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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.

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

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

Introduction

Prostate cancer (PCa) is the fifth leading cause of cancer death in men worldwide, with an estimated 1.4 million cases diagnosed and an estimated 375,000 PCa deaths in 2020 [1]. However, important disparities in its incidence and mortality are seen among different populations around the world [1]. Due to the lack of recommendation for PCa screening, and the increased sensitivity of imaging that identifies metastases in a higher number of men affected with PCa, leading to stage migration phenomenon, the incidence of PCa diagnosed at a metastatic stage has increased for the 20 last years [2]. If the most common site of PCa metastasis is the bone (>80%), the visceral metastases, which used to be infrequently observed, have become more common. Their most frequent locations are in the lung and liver, each representing approximately 40% of visceral metastases. Their incidence in men who died from metastatic PCa significantly increased from 26% in 2009 to 40% in 2016 [3]. Thanks to multiple systemic therapies approved since 2010 with proven survival benefits, men with metastatic PCa are living longer than ever [4], but thus may later develop visceral metastases. The increase in the number of lines of therapy may also be behind the increase in visceral disease. After progression under androgen deprivation therapy, visceral metastases confer worse median survival duration [5].

The lethality of PCa is related to the evolution of metastatic castrate-resistant clones [6]. The landscape of genomic alterations reported in metastatic PCa was established from analyses of germline [7] and tumour [6, 8-10] samples. The most frequent germline mutations carried by patients with metastatic PCa are in the *BRCA2* and *ATM* genes, with a prevalence estimated across different reports to be approximately 5% and 2%, respectively [11]. In tumours from patients with metastatic castration-resistant PCa (mCRPC), the most common genomic alterations are observed in the *AR* and *TP53* genes, with frequencies above 40% [12]. The characterization of

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

Patients and Methods

Patients

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

Genomic analyses

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

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

Statistical analyses

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

Results

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

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

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

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

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;

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

Discussion

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

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

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

On its side, *BRCA2* is known to be the gene with the highest rate of germline mutations in PCa, with a frequency reaching 2-6% in metastatic PCa patients [11]. Somatic mutations in this gene only occur in approximately 5-10% of metastatic PCa, of which 80% correspond to biallelic inactivation, and more than 50% are linked to germline mutations [28]. Concordantly, we found 5% of *BRCA2* mutations in our study, of whom 56% were germline ones. Our results therefore supported that *BRCA2* is the most frequently mutated gene at the germline level in metastatic PCa and, that mutations in *BRCA2*, *ATM* and *CDK12* genes are mutually exclusive [8]. Germline mutations in the *BRCA2* gene were reported to be associated with distant metastasis at diagnosis and poor survival [28]. In our study including

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

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

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

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

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

Conclusions

Recent developments of targeted therapies for advanced PCa have focused attention on genes involved in homologous recombination deficiency and microsatellite instability. However, our results confirmed that mutations in the *TP53* gene and genes (*APC*/*CTNNB1)* related to the Wnt-pathway are associated with unfavourable outcome with metastatic visceral dissemination and early death. These genomic alterations could be considered as markers to identify PCa patients at high risk of life-threatening disease who might benefit from more intensified treatment or new targeted therapies.

References

[1] Hinata N, Fujisawa M. Racial Differences in Prostate Cancer Characteristics and Cancer-Specific Mortality: An Overview. World J Mens Health 2022;40:217-27.

[2] Desai MM, Cacciamani GE, Gill K, et al. Trends in Incidence of Metastatic Prostate Cancer in the US. JAMA Netw Open 2022;5:e222246.

[3] Nafissi NN, Kosiorek HE, Butterfield RJ, et al. Evolving Natural History of Metastatic Prostate Cancer. Cureus 2020;12:e11484.

[4] Drake CG. Visceral metastases and prostate cancer treatment: 'die hard,' 'tough neighborhoods,' or 'evil humors'? Oncology (Williston Park) 2014;28:974-80.

[5] Halabi S, Kelly WK, Ma H, et al. Meta-Analysis Evaluating the Impact of Site of Metastasis on Overall Survival in Men With Castration-Resistant Prostate Cancer. J Clin Oncol 2016;34:1652-9.

[6] Woodcock DJ, Riabchenko E, Taavitsainen S, et al. Prostate cancer evolution from multilineage primary to single lineage metastases with implications for liquid biopsy. Nat Commun 2020;11:5070.

[7] Plym A, Dióssy M, Szallasi Z, Sartor O, et al. DNA Repair Pathways and Their Association With Lethal Prostate Cancer in African American and European American Men. JNCI Cancer Spectr 2021;6:pkab097.

[8] Quigley DA, Dang HX, Zhao SG, et al. Genomic Hallmarks and Structural Variation in Metastatic Prostate Cancer Cell 2018;175:889.

[9] Mahal BA, Alshalalfa M, Kensler KH, et al. Racial Differences in Genomic Profiling of Prostate Cancer. N Engl J Med 2020;383:1083-5. PMID: 32905685.

[10] Mateo J, Seed G, Bertan C, Rescigno P, et al. Genomics of lethal prostate cancer at diagnosis and castration resistance. J Clin Invest 2020;130:1743-51.

[11] Abdi B, Basset N, Perrot E, et al. DNA damage repair gene germline profiling for metastatic prostate cancer patients of different ancestries. Prostate 2022;82:1196-201.

[12] Abida W, Armenia J, Gopalan A, et al. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. JCO Precis Oncol 2017;2017:PO.17.00029.

[13] Castro E, Mateo J, Olmos D, de Bono JS. Targeting DNA Repair: The Role of PARP Inhibition in the Treatment of Castration-Resistant Prostate Cancer. Cancer J 2016;22:353-6.

[14] Graf RP, Fisher V, Weberpals J, Gjoerup O, et al. Comparative Effectiveness of Immune Checkpoint Inhibitors vs Chemotherapy by Tumor Mutational Burden in Metastatic Castration-Resistant Prostate Cancer. JAMA Netw Open 2022;5:e225394.

[15] Crabb SJ, Griffiths G, Dunkley D, et al. Overall Survival Update for Patients with Metastatic Castration-resistant Prostate Cancer Treated with Capivasertib and Docetaxel in the Phase 2 ProCAID Clinical Trial. Eur Urol 2022;82:512-5.

[16] Ji Y, Huang PH, Woolfenden S, Myers A. Model-based dose selection to inform translational clinical oncology development of WNT974, a first-in-class Porcupine inhibitor. Clin Transl Sci 2022;15:1713-22.

[17] Patel JN, Braicu I, Timms KM, et al. Characterisation of homologous recombination deficiency in paired primary and recurrent high-grade serous ovarian cancer. Br J Cancer 2018;119:1060-6.

[18] Budczies J, Kluck K, Beck S, et al. Homologous recombination deficiency is inversely correlated with microsatellite instability and identifies immunologically cold tumors in most cancer types. J Pathol Clin Res 2022;8:371-82.

[19] Paner GP, Stadler WM, Hansel DE, Montironi R, Lin DW, Amin MB. Updates in the Eighth Edition of the Tumor-Node-Metastasis Staging Classification for Urologic Cancers. Eur Urol 2018;73:560-569.

[20] Hussain M, Corcoran C, Sibilla C, et al. Tumor Genomic Testing for >4,000 Men with Metastatic Castration-resistant Prostate Cancer in the Phase III Trial PROfound (Olaparib). Clin Cancer Res 2022;28:1518-30.

[21] Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC. p53 is mutated in a subset of advanced-stage prostate cancers. Cancer Res 1993;53:3369-73.

[22] Maxwell KN, Cheng HH, Powers J, et al. Inherited TP53 Variants and Risk of Prostate Cancer. Eur Urol 2022;81:243-50.

[23] Nientiedt C, Budczies J, Endris V, et al. Mutations in TP53 or DNA damage repair genes define poor prognostic subgroups in primary prostate cancer. Urol Oncol 2022;40:8.e11-8.

[24] Kamoun A, Cancel-Tassin G, Fromont G, et al. Comprehensive molecular classification of localized prostate adenocarcinoma reveals a tumour subtype predictive of non-aggressive disease. Ann Oncol 2018;29:1814-21.

[25] Tonon L, Fromont G, Boyault S, et al. Mutational Profile of Aggressive, Localised Prostate Cancer from African Caribbean Men Versus European Ancestry Men. Eur Urol 2019;75:11-5.

[26] Nyquist MD, Corella A, Coleman I, et al. Combined TP53 and RB1 Loss Promotes Prostate Cancer Resistance to a Spectrum of Therapeutics and Confers Vulnerability to Replication Stress. Cell Rep 2020;31:107669.

[27] Alshalalfa M, Goglia AG, Swami N, Nguyen B, Hougen HY, Khan A, Kishan AU, Punnen 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.

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

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

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

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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	54.7 ± 47.8
Median (IQR)	37.0 (56.0)

SD: standard deviation; IQR: Interquartile range

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Figure legend:

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

Figure 2: Age at death or last news (years) according to the metastatic clinical status. M1a (blue): lymph node metastasis; M1b (green): bone metastasis, M1c (red): visceral metastasis.

Figure 3: Age at death or last news (years) according to the mutationnal status (0 not mutated; 1 mutated).

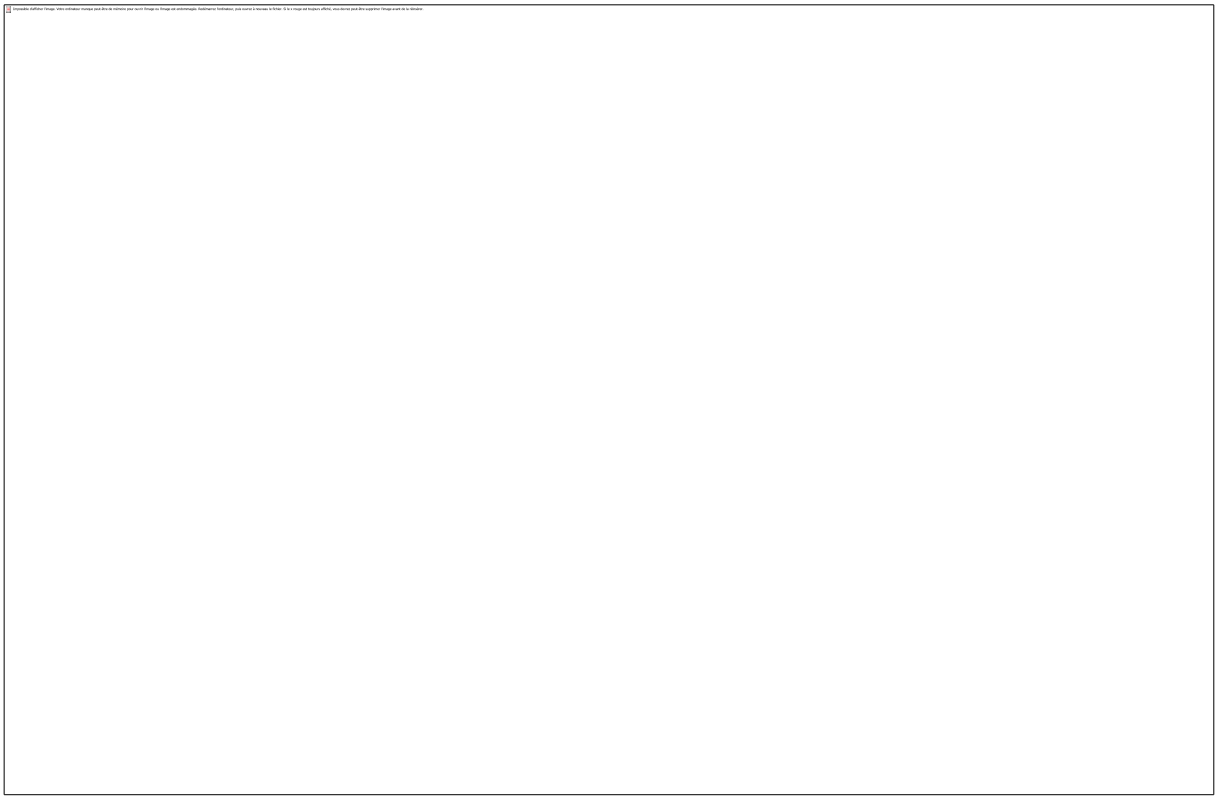
Figure 4: Correlation between HRD score and the metastatic clinical status. M1a: lymph node metastasis; M1b: bone metastasis, M1c: visceral metastasis.

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

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

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

401 Figure 1



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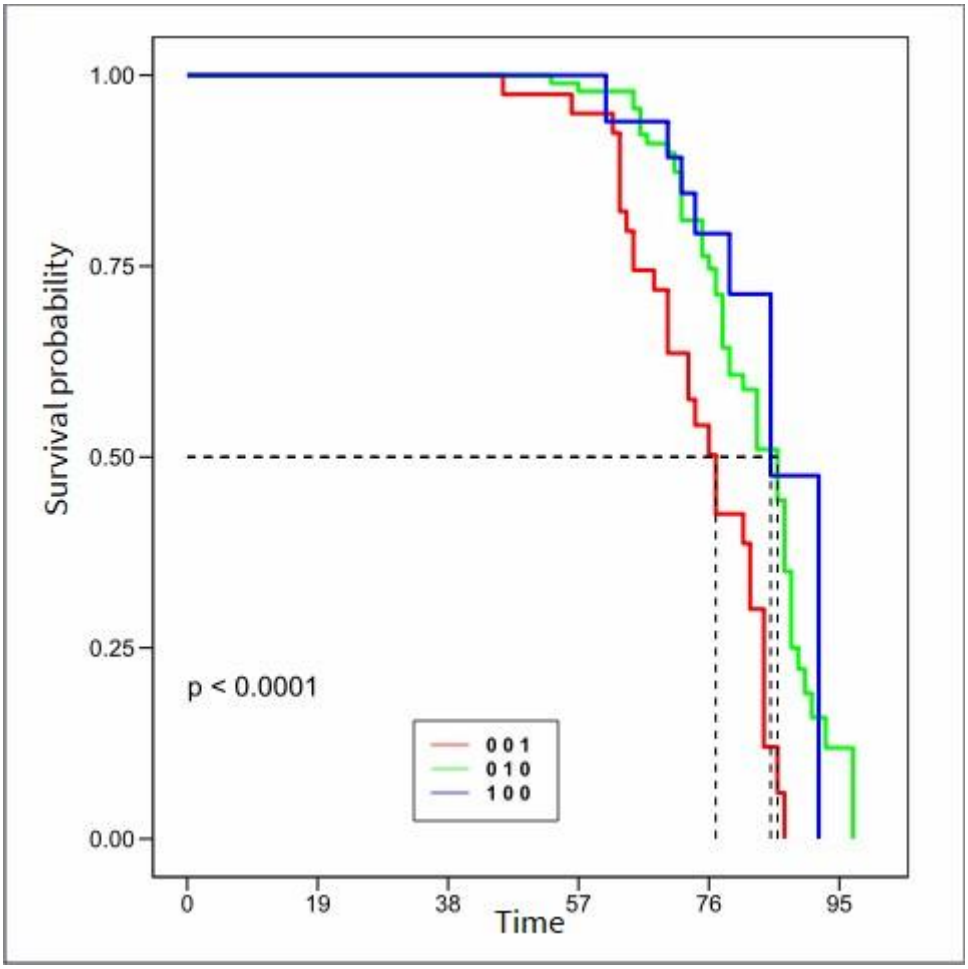
404 Figure 2



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407 Figure 3

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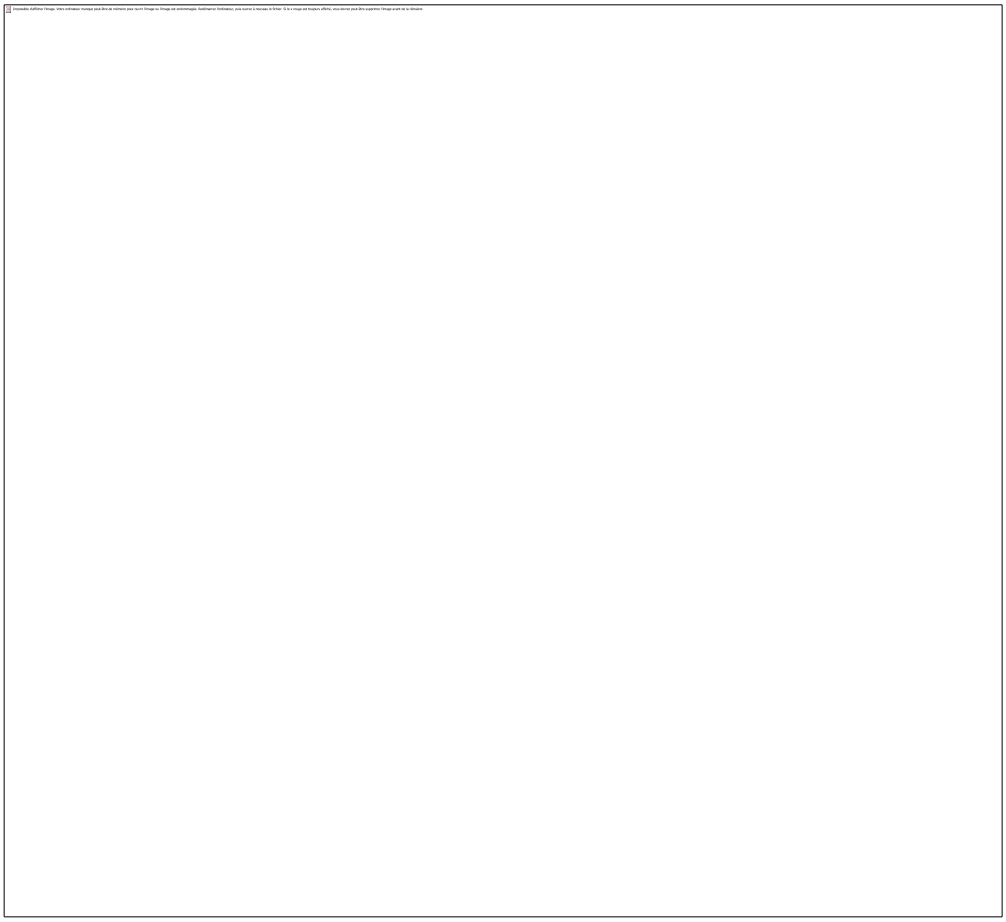
410 Figure 4



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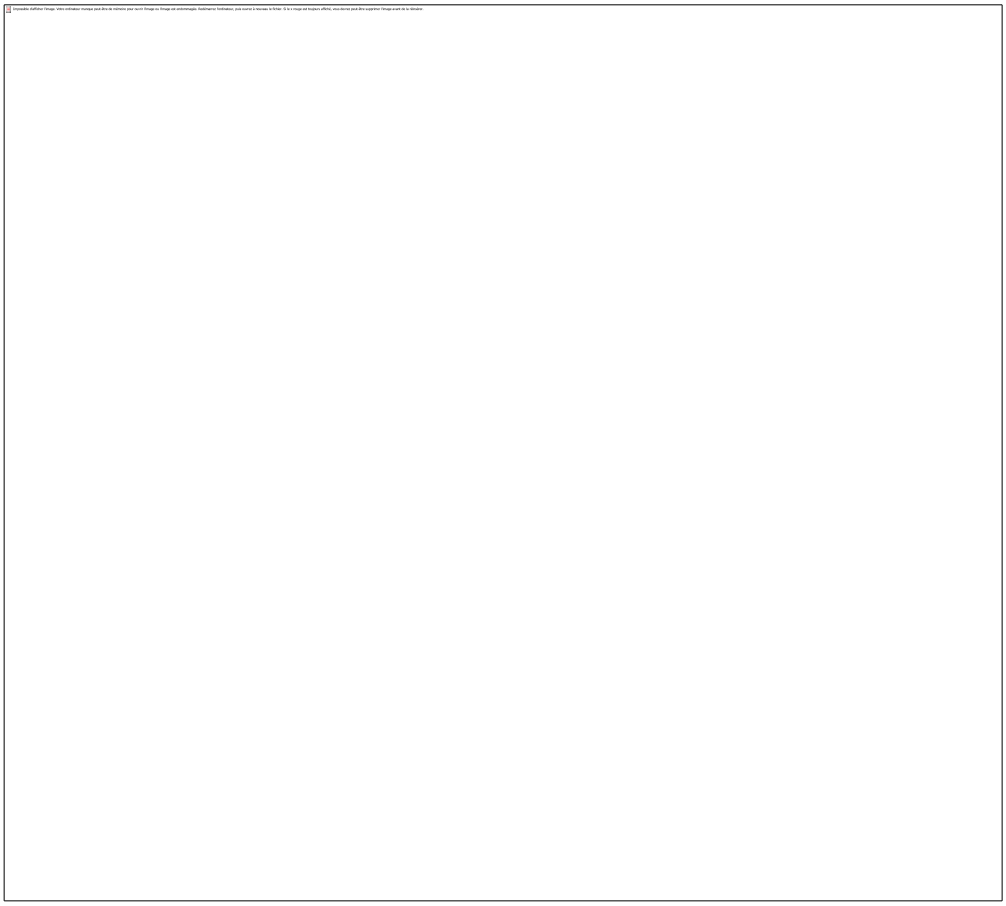
413 Figure 5



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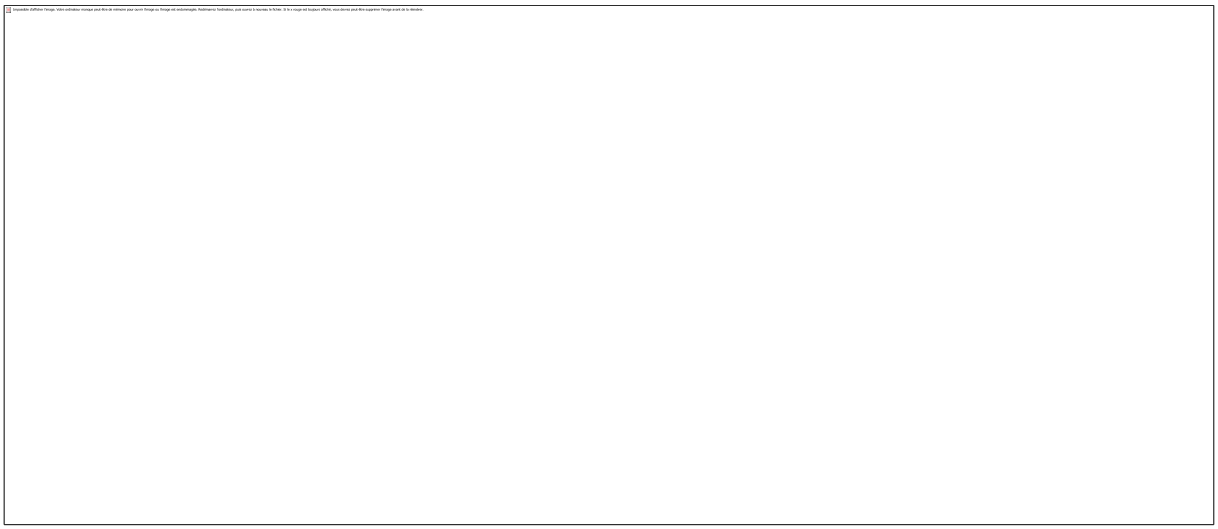
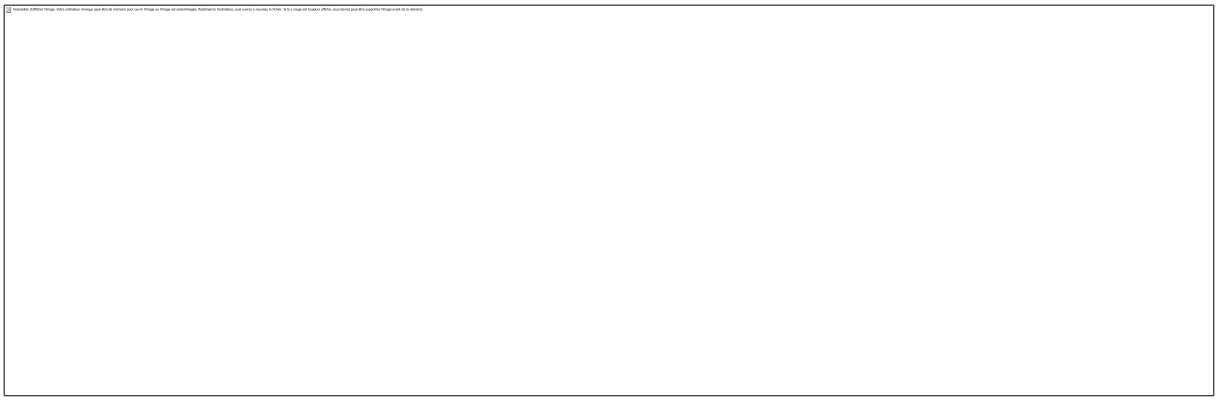
Supplementary figure 1.



Supplementary figure 2.



Supplementary Table S1: Patients with other cancers

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

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

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

Supplementary Table S3: Correlation between germline and somatic mutations

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

*Biallelic alterations

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

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