



HAL
open science

Tumour-based mutational profiles predict visceral metastasis outcome and early death in prostate cancers

Olivier Cussenot, Kirsten M Timms, Emmanuel Perrot, Pascal Blanchet, Laurent Brureau, Cara Solimeno, Gaele Fromont, Eva Comperat, Geraldine Cancel-Tassin

► To cite this version:

Olivier Cussenot, Kirsten M Timms, Emmanuel Perrot, Pascal Blanchet, Laurent Brureau, et al.. Tumour-based mutational profiles predict visceral metastasis outcome and early death in prostate cancers. *European Urology Oncology*, 2024, 10.1016/j.euo.2023.12.003 . hal-04385702

HAL Id: hal-04385702

<https://hal.sorbonne-universite.fr/hal-04385702>

Submitted on 10 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Tumour-based mutational profiles predict visceral metastasis outcome and early death in**
2 **prostate cancers**

3

4 Olivier Cussenot^{1,2}, Kirsten M. Timms³, Emmanuel Perrot^{4,5}, Pascal Blanchet^{4,5}, Laurent
5 Brureau^{4,5}, Cara Solimeno³, Gaelle Fromont^{1,6,7}, Eva Comperat^{1,8}, Geraldine Cancel-Tassin^{1,9*}

6

7 ¹CeRePP, Paris, France

8 ²Nuffield Department of Surgical Sciences, University of Oxford UK

9 ³Myriad Genetics, Salt Lake City, UT; USA

10 ⁴Department of Urology, CHU Pointe-a-Pitre/Abymes, Pointe a Pitre, Guadeloupe, France

11 ⁵Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S
12 1085, Pointe-à-Pitre, Guadeloupe

13 ⁶Inserm UMR1069 "Nutrition, Croissance et Cancer" Université François Rabelais, Faculté de
14 Médecine, Tours, France

15 ⁷CHRU Bretonneau, Departments of Pathology and Urology, Tours, France

16 ⁸Department of Pathology, Medical University of Vienna, 1090 Vienna, Austria

17 ⁹GRC n°5 Predictive Onco-Urology, Sorbonne Université, Paris, France

18

19 ***Corresponding author**

20 Geraldine Cancel-Tassin, PhD.

21 CeRePP/ GRC n°5, Hopital Tenon, 4 rue de la Chine, 75020 PARIS, FRANCE

22 Phone: +331 56 01 76 46 ; Fax : +331 56 01 76 47

23 Email: g.cancel@cerepp.org

24

25 **Keywords**

26 DNA repair; genes; germline; somatic; mutation; metastasis; prostate cancer

27

28 **Word count of text: 2578**

29 **Word count of the abstract: 300**

30

31 **Abstract**

32 **Background:** Visceral metastases are known to occur in advanced prostate cancer, usually
33 when the tumour is resistant to androgen deprivation and, have worse outcomes regardless
34 of therapies.

35 **Objective:** To analyse genomic alterations in tumour samples according to their lymphatic,
36 bone and visceral metastatic stages and overall survival.

37 **Design, Setting, and Participants:** We selected 200 patients with metastatic prostate cancer.
38 Genomic profiling of 111 genes and molecular signatures (Homologous recombination
39 deficiency: HRD; Microsatellite instability: MSI and Tumour burden mutation: TMB) was
40 performed with the MyChoice™ test (Myriads Genetics, Inc).

41 **Outcome Measurements and Statistical Analysis:** Association between genomic profiles and
42 visceral metastatic evolution was evaluated using logistic regression. Kaplan-Meier and Cox
43 proportional hazards analyses were used for analyses of early death.

44 **Results and limitations:** 173 (87%) genomic profiles were obtained. Eighty-four (49%)
45 patients died during the follow-up period (median duration = 76 months). *TP53* was the
46 most frequently mutated gene, followed by FANC genes, including *BRCA2*, and those of the
47 Wnt-pathway (*APC/CTNNB1*). *TP53* gene mutations were more frequent in patients of
48 European (42%) than African (16%) ancestry. An HRD score >25 was predictive of FANC
49 genes mutations. The mutational status of *TP53* ($p < 0.001$) and *APC* ($p = 0.002$) genes were
50 significantly associated with the risk of visceral metastases. The mutational status of *CTNNB1*
51 ($p = 0.001$), *TP53* ($p = 0.015$), *BRCA2* ($p = 0.027$), and *FANC* ($p = 0.005$) genes were significantly
52 associated with an earlier age at death. The limitations are the retrospective study design
53 based on a selection of genes and, the low frequency of certain molecular events.

54 **Conclusion:** Mutations in the *TP53* gene and genes (*APC/CTNNB1*) related to the Wnt-
55 pathway are associated with metastatic visceral dissemination and early death. These
56 genomic alterations could be considered as markers to identify prostate cancer patients at
57 high risk of life-threatening disease who might benefit from more intensified treatment or
58 new targeted therapies.

59 **Patient summary:** In this report, we evaluated relationships between genomic profiles (gene
60 mutations and molecular signatures) of tumour samples from patients with metastatic prostate
61 cancer and early death. We found that mutations of specific genes, notably *TP53* and
62 *APC/CTNNB1* related to the Wnt-pathway, are associated with visceral metastatic
63 progression and an earlier age at death.

64

65 **Introduction**

66 Prostate cancer (PCa) is the fifth leading cause of cancer death in men worldwide, with an
67 estimated 1.4 million cases diagnosed and an estimated 375,000 PCa deaths in 2020 [1].
68 However, important disparities in its incidence and mortality are seen among different
69 populations around the world [1]. Due to the lack of recommendation for PCa screening, and
70 the increased sensitivity of imaging that identifies metastases in a higher number of men
71 affected with PCa, leading to stage migration phenomenon, the incidence of PCa diagnosed
72 at a metastatic stage has increased for the 20 last years [2]. If the most common site of PCa
73 metastasis is the bone (>80%), the visceral metastases, which used to be infrequently
74 observed, have become more common. Their most frequent locations are in the lung and
75 liver, each representing approximately 40% of visceral metastases. Their incidence in men
76 who died from metastatic PCa significantly increased from 26% in 2009 to 40% in 2016 [3].
77 Thanks to multiple systemic therapies approved since 2010 with proven survival benefits,
78 men with metastatic PCa are living longer than ever [4], but thus may later develop visceral
79 metastases. The increase in the number of lines of therapy may also be behind the increase
80 in visceral disease. After progression under androgen deprivation therapy, visceral
81 metastases confer worse median survival duration [5].
82 The lethality of PCa is related to the evolution of metastatic castrate-resistant clones [6]. The
83 landscape of genomic alterations reported in metastatic PCa was established from analyses
84 of germline [7] and tumour [6, 8-10] samples. The most frequent germline mutations carried by
85 patients with metastatic PCa are in the *BRCA2* and *ATM* genes, with a prevalence estimated across
86 different reports to be approximately 5% and 2%, respectively [11]. In tumours from patients with
87 metastatic castration-resistant PCa (mCRPC), the most common genomic alterations are
88 observed in the *AR* and *TP53* genes, with frequencies above 40% [12]. The characterization of

89 some of these genomic alterations or their molecular signatures is used as new companion
90 diagnostic tests for targeted therapies such as PARP-1 inhibitors [13] and immune check-
91 point inhibitors [14], or for suggested new targeted therapies such as PI3K/AKT [15] and Wnt
92 [16] pathway inhibitors. Here we report the prevalence of specific mutational profiles and
93 functional signatures (Homologous Recombination Deficiency: HRD, Tumor Mutational
94 Burden: TMB, Microsatellite Instability: MSI) in PCa evolving with visceral metastasis, and
95 their impact on early death.

96

97 **Patients and Methods**

98 Patients

99 The 200 patients included in this study were selected based on metastatic status among the
100 patients from the PROGENE study. They all provided written informed consent to participate
101 in this study that complied with the Declaration of Helsinki and was approved by the CCP Ile
102 de France IV (IRB: 00003835). Ancestry was based on self-report and on skin phenotype
103 selected by the clinician in the medical questionnaire. Patients of Asian ancestry, from North
104 African origin, born in India, or too complex to be classified were annotated as “other” and
105 were excluded from comparison between patients from African and European ancestries.

106 Genomic analyses

107 Archival tumour tissues were provided from formalin-fixed paraffin-embedded blocks of
108 primary samples: 158 prostate biopsies, 28 transurethral resections of the prostate, 8 radical
109 prostatectomies, and of metastatic tissues: 2 lymphadenectomies and 4 metastatic biopsies.
110 After histopathological review, tumour tissues were submitted to Myriad Genetics, Inc.
111 Tumour DNA was extracted from areas containing >30% of cancerous cells as described in
112 [17]. Germline DNA was extracted from saliva or blood using standard protocol. From 200 ng

113 of tumor DNA (or all available DNA if total yield was <200 ng) or 100 ng of germline DNA,
114 gene mutation detection and SNP whole genome analysis were performed using a custom
115 hybridization capture method described in [17], with a panel targeting approximately 27,000
116 genome-wide single nucleotide polymorphisms and the coding regions of 111 genes. Next
117 Generation Sequencing (NGS) was performed on an Illumina HiSeq2500 using a 200 cycle
118 HiSeq Rapid SBS Kit v2 and a HiSeq Rapid PE Cluster Kit v2. The method used to calculate the
119 HRD score was previously described [17]. MSI status and TMB score (number of
120 mutations/Megabase) were also determined [18].

121 Statistical analyses

122 χ^2 Fisher test was used to compare qualitative variables. Correlations between
123 quantitative variables were assessed with Pearson correlation. Comparisons of quantitative
124 data in different subpopulations were performed using the Mann-Whitney and Kruskal-
125 Wallis tests. Logistic regression was used to estimate odds ratios. Receiver Operating
126 Characteristic (ROC) curve analysis was used to define the better threshold to predict genes
127 alterations. Kaplan-Meier and Cox proportional hazards analyses were used for analyses. Of
128 early death All tests were two-sided, and $p < 0.05$ was considered to indicate statistical
129 significance. These statistical analyses were carried out with the softwares R++ (Toulouse,
130 France) and XLSTAT v2022.3.2 (Addinsoft, Paris, France).

131

132 **Results**

133 Of the samples collected from the 200 patients, twenty-seven (14%) did not provide
134 genomic results, all were biopsy samples. Absence of genomic results was significantly
135 associated with an older sample age ($p < 0.001$) (Supplementary Figure 1). The median age of
136 the samples was 44 months (IQR=73.25), with 38 months (IQR=52.0) for the samples with

137 sequencing results, compared to 145 months (IQR=15.75) for samples without result. The
138 characteristics of the 173 patients with genomic results are presented in Table 1.

139 The median age at metastatic stage was 71.0 years old (interquartile range [IQR]=14.0), with
140 no difference between patients of European and African ancestry (68.5 vs 71.0, $p=0.5$). The
141 majority of patients (80%) were metastatic at the time of diagnosis. Typology of metastases
142 was ranged using the 8th edition Tumor-Node-Metastasis classification system [19], as (M1a)
143 only in extra pelvic lymph nodes (21%), (M1b) bone metastasis (56%) and (M1c) visceral
144 metastasis (23%). 90 (52%) of patients were castration-resistant at the end of their follow-
145 up, but only 16 (9%) of the samples used for the genomic analysis were collected from tissue
146 exposed to androgen deprivation therapy. Fifteen (9%) patients had a positive family history
147 of PCa, and 22 (13%) had a personal history of cancer, of which 17 (10%) ones had another
148 cancer and five (2.9%) two other cancers (Table 1, Supplementary Table S1). The risk of
149 visceral metastasis or early death (LogRank) was not significantly different between patients
150 with other cancers and those with only PCa. The median duration of patient follow-up was
151 76 months (IQR= 14.0). Eighty-four (49%) patients died during this follow-up period, their
152 median age at death was 76.0 years (IQR=14.0). Age at death was correlated with age at
153 diagnosis (Spearman correlation; $\rho=0.94$; $p<0.001$).

154 Germline mutations were observed in 19 of 111 analysed genes (Supplementary table S2).
155 The most frequently mutated genes at the germline level were *BRCA2*, *POLN*, and *ERAP2*,
156 with five (2.9%), three (1.7%) and two (1.2%) patients carrying a mutation, respectively. Each
157 mutation was observed in only one patient. Somatic mutations were observed in 64 of the
158 111 sequenced genes (Supplementary Table S3). No association was found between the
159 mutational status and the origin of the sample (primary prostate tissue or metastasis).

160 Eighteen genes had a mutation frequency higher than 2% (Figure 1). *TP53* was the most

161 frequently mutated one, with 56 patients (32%) carrying a somatic mutation in this gene.
162 The mutation frequency of the *TP53* gene was significantly different according to ancestry
163 (43% and 16% in patients of European and African ancestry, respectively; $p=0.002$). The
164 alterations in the Wnt-pathway genes (*APC* and *CTNNB1*) and *PTEN/AKT1* counted for 9%
165 and 7% of mutations, respectively, none of these mutations was found at the germline level.
166 Mutations in the genes belonging to the Fanconi Anemia complementation group (*FANCA*,
167 *BRCA2*, *FANCE*, *FANCI*, *BRIP1*, *FANCM*, *PALB2*, *BRCA1*, and *UBE2T*) were found in 18 patients
168 (10%), including 10 (56%) which were present in germline. The mutations in the *APC* and
169 *CTNNB1* genes, on one hand, and, those in the *BRCA2*, *ATM* and *CDK12* genes, on the other
170 hand, were mutually exclusive.

171 Using univariate analysis, castrate resistant status (Odds ratio [OR]: 2.935; 95% Confidence
172 Interval [95% CI] 1.289-6.685; $p=0.010$), *TP53* (OR: 4.734; 95% CI 2.243-9.995; $p<0.001$) and
173 *APC* mutational status (OR: 9.192; 95% CI 2.254-3748; $p=0.002$) were significantly associated
174 with the risk of visceral metastasis. In univariate analysis, using the Kaplan-Meier (Log-rank),
175 visceral metastasis status ($p<0.001$), *CTNNB1* ($p=0.001$), *TP53* ($p=0.015$), *BRCA2* ($p=0.027$),
176 and FANC genes ($p=0.005$) mutational status were significantly associated with an earlier age
177 at death (Figures 2 & 3). In Cox proportional hazards, visceral metastasis (HR: 3.777; 95% CI
178 1.709-8.345; $p=0.001$) and, for molecular events, mutations in genes *CTNNB1* (HR: 10.95;
179 95% CI 3.133-38.25; $p=0.003$), and *TP53* (HR: 1.833; 95% CI 1.011-3.322; $p=0.047$) were
180 significantly related to early death.

181 Regarding the molecular signatures, median HRD score increased significantly with the
182 metastatic clinical status ($p=0.010$): from M1a (15.0; IQR=10.3), M1b (21.0; IQR=14.0) to M1c
183 (24.0; IQR=13.5) (Figure 4). The median value of the HRD score was higher in the group of
184 tumours sharing FANC genes mutations than other ones (29.0; IQR=23.0 vs 20.0; IQR=14.0;

185 p=0.011) (Figure 5). Using HRD score, the Area Under the ROC Curve (AUC) to predict *BRCA2*
186 or FANC genes mutation were 0.69 and 0.71, respectively. The threshold with the best
187 predictive performance (71% sensitivity) was HRD score >25. 33% (46/139) of the tumours
188 have a HRD score > 25 (Supplementary Table S4). HRD score >25 was significantly associated
189 with bone metastasis (OR: 5.825; 95% CI 1.654-20.52; p=0.006), but not with early death.
190 Only seven tumours (4.0%) had a TMB score ≥ 10 (range: 13.34- 229.40, Supplementary Table
191 S5), including all MSI positive ones (3.0%).

192

193 Discussion

194 Prostate cancer is a heterogeneous disease with different molecular drivers involved in
195 worse outcomes [5]. New companion diagnostic tests based on these genomic alterations or
196 their molecular signatures have been suggested to be used for targeted therapies [13-14].
197 The PROFOUND study, one of the first studies to analyse the response to PARP inhibitors
198 according to these alterations, showed some limitations in the molecular screening of
199 tumours in clinical practice [20]. In our study, the failure rate of NGS from archival samples
200 was 14%, lower than that observed in the PROFOUND one [20]. A sample age above 10 years
201 was the main cause of failure.

202 In our study, *TP53* was the most frequently mutated gene (32%), with a higher mutation
203 frequency in metastatic PCa patients of European ancestry compared to those of African
204 origin (42% versus 16%). This disparity is consistent with the results reported by Mahal et al.
205 [9]. Together, mutations in *BRCA2* or *ATM* genes accounted for 10% of cases, as did those in
206 the FANC group of genes. Alterations in Wnt-pathway genes (*APC* or *CTNNB1*) were also
207 frequently observed (9%). No difference in mutation frequency was found between patients
208 of different ancestry for genes other than *TP53*.

209 It is now widely recognized that inactivation of *TP53* is associated with genetic instability
210 with inverted rearrangements and a complex catastrophic disorganization of the genome
211 named chromotrypsis [8]. Defective *TP53* was initially described as a late event during the
212 natural history of PCa [21]. Germline *TP53* mutations were notably reported to be very rare
213 in PCa cohorts [22], which is consistent with the absence of germline *TP53* mutations that
214 we observed in our study. Recent extensive sequencing showed that tumors from patients
215 with metastatic PCa had the highest rates of *TP53* mutations, but a relatively high frequency
216 of *TP53* mutations was also found in aggressive primary prostate cancers [23]. Concordantly,
217 we previously reported a higher frequency of *TP53* alterations in tumor samples from 25
218 cases of aggressive localized PCa, compared to those from 132 patients with localized PCa
219 (52% vs. 12%) [24-25]. Moreover, 33% of tumours with biallelic alterations and 32% with a
220 single copy loss or pathogenic mutation of this gene were observed in samples from 410
221 mCRPC patients [26]. We found that *TP53* mutations in PCa tissues were associated with the
222 risk of visceral metastases and early age at death, confirming that these alterations are
223 predictive of worse metastatic and lethal outcome [26, 27].

224 On its side, *BRCA2* is known to be the gene with the highest rate of germline mutations in
225 PCa, with a frequency reaching 2-6% in metastatic PCa patients [11]. Somatic mutations in
226 this gene only occur in approximately 5-10% of metastatic PCa, of which 80% correspond to
227 biallelic inactivation, and more than 50% are linked to germline mutations [28].

228 Concordantly, we found 5% of *BRCA2* mutations in our study, of whom 56% were germline
229 ones. Our results therefore supported that *BRCA2* is the most frequently mutated gene at
230 the germline level in metastatic PCa and, that mutations in *BRCA2*, *ATM* and *CDK12* genes
231 are mutually exclusive [8]. Germline mutations in the *BRCA2* gene were reported to be
232 associated with distant metastasis at diagnosis and poor survival [28]. In our study including

233 only metastatic PCa patients, we did not find significant association between *BRCA2* or *FANC*
234 genes mutations and a specific site of metastasis. However, in univariate analyses, they were
235 both associated with an earlier age at death.

236 Molecular signatures such as HRD score [17], TMB score [14] and MSI status [18] allow
237 functional assessment of the DNA repair pathway. They already are companion theragnostic
238 markers of PARP or immune checkpoint inhibitors. From the experience of ovarian cancer,
239 HRD score >42 and TMB score ≥ 10 or MSI status are supposed to be relevant for therapeutic
240 choices [18]. However, recent reports suggested that the appropriate threshold for prostate
241 tumours will be around 20 [29]. We indeed showed that HRD score >25 has the best
242 sensitivity (Se) and specificity (Sp) values to predict *BRCA2* (Se=71%, Sp=69%) and *FANC*
243 (Se=71%, Sp=71%) genes mutations. In our study, high HRD and TMB scores were not
244 completely mutually exclusive, with one patient carrying a germline mutation in the *MSH2*
245 gene whose tumor was MSI positive, had a TMB and HRD score of 19.33 and 28,
246 respectively. Furthermore, we observed an increase in the HRD score with the typology of
247 metastases and, that an HRD score >25 was associated with the risk of bone metastases, but
248 not with early death.

249 Although mutations in Wnt-pathway genes (*APC* and *CTNNB1*) are generally rare in primary
250 PCa (<10%), they are relatively more common in metastatic PCa [30]. Additionally, *APC*
251 hypermethylation, observed in at least 30% of PCa cases, was associated with worse
252 outcomes in CRPC patients [31]. Dysregulation of the Wnt-pathway was shown to promote
253 metastatic dissemination and treatment resistance, and notably, somatic Wnt-activating
254 mutations were associated with first-line resistance to abiraterone/enzatumamide [31]. Our
255 study confirmed, in one hand, that *APC* mutations were associated with the risk of visceral
256 metastases, and on the other hand, that *CTNNB1* mutations are associated, after

257 multivariable adjustments for concurrent alterations in other mutated genes, with an earlier
258 death.

259 Our study has certain limitations, it is a retrospective study with real data, based on a
260 selection of 111 genes which did not include some genes important for PCa, such as *AR* [9,
261 12, 21], *RB1* [9] or *ZNRF3* of the Wnt-pathway [32], and due to the number of tumors
262 analysed, certain molecular events are rare. However, the results are consistent with
263 previous reports and reinforce the role of genes involved in the Wnt-pathway in determining
264 worse outcomes.

265

266 **Conclusions**

267 Recent developments of targeted therapies for advanced PCa have focused attention on
268 genes involved in homologous recombination deficiency and microsatellite instability.

269 However, our results confirmed that mutations in the *TP53* gene and genes (*APC/CTNNB1*)
270 related to the Wnt-pathway are associated with unfavourable outcome with metastatic
271 visceral dissemination and early death. These genomic alterations could be considered as
272 markers to identify PCa patients at high risk of life-threatening disease who might benefit
273 from more intensified treatment or new targeted therapies.

274 **References**

- 275 [1] Hinata N, Fujisawa M. Racial Differences in Prostate Cancer Characteristics and Cancer-
276 Specific Mortality: An Overview. *World J Mens Health* 2022;40:217-27.
- 277 [2] Desai MM, Cacciamani GE, Gill K, et al. Trends in Incidence of Metastatic Prostate Cancer
278 in the US. *JAMA Netw Open* 2022;5:e222246.
- 279 [3] Nafissi NN, Kosiorek HE, Butterfield RJ, et al. Evolving Natural History of Metastatic
280 Prostate Cancer. *Cureus* 2020;12:e11484.
- 281 [4] Drake CG. Visceral metastases and prostate cancer treatment: 'die hard,' 'tough
282 neighborhoods,' or 'evil humors'? *Oncology (Williston Park)* 2014;28:974-80.
- 283 [5] Halabi S, Kelly WK, Ma H, et al. Meta-Analysis Evaluating the Impact of Site of Metastasis
284 on Overall Survival in Men With Castration-Resistant Prostate Cancer. *J Clin Oncol*
285 2016;34:1652-9.
- 286 [6] Woodcock DJ, Riabchenko E, Taavitsainen S, et al. Prostate cancer evolution from
287 multilineage primary to single lineage metastases with implications for liquid biopsy. *Nat*
288 *Commun* 2020;11:5070.
- 289 [7] Plym A, Dióssy M, Szallasi Z, Sartor O, et al. DNA Repair Pathways and Their Association
290 With Lethal Prostate Cancer in African American and European American Men. *JNCI*
291 *Cancer Spectr* 2021;6:pkab097.
- 292 [8] Quigley DA, Dang HX, Zhao SG, et al. Genomic Hallmarks and Structural Variation in
293 Metastatic Prostate Cancer *Cell* 2018;175:889.
- 294 [9] Mahal BA, Alshalalfa M, Kensler KH, et al. Racial Differences in Genomic Profiling of
295 Prostate Cancer. *N Engl J Med* 2020;383:1083-5. PMID: 32905685.
- 296 [10] Mateo J, Seed G, Bertan C, Rescigno P, et al. Genomics of lethal prostate cancer at
297 diagnosis and castration resistance. *J Clin Invest* 2020;130:1743-51.

- 298 [11] Abdi B, Basset N, Perrot E, et al. DNA damage repair gene germline profiling for
299 metastatic prostate cancer patients of different ancestries. *Prostate* 2022;82:1196-201.
- 300 [12] Abida W, Armenia J, Gopalan A, et al. Prospective Genomic Profiling of Prostate
301 Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect
302 Clinical Decision Making. *JCO Precis Oncol* 2017;2017:PO.17.00029.
- 303 [13] Castro E, Mateo J, Olmos D, de Bono JS. Targeting DNA Repair: The Role of PARP
304 Inhibition in the Treatment of Castration-Resistant Prostate Cancer. *Cancer J*
305 2016;22:353-6.
- 306 [14] Graf RP, Fisher V, Weberpals J, Gjoerup O, et al. Comparative Effectiveness of
307 Immune Checkpoint Inhibitors vs Chemotherapy by Tumor Mutational Burden in
308 Metastatic Castration-Resistant Prostate Cancer. *JAMA Netw Open* 2022;5:e225394.
- 309 [15] Crabb SJ, Griffiths G, Dunkley D, et al. Overall Survival Update for Patients with
310 Metastatic Castration-resistant Prostate Cancer Treated with Capivasertib and Docetaxel
311 in the Phase 2 ProCAID Clinical Trial. *Eur Urol* 2022;82:512-5.
- 312 [16] Ji Y, Huang PH, Woolfenden S, Myers A. Model-based dose selection to inform
313 translational clinical oncology development of WNT974, a first-in-class Porcupine
314 inhibitor. *Clin Transl Sci* 2022;15:1713-22.
- 315 [17] Patel JN, Braicu I, Timms KM, et al. Characterisation of homologous recombination
316 deficiency in paired primary and recurrent high-grade serous ovarian cancer. *Br J Cancer*
317 2018;119:1060-6.
- 318 [18] Budczies J, Kluck K, Beck S, et al. Homologous recombination deficiency is inversely
319 correlated with microsatellite instability and identifies immunologically cold tumors in
320 most cancer types. *J Pathol Clin Res* 2022;8:371-82.

- 321 [19] Paner GP, Stadler WM, Hansel DE, Montironi R, Lin DW, Amin MB. Updates in the
322 Eighth Edition of the Tumor-Node-Metastasis Staging Classification for Urologic Cancers.
323 Eur Urol 2018;73:560-569.
- 324 [20] Hussain M, Corcoran C, Sibilla C, et al. Tumor Genomic Testing for >4,000 Men with
325 Metastatic Castration-resistant Prostate Cancer in the Phase III Trial PROfound
326 (Olaparib). Clin Cancer Res 2022;28:1518-30.
- 327 [21] Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC. p53 is mutated in a
328 subset of advanced-stage prostate cancers. Cancer Res 1993;53:3369-73.
- 329 [22] Maxwell KN, Cheng HH, Powers J, et al. Inherited TP53 Variants and Risk of Prostate
330 Cancer. Eur Urol 2022;81:243-50.
- 331 [23] Nientiedt C, Budczies J, Endris V, et al. Mutations in TP53 or DNA damage repair
332 genes define poor prognostic subgroups in primary prostate cancer. Urol Oncol
333 2022;40:8.e11-8.
- 334 [24] Kamoun A, Cancel-Tassin G, Fromont G, et al. Comprehensive molecular classification
335 of localized prostate adenocarcinoma reveals a tumour subtype predictive of non-
336 aggressive disease. Ann Oncol 2018;29:1814-21.
- 337 [25] Tonon L, Fromont G, Boyault S, et al. Mutational Profile of Aggressive, Localised
338 Prostate Cancer from African Caribbean Men Versus European Ancestry Men. Eur Urol
339 2019;75:11-5.
- 340 [26] Nyquist MD, Corella A, Coleman I, et al. Combined TP53 and RB1 Loss Promotes
341 Prostate Cancer Resistance to a Spectrum of Therapeutics and Confers Vulnerability to
342 Replication Stress. Cell Rep 2020;31:107669.
- 343 [27] Alshalalfa M, Goglia AG, Swami N, Nguyen B, Hougen HY, Khan A, Kishan AU, Punnen
344 S, Nguyen PL, Mahal BA, Dee EC. Determinants of widespread metastases and of

- 345 metastatic tropism in patients with prostate cancer: A genomic analysis of primary and
346 metastatic tumors. *Urol Oncol.* 2023;41:253.e21-6.
- 347 [28] De Sarkar N, Dasgupta S, Chatterjee P, et al. Genomic attributes of homology-
348 directed DNA repair deficiency in metastatic prostate cancer. *JCI Insight* 2021;6:e152789.
- 349 [29] Leibowitz BD, Dougherty BV, Bell JSK, Kapilivsky J, Michuda J, Sedgewick AJ, Munson
350 WA, Chandra TA, Dry JR, Beaubier N, Igartua C, Taxter T. Validation of genomic and
351 transcriptomic models of homologous recombination deficiency in a real-world pan-
352 cancer cohort. *BMC Cancer.* 2022;22:587.
- 353 [30] Isaacsson Velho P, Fu W, et al. Wnt-pathway Activating Mutations Are Associated
354 with Resistance to First-line Abiraterone and Enzalutamide in Castration-resistant
355 Prostate Cancer. *Eur Urol* 2020;77:14-21.
- 356 [31] Hendriks RJ, Dijkstra S, Smit FP, et al. Epigenetic markers in circulating cell-free DNA
357 as prognostic markers for survival of castration-resistant prostate cancer patients.
358 *Prostate* 2018;78:336-42.
- 359 [32] Fraser M, Livingstone J, Wrana JL, Finelli A, et al. Somatic driver mutation prevalence
360 in 1844 prostate cancers identifies ZNRF3 loss as a predictor of metastatic relapse. *Nat*
361 *Commun* 2021;12:6248.

362 **Declaration of Interest statement:** Kirstens M Timms and Cara Solimeno are employed in Myriad
363 Genetics, Inc. Pr Olivier Cussenot reports receiving speaker honoraria from Myriad Genetics, Inc. The
364 other authors have no conflicts of interest to declare that are relevant to the content of this
365 article.

366 **Data Access and Responsibility:** Olivier Cussenot had full access to all the data in the study
367 and takes responsibility for the integrity of the data and the accuracy of the data analysis.

368 **Data Sharing Policy:** Data are available for bona fide researchers who request it from the
369 authors

370 **Funding/Support and Role of the Sponsor:** This work was supported by grant from the
371 French National Cancer Institute (INCa) [grant number: 2017-016] and from the Fonds de
372 Dotation pour l'Innovation dans la prise en charge du Cancer de Prostate (FDCP). The study
373 funders had no role in the study design, data collection, analysis and interpretation, or
374 writing of the report, and in the decision to submit the article for publication.

375 **Acknowledgements:** We thank the patients for their participation in this study, and Cecile
376 Gaffory and Valerie Ondet for their technical assistance.

377 **Table 1: Characteristics of the patients**

Age at diagnosis (years)	
Mean \pm SD	70.1 \pm 10.3
Median (IQR)	71.0 (14.8)
Age at metastatic stage (years)	
Mean \pm SD	70.9 \pm 10.0
Median (IQR)	71.0 (14.0)
PSA level at diagnosis (ng/ml)	
Mean \pm SD	271.1 \pm 609.0
Median (IQR)	67.5 (229.3)
PSA level at metastatic stage (ng/ml)	
Mean \pm SD	262.5 \pm 605.7
Median (IQR)	57.4 (229.2)
ISUP at diagnosis	
1	7 (4.0%)
2	11 (6.3%)
3	24 (13.9%)
4	22 (12.7%)
5	106 (61.3%)
Unknown	3 (1.7%)
Castrate resistant prostate cancers	
No	83 (48.0%)
Yes	90 (52.0%)

Typology Metastasis	
Extra pelvic lymph node (M1a)	37 (21.4%)
Bone (M1b)	96 (55.5%)
Visceral (M1c)	40 (23.1%)
Ancestry	
European	80 (46.2%)
African	49 (28.3%)
Other	44 (25.4%)
Familial History Cancer	
No	158 (91.3%)
Yes	15 (8.7%)
Personal History of Other Cancer	
0	151 (87.3%)
1	17 (9.8%)
2	5 (2.9%)
Death	
No	89 (51.4%)
Yes	84 (48.6%)
Follow-up duration from diagnosis to death or last news (months)	54.7 ± 47.8
Mean ± SD	37.0 (56.0)
Median (IQR)	

378 SD: standard deviation; IQR: Interquartile range

379

381

382 **Figure legend:**

383 Figure 1: Distribution of the genes with a mutation frequency higher than 2%

384

385 Figure 2: Age at death or last news (years) according to the metastatic clinical status. M1a

386 (blue): lymph node metastasis; M1b (green): bone metastasis, M1c (red): visceral metastasis.

387

388 Figure 3: Age at death or last news (years) according to the mutational status (0 not

389 mutated; 1 mutated).

390

391 Figure 4: Correlation between HRD score and the metastatic clinical status. M1a: lymph node

392 metastasis; M1b: bone metastasis, M1c: visceral metastasis.

393

394 Figure 5: Correlation between HRD score and FANC genes mutational status

395

396 Supplementary figure 1. Distribution of the age of samples according to organ sources

397

398 Supplementary figure 2. Distribution of the age of samples according to the result of DNA

399 sequencing

400

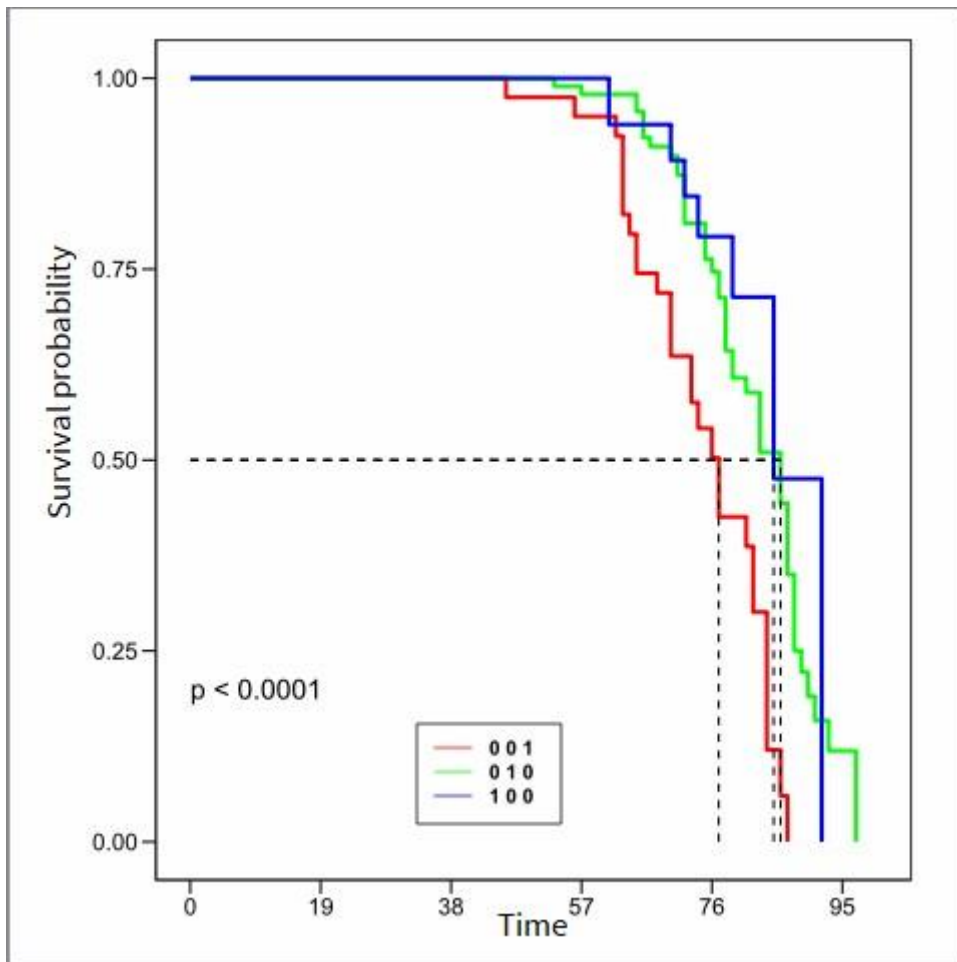
401 Figure 1



402

403

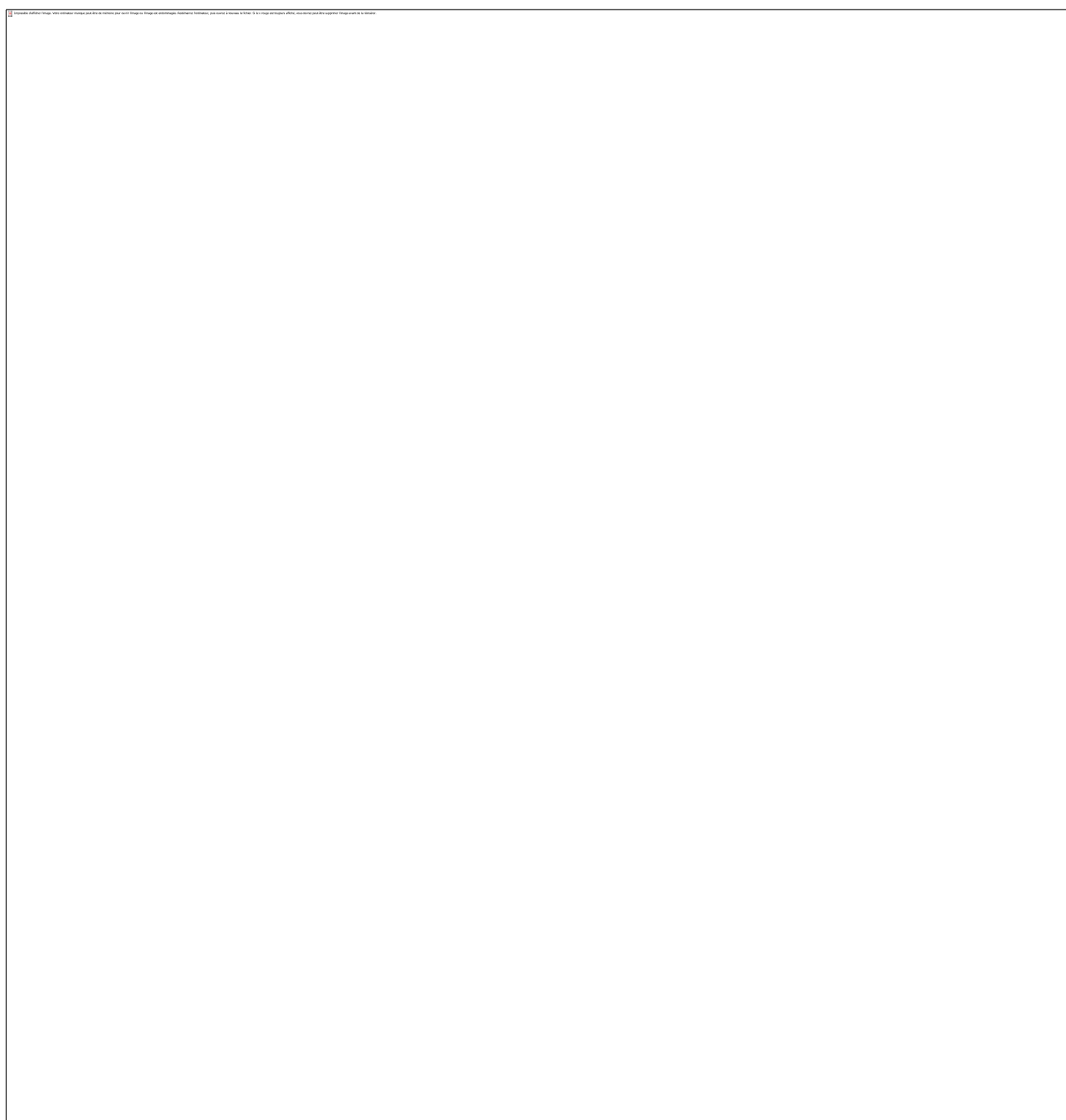
404 Figure 2



405

406

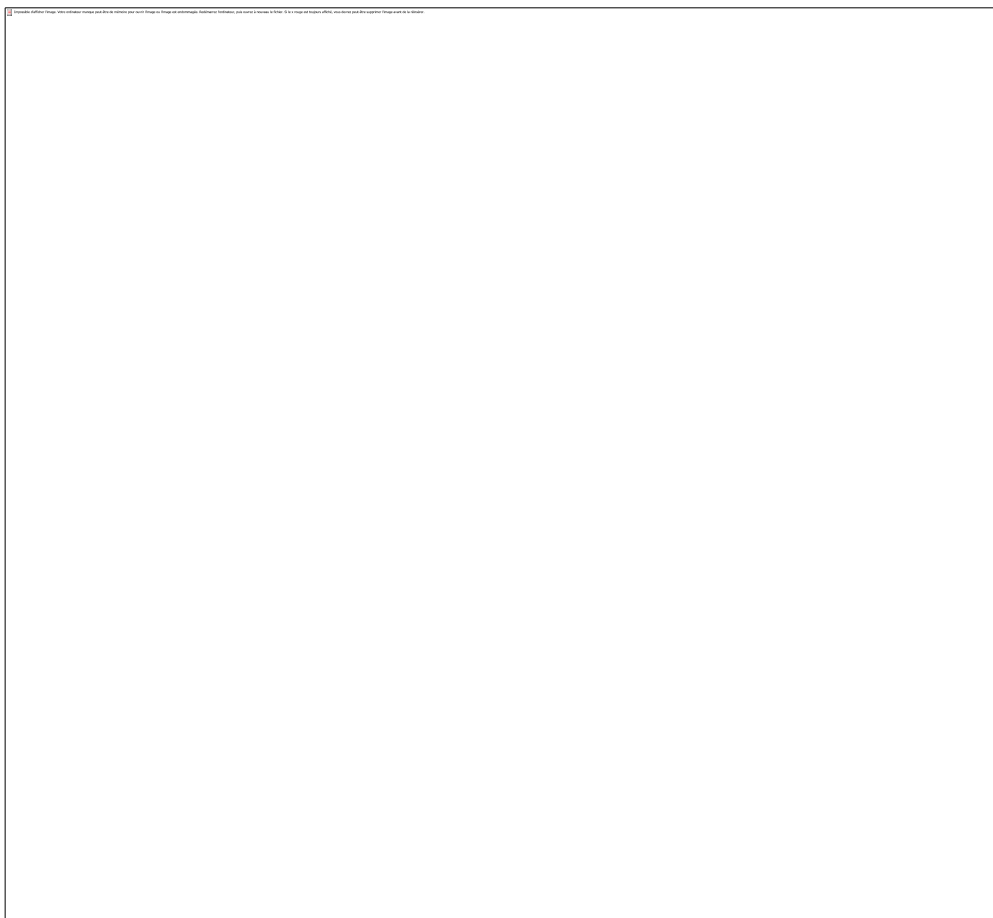
407 Figure 3



408

409

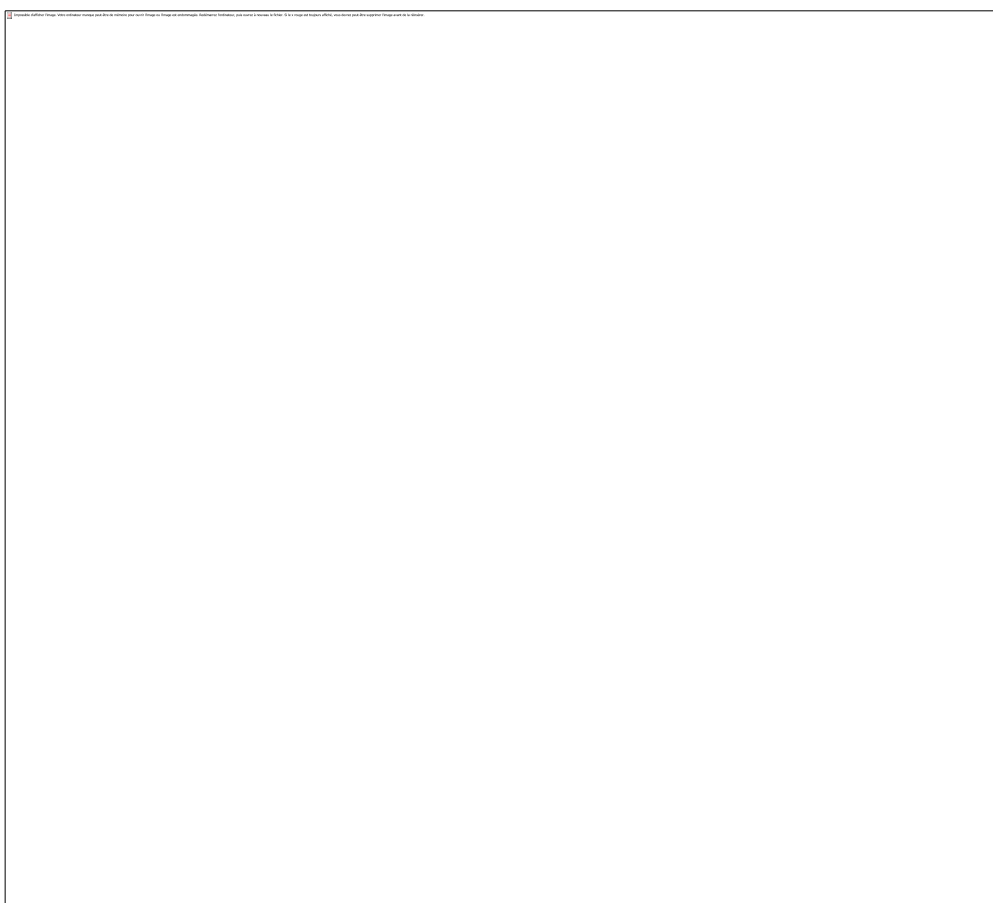
410 Figure 4



411

412

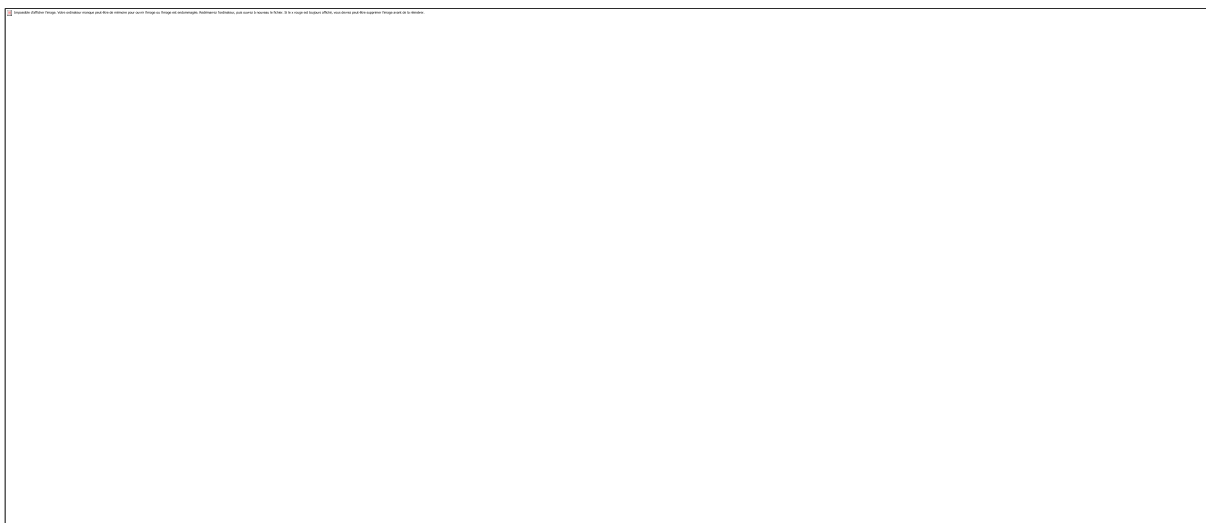
413 Figure 5



414

415

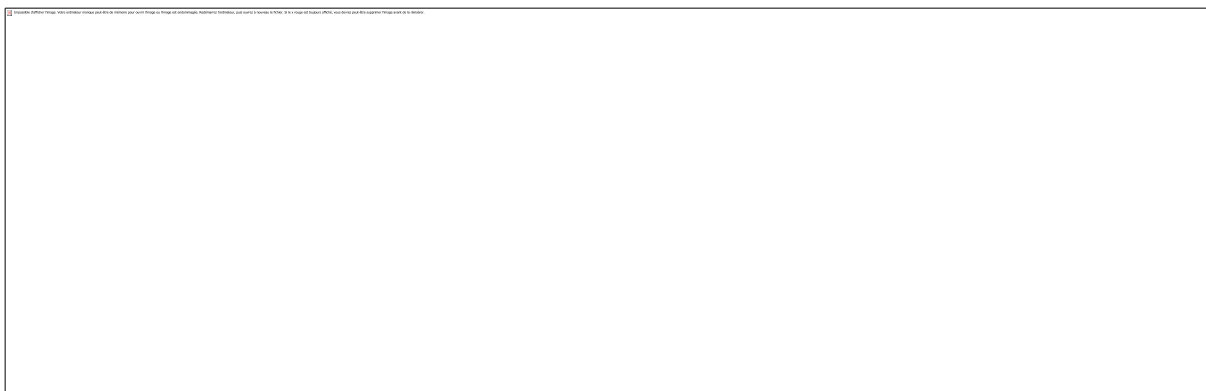
416 Supplementary figure 1.



417

418

419 Supplementary figure 2.



420

421

422 Supplementary Table S1: Patients with other cancers

423

First cancer	Second cancer	Third cancer	Germline mutation	Ancestry
Breast	Prostate	Lung	<i>FANCE c.1096del (p.Ser366Alafs*10)</i>	European
Breast	Prostate			Other
Colon	Prostate	Thyroid	<i>MSH2 c.1165C>T (p.Arg389*)</i>	European
Colon	Prostate			European
Colon	Prostate			Other
Colon	Prostate			Other
Kidney	Prostate			European
Kidney	Prostate			European
Leukemia	Prostate			Other
Lung	Prostate		<i>POLG c.2924del (p.Gln975Argfs*31)</i>	European
Melanoma	Prostate	Bladder		European
Melanoma	Prostate			African
Oesophagus	Prostate			European
Prostate	Bladder			European
Prostate	Colon		<i>POLN c.1609C>T (p.Gln537*)</i>	African
Prostate	Colon			Other
Prostate	Colon		<i>BARD1 c.176_177del (p.Glu59Alafs*8)</i>	European
Prostate	Colon			Other
Prostate	Lung	Colon		European
Prostate	Lung		<i>POLN c.2586G>A (p.Trp862*)</i>	African
Prostate	Pancreas			African
Upper Urinary Tract	Bladder	Prostate	<i>POLG c.1586-2dupA</i>	African

424

425 Supplementary Table S2: List of germline mutations identified in the 173 patients with
426 metastatic prostate cancer.

427

Gene	HGVS_Name
BARD1	c.176_177del (p.Glu59Alafs*8)
BRCA1	c.815_824dup (p.Thr276Alafs*14)
BRCA2	c.2283T>G (p.Tyr761*)
BRCA2	c.1626dupA (p.His543Thrfs*17)
BRCA2	c.4022del (p.Ser1341*)
BRCA2	c.3075_3076delinsTT (p.Lys1026*)
BRCA2	c.6486_6489del (p.Lys2162Asnfs*5)
BRIP1	del exon 15
CHEK2	c.349A>G (p.Arg117Gly)
ERAP1	c.1320+4A>G
ERAP2	c.788_789del (p.Leu263Argfs*7)
ERAP2	c.371C>A (p.Ser124*)
FANCA	c.1115_1118del (p.Val372Alafs*42)

FANCE	c.1096del (p.Ser366Alafs*10)
FANCI	c.1993-17C>G
MSH2	c.1165C>T (p.Arg389*)
NBN	c.1903A>T (p.Lys635*)
PDCD1LG2	c.661del (p.Trp221Glyfs*17)
POLE	c.5811+5G>A
POLG	c.2924del (p.Gln975Argfs*31)
CHEK2	c.444+1G>A
POLN	c.1609C>T (p.Gln537*)
POLN	c.2586G>A (p.Trp862*)
POLQ	c.3200del (p.Leu1067Tyrfs*18)
RAD54B	c.1678C>T (p.Arg560*)
XRCC3	c.194-14T>G

428

429 Supplementary Table S3: Correlation between germline and somatic mutations

430

Germline mutated gene in a patient	Somatic mutated genes in the same patient
BARD1	AKT1
BRCA1	
BRCA2	KRAS
BRCA2	TP53 + CTNNB1
BRCA2	TP53
BRCA2	APC
BRCA2	
BRIP1	TP53
CHEK2	
ERAP1	
ERAP2	
ERAP2 + POLN	
FANCA	AKT1
FANCE	KRAS
FANCI	
MSH2	APC* + ATM + BLM + MSH3 + CHEK1 + FANCI + POLQ + ERBB2 + MLH3 + MSH6 + RAD50
NBN	
PDCD1LG2	
POLE	CDK12*
POLG	
POLG	
POLN	
POLN	ERAP2 + TP53
POLQ	TP53
RAD54B	
XRCC3	
	NBN

	TP53
	TP53
	PTEN
	MSH3 + TP53
	MSH2 + MSH3 + PMS2 + POLE + PTEN
	APC + TP53
	ATM
	TP53
	TP53
	TP53
	TP53
	PPP2R2A + CTNNB1 + TP53
	ATM + ATR + CTNNB1 + MSH3 + PRKDC + MSH6 + POLD1 + TP53BP1 + UBE2T + MLH1 + PIK3CA + TP53
	TP53
	TP53
	B2M
	MSH2 + RAD50 + PRKDC + MYH + NLRC5 + RAD52+ EGFR
	TP53 + CTNNB1
	PTEN
	PTEN
	CDK12*
	ATM* + CHEK2
	KRAS
	CTNNB1
	TP53
	CDK12* + ALK
	TP53
	POLQ + TP53
	PIK3CA
	PPP2R2A + PALB2*
	ATM + BRAF + CDH1
	PTEN + TP53
	APC + RAD54L
	TP53
	KRAS
	TP53
	UIMC1
	POLN + AKT1
	ATM + PRKDC + POLE + MSH2 + MSH3 + TP53BP1 + TAP2
	TP53
	PMS2 + TP53
	TP53

	TP53
	MAP2K1
	TP53
	APC + TP53
	TP53
	CDK12*
	ATR + BRCA2 + RAD50 + PRKDC*+CDH1*
	APC + AKT1
	BRCA2
	APC + TP53
	TP53 + CDH1
	TP53
	TP53
	CHEK1 + MSH2 + MSH3 + POLE + POLD1 + PRKDC + RAD50 + TAP2 + TP53 + EPCAM + ERCC5 + FANCM + FOXL2
	TP53
	TP53BP1
	PTEN + TP53* + BMPR1A
	TP53
	TP53
	TP53
	POLE
	ATR
	BRCA2
	ATR + ATM
	APC
	PIK3CA + TP53
	BRCA2
	TP53
	CHEK2
	CTNNB1
	TP53 + POLQ
	ATM
	PRKDC
	CDK12
	TP53
	CDK12* + BRAF
	CDK12 + APC + POLH
	TP53
	POLQ + TP53
	BLM + BAP1 + PTEN + TP53
	PDCD1LG2 + TP53
	TP53
	TP53
	BMPR1A + TP53

	PTEN + APC* + CHEK2 + TP53
	ATM
	TP53
	CDK12
	TP53
	TP53

431 *Biallelic alterations

432

433 Supplementary Table S4: Genomic characteristics of the tumours with HRD score > 25

434

Germline mutated genes	Somatic mutated genes	HRD Score	MSI Status	TMB
	TP53	26	Negative	1.39
	BRCA2	26	Negative	1.63
	TP53	27	Negative	3.29
	PTEN	27	Negative	0.92
		27	Negative	2.2
BRCA2	TP53 + CTNNB1	27	ND	4.94
	TP53	27	Negative	0.83
	BMP1A + TP53	27	Negative	0.64
	PTEN + APC* + CHEK2 + TP53	27	Negative	0
		27	Negative	1.92
MSH2	APC* + ATM + BLM + MSH3 + CHEK1 + FANCI + POLQ + ERBB2 + MLH3 + MSH6 + RAD50	28	Positive	19.32
	TP53	28	Negative	0.68
		28	Negative	4.97
	CDK12* + BRAF	28	Negative	2.26
	ATM	28	Negative	1.27
		29	Negative	ND
	TP53	30	Negative	1.27
POLN	ERAP2 + TP53	30	Negative	3.31
FANCI		30	Negative	0
	TP53	30	Negative	2.55
	PTEN	30	Negative	2.02
	TP53	31	Negative	0
	TP53	31	Negative	1.29
ERAP2		31	Negative	1.35
	ATM	33	Negative	3.24
	ATR + ATM	33	Negative	1.90
		33	Negative	1.88
	TP53	33	Negative	4.48
	TP53	33	Negative	1.28
		34	Negative	2.20

	APC + TP53	34	Negative	0.69
FANCE	KRAS	36	Negative	2.50
	TP53	36	Negative	0
	TP53BP1	37	Negative	4.73
	PPP2R2A + PALB2*	38	Negative	1.55
	CDK12	38	Negative	3.78
		38	Negative	4.46
	TP53	40	Negative	1.35
	TP53 + CDH1	42	ND	ND
BRIP1	TP53	42	Negative	0.66
	B2M	42	Negative	5.37
BRCA2	APC	45	Negative	3.18
	BRCA2	50	Negative	2.53
POLQ	TP53	50	Negative	1.58
		51	Negative	3.89
BRCA2	TP53	67	Negative	3.54

435 *Biallelic alterations

436 HRD: Homologous Recombination Deficiency; MSI: Microsatellite Instability; TMB: Tumor
437 Mutational Burden

438

439

440 Supplementary Table S5: Genomic characteristics of the tumours with TMB score > 10

441

Germline mutated genes	Somatic mutated genes	MSI status	TMB score	HRD score
	MSH2 + MSH3 + PMS2 + POLE + PTEN	Positive	13.34	6
	MSH2+RAD50+PRKDC+MYH+NLRC5+ RAD52+EGFR	Negative	13.61	4
	ATM + ATR + CTNNB1 + MSH3 + PRKDC + MSH6 + POLD1 + TP53BP1 + UBE2T + MLH1 + PIK3CA + TP53	Negative	14.48	10
	ATR + BRCA2 + RAD50 + PRKDC* + CDH1*	Positive	14.93	15
MSH2	APC * + ATM + BLM + MSH3 + CHEK1 + FANCI + POLQ + ERBB2 + MLH3 + MSH6 + RAD50	Positive	19.32	28
	ATM + PRKDC + POLE + MSH2 + MSH3 + TP53BP1 + TAP2	Positive	19.42	1
	CHEK1 + MSH2 + MSH3 + POLE + POLD1 + PRKDC + RAD50 + TAP2 + TP53 + EPCAM + ERCC5 + FANCM + FOXL2	Positive	229.40	2

442 *Biallelic alterations

443 HRD: Homologous Recombination Deficiency; MSI: Microsatellite Instability; TMB: Tumor
444 Mutational Burden

445

