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, Gaelle Fromont, Eva Comperat, Geraldine Cancel-Tassin

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2 **prostate cancers**

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4 Olivier Cussenot[1,2], Kirsten M. Timms[3], Emmanuel Perrot[4,5], Pascal Blanchet[4,5], Laurent

5 Brureau[4,5], Cara Solimeno[3], Gaelle Fromont[1,6,7], Eva Comperat[1,8], Geraldine Cancel-Tassin[1,9*]

6

7 [1]CeRePP, Paris, France

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

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

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

11 [5]Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S

12 1085, Pointe-à-Pitre, Guadeloupe

13 [6]Inserm UMR1069 "Nutrition, Croissance et Cancer" Université François Rabelais, Faculté de

14 Médecine, Tours, France

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

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

17 [9]GRC 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

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27

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Abstract

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

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

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

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

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

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   Khi^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/enzatulamide [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.

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

Age at diagnosis (years)	
Mean ± SD	70.1 ± 10.3
Median (IQR)	71.0 (14.8)
Age at metastatic stage (years)	
Mean ± SD	70.9 ± 10.0
Median (IQR)	71.0 (14.0)
PSA level at diagnosis (ng/ml)	
Mean ± SD	271.1 ± 609.0
Median (IQR)	67.5 (229.3)
PSA level at metastatic stage (ng/ml)	
Mean ± SD	262.5 ± 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) Mean ± SD Median (IQR)	54.7 ± 47.8 37.0 (56.0)

378   SD: standard deviation; IQR: Interquartile range

379

380

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 mutationnal 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

Impossible d'afficher l'image. Votre ordinateur manque peut-être de mémoire pour ouvrir l'image ou l'image est endommagée. Redémarrez l'ordinateur, puis ouvrez à nouveau le fichier. Si le x rouge est toujours affiché, vous devrez peut-être supprimer l'image avant de la réinsérer.
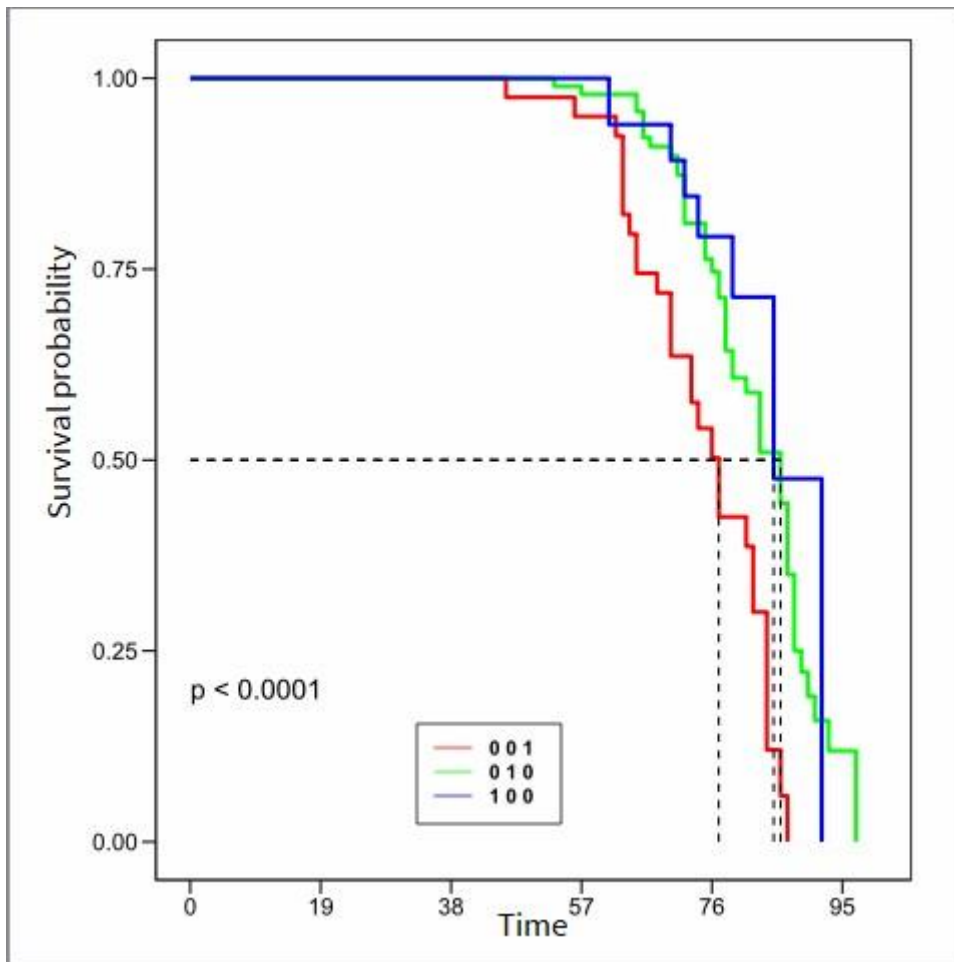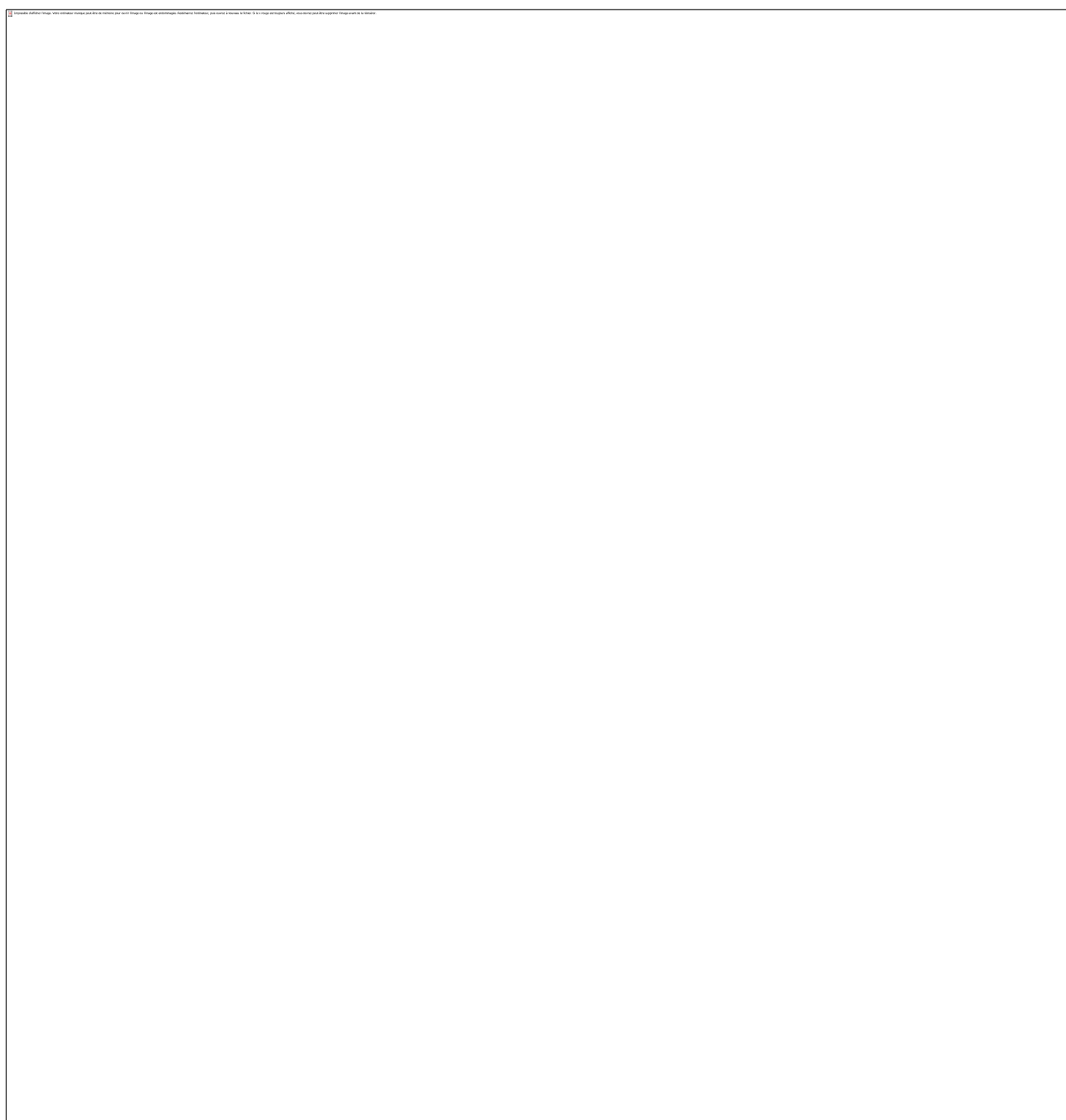
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410   Figure 4
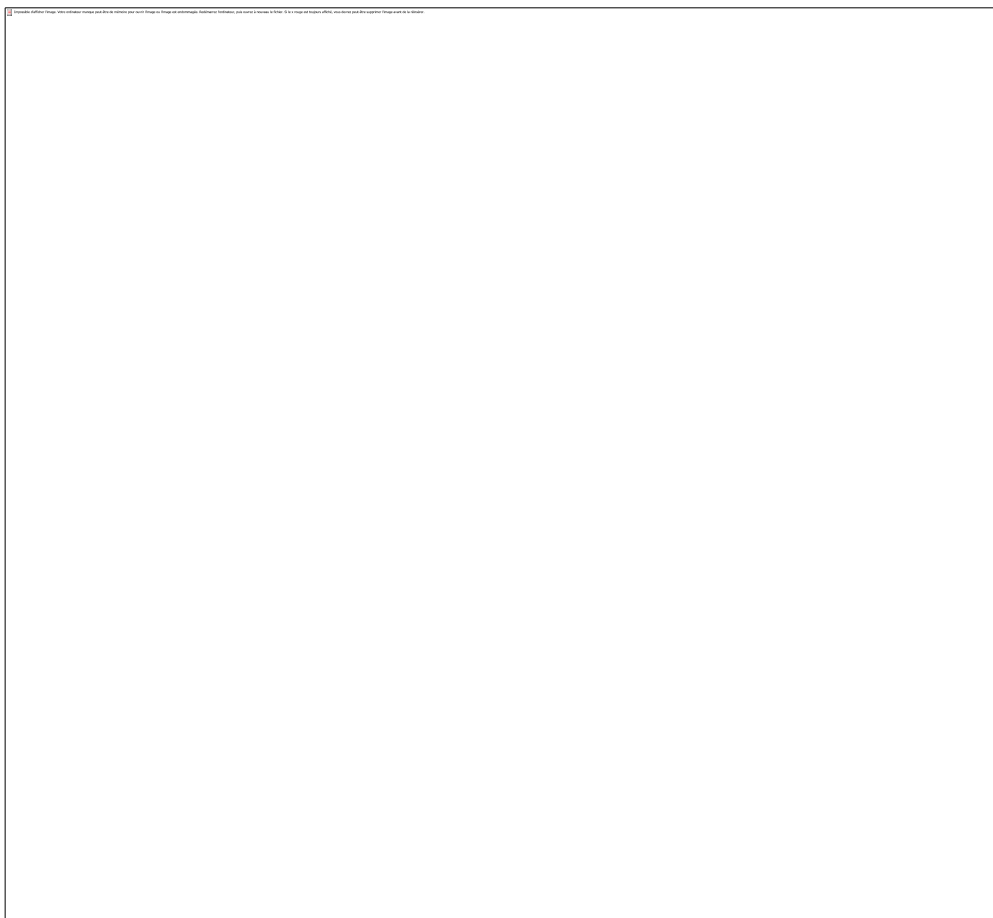
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413   Figure 5

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416   Supplementary figure 1.

417

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419   Supplementary figure 2.

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421

422   Supplementary Table S1: Patients with other cancers

423

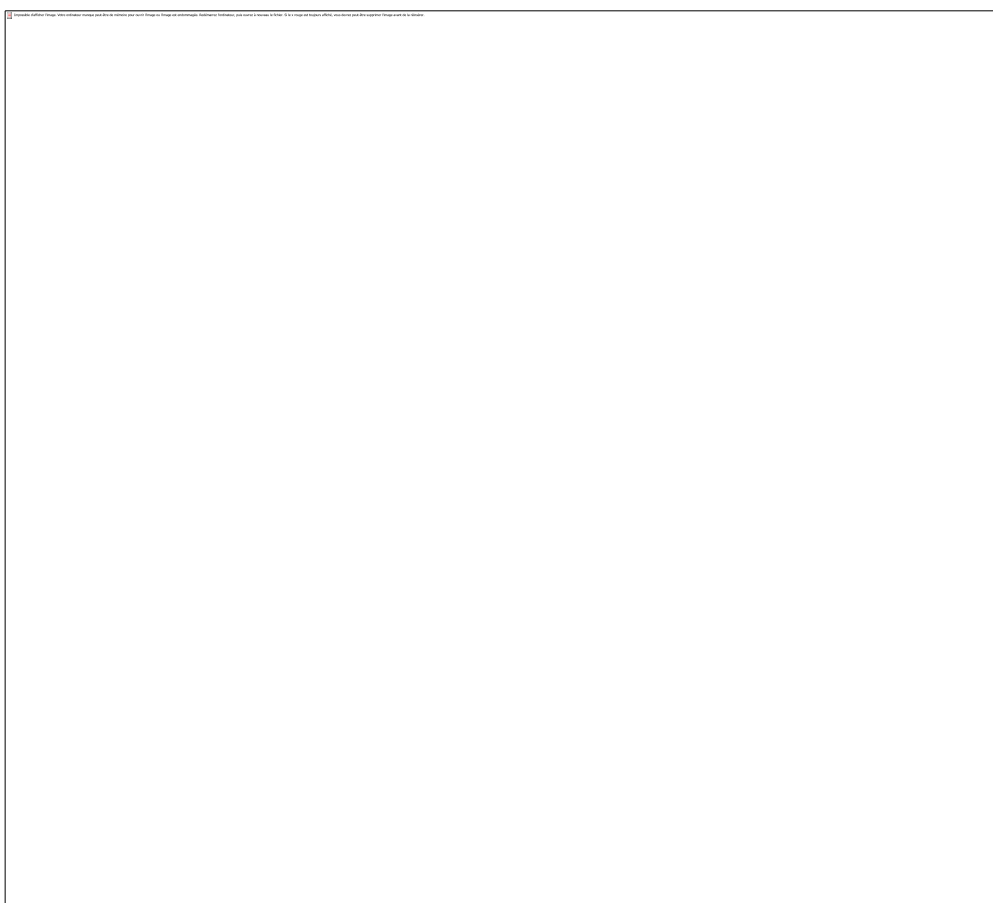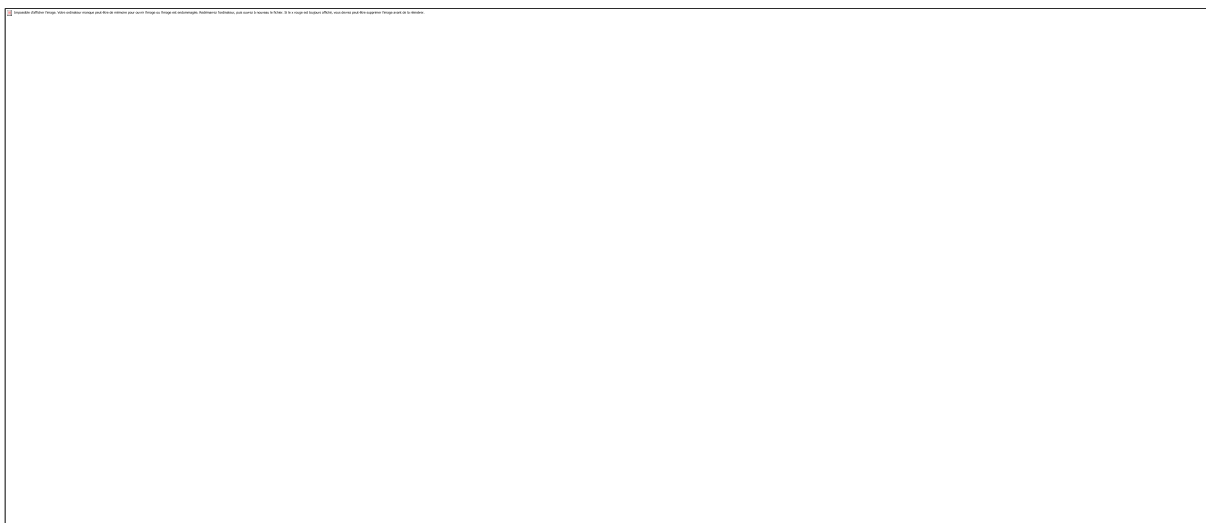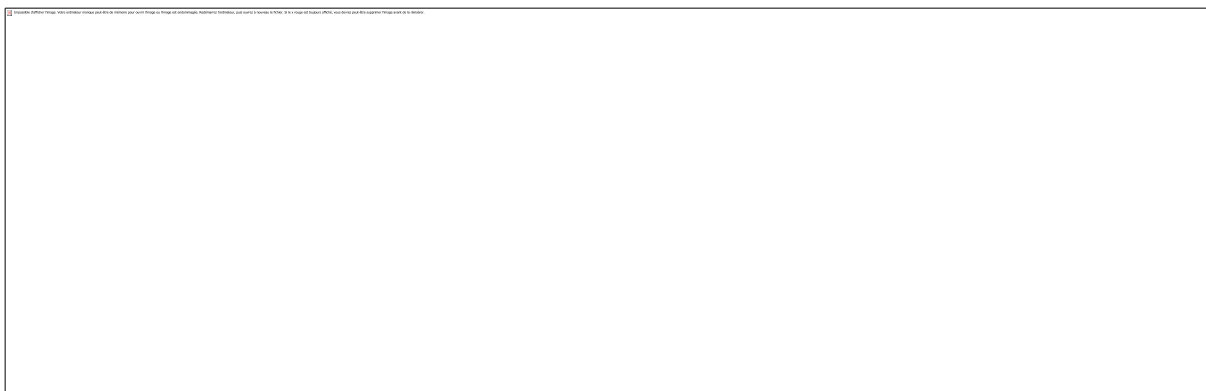| First cancer	Second cancer	Third cancer	Germline mutation	Ancestry
Breast	Prostate	Lung	*FANCE c.1096del (p.Ser366Alafs*10)*	European
Breast	Prostate			Other
Colon	Prostate	Thyroid	*MSH2 c.1165C>T (p.Arg389*)*	European
Colon	Prostate			European
Colon	Prostate			Other
Colon	Prostate			Other
Kidney	Prostate			European
Kidney	Prostate			European
Leukemia	Prostate			Other
Lung	Prostate		*POLG c.2924del (p.Gln975Argfs*31)*	European
Melanoma	Prostate	Bladder		European
Melanoma	Prostate			African
Oesophagus	Prostate			European
Prostate	Bladder			European
Prostate	Colon		*POLN c.1609C>T (p.Gln537*)*	African
Prostate	Colon			Other
Prostate	Colon		*BARD1 c.176_177del (p.Glu59Alafs*8)*	European
Prostate	Colon			Other
Prostate	Lung	Colon		European
Prostate	Lung		*POLN c.2586G>A (p.Trp862*)*	African
Prostate	Pancreas			African
Upper Urinary Tract	Bladder	Prostate	*POLG c.1586-2dupA*	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
	BMPR1A + 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

*Biallelic alterations

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

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

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

*Biallelic alterations

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

446