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Impact of DNA damage repair defects and aggressive variant features on response to carboplatin-based chemotherapy in metastatic castration-resistant prostate cancer

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Abstract

Platinum-based chemotherapy is not standard of care for unselected or genetically selected metastatic castration-resistant prostate cancer (mCRPC) patients. A retrospective assessment of 71 patients was performed on platinum use in the Netherlands. Genetically unselected patients yielded low response rates. For a predefined subanalysis of all patients with comprehensive next-generation sequencing, 30 patients were grouped based on the presence of pathogenic aberrations in genes associated with DNA damage repair (DDR) or aggressive variant prostate cancer (AVPC). Fourteen patients (47%) were DDR deficient (DDRd), of which seven with inactivated *BRCA2* (BRCA2mut). Six patients classified as AVPC. DDRd patients showed beneficial biochemical response to carboplatin, largely driven by all BRCA2mut patients having >50% prostate-specific antigen (PSA) decline and objective radiographic response. In the wild-type *BRCA2* subgroup, 35% had a >50% PSA decline ($P = .006$) and 16% radiographic response ($P < .001$). Median overall survival was 21 months for BRCA2mut patients vs 7 months ($P = .041$) for those with functional *BRCA2*. AVPC patients demonstrated comparable responses to non-AVPC, including a similar overall survival, despite the poor prognosis for this subgroup. In the scope of the registration of poly-(ADP)-ribose polymerase inhibitors (PARPi) for mCRPC, we provide initial insights on cross-resistance between PARPi and platinum compounds. By combining the literature and our study, we identified 18 patients who received both agents. In this cohort, only BRCA2mut patients treated with

Abbreviations: ALP, alkaline phosphatase; AVPC(-MS), aggressive variant prostate cancer (molecular signature); BRCA2mut, inactivated BRCA2; BRCA2wt, BRCA2 wild-type subgroup; CAPRI, Castration-Resistant Prostate Cancer Registry; CPCT, Center for Personalized Cancer Treatment; DDR, DNA damage repair; DDRd, DDR deficient; DDRp, DDR proficient; FMI, Foundation Medicine; IQR, interquartile range; mCRPC, metastatic Castration-Resistant Prostate Cancer; MMR, mismatch repair; OS, overall survival; PARP, poly-(ADP)-ribose polymerase; PARPi, PARP inhibitors; PSA, prostate-specific antigen; RECIST 1.1, Response Evaluation Criteria in Solid Tumours 1.1; SC-NEPC, small cell (neuroendocrine) prostate cancer; VUS, variant of unknown significance.

platinum first (n = 4), responded to both agents. We confirm that *BRCA2* inactivation is associated with meaningful responses to carboplatin, suggesting a role for both PARPi and platinum-based chemotherapy in preselected mCRPC patients.

KEYWORDS

aggressive variant prostate cancer, carboplatin, DNA repair, homologous recombination, metastatic castration-resistant prostate cancer

1 | BACKGROUND

Treatment options for metastatic castration-resistant prostate cancer (mCRPC) have expanded over the past decades, but long-term overall survival remains limited, with only a minority of patients surviving longer than 3 years after the development of castration resistance.[1] Taxane chemotherapy (docetaxel and more recently cabazitaxel) has shown considerable survival benefit, thus became part of the standard care in patients with mCRPC alongside the next-generation hormonal agents abiraterone and enzalutamide. Unfortunately, therapy resistance inevitably occurs, and novel therapeutic regimens are heavily sought after. After the results from the Phase III PROfound trial, the first poly-(ADP)-ribose polymerase (PARP) inhibitor has recently received FDA approval to be introduced for mCRPC patients harbouring pathogenic defects in DNA damage repair genes [DDR deficient (DDRd)].[2] DDRd is present in up to 20% to 30% of the mCRPC patients.[3] These aberrations are common in genes (in)directly involved in the homologous recombination pathway, such as *BRCA1/2*, *ATM* and *CHEK2* (amongst others). Inherited or somatic mutations in these genes involved in the detection and repair of DNA strand breaks lead to genomic instability, commonly resulting in a more aggressive disease and worse clinical outcomes after treatment with next-generation hormonal agents or taxanes.[3] In this DDRd population, clinical trials are investigating whether platinum compounds may also be effective (NCT03652493, NCT02985021, NCT04038502, NCT02598895, NCT03442556).

Platinum compounds in mCRPC have been evaluated in clinical trials over the last three decades. A recent systematic review and meta-analysis illustrated the use of platinum-based chemotherapy, commonly in combination with taxanes.[4] Results from a limited number of randomised controlled trials with platinum compounds, with unselected patients, have shown no overall survival benefit when compared to standard of care chemotherapies.[5-7] Therefore, platinum-containing regimens are not current standard of care for molecularly unselected patients with mCRPC. In patients with DDRd tumours, multiple retrospective studies and case series have shown clinical meaningful responses to platinum compounds.[8-10] A recent study demonstrated biochemical responses in half of the patients with DDRd, particularly those with *BRCA2* aberrations, as well as those with alterations in *PALB2*, *FANCA* or *CDK12*.[11] Patients with small cell (neuroendocrine) prostate cancer (SC-NEPC) appear to show superior disease control with platinum-based regimens over standard of care taxane chemotherapy for mCRPC.[12,13] A recent study identified patients with aggressive variant prostate cancer (AVPC), as a subtype defined as either SC-NEPC or prostate adenocarcinoma with clinicopathological features associated with poor outcome, to benefit most from platinum-based regimens.[14] This subtype is molecularly characterised by combined alterations in *RB1*, *TP53*, and/or *PTEN*.[15] The beneficial responses of distinct subtypes underscore that platinum-based chemotherapy may have a role in postponing progression and improving quality of life in selected patient groups.

In this report, we assessed real-world use, and outcomes from platinum-based chemotherapy in the Netherlands. Next, we examined the association between DDR alterations and platinum sensitivity in a retrospective study cohort with comprehensive tumour and/or germline sequencing data available. We describe responsiveness of patients with germline and/or somatic alterations in a range of DDR genes, focusing on alterations in *BRCA2*, molecular features of AVPC and exceptional responders. Lastly, in the wake of registration of multiple PARP inhibitors (PARPi) for mCRPC, it is important to investigate whether antitumour efficacy remains to PARPi after exposure and resistance to platinum, and vice versa. We provide new data on this cross-resistance between platinum chemotherapy and PARPi.

What's new?

Platinum-based chemotherapy is not standard of care for unselected or genetically-selected patients with metastatic castration-resistant prostate cancer (mCRPC). However, several studies have shown that platinum-based chemotherapy may still have a role in postponing progression in selected patient groups. This new study investigating DNA damage repair gene alterations and response to platinum-based chemotherapy provides evidence that deep and durable responses are primarily associated with patients harbouring *BRCA2* inactivation. Based on these data and the limited available literature, platinum-based chemotherapy followed by PARP inhibition is potentially emerging as the optimal treatment sequence in pre-selected mCRPC patients.

2 | METHODS

2.1 | Patient population and study design

The main study population of this retrospective cohort study consists of all mCRPC patients who started with platinum-based chemotherapy in the Radboudumc between July 2014 and January 1, 2020 (n = 36). The primary objective was to evaluate platinum responsiveness in patients with diverse alterations in genes directly or indirectly involved in DDR. All 30 patients were included that had undergone comprehensive genetic analysis by targeted or whole-genome sequencing of primary or metastatic tumour tissue. For the comparison of outcome from platinum-based chemotherapy, between a tertiary referral centre cohort (Radboudumc) and a real-world CRPC cohort, 36 patients in the Castration-Resistant Prostate Cancer Registry (CAPRI) were identified. One patient was present in both cohorts. CAPRI is a population-based, observational, retrospective registry based on pseudonymised patient files from CRPC patients from 20 hospitals in the Netherlands, balanced geographically and by type of hospital.[1] Identified patients from CAPRI received platinum-based chemotherapy somewhere between January 2010 and December 2017, with follow-up until January 2019. CAPRI is registered in the Dutch Trial Registry as NTR3591.

Demographic, clinical and histopathological data as well as diagnostic parameters were extracted from the electronic patient records. In the Radboudumc cohort, a structured analysis was performed based on the presence of at least one (likely) pathogenic somatic and/or germline aberration in *BRCA2* (BRCA2mut) as well as a predefined panel of genes directly or indirectly involved in DDR (DDRd), and a DDR proficient group (DDRp). All data were stored anonymously. Patient follow-up for the Radboudumc was until the May 01, 2020, lost-to-follow-up or death.

The Dutch cohort of patients from CAPRI was compared to the Radboudumc cohort. An AVPC population was identified within the Radboudumc cohort based on a molecular signature (AVPC-MS). Cases with exceptional responses, defined as a prostate-specific antigen (PSA) decline of more than 90% after platinum-based chemotherapy, were analysed more comprehensively with additional tests. In addition, patients who received both PARPi and platinum compounds were identified for exploratory analysis and included until June 2020.

2.2 | Genetic testing

Archived or fresh tumour material was sequenced by either a non-profit service provider (Center for Personalized Cancer Treatment [CPCT]), or fee for service provider (Foundation Medicine [FMI]) and often additionally sequenced by a custom in-house NGS platform.[16,17] The Pathogenicity of the alterations was analysed (Peter H. J. Slootbeek) and reassessed in a blinded manner by an experienced clinical molecular biologist (Leonie I. Kroeze) at the Department of Pathology, according to the guidelines for sequence variants.[18] Gene disruptions were classified as variant of unknown significance (VUS). The predefined genes of interest for the DDRd group consisted of: *ATM*, *ATR*, *BAP1*, *BARD1*, *BRCA1*, *BRCA2*, *BARD1*, *BRIP1*, *CDH1*, *CDK12*, *CHEK1*, *CHEK2*, *ERCC2*, *ERCC4*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *MRE11A*, *NBN*, *PALB2*, *PARP1*, *PARP2*, *PARP3*, *PPP2R2A*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD52*, *RAD54L* and *RPA1*. To meet the AVPC-MS, deleterious aberrations in at least two of *TP53*, *RB1* or *PTEN* needed to be present in the biopsied tumour material.[15] The DNA mismatch repair (MMR) genes *MSH2*, *MSH6*, *MLH1* and *PMS2*, not in the predefined DDRd list, were nevertheless included in the results, to give full insight on the DDRd genomic profile of the study subjects, even though MMR deficiency alone is not associated with response to platinum or PARPi.

2.3 | Study outcomes

Biochemical (PSA) response to platinum-based chemotherapy was the primary outcome of this study. Patients with baseline PSA values below 2 μg/L were excluded for biochemical analyses. PSA responses were assessed according to the Prostate Cancer Clinical Trials Working Group criteria,[19] with assessment of declines of >50% (PSA_{50}) and >90% (PSA_{90}). If PSA declined at any time from initiation of platinum-based therapy until next therapy, this value was used to calculate maximal change. If possible, early PSA rises before 12 weeks of therapy were ignored. Patients with early biochemical, radiological or clinical progression, and therefore a missing 12-week PSA, were defined as PSA_{50} nonresponders in the analyses of PSA change at 12 (±2) weeks. Secondary outcomes were overall survival (OS) and best radiographic response. OS was defined as time from initiation of platinum-based chemotherapy trough date of death from any cause or censored at last date of follow-up if alive. Best radiographic response was classified according to Response Evaluation Criteria in Solid Tumours (RECIST 1.1) criteria.[20]

2.4 | Statistical analyses

Baseline characteristics and biochemical outcomes between the subgroups were compared using the Pearson chi-square or Fisher exact test for categorical variables and the nonparametric Mann-Whitney *U* test for continuous variables. Kaplan-Meier curves were used to visualise the survival and if adequate, OS was compared using univariable Cox proportional hazards models. A two-sided *P* value <.05 was considered significant. Factors associated with response were identified using a univariable and multivariable linear regression. To adhere to the presumptions of the regression model, the maximal PSA change underwent additive and square root transformation. Variables with a *P* value <.20 in the univariable analyses were included for a multivariable linear regression analysis. The multivariable model was fitted by including variables in the model with a forward selection strategy with an entry level of 0.05 at every step. Statistical tests were performed using SPSS software (v25).

3 | RESULTS

3.1 | Baseline characteristics and efficacy of platinum chemotherapy in the CAPRI and Radboudumc cohorts

In the Dutch population-based CAPRI, 36 patients were treated with platinum-based chemotherapy in a time frame of 8 years. No data were available on their molecular underpinnings. From the Radboudumc, the same number of patients (36) was included. One patient treated in the Radboudumc was also registered in CAPRI. The CAPRI and the Radboudumc cohort were considered unmatched populations (Table S1). Baseline alkaline phosphatase (ALP) and PSA were higher in the Radboudumc cohort when compared to the CAPRI cohort (ALP: 234 vs 126 U/L; PSA: 105 vs 52 μg/L).

For the CAPRI cohort, the median age at start of platinum-based chemotherapy was 70 years. Patients were treated with cisplatin in 19% of the cases and with carboplatin in 81%, two patients received the latter as monotherapy. The most frequent combination partners in the remaining cases were docetaxel (33%) and etoposide (33%) (Table S2). The proportion of patients with a PSA_{50} response was 16.7% in this cohort (Table S3). Median OS from start of platinum-based chemotherapy was 7.0 months (Figure S1).

The median age of the Radboudumc cohort was 62 years at initiation of platinum-based chemotherapy. All patients were treated with carboplatin, 61% in combination with cabazitaxel, 11% received this

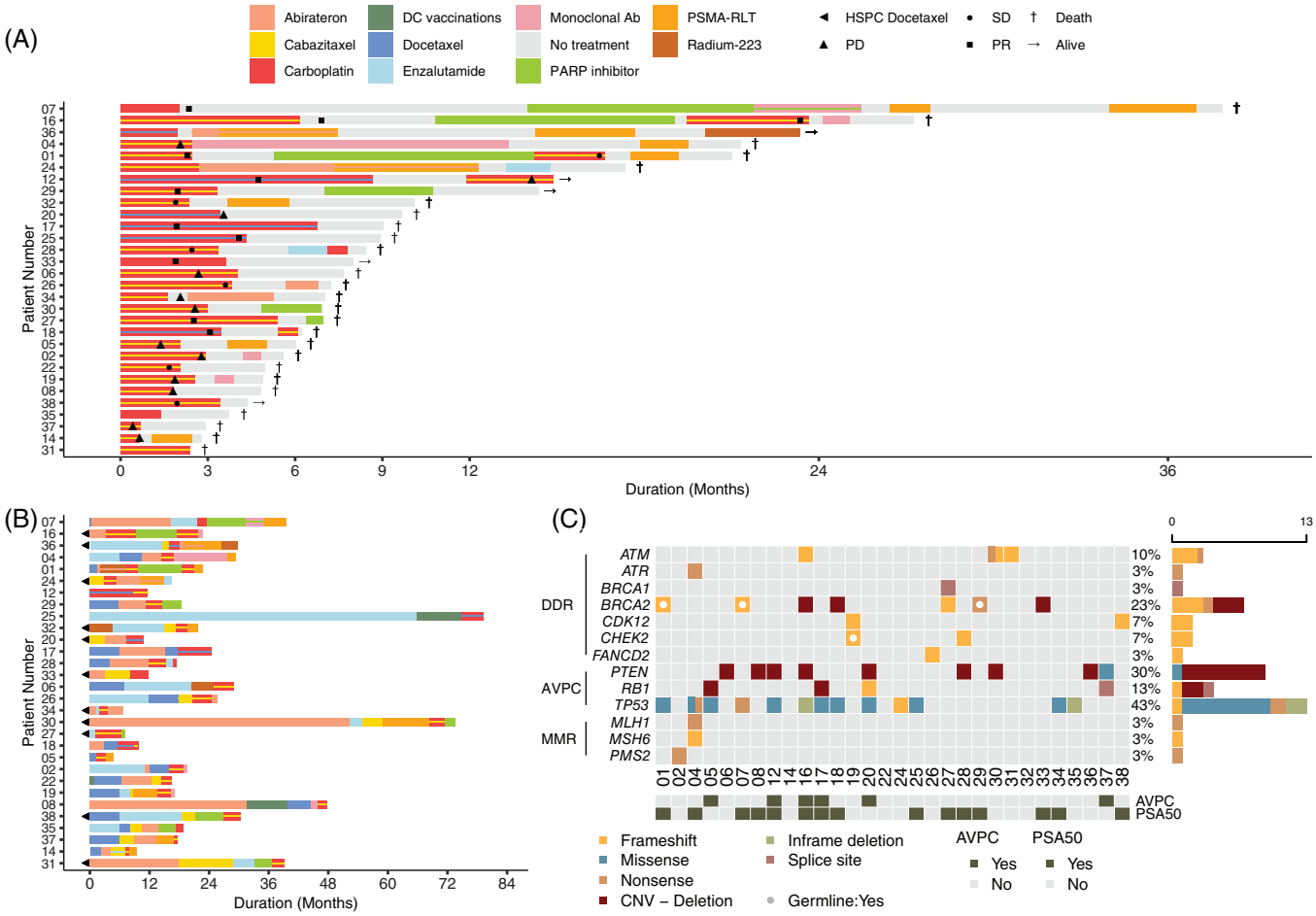

FIGURE 1 Treatments and mutational landscape of the Radboudumc cohort. A, Swimmerplot presenting the duration on platinum-based chemotherapies and subsequent therapies until death or last follow-up. The colour scheme represents all therapies and the symbols indicate best radiographic response or status at last follow-up. B, Swimmerplot starting at date of mCRPC and visualising only the period on treatment for each therapy given. The symbol indicates the administration of upfront docetaxel in hormone sensitive setting. Study subject 30 received a trial with docetaxel and an inhibitor of cytotoxic T-lymphocyte-associated Antigen 4 in hormone sensitive setting. C, Oncoplot showing the pathogenic or likely pathogenic alterations of the Radboudumc cohort in predefined genes directly or indirectly involved with DNA damage repair (DDR), genes in the aggressive-variant prostate cancer molecular signature (AVPC-MS), and mismatch repair (MMR) genes. The colour of the boxes represent the effect of the mutation. Split boxes indicate two different alterations in the same gene. Germline mutations are accentuated. The AVPC bar identifies patients meeting the AVPC-MS criteria and PSA50 identifies patients having a >50% PSA decline to carboplatin. CNV, copy number variant; PD, progressive disease; PR, partial response; SD, stable disease [Color figure can be viewed at wileyonlinelibrary.com]

as monotherapy. PSA_{50} response was observed in 47.1% of the patients from the Radboudumc cohort. OS was comparable to the CAPRI cohort with a median of 7.3 months.

3.2 | Molecular landscape of patients included in the Radboudumc cohort

Of the 36 patients in the Radboudumc cohort, 30 underwent comprehensive genetic analysis. All therapies since diagnosis of castration-resistance for the molecularly profiled patients are depicted in Figure 1. In general, patients were heavily pretreated and received a median of three lines of systemic therapies between castration-resistance and initiation of platinum-based therapy. All but two (Subjects 12 and 25) underwent prior treatment with a taxane. Fourteen of the 30 evaluable patients (47%) harboured deleterious genetic alterations in the predefined genes directly or indirectly involved in DDR (DDRd), half of these patients had a (likely) pathogenic *BRCA2* alteration (BRCA2mut) (Figure 1C). Other pathogenic alterations were identified in *BRCA1* (n = 1), *ATM* (n = 3), *ATR* (n = 1), *CDK12* (n = 2), *CHEK2* (n = 2), *FANCD2* (n = 1). The remaining 16 patients (53%) were genetically DDRp, of which in five patients a VUS was detected in one of the predefined DDR genes (Table S4). Six patients (20%) showed a molecular signature of AVPC (AVPC-MS) with inactivation of ≥2 associated genes (*TP53*, *RB1*, *PTEN*), of which subject 16 was also *BRCA2* deficient. Two patients (7%) showed inactivation of one or more MMR genes and one of them was also DDRd. Microsatellite instability was detected in both patients. Out of the 48 alterations depicted in Figure 1C, 35 (73%) were proven to be somatic. Three mutations in *BRCA2* and one in *CHEK2* were germline. For the remaining nine alterations the germline status was not assessed (Table S4). Study subject 18 had a loss of one allele of *BRCA2* and the zygosity of the *BRCA2* loss of study subject 33 was unknown. All other copy number variants were homozygous deletions.

3.3 | Efficacy of carboplatin-based chemotherapy in molecular subtypes of mCRPC

3.3.1 | Baseline comparison DDRd vs DDRp subgroups

At baseline, clinical characteristics and demographics did not differ significantly between the DDRd and DDRp subgroups (Table S5). Median PSA and ALP at start of therapy were higher in the DDRd subgroup when compared to DDRp patients (PSA: 300 vs 100 μg/L, P = .031; ALP 278 vs 146 U/L, P = .036, respectively). In both subgroups, cabazitaxel was the preferred combination partner of platinum (Table S2). For the BRCA2mut subgroup, the median time from initial diagnosis to initiation of platinum-based therapy was 30 months, when compared to 56 months for the *BRCA2* wild-type (BRCA2wt) subgroup (P = .037). At baseline, these subgroups were not statistically different.

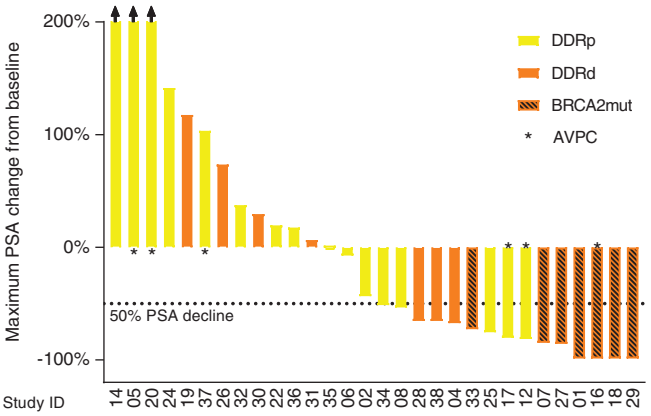

FIGURE 2 Waterfallplot of the best PSA change to carboplatin. Colour indicates whether a study subject is in the DDRd or DDRp subgroup. BRCA2mut and AVPC patients are marked accordingly. AVPC, aggressive-variant prostate cancer; DDRd, DNA damage repair deficient; DDRp, DNA damage repair proficient; PSA, prostate-specific antigen [Color figure can be viewed at wileyonlinelibrary.com]

3.3.2 | Outcome

Of the DDRd patients, 10 (71%) had a PSA_{50} response when compared to 5 (31%) in the DDRp subgroup (P = .028, Figure 2). All other PSA outcome metrics also demonstrated a more beneficial response to carboplatin for the DDRd subgroup (Table 1). Best radiographic response according to RECIST 1.1 criteria (Table 2) and OS did not statistically differ between the DDRd and DDRp subgroups (OS 8.4 vs 7.0 months, respectively; hazard ratio [HR] 1.720; 95% confidence interval [CI] 0.732-4.043; P = .214, Figure 3).

Next, we focussed on the *BRCA2* status of the 30 molecularly profiled patients and compared the seven BRCA2mut patients to the 23 BRCA2wt patients. All outcome measures significantly favoured the BRCA2mut subgroup (Table 1). All patients in the BRCA2mut subgroup witnessed a PSA_{50} response, while this was 35% in the BRCA2wt subgroup, P = .006. Moreover, all seven BRCA2mut patients showed a radiographic partial response when compared to 16% in the BRCA2wt subgroup (P < .001, Table 2). Median OS from start of carboplatin was almost 14 months longer for the BRCA2mut subgroup when compared to the BRCA2wt subgroup (21.1 vs 7.3 months; HR 3.588; 95% CI 1.051-12.248; P = .041, Figure 3). In a multivariable analyses *BRCA2* status emerged as the independent predictor for PSA response (Table S6).

Of the 15 patients in the Radboudumc cohort with a PSA_{50}, 27% (n = 4) were defined as exceptional responders, with a PSA_{90} response. All harboured at least one pathogenic *BRCA2* aberration. Their median radiographic progression-free survival was 6 months (range 3-10 months). Subject 18 did not classify as BRCA2mut based on the routine next-generation sequencing, but a loss of heterozygosity of *BRCA2* was implied. An additional genetic analyses with multiplex ligation-dependent probe amplification confirmed a loss of one allele of *BRCA2*.

TABLE 1 PSA response to carboplatin, by DDR and *BRCA2* status

	Number of patients (valid %) or median [IQR]			Number of patients (valid %) or median [IQR]		
	DDRd, N = 14	DDRp, N = 16	*P* value	BRCA2mut, N = 7	BRCA2wt, N = 23	*P* value
Maximal PSA change (%)	−70 [−99 to +13]	8 [−53 to +133]	**.013**	−99 [−99 to −85]	7 [−66 to +103]	**<.001**
Patients with >50% maximal PSA decline	10 (71.4)	5 (31.3)	**.028**	7 (100.0)	8 (34.8)	**.006**
Patients with >90% maximal PSA decline	4 (28.6)	0 (0.0)	**.037**	4 (57.1)	0 (0.0)	**.001**
PSA change at 12 weeks (%)	−66 [−97 to −14]	0 [−61 to +186]	**.021**	−96 [−99 to −62]	0 [−65 to +131]	**.002**
Patients with >50% PSA decline at 12 weeks	10 (71.4)	3 (21.4)	**.008**	7 (100.0)	6 (28.6)	**.001**

Abbreviations: BRCA2mut, inactivated *BRCA2*; BRCA2wt, *BRCA2* wild-type subgroup; DDR, DNA damage repair; DDRd, DDR deficient; DDRp, DDR proficient; IQR, interquartile range; PSA, prostate-specific antigen. Values in bold are significant (*P* < .05).

TABLE 2 Best radiographic response to carboplatin, by DDR, *BRCA2* and AVPC status

Subgroups	Best radiographic response[a]	Number of patients (valid %)	*P* value
DDRd vs DDRp	Partial response	7 (58.3) vs 3 (21.4)	.122
	Stable disease	2 (16.7) vs 2 (14.3)	
	Progressive disease	3 (25.0) vs 9 (64.3)	
BRCA2mut vs BRCA2wt	Partial response	7 (100.0) vs 3 (15.8)	**<.001**
	Stable disease	- vs 4 (21.1)	
	Progressive disease	- vs 12 (63.2)	
AVPC vs non-AVPC	Partial response	3 (50.0) vs 7 (35.0)	.690
	Stable disease	- vs 4 (20.0)	
	Progressive disease	3 (50.0) vs 9 (45.0)	

Abbreviations: AVPC, aggressive-variant prostate cancer; BRCA2mut, inactivated *BRCA2*; BRCA2wt, *BRCA2* wild-type subgroup; DDR, DNA damage repair; DDRd, DDR deficient; DDRp, DDR proficient. Values in bold are significant (*P* < .05).
[a]According to Response Evaluation Criteria in Solid Tumours (RECIST1.1).

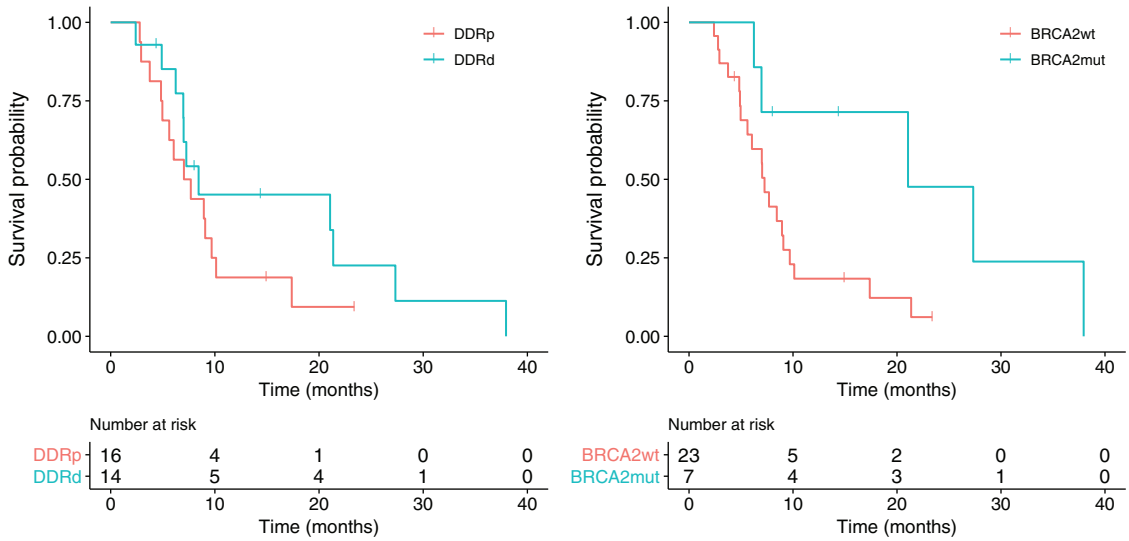

FIGURE 3 Kaplan–Meier curves visualising overall survival (OS) for DDRp vs DDRd (left) and BRCA2wt vs BRCA2mut (right). BRCA2mut, inactivated *BRCA2*; BRCA2wt, *BRCA2* wild-type subgroup; DDRd, DNA damage repair deficient; DDRp, DNA damage repair proficient [Color figure can be viewed at wileyonlinelibrary.com]

3.3.3 | Aggressive variant prostate cancer

We identified six AVPC patients, defined by molecular signatures, with inactivation of *TP53*, *RB1* and *PTEN* (n = 1), *TP53* and *RB1* (n = 2), *TP53* and *PTEN* (n = 2) and *PTEN* and *RB1* (n = 1). These six AVPC patients were of younger age compared to the 24 patients in the non-AVPC subgroup (median 57 vs 63 years, P = .029). Other baseline characteristics were comparable (Table S7). The duration of platinum-based treatment did not differ significantly (Table S8).

PSA responses to carboplatin-based chemotherapy were comparable between the AVPC and the non-AVPC subgroup (Table S9). In both groups, half of the patients had a PSA_{50} response to treatment with carboplatin. The AVPC subgroup showed a 1.8-month numerical median OS benefit over the non-AVPC subgroup (9.1 vs 7.3 months,

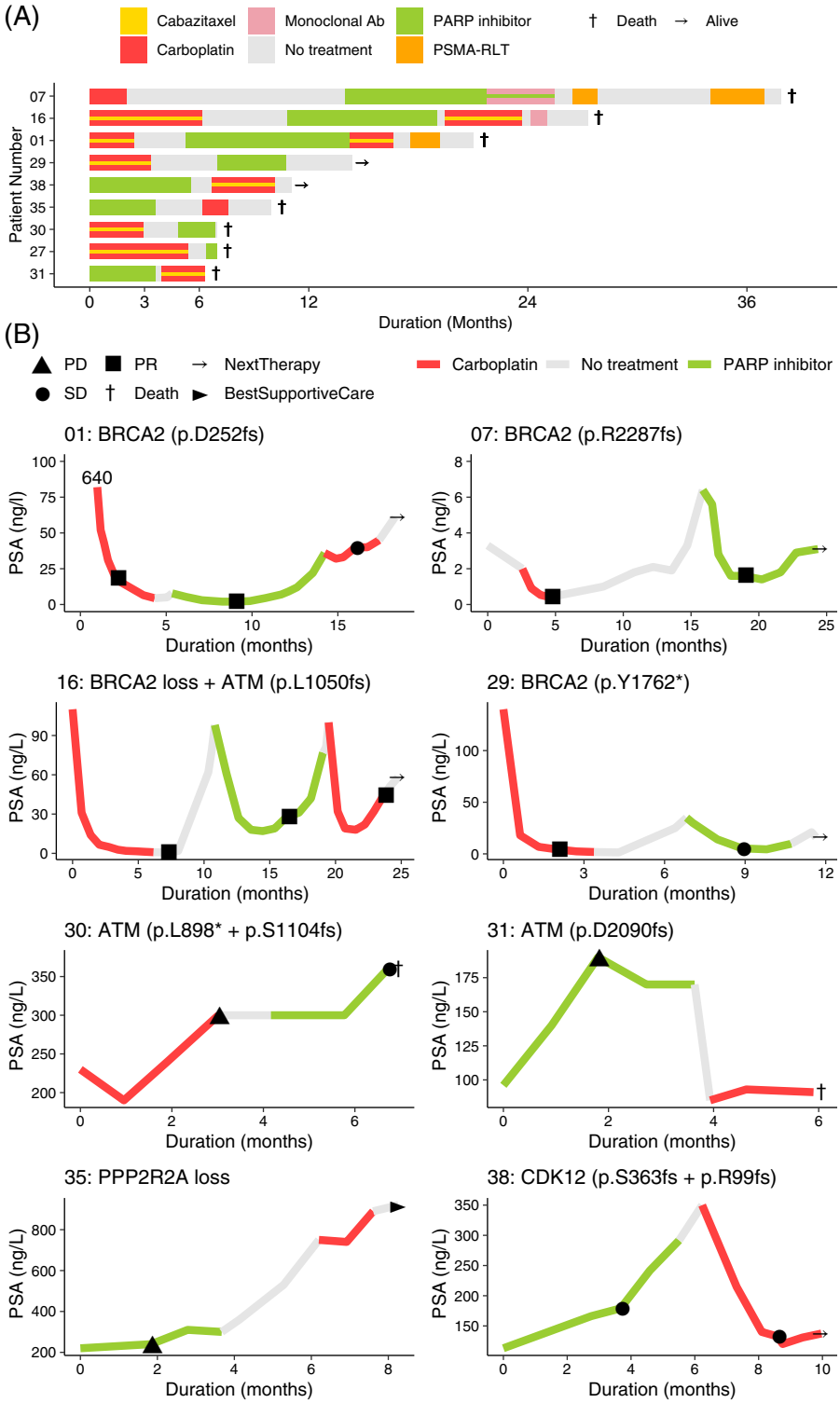

FIGURE 4 A, Swimmerplot showing a sub cohort of patients who received both platinum compounds and PARP inhibitors. The colour scheme represents therapies. B, Individual patient plots visualising the PSA kinetics starting from first targeted therapy till discontinuation of sequential therapy, for patients who received both platinum compound and PARP inhibitors (PARPi). Above each plot, the subject ID and mutation for enrolment to PARPi are indicated. The line is coloured coded based on treatment and the symbols indicate best radiographic response or further disease course. The PSA of study subject 01 starts at 640 ng/L. *Note*: Not all *y*-axes extend to zero. PARP, poly-(ADP)-ribose polymerase; PD, progressive disease; PR, partial response; PSA, prostate-specific antigen; SD, stable disease [Color figure can be viewed at wileyonlinelibrary.com]

respectively; Figure S1). Best radiographic response was comparable between both subgroups (Table 2). These comparable responses to carboplatin-based chemotherapy are in contrast with the tendency of AVPC patients to show worse outcome metrics to standard of care therapy, and a poorer survival compared to non-AVPC patients.

3.4 | Responses in patients with both PARP inhibitors and platinum-based therapy

In the scope of registration of PARPi for mCRPC, it is key to have insight in possible cross-resistance between platinum compounds and PARPi. Here, we evaluated response rates to carboplatin and PARPi in a small subcohort; nine patients from the Radboudumc cohort were treated with PARPi during the course of their disease, all subsequent or prior to treatment with carboplatin (Figure 4A). Subject 27 was excluded from the analyses due to a lack of adequate follow-up after PARPi. The remaining eight patients harboured alterations in *BRCA2* (n = 4), *ATM* (n = 2), *PPP2R2A* (n = 1, not in predefined DDR panel) and *CDK12* (n = 1). Subject 16 harboured pathogenic alterations in *BRCA2* as well as *ATM*. All four BRCA2mut patients with initial PSA_{50} response to carboplatin, also showed a PSA_{50} response to subsequent PARPi (Figure 4B). Moreover, every BRCA2mut patient had a radiographic response to initial platinum therapy and three out of four also to subsequent PARPi therapy. Notably, study Subject 16 (*BRCA2* loss and *ATM* frame shift mutation) showed outstanding response to consecutively carboplatin, PARPi and carboplatin rechallenge, with maximum PSA decline of 99%, 83% and 82%, respectively. Radiographically, partial response was observed following all three therapies in this patient.

We assessed the literature for additional patient data, and extended the inclusion period for patients within our institute for an exploratory analysis of cross-resistance between these compounds (Table S10). In total, we could identify 18 patients, of which 2 patients from our institute who received their first dose of platinum-based chemotherapy after the inclusion period of our main study. PSA response was missing for one patient and radiographic response for four. Eight patients (47%) showed differential responses, showing either PSA_{50} responsiveness to platinum or PARPi, but not to the second agent. There were five patients (29%) with concordance in nonresponsiveness and four (24%) with concordance in responsiveness, of those all four BRCA2mut patients treated with platinum first and subsequently with PARPi. In the reverse sequence (PARPi followed by platinum-based chemotherapy) none of the five patients with response to PARPi responded to sequential platinum-based chemotherapy, while 43% (3/7) of the patients without response to PARPi responded to platinum. Only one of the four patients with an *ATM* mutation had a PSA_{50} response to PARPi and none had such response to platinum.

4 | DISCUSSION

We have described the real-world use and outcomes of treatment with platinum compounds in mCRPC patients in the Netherlands. A molecularly unselected patient population of the nationwide Dutch CAPRI cohort yielded low response rates to platinum-based treatment. The CAPRI and the Radboudumc cohort were considered unmatched populations. It cannot be excluded that differences in response to platinum-based chemotherapy might be influenced by confounders like age at start of platinum-based therapy or the combination partner of the platinum agent. In the Radboudumc cohort, we specifically investigated the response of patients with or without DNA damage repair defects or a molecular signature of AVPC. These results supported existing evidence on antitumour activity of platinum combination therapy in patients with DDRd prostate cancer. More than 70% of the patients in the DDRd subgroup demonstrated a biochemical response to carboplatin. Half of the DDRd patients harboured a pathogenic *BRCA2* alteration, and particularly these patients benefitted from carboplatin-based chemotherapy as shown by higher objective radiographic response rates and longer OS on top of a PSA_{50} response for all BRCA2mut patients. Despite their poor prognosis, AVPC patients had comparable outcomes as non-AVPC patients, suggesting a possible benefit of platinum-based chemotherapy.

DDRd, and particularly genes associated with homologous recombination, are suggested as plausible biomarkers for platinum responses in mCRPC.[10] A recent study by Mota et al. described a higher proportion of PSA_{50} responses to platinum-based chemotherapy in the DDRd group when compared to DDRp patients.[11] In our study, a similar outcome was found, although the biochemical response of DDRd patients to carboplatin was largely driven by the BRCA2mut subgroup, with all seven BRCA2mut patients showing a PSA_{50} response. In comparison, four out of six (67%) BRCA2mut patients in the study of Mota et al. had a similar response. This exceptional responsiveness of *BRCA2* inactivated patients to platinum-based chemotherapy was previously described in a case series of three patients by Cheng et al, supported by an in vitro and in vivo study.[8,9] We strongly underline these findings, with all BRCA2mut patients in our study also demonstrating an objective radiological response with clinical benefit suggested by a significant longer OS witnessed in this subgroup.

Novel opportunities for patients with mCRPC have arisen due to advances in molecular characterisation and personalised medicine. One avenue is on targeting DDRd. Besides platinum compounds, PARPi have shown very promising results in Phase 1 and 2 trials.[21-23] Recently, these findings were validated in the Phase III PROfound trial.[2] Patients with *BRCA2* alterations seemed to benefit most from the inhibition and trapping of the PARP-1 and PARP-2 enzymes. Through the inhibition of repair of single strand DNA breaks, PARPi add to the formation of lethal double strand breaks, in the presence of DDRd, leading to apoptosis and programmed cell death. The potential benefit of the combination of PARPi and platinum compounds over PARPi alone appears encouraging; however, this is not yet investigated in mCRPC.[24]

At this moment, the question remains how-to best sequence platinum compounds with PARPi in DDRd patients with mCRPC. Particularly, after registration of PARP inhibitors, it is expected that PARPi

will be favoured over platinum compounds. Issues of cross-resistance need to be adequately addressed. We evaluated responses to these therapies in patients from our institute and from Mota et al.[11] Only four patients had a PSA_{50} response to both therapies, all *BRCA2* mutated and treated with platinum first and subsequently PARPi. In the reverse sequence (PARPi – platinum), all patients who responded to PARPi did not respond to platinum compounds, but almost half of the nonresponders did remain responsive to platinum. Notably, *ATM* mutated patients yielded low response to both PARPi and platinum-based chemotherapy. This is in line with early data suggesting that response to platinum and PARPi is more commonly seen in *BRCA2* mutated patients when compared to *ATM* mutated patients.[25] Our combined sample size of 18 patients was relatively small and insufficient to draw conclusions on the order of applying these two types of therapy. More data are needed, preferably from randomised trials with platinum-based chemotherapy vs PARPi with crossover design, to provide recommendations on optimal treatment planning on a DDRd gene-specific basis.

In end-stage castration-resistant disease, patients commonly develop poor prognostic disease features associated with AVPC, for which effective therapy is much sought after. Recently, Corn et al have shown that multimodality chemotherapy of carboplatin in combination with cabazitaxel, improved radiographic progression-free survival, but not OS, over cabazitaxel alone in a molecularly unselected patient cohort.[14] Post hoc analyses suggested that AVPC-MS-positive patients derived most benefit from the combination, indicated by both radiographic progression-free survival (2.2 vs 6.0 months, $P < .05$) and OS benefit (9.9 vs 17.4 months, $P < .05$). Our results were in line with this study, demonstrating comparable OS and PSA responses for AVPC and non-AVPC patients treated with carboplatin. In contrast to our study, no clear benefit for *BRCA2*-deficient patients was found in the study by Corn et al, possibly due to challenges and difficulties in utilising ctDNA for the detection of the full spectrum of *BRCA2* alterations, particularly deletions.

AVPC and DDRd patients may both benefit from platinum-based chemotherapy trough a similar method of action. *TP53* and *PTEN* are part of the AVPC-MS. P53 is a mediator of DDR, since it promotes cell cycle arrest and provides time for the DDR machinery to repair the damage, or induces senescence/cell death in response to DNA damage.[26,27] Although not yet confirmed in a clinical setting, *PTEN* alterations in an in vitro setting appear to influence homologous repair, in such a way that *PTEN*-deficient prostate cancer cells are susceptible to treatment with PARPi.[28,29] Moreover, a preclinical study demonstrated in vitro and in vivo, that a senescence response to PARPi triggered by *PTEN* deficiency in prostate cancer cells, transformed to apoptosis in case of additional p53 dysfunction.[30] These two molecular subtypes may both have an increased vulnerability to the DNA crosslinking and strand breakage of platinum compounds through the accumulation of cell damage caused by the inability to correctly restore the DNA sequence.

Recently, SLFN11 expression has also been suggested as a predictive biomarker for response to DNA-damaging agents. This DNA/RNA helicase, overexpressed in approximately 45% of the mCRPC tumours, was associated with a longer progression-free survival in CRPC patients upon treatment with platinum-based chemotherapy in a recent study by Conteduca et al..[31] In a multivariable analysis it emerged as an independent predictor for a longer progression-free survival; no detailed breakdown on gene level was given of the 17 patients with DDRd, therefore it is hard to interpret whether SLFN11 may outperform *BRCA2*. Homologous recombination deficiency signatures may also outperform *BRCA2* as predictive biomarker, as *BRCA1/2* may be silenced by methylation or posttranslational processes. Approximately 30% of mCRPC patients with a homologous recombination deficiency signature lacked alterations in *BRCA2* and other key homologous recombination genes.[32] Also, the neutrophil to lymphocyte ratio, a well-known prognostic factor in mCRPC was shown to be independently associated with worse outcomes after platinum therapy.[33]

Our results should be viewed in the context of several limitations. The retrospective nature of this study allows for selection bias. No prior power analysis was performed, and the sample size is based on consecutively enrolling patients treated with platinum-based chemotherapy. The size of the cohort and lack of randomisation allow baseline imbalances such as higher PSA and ALP levels for DDRd patients, which might influence the response measures. Genetic analysis was performed by three different next generation sequencing platforms. We tried to mitigate differences in pathogenicity reporting, by reassessing the pathogenicity from third-party providers, which was performed in a blinded manner by an experienced clinical molecular biologist. Due to the nature of our retrospective study, which lacked a comparator arm without use of platinum chemotherapy, the specified OS results and intergroup comparison were in the context of DDRd gene-specific prognosis.

5 | CONCLUSIONS

Our study adds to a body of evidence that platinum compounds are beneficial for mCRPC patients pre-selected for *BRCA2* alterations. These data are of importance to help protect patients from unnecessary toxicity and adverse events, with platinum-based drugs commonly administered as a last resort therapy. The individual benefit of infrequent alterations in DNA damage repair genes, including *PALB2*, *BRIP1* and others, to platinum chemotherapy is unknown. Carboplatin, when given prior to PARPi, did not appear to diminish efficacy to subsequent PARPi, however more data is required to gain insight on cross-resistance. AVPC patients also seemed to benefit from platinum compounds indicated by comparable responses for this prognostic poor subgroup. This study provides novel data which may help guide the path towards improved precision medicine for patients with mCRPC.

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CONFLICT OF INTERESTS

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DATA AVAILABILITY STATEMENT

The NGS data for all patients included in this study are not available in a structured public repository. The whole-genome sequencing data, for which we will supply identifiers to help in this request, are freely available for academic use from the Hartwig Medical Foundation through standardised procedures and request forms can be found at https://www.hartwigmedicalfoundation.nl. All other NGS data are available upon reasonable request from the corresponding author, Niven Mehra. The (likely) pathogenic variants and variants of unknown significance of all DDR genes identified in this study and other data supporting the findings of this study are available as supplementary material.

ETHICS STATEMENT

This study (CMO-2019-5103) was approved by the Medical Review Ethics Committee Arnhem-Nijmegen, the Netherlands. All sequenced patients provided written informed consent. The CAPRI study was approved by their local medical ethics committee and hospital board.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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