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Original Research

Impact of concurrent tumour events on the prostate cancer outcomes of germline *BRCA2* mutation carriers

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Abstract ***Background:*** Several studies have reported the association of germline *BRCA2* (g*BRCA2*) mutations with poor clinical outcomes in prostate cancer (PCa), but the impact of concurrent somatic events on g*BRCA2* carriers survival and disease progression is unknown.
Patients and methods: To ascertain the role of frequent somatic genomic alterations and histology subtypes in the outcomes of g*BRCA2* mutation carriers and non-carriers, we correlated the tumour characteristics and clinical outcomes of 73 g*BRCA2* and 127 non-carriers. Fluorescent *in-situ* hybridisation and next-generation sequencing were used to detect copy number variations in *BRCA2*, *RB1*, *MYC* and *PTEN*. Presence of intraductal and cribriform subtypes was also assessed. The independent impact of these events on cause-specific survival (CSS), metastasis-free survival and time to castration-resistant disease was assessed using cox-regression models.
Results: Somatic *BRCA2-RB1* co-deletion (41% versus 12%, $p < 0.001$) and *MYC* amplification (53.4% versus 18.8%, $p < 0.001$) were enriched in *gBRCA2* compared to sporadic tumours. Median CSS from diagnosis of PCa was 9.1 versus 17.6 years in *gBRCA2* carriers and non-carriers, respectively (HR 2.12; $p = 0.002$), Median CSS in *gBRCA2* carriers increased to 11.3 and 13.4 years in the absence of *BRCA2-RB1* deletion or *MYC* amplification, respectively. Median CSS of non-carriers decreased to 8 and 2.6 years if *BRCA2-RB1* deletion or *MYC* amplification were detected.
Conclusions: g*BRCA2*-related prostate tumours are enriched for aggressive genomic features, such as *BRCA2-RB1* co-deletion and *MYC* amplification. The presence or absence of these events modify the outcomes of *gBRCA2* carriers.

1. Introduction

Prostate cancer (PCa) is a heterogeneous disease driven by multiple genomic events [1,2]. Alterations in *BRCA2* have been described in 3–5% of localised tumours and in up to 9% of metastatic PCa [1–3] being usually early events already present in the primary tumour [4]. Importantly, half of them are germline in origin [5,6]. Both germline and somatic *BRCA2* alterations predict favourable responses to PARP inhibitors [3,7], but while the prognostic implications of somatic *BRCA2* alterations remain unclear [8–12], germline *BRCA2* (g*BRCA2*) mutations have been consistently identified as a marker of poor outcomes in PCa. g*BRCA2* mutations have been associated with frequent Gleason grade group reclassification during active surveillance [8]; short metastasis-free survival (MFS) in patients with localised disease [10]; early development of castration resistance [11,12] and reduced cause-specific survival (CSS) [9,11,12]. The biological underpinnings of this aggressive behaviour have not been elucidated but could be related to the presence of certain histology subtypes and/or concurrent somatic events linked to genomic instability and poor PCa outcomes. g*BRCA2*-related PCa has been associated with intraductal (IDC) [13,14] and cribriform (CRIB) histology subtypes. [14] Copy number variations (CNV) predominantly deletions, are the most frequent genomic events in *BRCA2*-deficient tumours [15–17]. In an earlier report, we observed an enrichment in somatic *BRCA2*, *RB1* and *PTEN* deletions and *MYC* amplification in *gBRCA2*-related PCa using high-resolution comparative genomic hybridisation arrays [15]. A high incidence of somatic *BRCA2* loss, *RB1* deletions and *MYC* amplification in these tumours has also been reported by other groups [5,17–19].

However, none of the studies addressing the prognostic impact and clinical implications of g*BRCA2*

mutations in PCa [9–12,20,21] have taken into consideration histology subtypes or concurrent genomic events.

2. Materials and methods

2.1. Study design

PROREPAIR-A is a multicentre observational study that enrolled PCa patients previously screened for germline mutations in DNA damage and repair (DDR) genes in the context of other research protocols or as routine clinical practice. The study includes known carriers and non-carriers, irrespective of disease stage at diagnosis. Only patients harbouring pathogenic or likely pathogenic variants in *BRCA2* according to the American College of Medical Genetics and Genomics guidelines and ClinVar annotations were considered for this analysis (Suppl. Table 1). Each *gBRCA2* carrier was initially matched with two sporadic cases (without germline DDR mutations) by Gleason grade group and presence/absence of metastases at diagnosis. Eligibility required availability of archival diagnostic formalin-fixed paraffin-embedded (FFPE) material, baseline diagnostic characteristics and outcomes (Fig 1).

The primary aim of the study was to confirm the prognostic value of *gBRCA2* for CSS, defined as time from diagnosis of PCa to death from the disease. Secondary objectives intended to establish the association between *gBRCA2* and CNV in *BRCA2*, *RB1*, *PTEN* and *MYC*, as well as the impact of these somatic events in CSS, MFS and time to castration-resistant disease (TTCR) for *gBRCA2* carriers and non-carriers.

The study commenced in January 2013. Patient outcomes were retrospectively collected until July 2016 and prospectively collected afterwards, until the data cut-off in March 2020. The study was granted approval by the local institutional review boards at the participating sites.

2.2. Molecular and histological characterisation of tumour samples

Tumour blocks were collected under the study protocol and centrally reviewed by two pathologists (AGP, PGP) who marked tumour areas amenable for subsequent studies. These were prioritised according to availability for: i) cytogenetic studies; ii) next-generation sequencing (NGS).

We determined *BRCA2*, *RB1*, *PTEN* and *MYC* somatic copy number status by fluorescence *in situ* hybridisation (FISH) using the methods previously described [23–25] with directly labelled bacterial artificial chromosomes from previously published assays and/or commercial diagnostic probes (Suppl. Figure 1). Then, multi-colour high-resolution images were obtained from the hybridised slides using the ARIOL SL-50 platform (Leica) and scored by three trained operators (EC, RL, FLC) in a minimum of 100 nuclei per slide. *BRCA2*, *RB1*, and *PTEN* genes were classified as mono- or bi-allelic loss if 1 or 2 copies were deleted in at least 50% of evaluable cells. *MYC* gain was defined as a *MYC*:CEP8 signal ratio of ≥ 1.5:1 and *MYC* amplification as *MYC*:CEP8 ≥ 2.2:1 in > 20% of cells, respectively [26].

We compared CNV in *BRCA2*, *RB1*, *MYC and PTEN* determined by FISH and NGS in samples with good DNA quality and quantity amenable for whole-exome sequencing or targeted sequencing with the UW-OncoPlex panel [27] (Fig. 1, Suppl. Table 2).

Finally, two expert uropathologists (DSC and TLL) blinded to mutational status independently scored those cases with tumour tissue available (n = 151) for the presence of IDC and CRIB patterns with the support of immunostaining for basal cell markers [14].

2.3. Statistical methods

The required sample size was calculated based on the expected odds ratio for the 10-year CSS rate in g*BRCA2* carriers and non-carriers [28]. We estimated a 10-year CSS rate of ≤ 40% and ≥ 70% for g*BRCA2* carriers and non-carriers, respectively [11]. Considering a two-sided significance level of 5%, a power of 90% and a 1 carrier: 2 non-carriers matching ratio, at least 141 patients were required for the primary endpoint analysis. Initially, 240 patients were enrolled, but tissue and/or follow up data were not available for 40 patients who were excluded from the study. Nonetheless, this attrition in cases did not result in significant imbalances (Table 1).

Descriptive statistics were used to summarise patients and samples characteristics. The association, correlation and concordance between germline status, presence of somatic CNV and histology subtypes were analysed using the Chi-squared test, Pearson correlation and Cohen's Kappa, respectively. Other associations between patient/tumour characteristics and germline status were analysed using chi-squared, Mantel-Haenszel linear-trend or the Mann-Whitney U tests, as appropriate. All time-to-events were defined from initial PCa diagnosis and assessed using the Kaplan-Meier method. The resulting survival curves were compared using a log-rank test. Univariable and multivariable HR were calculated using Cox proportional-hazards models. All p values were two-sided. Analyses were performed using Statistical Package for the Social Sciences for Windows version 19 (SPSS, Chicago, IL).

3. Results

A total of 200 patients were included (73 g*BRCA2* and 127 non-carriers) of which 24.8% presented metastasis at diagnosis (28.8% carriers versus 22% non-carriers,

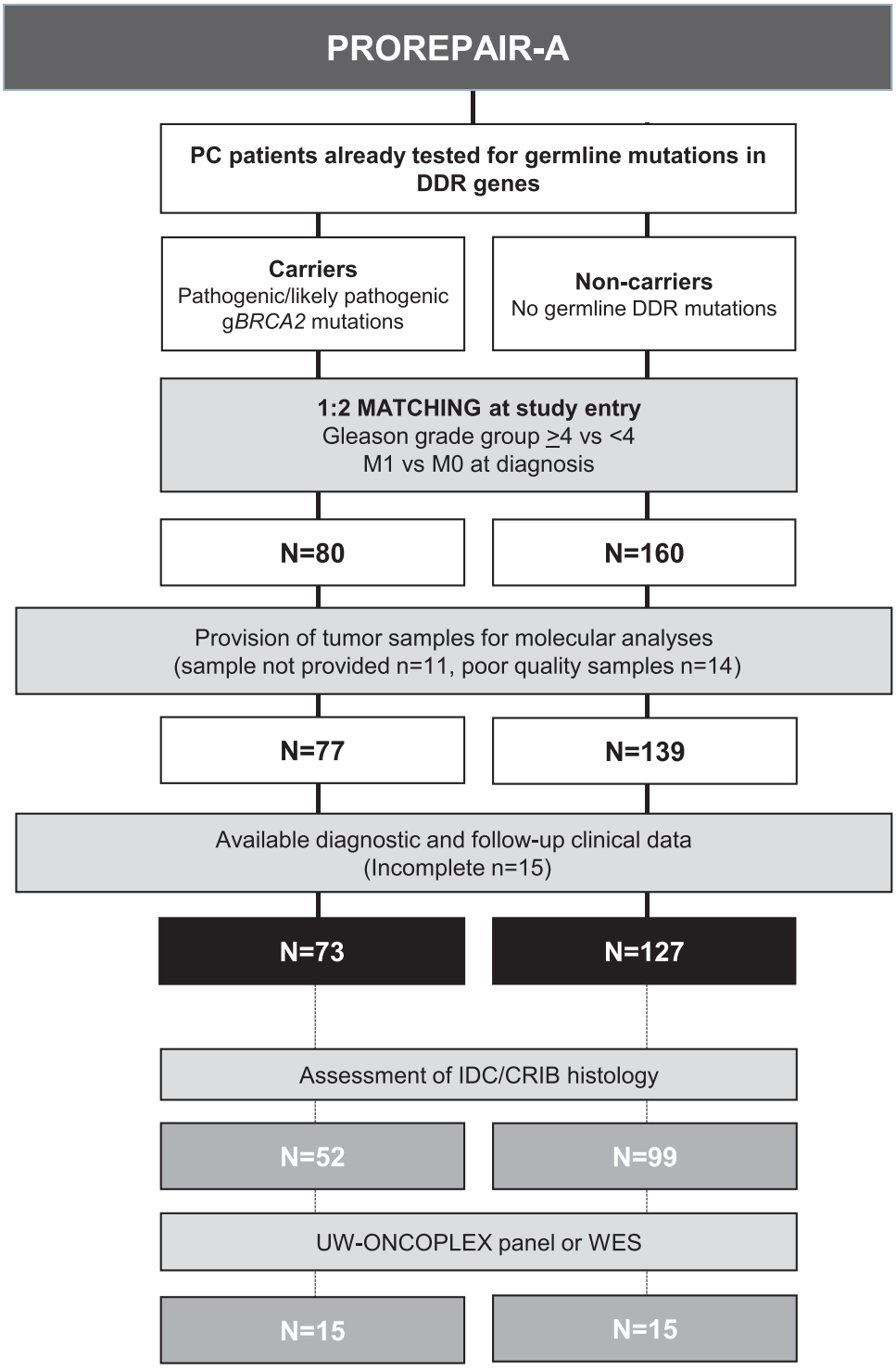

Fig. 1. Study flow-chart. CRIB = cribriform; g g*BRCA2* = germline *BRCA2*; IDC = intraductal; PCa = prostate cancer; WES = whole exome sequencing. UW-OncoPlex™ Cancer gene Panel https://testguide.labmed.uw.edu/public/view/OPX.

p = 0.287). The only significant differences between carriers and non-carriers were median age at diagnosis (64.5 versus 62.6 years, p = 0.028) and a higher frequency of T3/T4 stage among g*BRCA2* carriers (31.5% versus 9.4%; p < 0.001). Patients' characteristics are summarised in Table 1.

3.1. Molecular characteristics and histology of tumours from gBRCA2 carriers and non-carriers

Somatic *BRCA2* deletions as detected by FISH were present in 31 tumours from g*BRCA2* carriers (42.5%, 29 heterozygous and 2 homozygous) and 15 from non-

Table 1
Baseline characteristics of patients included in the study.

	Non-carriers (N = 127)	*gBRCA2* (N = 73)	p Value
Age at diagnosis			
Median, years (range)	64.5 (51.1–82.7)	62.6 (43.9–82.1)	0.028
PSA at diagnosis			
Median, ng/mL (range)	12.9 (1.5–578.0)	9.0 (0–3380)	0.077
Clinical/pathological stage			
T1/T2	115 (90.6%)	50 (68.5%)	< 0.001
T3/T4	12 (9.4%)	23 (31.5%)	
Node involvement			
N0	122 (96.1%)	65 (89%)	0.073
N1	5 (3.9%)	8 (11%)	
Metastases at diagnosis			
M0	99 (78.0%)	52 (71.2%)	0.287
M1	28 (22%)	21 (28.8%)	
Gleason grade group			
≤ 3	55 (43.3%)	31 (42.5%)	0.908
≥ 4	72 (56.7%)	42 (57.5%)	
Local treatment			
No primary therapy	25 (19.7%)	22 (30.1%)	0.137
RP	84 (66.1%)	37 (50.7%)	
RT	18 (14.2%)	11 (15.1%)	
Unknown	0	3 (4.1%)	
Somatic *BRCA2* deletion by FISH			
No alteration	112 (88.2%)	40 (54.8%)	< 0.001
BRCA2 deletion	15 (11.8%)	31 (42.5%)	
Heterozygous	13 (10.2%)	29 (39.7%)	
Homozygous	2 (1.6%)	2 (2.7%)	
Undetermined	0	2 (2.7%)	
RB1 status by FISH			
No alteration	100 (78.7%)	33 (45.2%)	< 0.001
RB1 deletion	27 (21.3%)	40 (54.8%)	
Heterozygous	18 (14.2%)	36 (49.3%)	
Homozygous	9 (7.1%)	4 (5.5%)	
BRCA2-RB1 co-deletion by FISH			
No	100 (78.7%)	32 (43.8%)	< 0.001
BRCA2 deletion only	0	1 (1.4%)	
RB1 deletion only	12 (9.4%)	8 (11.0%)	
BRCA2-RB1 co-deletion	15 (11.8%)	30 (41.1%)	
Undetermined	0	2 (2.7%)	
MYC status by FISH			
No alteration	103 (81.1%)	32 (43.9%)	< 0.001
MYC amplification	12 (9.4%)	35 (47.9%)	
MYC gain	12 (9.4%)	4 (5.5%)	
Undetermined	0	2 (2.7%)	
PTEN status by FISH			
No alteration or heterozygous deletion	93 (73.2%)	46 (63.1%)	0.213
PTEN homozygous deletion	34 (26.8%)	25 (34.2%)	
Undetermined	0	2 (2.7%)	
Histology features (n = 151)	(n = 99)	(n = 52)	
Intraductal	45 (45.5%)	21 (40.4%)	0.550
Cribriform	44 (44.4%)	28 (53.8%)	0.272
Intraductal and/or cribriform	56 (56.6%)	30 (57.7%)	0.894

Percentage distribution across each variable include patients with unknown or missing values who were excluded for statistical hypothesis testing patients.
FISH, fluorescence in situ hybridisation; *gBRCA2*, germline *BRCA2*; N/A, not applicable; PSA, prostate-specific A antigen; RP, radical prostatectomy; RT, radiotherapy.

carriers (11.8%, 13 heterozygous and 2 homozygous) ($p < 0.001$). *RB1* deletions (54.8% versus 21.3%, $p < 0.001$) and *MYC* amplification (53.4% versus 18.8%, $p < 0.001$) were also more frequent in *gBRCA2* than in sporadic tumours (Table 1).

BRCA2 and *RB1* were frequently co-deleted in all groups. In 49 out of 51 tumours with somatic *BRCA2* deletion a concurrent *RB1* deletion was noted, with a strong correlation between these two alterations ($p = 0.001$, concordance Kappa index 0.74, Suppl. Table 3). *BRCA2-RB1* co-deletion was more frequent in *gBRCA2* than in sporadic tumours (41.1% versus 11.8%, $p < 0.001$) (Table 1). Primary tumours of patients presenting with metastatic disease at diagnosis (from carriers and non-carriers) were enriched for somatic *BRCA2-RB1* co-deletion (34% versus 21%, $p < 0.01$) and *MYC* amplification (42% versus 16%, $p < 0.001$) compared with those who presented with localised disease (Suppl. Figure 2).

The concordance in CNV detected by FISH and NGS was analysed in a subset of 30 tumours using the Cohen's Kappa concordance index. Kappa's linear weighted values ranged from substantial to almost perfect agreement for the genes explored: 0.801 (IC 95% 0.584–1.000) for somatic *BRCA2* deletions, 0.708 (IC 95% 0.483–0.934) for *RB1* deletions, 0.694 (IC 95% 0.483–0.905) for *PTEN* deletions and 0.627 (IC 95% 0.350–0.904) for *MYC* alterations (Suppl. Table 2).

The presence of IDC and CRIB patterns was assessed in 151 tumours (52 g*BRCA2* and 99 sporadic tumours). IDC and/or CRIB were present in 57.7% of g*BRCA2* and 56.6% of sporadic tumours. IDC was frequently associated with somatic *PTEN* loss, whilst CRIB was associated with somatic *BRCA2* and *RB1* loss as well as *MYC* amplification (Suppl. Table 3). IDC and/or CRIB morphologies were significantly more frequent in tumours with the *BRCA2-RB1* co-deletion (67.6% versus 41.9%, $p = 0.008$).

3.2. Clinical outcomes based on gBRCA2 status

After a median follow-up of 12.0 years (95% CI, 11.5–12.6), 86 PCa-related deaths occurred: 45 in g*BRCA2* carriers and 34 in non-carriers. At the time of data cut-off, 142 patients (excluding censored carriers and non-carriers) were eligible for the primary endpoint analysis. The 10-year CSS rate was significantly inferior in *gBRCA2* patients than in non-carriers (26.8% versus 66.1%, $p < 0.001$). Median CSS from diagnosis of PCa was significantly shorter in g*BRCA2* carriers than in non-carriers when all patients were considered (9.1 versus 17.6 years; HR 2.12; 95% CI 1.33–3.33; $p = 0.002$), but also when the analysis was limited to M0 patients (11.3 years versus not-reached, HR 3.71 95%CI 1.87–7.36, $p < 0.001$) (Table 2, Fig. 2).

During the follow-up, 29.8% of patients with M0 disease at diagnosis developed metastases. This occurred significantly earlier in *gBRCA2* carriers (8.6 years versus not-reached, HR 3.94 95%CI 2.12–7.32, $p < 0.001$). Likewise, TTCR was shorter in *gBRCA2* carriers (8.8 years versus not-reached, HR 1.88, 95%CI 1.20–2.96; $p = 0.005$ (Table 2, Fig. 2).

3.3. Clinical outcomes based on somatic alterations and histology subtypes

Somatic *BRCA2*, *RB1* deletions, *BRCA2-RB1* co-deletion, as well as *MYC* amplification and *MYC* gain determined by FISH were significantly associated with shorter CSS and TTCR in the univariate analysis of the entire study population (gBRCA2 and sporadic tumours). Likewise, these genomic events and *PTEN* loss were also correlated with CSS, TTCR and MFS in the group of patients with localised disease at diagnosis (Table 2, Suppl. Table 5). Similar association with poor outcomes, in the entire cohort and in patients with localised disease only was observed in cases with either IDC or CRIB patterns (Table 2, Suppl. Table 5).

3.4. Multivariable cox-regression analyses

Multivariable analyses (MVA) confirmed the independent prognostic value of g*BRCA2* mutations as predictor of CSS (HR 3.92, $p = 0.009$) in the entire cohort. Other variables independently associated with shorter CSS were somatic *BRCA2-RB1* co-deletion (HR 4.0, $p = 0.009$), *MYC* amplification (HR 2.57, $p = 0.037$), metastasis at diagnosis (HR 12.37, $p < 0.001$) and Gleason grade group ≥ 4 (HR 6.0, $p < 0.001$) (Table 3). Among M0 patients, *gBRCA2* (HR 6.30, $p = 0.009$), *BRCA2-RB1* codeletion (HR 7.49, $p = 0.004$) and Gleason grade group≥ 4 (HR 7.85, $p = 0.001$) were also associated CSS. IDC and CRIB patterns were not associated with CSS in the MVA (Suppl. Table 6).

Independent prognostic factors for MFS in the M0 cohort included g*BRCA2* mutations (HR 5.56, $p < 0.001$), somatic *BRCA2-RB1* co-deletion (HR 5.99, $p < 0.001$) Gleason grade group ≥ 4 ($p = 0.001$), T3/T4 ($p = 0.019$), N1 (HR 2.63, $p = 0.029$) and CRIB (HR 3.78, $p = 0.028$) g*BRCA2* mutations (HR 3.73,$p = 0.011$) and *BRCA2-RB1* co-deletion (HR 2.92 $p = 0.048$) also predicted shorter TTCR. Other poor prognostic factors for TTCR included Gleason grade group ≥ 4 (HR 2.72, $p = 0.002$), high PSA levels at diagnosis (HR 2.72 $p = 0.021$) and metastatic stage (9.38, $p < 0.001$) (Table 3, Supl.Table_5).

3.5. Impact of somatic BRCA2-RB1 co-deletion and MYC amplification on Cause Specific Survival by gBRCA2 status

As both, *BRCA2-RB1* co-deletion and *MYC* amplification, were independently associated with shorter CSS,

Table 2
Univariate analysis of outcomes by group. All patients and non-metastatic (M0).

Variable in the UVA	All stages patients (n = 200)						Non-metastatic (M0) only (n = 151)					
	Cause-specific survival			Time to castration resistance			Cause-specific survival in M0			Metastases-free survival		
	Median CSS	UVA HR (95% CI)	p Value	Median TTCR	UVA HR (95% CI)	p Value	Median TTCR	UVA HR (95% CI)	p Value	Median MFS	UVA HR (95% CI)	p Value
Germline BRCA2 status												
g*BRCA2* versus non-carrier	9.1 versus 17.5	2.06 (1.30–3.27)	0.002	8.8 versus NR	1.88 (1.20–2.96)	0.005	11.3 versus NR	3.71 (1.87–7.36)	< 0.001	8.6 versus NR	3.94 (2.12–7.32)	< 0.001
Somatic *BRCA2* status												
BRCA2 deletion versus no	6.3 versus 16.9	3.04 (1.90–4.86)	< 0.001	4.7 versus NR	3.04 (1.91–4.86)	< 0.001	11.3 versus NR	4.56 (2.26–9.20)	< 0.001	8.1 versus NR	4.71 (2.48–8.93)	< 0.001
Heterozygous deletion versus normal	6.6 versus 16.9	2.90 (1.73–4.85)	< 0.001	5.0 versus NR	2.65 (1.29–5.44)	< 0.001	12.6 versus NR	5.06 (2.04–12.56)	< 0.001	8.5 versus NR	5.15 (2.10–12.67)	< 0.001
Homozygous deletion versus normal	5.7 versus 16.9	2.02 (1.05–3.90)	0.035	4.8 versus NR	2.42 (0.94–6.20)	0.066	11.0 versus NR	1.45 (0.54–3.88)	0.461	7.8 versus NR	1.65 (0.71–3.82)	0.246
RB1 status												
RB1 loss versus no	9.8 versus 16.9	2.05 (1.31–3.20)	0.002	9.0 versus NR	1.89 (1.21–2.95)	0.005	11.8 versus 17.6	3.19 (1.65–6.16)	0.001	9.3 versus NR	3.70 (2.04–6.68)	< 0.001
Heterozygous deletion versus normal	9.9 versus 16.9	1.83 (1.15–2.90)	0.010	9.0 versus 15.5	1.72 (1.01–2.73)	0.020	11.8 versus 17.6	3.36 (1.67–6.75)	0.001	9.5 versus NR	3.45 (1.91–6.24)	< 0.001
Homozygous deletion versus normal	10.9 versus 16.9	1.89 (0.86–4.19)	0.117	8.7 versus 15.5	1.77 (0.80–3.90)	0.159	11.3 versus 17.6	2.58 (0.75–8.91)	0.134	10.2 versus NR	1.97 (0.59–6.55)	0.272
BRCA2-RB1 co-deletion												
BRCA2-RB1 co-deletion versus no	6.3 versus 16.9	2.95 (1.84–4.73)	< 0.001	4.7 versus NR	3.01 (1.88–4.82)	< 0.001	9.9 versus NR	4.63 (2.29–9.33)	< 0.001	8.1 versus NR	4.60 (2.44–8.67)	< 0.001
MYC status												
MYC amplification versus no	6.0 versus 17.6	5.25 (3.25–8.50)	< 0.001	2.9 versus NR	4.78 (2.95–7.76)	< 0.001	9.0 versus NR	14.70 (6.24–36.62)	< 0.001	8.8 versus NR	4.83 (2.51–9.31)	< 0.001
MYC gain versus no	12.6 versus 17.6	2.56 (1.31–4.99)	0.006	10.7 versus NR	2.79 (1.47–5.30)	0.002	12.6 versus NR	2.66 (0.97–7.28)	0.057	10.7 versus NR	2.53 (1.04–6.14)	0.003
PTEN status												

(*continued on next page*)

Table 2 (*continued*)

Variable in the UVA	All stages patients (n = 200)						Non-metastatic (M0) only (n = 151)					
	Cause-specific survival			Time to castration resistance			Cause-specific survival in M0			Metastases-free survival		
	Median CSS	UVA HR (95% CI)	p Value	Median TTCR	UVA HR (95% CI)	p Value	Median TTCR	UVA HR (95% CI)	p Value	Median MFS	UVA HR (95% CI)	p Value
Homozygous deletion versus no	10.7 versus 17.6	1.46 (0.93–2.30)	0.104	10.7 versus NR	1.51 (0.96–2.36)	0.076	12.6 versus NR	2.57 (1.33–4.95)	0.005	12.6 versus NR	1.91 (1.06–3.45)	0.033
Heterozygous deletion versus normal	15.0 versus NR	0.52 (0.24–1.11)	0.092	NR versus NR	*0.88* (0.24–1.10)	0.516	17.6 versus NR	1.04 (0.39–2.81)	0.939	NR versus NR	1.62 (0.73–3.59)	0.239
Age at diagnosis												
> 65 versus ≤ 65 years	12.6 versus 16.9	1.52 (0.97–2.37)	0.068	NR versus NR	1.16 (0.74–1.80)	0.523	12.5 versus 16.3	1.59 (1.04–2.45)	0.034	NR versus NR	1.13 (0.63–2.03)	0.695
PSA at diagnosis												
Above median versus below median	16.2 versus NR	2.36 (1.16–4.19)	0.015	15.5 versus NR	3.98 (1.84–8.58)	< 0.001	16.9 versus NR	2.09 (1.05–4.17)	0.036	16.4 versus NR	1.39 (0.71–2.71)	0.335
Gleason grade group												
≥ 4 versus ≤ 3	8.0 versus 17.6	6.15 (3.32–11.4)	< 0.001	4.7 versus NR	6.10 (3.36–11.07)	< 0.001	12.6 versus NR	4.79 (2.18–10.54)	< 0.001	12.3 versus NR	3.09 (1.64–5.81)	< 0.001
N1 at diagnosis												
Yes versus no	5.6 versus 14.9	2.31 (1.15–4.64)	0.019	3.6 versus 15.0	1.90 (0.91–3.94)	0.086	7.6 versus NR	4.30 (1.77–10.45)	0.001	6.5 versus NR	2.86 (1.21–6.76)	0.017
Clinical/ pathological stage												
T3/T4 versus T1/T2	11.3 versus 16.9	1.47 (0.87–2.49)	0.155	8.8 versus 15.5	1.52 (0.90–2.57)	0.120	12.7 versus NR	2.68 (1.33–5.42)	0.006	8.8 versus NR	4.06 (2.22 versus 7.40)	< 0.001
M1 at diagnosis												
Yes versus no	3.1 versus 17.6	20.75 (11.83–36.38)	< 0.001	1.2 versus NR	24.1 (13.67–42.50)	< 0.001	–	–	–	–	–	–
Histology variants	All stages patients (n = 151)						Non-metastatic (M0) only (n = 122)					
IDC												
Yes versus no	11.8 versus 17.6	1.92 (1.13–3.28)	0.016	13.0 versus NR	1.91 (1.11–3.27)	0.019	14.9 versus 17.6	2.36 (1.09–5.13)	0.029	13.0 versus NR	2.17 (1.10–4.27)	0.025
CRIB												
Yes versus no	11.3 versus NR	2.55 (1.43–4.54)	0.001	9.0 versus NR	2.71 (1.52–4.82)	0.001	14.9 versus NR	4.90 (1.84–13.04)	0.001	10.0 versus NR	4.97 (2.25–10.96)	< 0.001

CRIB, cribriform; g*BRCA2,* germline *BRCA2*; CSS, cause-specific survival; IDC, intraductal; MFS, metastasis-free survival; NR, not reached; PSA, prostate-specific antigen; TTCR, time to castration resistant disease.

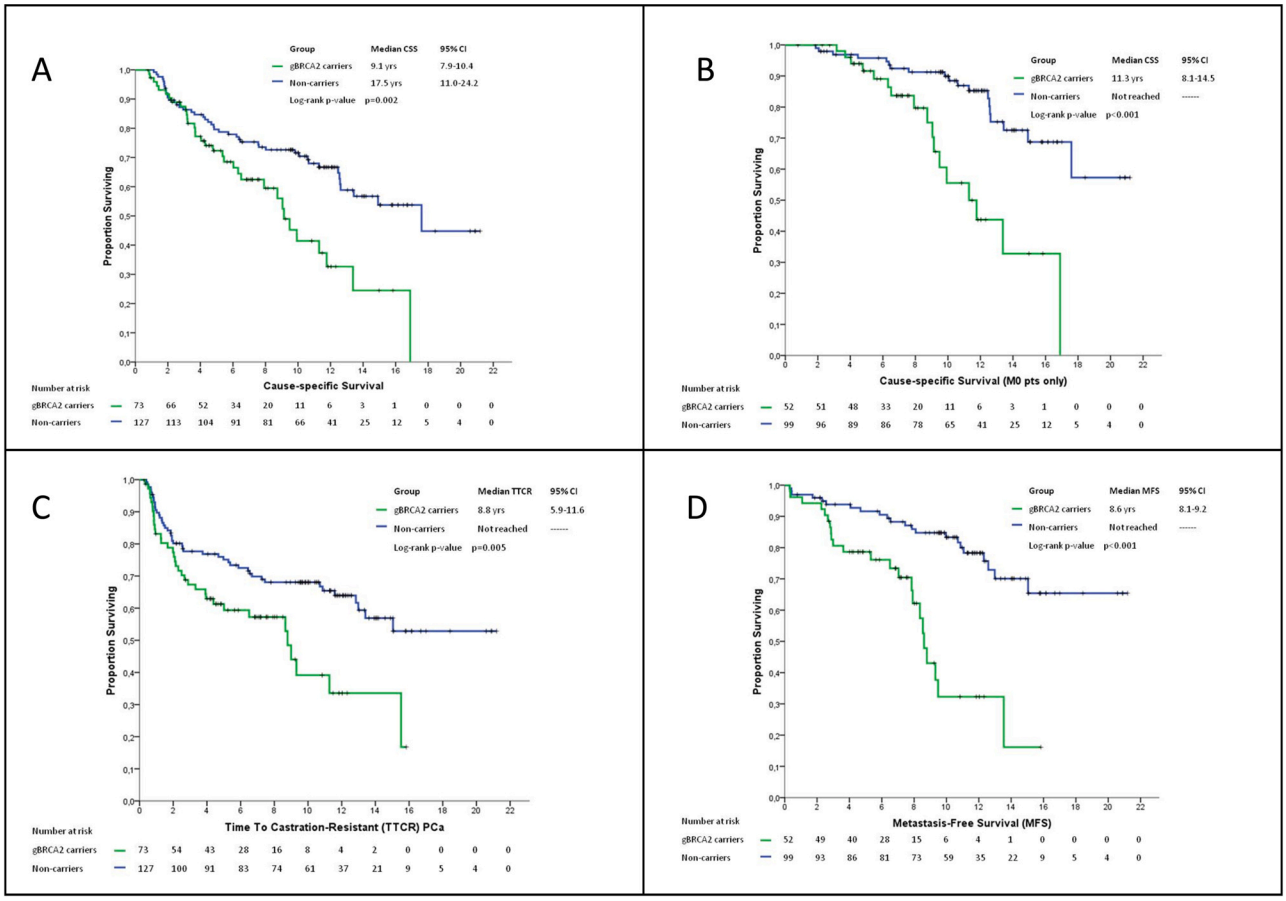

Fig. 2. Cause-specific survival (CSS), metastases-free survival (MFS) and time to castration-resistant disease (TTCR) from diagnosis of prostate cancer in gBRCA2 mutation carriers and non-carriers. Kaplan-Meier curves in g*BRCA2* versus non-carriers for: (A) CSS; (B) CSS in M0 patients; (C) TTCR; (D) MFS in M0 patients. g*BRCA2* = germline *BRCA2*; M0 = patients with no evidence of distant metastases at diagnosis.

we analysed whether these somatic events may affect the outcomes of PCa patients by g*BRCA2* status.

Among *gBRCA2* patients the presence of somatic *BRCA2-RB1* co-deletion (6.3 versus 11.3 years, $p = 0.041$, Fig. 3A) or *MYC* amplification (6.0 versus 13.4 years, $p < 0.001$, Fig. 3B) was associated with shorter CSS. Similar associations were also noted in the non-carrier population for *BRCA2-RB1* co-deletion (8 years versus NR years, $p < 0.001$, Fig. 3C) and *MYC* amplification (2.6 years versus NR, $p < 0.001$, Fig. 3D).

4. Discussion

Our results confirm the negative prognostic value of g*BRCA2* mutations for MFS, TTCR and CSS and the enrichment of somatic *BRCA2* loss, *RB1* loss, *BRCA2-RB1* co-deletion and *MYC* amplification in g*BRCA2*-related PC, suggesting that g*BRCA2* mutations associate with an aggressive tumour genotype. Importantly, we have observed that the presence/absence of concurrent genomic events modify the prognosis of *gBRCA2* carriers. Median CSS of *gBRCA2* carriers in our series was 9.1 years, but it rose to 11.3 and 13.4 years in the absence of *BRCA2-RB1* deletion or *MYC* amplification, respectively. Likewise, median CSS in non-carriers was 17.6 years, but decreased to 8 and 2.6 years if *BRCA2-RB1* co-deletion or *MYC* amplification were detected. Our data suggest that the outcomes of carriers and non-carriers seem to be remarkably more similar when tumour variables associated with aggressive PCa phenotypes are considered.

BRCA2 and *RB1* are located on chromosome 13q, 16 Mb apart, and concomitant deletion (homozygous and heterozygous) of the two genes is frequently reported in PCa [19,29]. *BRCA2-RB1* co-deletion has been associated with aggressive biology and enhanced genome instability in pre-clinical models [24]. Here, we show for the first time that this event correlates with shorter CSS, MFS and TTCR in PCa and that it is significantly more frequent in *gBRCA2*-related tumours.

Risbridger et al. [13] have described an increased incidence of IDC in *gBRCA2*-related PCa that we were not able to confirm in a larger series [14], although we noted an association between the presence of IDC and/or CRIB histologies and bi-allelic *BRCA2* alterations regardless of their somatic or germline origen [14]. In the

Table 3
Multivariate analysis (MVA) for Cause specific survival, metastasis free survival and time to castration resistance. All patients and non-metastatic (M0).

Variable in the MVA	All stages patients (n = 198*)				Non-metastatic (M0) patients (n = 151)			
	Cause-specific survival		Time to castration resistance		Cause-specific survival		Metastasis-free survival	
	MVA HR (95% CI)	p Value	MVA HR (95% CI)	p Value	MVA HR (95% CI)	p Value	MVA HR (95% CI)	p Value
Germline *BRCA2* status								
g*BRCA2* versus non-carrier	3.92 (1.40–10.93)	0.009	3.73 (1.29–10.77	0.015	6.30 (1.59–24.92)	0.009	5.56 (1.57–19.75)	0.008
RB1 status								
RB1 deletion versus no	0.91 (0.18–4.58)	0.914	0.77 (0.16–3.89)	0.758	0.59 (0.07–5.35)	0.643	0.45 (0.05–3.96)	0.470
BRCA2-RB1 co-del								
BRCA2-RB1 co-del versus no	4.01 (1.42–11.34)	0.009	2.92 (1.01–8.45)	0.048	7.49 (1.89–29.78)	0.004	5.99 (1.63–22.08)	0.007
MYC status								
MYC amplification versus no	2.57 (1.06–6.25)	0.037	2.21 (0.85–5.73)	0.102	2.74 (0.79–9.53)	0.114	1.91 (0.61–5.95)	0.263
MYC gain versus no	1.82 (0.59–5.66)	0.299	2.21 (0.73–6.73)	0.162	1.51 (0.35–6.42)	0.578	1.95 (0.48–7.87)	0.351
PTEN loss								
PTEN homdel versus no	1.03 (0.51–2.07)	0.930	1.07 (0.51–2.23)	0.858	1.08 (0.44–2.66)	0.868	1.95 (0.48–2.72)	0.769
Age at diagnosis								
> 65 versus ≤ 65 years	0.87 (0.45–1.68)	0.684	0.99 (0.95–1.03)	0.642	0.91 (0.40–2.09)	0.831	0.58 (0.12–2.87)	0.506
PSA at diagnosis								
Above versus below median	1.46 (0.65–3.26)	0.355	2.72 (1.16–6.35)	0.021	1.94 (0.79–4.77)	0.148	2.19 (0.85–5.65)	0.103
Gleason grade group								
≥ 4 versus ≤ 3	6.00 (2.21–16.27)	< 0.001	4.51 (1.72–11.83)	0.002	7.85 (2.41–25.61)	0.001	5.96 (2.34–15.17)	< 0.001
Clinical/pathological stage								
T3/T4 versus T1/T2	0.99 (0.44–2.22)	0.977	1.00 (0.44–2.25)	0.994	1.61 (0.62–4.16)	0.331	3.01 (1.33–6.82)	0.008
N1 at diagnosis								
Yes versus no	0.96 (0.30–3.10)	0.952	0.84 (0.25–2.75)	0.767	1.14 (0.24–5.52)	0.867	2.63 (1.11–6.26)	0.029
M1 at diagnosis								
Yes versus no	12.37 (4.06–37.67)	< 0.001	9.38 (3.31–26.57)	< 0.001	–	–	–	–

co-del, co-deletion; g*BRCA2*, germline *BRCA2*; homdel, homozygous deletion; MVA, multivariate analysis; PSA, prostate-specific antigen.
*Two patients excluded from MVA due to incomplete data for the variables included in the MVA.

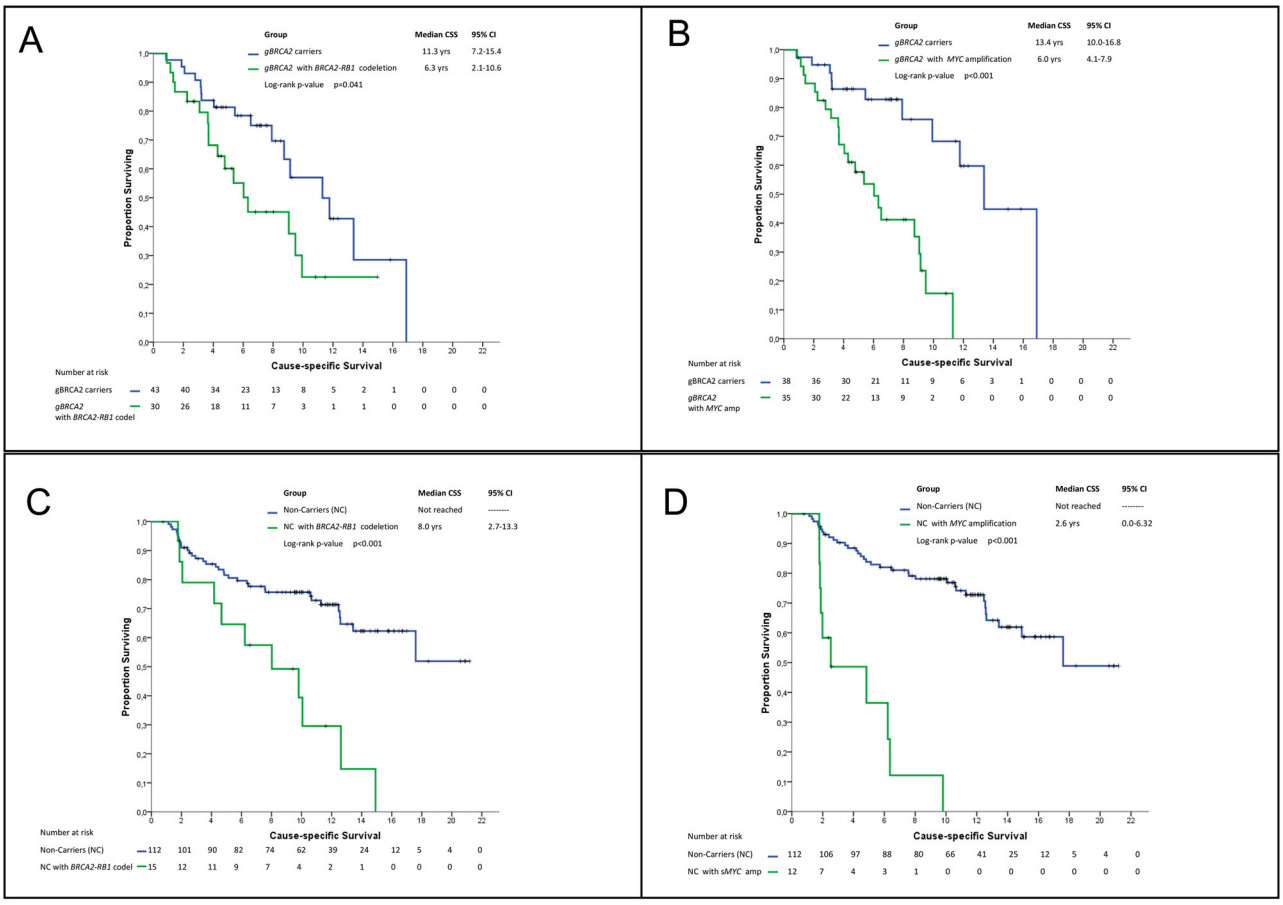

Fig. 3. Cause-specific survival (CSS) from diagnosis of prostate cancer in g*BRCA2* carriers and non-carriers by somatic *BRCA2-RB1* co-deletion and *MYC* amplification. Kaplan-Meier curves for CSS: (A) g*BRCA2* carriers with and without somatic *BRCA2-RB1* co-deletion; (B) g*BRCA2* carriers with and without *MYC* amplification; (C) non-carriers with and without somatic *BRCA2-RB1* co-deletion; (D) non-carriers with and without *MYC* amplification. amp = amplification; co-del = co-deletion; g*BRCA2* = germline *BRCA2*; NC = non-carriers.

current study, we have observed that IDC and CRIB patterns are enriched in tumours with *BRCA2-RB1* co-deletion or with *MYC* amplification in both carriers and non-carriers. All these findings are in line with previous reports of an association between genomic instability and presence of IDC and CRIB in PCa [30]. Furthermore, IDC and CRIB histologies are poor prognosis factors in PCa [31] and Risbridger et al. [13] have already reported a negative impact of IDC on the survival of *gBRCA2* carriers. In our series, IDC and CRIB were both related with shorter CSS, MFS and TTCR in the univariate analysis; however, these associations did not remain significant when other factors, such as *BRCA2-RB1* co-deletion and *MYC* amplification, were considered in the multivariate analyses.

It has consistently been reported that 30−50% of archival FFPE samples fail NGS [32] and copy number calling is challenging in plasma samples with low circulating free DNA tumour fractions [33]. Thus, different approaches for genomic tumour profiling need to be explored. Before NGS became broadly available, FISH was routinely used to assess CNV and it was the method of choice to validate copy number calls in early NGS studies [22]. FISH has recently been used to assess *RB1* CNVs [23] and *BRCA2-RB1* co-deletion [24]. Using FISH, we have been able to analyse CNV in the genes of interest in 94% of our samples. Concordance between NGS and FISH is affected by multiple parameters, including sequencing read depth and the variation size. We compared FISH and NGS results in a subset of tumours (n = 30), and found a strong concordance between both methods for the detection of *BRCA2* deletions and *BRCA2-RB1* co-deletion. This observation warrants further study as FISH could be a simple, fast and low-cost technique to identify *BRCA2* gene deletions which could be missed with other analytical approaches such as NGS from circulating tumour DNA if the tumour fraction is low.

Previous reports have described CNV as the most frequent event in g*BRCA2*-related PCa with enrichment in *BRCA2* and *RB1* deletions and *MYC* amplification [15–17]. A limitation of our study is that we did not analyse other alterations in these genes that could also result in a loss of function. Furthermore, an assessment

of global genomic instability would have been required for a more accurate analysis of the associations and correlations between genomic events. Future studies will be needed to understand how other tumour events affect the outcomes of g*BRCA2* carriers (i.e. *TP53* mutations [35], methylation patterns [17]).

In conclusion, our data suggest that the PCa outcomes of g*BRCA2* carriers are influenced by the presence/absence of concurrent tumour events known to impact PCa prognosis. When these events are considered, the prognosis of g*BRCA2* carriers and non-carriers seem to be more alike than previously reported. Integration of germline and somatic information would refine prognosis estimations and may contribute to design personalised management strategies for *gBRCA2* mutation carriers diagnosed with PCa.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: R.L. declares speaker fees from Roche, Janssen, Sanofi and Bayer, and travel support from Roche, Janssen, Sanofi and Astellas Pharma, E.C. declares honoraria from AstraZeneca, Bayer, Clovis, Janssen, Pfizer and Roche, consulting or Advisory Roles for AstraZeneca, Bayer, Janssen, MSD and Pfizer, research funding from AstraZeneca (Inst), Bayer (Inst) and Janssen (Inst) and travel support from Astra Zeneca, Bayer and Janssen, F.L-C. declares consulting or Advisory Role from Astella Pharma, speaker fees from Janseen, Astellas Pharma, Research Funding from Astellas Pharma (Inst) and travel support from Astellas Pharma and Janssen, C.L. declares honoraria from Roche and travel support from Astellas Pharma and Angelini, N.R-L. declares speaker fees from MSD, consulting or Advisory Role from Ipsen, Astellas Pharma, Bayer, Tesaro, AstraZeneca and Sanofi, research funding from Janssen (Inst), and Pfizer (Inst) and travel support from Janssen, D.L. declares speaker fees from Janssen, Bayer, Astellas Pharma, Sanofi, Pfizer and BMS, consulting or Advisory Role from Sanofi, and travel support from Janssen, and Astellas Pharma, F.Z. declares expert testimony for Sanofi and travel support from Ipsen, J.M. declares consulting or advisory role from Astra Zeneca, Janssen and Roche, speaker's bureau from Astellas Pharma, AstraZeneca and Sanofi and travel support from AstraZeneca, Ipsen and Sanofi, C.C.P. declares consulting or Advisory Role from AstraZeneca and Promega, E.S.A. declares honoraria and consulting or advisory role from Astellas, AstraZeneca, Clovis Oncology, Dendreon, ESSA, Janssen Biothec, Medivation, Merck, Sanofi; Research Funding from Aragon Pharmaceuticals (Inst), Astellas (Inst), AstraZeneca (Inst), Clovis Oncology (Inst), Constellation Pharmaceuticals (Inst), Dendreon (Inst), Exelixis (Inst), Genetech (Inst), Janssen Biotech (Inst), Johnson&Johnson (Inst), Merck (Inst), Millennium (Inst), Novartis (Inst), Sanofi (Inst) and Tokai pharmaceuticals (Inst); co-inventor of a biomarker technology licensed to Quiagen, T.L. declares consulting or advisory role for Janssen and research funding from DeepBio and Ventana Medical Systems, J.R-B. declares consulting or Advisory Role from Janssen, Astellas Pharma and Bayer, and Research Funding from HealthMDx (Inst), S.S. declares honoraria from Amgen, Bristol-Meyers Squibb, Merck, Merck Serono consulting or Advisory Role from AstraZeneca and Merck, research funding from AstraZeneca, Merck Sharp and Dohme, Amgen and Endocyte.

D.O. declares honoraria from Bayer, Janssen and Sanofi, consulting or Advisory Role from AstraZeneca, Bayer, Clovis, Daiichi-Sankyo, Janssen, MSD and Roche, research funding from Astellas (Inst), AstraZeneca (Inst), Bayer (Inst), Genentech (Inst), Janssen (Inst), Medivation (Inst), MSD (Inst), Pfizer (Inst), F. Hoffman-Roche (Inst) and Tokai Pharmaceutics (Inst) and travel support from Bayer, Ipsen, Janssen and Roche.

All remaining authors have declared no conflicts of interest.

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CRediT authorship contribution statement

RL, EC, and DO conceived and designed the study. RL, EC, FL-C, HT, MR-B, NR-L, DL, AC, CA, UA, SA-L, JB, IC, MJ JF, GL, TRyC, EA, BH, JR-B, SS and DO acquired the tissue samples and clinical data. AGP and PGP marked slides amenable for the histologic analysis and genomic studies. DCS and TLL scored samples for the presence of IDC and CRIB patters. EC, IMA, YC-F, DA, PL-C, JM and CCP prepared the samples and/or interpreted sequencing data. RL, FL-C and EC conducted and interpreted FISH results. RL, DL and DO performed the statistical analyses. EC, DO and RL wrote the manuscript in consultation with TL, EA, SS, JM and CCP. All authors provided critical feedback and approved the final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.02.022.

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