethylLight Assay	APC, GSTP1, CRIP3, HOXD8	No	Yes	No	60%	81%
Vera Constâncio [12]	2019	121	121 PCa	NA	Plasma	Multiplex qMSP	APC, FOXA1, GSTP1, HOXD3, RARß2, RASSF1A, SEPT9, SOX17	Yes	Yes	No	72%	72%
Jacob J. K. Carson[17]	2020	ND	small cohort of patients of men with	NA	Serum/Plasma	mDETECT	Methylation DETection of Circulating Tumor DNA (mDETECT)	No	Yes	No	NA	NA

			BCR									
Marianne Trier Bjerre [14]	2020	264	102 lPCa, 65 de novo mPCa	36 Healthy control, 61 BPH	Plasma	MS-ddPCR	DOCK2, HAPLN3, and FBXO30	Yes	Yes	Yes	80%–100%	75%–94%
Himisha Beltran [15]	2020	62	10 mPCa, 35 CRPC-Adeno, 17 CRPC-NE	NA	Plasma	WGBS	relevant from panel of genes: ASXL3, SPDEF, INSM1, CDH2, TP53, RB1, CYLD, AR	Yes	No	Yes	NA	NA
Anjui Wu[18]	2020	25	25 mCRP	NA	Plasma	NGS	next-generation sequencing (NGS) on plasma DNA	Yes	No	No	NA	NA
Romina Silva[9]	2020	4	4 mCRP	NA	Urine and plasma	MethyLight qPCR and Infinium® MethylationEPIC BeadChip	Methylation array chip	Yes	No	Yes	NA	NA

AS= active surveillance; BCR=biochemical recurrence; BPH= benign prostatic hyperplasia; CRPC-Adeno= castration-resistant adenocarcinoma; ddPCR= digital droplet PCR; DRE= digital rectal examination; FV= first morning void; lPCa= localized prostate cancer; qPCR= quantitative PCR; qMPS= quantitative Methylation specific PCR; mCRPC-NE=metastatic castration-resistant neuroendocrine prostate cancer; mCRP= metastatic castration resistant prostate cancer; mDETECT= Methylation DETection of Circulating Tumor DNA; mPCa= hormone-naive metastatic prostate adenocarcinoma; PCa = prostate cancer; MS-SNuPE= Microarray-based methylation-sensitive single-nucleotide primer extension; MS-ddPCR= methylation specific digital droplet PCR; MS-qPCR= methylation specific quantitative PCR; Multiplex qMSP= multiplex quantitative methylation-specific PCR; NA= not assessed; ND= not defined; NGS= next-generation sequencing; OBBPA-ddPCR= optimized bias-based pre-amplification-digital droplet PCR; WGBS=Whole-genome bisulfite sequencing.

186 **CONCLUSION**

187 The ctDNA can be easily identified from the quote of cfDNA release from cancer cells
188 in bloodstream or in urine. It is important to note that the total level of cfDNA in plasma
189 did not relate with presence of PCA, although higher values of cfDNA have been found
190 in advanced disease patients [14]. However, the measure of cfDNA level alone remains
191 a poor predictor of the disease. Methylation of specific biomarkers and, in particular, the
192 number of methylated biomarkers in a panel can provide useful tools for clinicians
193 either to manage the risk of asymptomatic patients or to predict the progression.
194 Molecular tests are more expensive than PSA, however the reduction of improper
195 prostate biopsies, the precise identification of patients with risk should fully balance the
196 initial screening test costs, and prospectively save costs and suffering. Reducing the
197 number of unnecessary biopsies will be one of the best targets that we can achieve in
198 PCa. The finding of low-grade PCa and an increase demand of AS need to be supported
199 by reliable and precise test able to define an upgrading or upstaging of PCa. The
200 reduction of costs associated to unnecessary treatment will be devolved for more precise
201 screening and diagnosis able to improve the quality of life of our patients.
202
203 Some of the biomarkers are candidates for identifying more aggressive forms such as
204 HOXD8rc, CXCL14, SLC16A5rc and GRASP or to predict progression such as
205 DOCKK2, HAPLN3 and FBXO30. It is important to note that methylation of some
206 biomarkers that can be found in tissues, cannot be found in ctDNA [14]. This is because
207 PCa does not release cancer cells if it is at the early stages. Thus, the analysis of cfDNA
208 methylation associated to the study of PCa tissues, remains a very important task to
209 deepen the role of cfDNA biomarkers methylation.
210
211 In conclusion the studies showed the possibility to analyze the methylation of cfDNA
212 biomarkers either from plasma or urine, thus opening more possibilities to monitor PCa
213 patients and, possibly to develop screening programs. A challenge is to study the

© The Author(s) 2018. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

integration of the anamnestic data, the PSA and the molecular methylation status on cfDNA to personalize the patient's care.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception, data search, design and writing of the review: Pavan N and Scaggiante B

Performed critical revision: Grassi G

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA A Cancer J Clin 2018;69:7-34.

2. Goering W, Kloth M, Schulz WA. DNA Methylation Changes in Prostate Cancer. In: Dumitrescu RG, Verma M, editors. Cancer Epigenetics. Totowa: Humana Press; 2012. pp. 47-66.

247 3. Sobhani N, Sirico M, Generali D, Zanconati F, Scaggiante B. Circulating cell-free
248 nucleic acids as prognostic and therapy predictive tools for metastatic castrate-resistant
249 prostate cancer. World J Clin Oncol 2020;11:450-63.
250 4. Brikun I, Nusskern D, Freije D. An expanded biomarker panel for the detection of
251 prostate cancer from urine DNA. Exp Hematol Oncol 2019;8:13.
252 5. Brikun I, Nusskern D, Decatus A, Harvey E, Li L, Freije D. A panel of DNA
253 methylation markers for the detection of prostate cancer from FV and DRE urine DNA.
254 Clin Epigenetics 2018;10:91.
255 6. Nekrasov KA, Vikarchuk MV, Rudenko EE, et al. 6-gene promoter methylation assay
256 is potentially applicable for prostate cancer clinical staging based on urine collection
257 following prostatic massage. Oncol Lett 2019;18:6917-25.
258 7. Connell SP, O'Reilly E, Tuzova A, et al. Development of a multivariable risk model
259 integrating urinary cell DNA methylation and cell-free RNA data for the detection of
260 significant prostate cancer. Prostate 2020;80:547-58.
261 8. Zhao F, Vesprini D, Liu RSC, et al. Combining urinary DNA methylation and
262 cell-free microRNA biomarkers for improved monitoring of prostate cancer patients on
263 active surveillance. Urol Oncol 2019;37:297.e9-297.e17.
264 9. Silva R, Moran B, Russell NM, et al. Evaluating liquid biopsies for methylomic
265 profiling of prostate cancer. Epigenetics 2020;15:715-27.
266 10. Haldrup C, Pedersen AL, Øgaard N, et al. Biomarker potential of ST6GALNAC3
267 and ZNF660 promoter hypermethylation in prostate cancer tissue and liquid biopsies.
268 Mol Oncol 2018;12:545-60.
269 11. Reis IM, Ramachandran K, Speer C, Gordian E, Singal R. Serum GADD45a
270 methylation is a useful biomarker to distinguish benign vs malignant prostate disease.
271 Br J Cancer 2015;113:460-8.
272 12. Constâncio V, Nunes SP, Moreira-Barbosa C, et al. Early detection of the major
273 male cancer types in blood-based liquid biopsies using a DNA methylation panel. Clin
274 Epigenetics 2019;11:175.
275 13. Menschikowski M, Jandeck C, Friedemann M, et al. Identification of rare levels of
276 methylated tumor DNA fragments using an optimized bias based
277 pre-amplification-digital droplet PCR (OBBPA-ddPCR). Oncotarget 2018;9:36137-50.
278 14. Bjerre MT, Nørgaard M, Larsen OH, et al. Epigenetic Analysis of Circulating Tumor
279 DNA in Localized and Metastatic Prostate Cancer: Evaluation of Clinical Biomarker
280 Potential. Cells 2020;9:1362.

281 15. Beltran H, Romanel A, Conteduca V, et al. Circulating tumor DNA profile
282 recognizes transformation to castration-resistant neuroendocrine prostate cancer. J Clin
283 Invest 2020;130:1653-68.
284 16. O'Reilly E, Tuzova AV, Walsh AL, et al. epiCaPture: A Urine DNA Methylation Test
285 for Early Detection of Aggressive Prostate Cancer. JCO Precis Oncol 2019;2019.
286 17. Carson JJK, Di Lena MA, Berman DM, Siemens DR, Mueller CR. Development
287 and initial clinical correlation of a DNA methylation-based blood test for prostate cancer.
288 Prostate 2020;80:1038-42.
289 18. Wu A, Cremaschi P, Wetterskog D, et al. Genome-wide plasma DNA methylation
290 features of metastatic prostate cancer. J Clin Invest 2020;130:1991-2000.
291