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An observational Study of the Impact of somatic DNA Repair Mutations on the Clinical Outcomes of bone metastases in Patients with Metastatic Castration-Resistant Prostate Cancer.

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Introduction

Prostate cancer (PCa) represents the most prevalent cancer type in men with an incidence of 1,100,000 new cases worldwide every year [1].

Many patients (approximately 15–30%) who experience prostate-specific antigen (PSA) recurrence after primary treatment for PCa (surgery and/or radiotherapy) receive androgen-deprivation therapy (ADT) [2–4]. Until prostate cancer continues to respond to ADT, it is defined as hormone-sensitive (HSPC). Metastatic hormone-sensitive prostate cancer (mHSPC) includes patients with synchronous metastases as well as patients who develop metastases after months or years from diagnosis and treatment of the primary tumor. The progression of PCa despite ADT and castrate testosterone level is defined as castration-resistant prostate cancer (CRPC); this condition can be observed both in patients with metastatic (mCRPC) and non metastatic (nmCRPC) lesions.

In patients with both mHSPC and mCRPC, bone represents the most preferential target site of metastases with an incidence of nearly 75%; autopsy data indicate that the incidence of metastatic bone lesions is 65-75% in PCa patients [5,6].

Bone metastases led to the disruption of normal bone homeostasis; the development of bone metastases is the result of complex interactions between tumor cells, bone marrow cells, and resident bone cells [7].

“Bone remodelling units” are specialized skeleton sites responsible for the physiological remodelling process by regulating osteoclasts and osteoblasts functions and, consequently, maintaining the balance between bone formation and degradation in order to preserve skeletal integrity [8].

Tumour cells are attracted to skeletal tissue by chemotactic cytokines which normally regulate the migration of Hematopoietic Stem Cells (HSCs) into the hematopoietic stem cell niche: in fact, osteoblastic-induced stromal-derivedfactor-1 (SDF-1 or CXCL12) binds CXCR4 receptor expressed both by HSCs and PCa cells. The competitive binding of SDF-1/CXCR4 of HSCs and PCa cells leads to the formation of the “onco-niche” tumor cells. PCa cells which migrated in these niches may be quiescent or active: in the latter case, they can damage physiological bone remodelling by interfering with normal osteoclastic and osteoblastic activity processes through secretion of paracrine factors such as transforming growth factor β1 (TGF β1), parathyroid-hormone-related peptide (PTHrP) and interleukin 6 (IL-6). The result is an aberrant activation of the RANK/RANK ligand (RANKL) pathway and, consequently, abnormal stimulation of bone resorption [7,8]. In PCa bone metastasis, after the first enhanced osteolysis, there is a strong osteoblastic stimulation resulting in an excessive abnormal bone apposition. “Bone hunger syndrome” is a

hyperparathyroidism condition due to calcium entrapment in skeletal tissue and serum calcium deficiency due to the enhanced osteoclastic activity . This compensatory hyperparathyroidism leads to osteoclasts activation also at distant sites resulting in a generalized state of osteoclast ogenesis [7,8].

PCa bone metastases affect the quality of life (QoL) because of the risk of bone pain as well as the development of clinical complications defined as "skeletal-related events" (SREs) [9,10]. Pathologic bone fractures, hypercalcemia, spinal cord compression, surgery to bone, and radiotherapy to bone are the five events defined SREs by Food and Drug Administration (FDA)[11]. The frequency and type of SREs depend on their location and number as well as on their osteolytic or osteoblastic nature [12,13]. SREs negatively correlate with survival [14,15]: pathologic fractures and metastatic spinal cord compressions are associated with a significantly increased risk of death [16,17,18].

In HSPC, ADT pressure causes the development of adaptive survival mechanisms in cancer cells that lead to the transition to CRPC. These processes involve genomic alterations (GA) which may be associated with the androgen receptor (AR) pathway as well as AR-independent molecular mechanisms [19,20]. AR pathway alterations result in persisting AR activation and represent the most frequent biological events in CRPC. AR pathway alterations include amplifications, mutations, AR splice variants, intratumoral androgen synthesis, and AR enhancer amplification, among others. In the last two years, the treatment landscape of mCRPC and nmCRPC has radically changed. Recently European Medicines Agency (EMA) and FDA approved Apalutamide, darolutamide, and enzalutamide for the treatment of nmCRCP [21]. Apalutamide, darolutamide and enzalutamide are AR signaling inhibitors (ARSI), new-generation antiandrogens that competitively inhibit the AR ligand-binding domain with higher affinity than first-generation agents, but they also impair AR translocation to the nucleus and obstruct AR-mediated transcription.

In mCRPC enzalutamide and abiraterone, a new-generation ARSI, demonstrated benefit in terms of overall survival (OS) and progression-free survival (PFS). Taxane-based chemotherapy has demonstrated efficacy prior to and after progression on abiraterone or enzalutamide, yet the 3-year overall survival rate for mCRPC remains under 50%[21,23,24,25,25,26,27].

In the last years, some GAs emerged as potential therapeutic targets. In this regard several efforts have been made to study GAs related to genomic instability, such as Breast Related Cancer Antigens (BRCA) 1 and BRCA2 alterations in several diseases, including PCa.

Defects in BRCA1 or BRCA2 result in impairments of DNA damage repair (DDR) deficiency by the error-free mechanism of homologous recombination repair (HRR) [28,29,30]. These defects cause single-strand annealing and non-homologous end joining and, consequently, genomic instability. BRCA-deficient cells are sensitive to inhibition of poly (ADP-ribose) polymerase (PARP), which results in irreversible DNA damage, cell cycle arrest, and cell death [31,32,33,34]. PARP is an important mediator of the base excision repair pathway: its loss causes an increase in RAD51 foci and sister chromatid exchanges, but it also increases the number of lesions which are usually repaired by HRR. This might be lethal in a BRCA-defective background and, consequently, PARP inhibition was found to be effective in BRCA-deficient cells [35]. Beyond BRCA1/2, deleterious alterations in other genes have been associated to DDR deficiency: ATM and CHEK2 (sensors of DNA damage), CDK12 (positive regulator of BRCA genes), and PALB2 and FANCA (which interact with BRCA1 and/or BRCA2 during DNA repair) [36,37,38,39,40].

The most recent advances in mCRPC therapy target DNA repair defects in mCRPC using PARP inhibitors (PARPi) [41,42,43, 44]. Olaparib has been EMA and FDA approved for the treatment of mCRPC with germline or somatic BRCA1/2 alterations. Germline alterations in HRR genes BRCA1/2 are a hereditary risk factor for prostate cancer; mCRPC samples show enrichment in BRCA2 alterations compared with primary tumors, suggesting that the loss of HRR is a therapeutically relevant driver of aggressive disease [41,42,43, 44]. Loss-of-function alterations in other DDR genes are also enriched in mCRPC and may be targetable with PARPis, but require further investigation [44,45]. The landscape of GAs in prostate cancer has been previously characterized using tissue biopsies and studied to identify mechanisms of resistance to ARSI [46,47,**Errore. L'origine riferimento non è stata trovata.**].Genomic profiling of the most recent specimens may be more valuable than archival tissues in capturing somatic alterations: despite the inherent advantages of profiling the latest available sample from a patient with advanced disease, in mCRPC patients the collection of a tissue specimen could be technically challenging because of metastases often confined only to the bones [46,47]. Bone biopsies are invasive and have high failure rates of DNA sequencing [46,47]. Blood-based liquid biopsy and genomic profiling of cell-

free circulating tumor DNA (ctDNA) from plasma provide a minimally invasive alternative method to profile mCRPC patients, with the added capability of detecting variants from multiple metastatic lesions that may have undergone clonal evolution.
This is a retrospective observational study evaluating the prevalence and effect of somatic DDR (sDDR) mutations on the clinical outcomes of bone metastases in patients with mCRPC.

Translational Relevance

Comprehensive genomic profiling (CGP) in mCRPC patients is of increasing value given the diversity of emerging treatment options. While CGP by tissue testing remains the gold standard, bone metastases are challenging to sample and analyze. Genomic profiling of plasma cell-free circulating tumor DNA (ctDNA) offers a compelling, minimally invasive complement to tissue testing. Advanced prostate cancer has a high shed rate into blood stream – in fact, ctDNA was detectable in 94% of patients. ctDNA profiling can overcome the technical difficulties and high failure rates associated with bone metastasis biopsy and help to guide precision therapy in advanced prostate cancer.

Methods

Study design and aims

The primary aim is to assess the impact of somatic DDR (sDDR) mutations on clinical course of bone metastases in mCRPC patients.
The impact of sDDR mutations on clinical outcomes of bone metastases is defined as:

- bone metastatic burden, defined as the number and sites (axial only vs non-axial) of bone metastasesat time of mCRPC diagnosis;
- bone metastases-specific survival, defined as the time from bone metastases onset to death for any cause;
- prevalence and type of SRE;
- time to first on-study SRE , defined as the time from bone metastases onset to first SRE;
- Bone pain, defined as necessity of opioid use for bone pain at the diagnosis of bone metastases in mCRPC.

As defined by FDA, SRE have been considered: Pathologic bone fractures, hypercalcemia, spinal cord compression, surgery to bone, and radiotherapy to bone.
The analysis of the impact of sDDR mutations have been performed according to 4 groups:

- BRCA2 and BCRCA1 only (Group 1);
- ATM / PALB2/RAD51/FANCA/ATM/CDK12/CHECK2 (Group 2);
- ATM/BRCA1/BRCA2/ RAD51/PALB2/FANCA/ATM/CDK12/CHECK2 (Group 3, all patients "positive" for genomic defects in DDR genes);
- No DDR carriers (Group 4, all patients negative for genomic defects in DDR genes)

However, for the final analysis, we considered only group 3 and group 4, which have therefore been renamed to group A and group B, respectively.
The secondary and point of the study is to evaluate the concordance between liquid and tissue biopsy in terms of presence or absence and type of molecular DDR alterations observed.
Patients were retrospectively included for the analysis at the "Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS Dino Amadori" (Meldola, FC Italy) from From January 2021 to September 2022 and prospectively observed until the data cut off 31.12.2022.

Patients

Patients enrolled were included in the biological observational prospective study IRSTB073 "Biomarker study: the next generation of prostate cancer biomarkers" (Identifier Code: L3P1380). Local Ethical Committee ("IRST Ethical Committee") approved the IRST B073 single centre prospective study. All patients provided written informed consent.
Main inclusion criteria were:

- histological or cytological confirmed diagnosis of prostate cancer or unequivocal increased of PSA;
- Patients must have metastatic and/or inoperable disease;
- Life expectancy of greater than 3 months;
- ECOG performance status <2;
- Age ≥ 18 years;
- no previous line of treatment for mCRPC
- sample tissue of the primary PCa tumor available for NGS analyses and/or blood sample baseline first line treatment for mCRPC

Study Procedures and somatic Variants Analyses

A 10-mL blood sample was drawn at study entry for somatic Cell-free DNA (cfDNA) extraction for FoundationOne LiquidCDx analysis,a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

Next generation sequencing using FoundationOne DX1 was performed on DNA from tumor-biopsy samples obtained at diagnoses of PCa, if available.

Through genomic testing of plasma and/or tumor tissue (archival, if available), patients were screened for the presence of a deleterious somatic alteration in BRCA1, BRCA2, ATM, CDK12, CHEK2, FANCA, PALB2, RAD51.

We considered DDR mutated all patients with at least one pathogenic mutation (according to the classification of American College of Medical Genetics and Genomics, ACMG) [48]. in one of the DDR genes evaluated (BRCA1/2, ATM, FANCA, CHEK2, RAD51, PALB2, and CDK12) in solid and/or liquid biopsy. Patients performing NGS analyses on solid sample and liquid biopsy were included in group B if no mutation was detected in both liquid and solid NGS analysis.

If a mutation in one or more DDR-related genes occurred in liquid biopsy and was not detected in solid biopsy (or vice versa), patient was included in group A.

Statistical analyses

The description of the cases was carried out through the use of descriptive statistics such as absolute frequencies and percentage frequencies for variables measured on a nominal or ordinal scale, medians, and intervals of variation for variables measured on a continuous scale.

Comparisons of median values of markers within different clinical features were obtained using the nonparametric Wilcoxon test of medians.

Time to skeletal event (SRE) was calculated as the time between the date of bone metastases onset and the date of the first SRE onset for patients who had at least one skeletal event and the difference between the date of bone metastases onset and last follow-up date for patients who did not have any SRE. Events are represented by patients who had at least one SRE.

The curves of the time-dependent variables were determined with the Kaplan-Meier limit product method and the relative comparisons were made according to the log-rank test.

All p-values were obtained considering 2-tailed tests and statistical analyzes were performed with SAS statistical software, version 9.4.

For each biomarker, concordance was defined as either positive or negative in both tumor and metastasis and discordance was defined as positivity at one site and negativity at the other or vice versa. For each receptor the discordance rate (DR) was calculated as the proportion of discordant

cases with respect to the total number of patients. The two-sided exact binomial 95% confidence interval (95% CI) was estimated.

The relation between the value and the level of agreement was first reported by Landis and Kock [49], with values indicating agreement as follows: 0.00–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement, and 0.81–1.00, almost perfect agreement (perfect agreement = 1.00).

Results

From January 2021 to September 2022, 150 mCRPC patients were enrolled. Plasma samples were obtained from 105 patients before starting first line treatment for mCRPC, whilst tissue samples from primary tumor were available for 104 patients. Liquid and tissue biopsy were both available for 61 patients. The baseline characteristics of all patients enrolled in the study are described in table 1.

Table 1. Baseline patients' characteristics

Variable	Number (%) Total =150
Age at diagnosis(years): Medianvalue (range)	65.5 (42-86)
Gleason score	
<8	47 (31.3%)
≥8	92 (61.4%)
Unknown	11 (7.3%)
Stage atdiagnosis	
Localized PCa	73 (48.8%)
mHSPC	64 (51.2%)
Type of mHSPC	
High risk and/or high volume	59 (39.3%)
No high risk/volume	66 (44%)
No mHSPC	25 (16.7%)

Variable	Number (%) Total =150
Sites of metastases in mCRPC	
Bone only	53 (35.4%)
Bone and visceral	11 (7.3%)
Bone and nodes	55 (36.6%)
Bone, visceral and nodes	2 (1.4%)
Visceral or nodes only	18 (12%)
No mCRPC	11 (7.3%)
Treatment for mHSPC	
LhRHanalogue	69 (46%)
LhRH analogue + docetaxel	31 (20.7%)
LhRH analogue + ARSI	25 (16.6%)
No mHSPC	25 (16.7%)
Tissue biopsy	
No	46 (30.6%)
Yes	104 (69.3%)
Liquid biopsy	
No	45 (30%)
Yes	105 (70%)
First line treatment for mCRPC	
Chemotherapy	13 (8.7%)
ARSI	126 (91.3%)

Table 1. Baseline patients' characteristics

The distribution of molecular alterations found in tissue and liquid biopsy is resumed in table 2.

Table 2.Distribution of molecular alterations found in tissue and liquid biopsy.

Gene	Tissuebiopsy (n=104)		Liquid biopsy (n=105)	
	Wild type no.	Mutated no.	Wild Type no.	Mutated no.
BRCA 2	94	10	93	12
BRCA 1	99	5	102	3
ATM	96	8	92	13
FANCA	103	1	102	3
RAD 51	103	1	104	1
CHECK 2	100	3	96	9
PALB 2	103	1	102	3
CDK12	100	4	95	10
Total mutations	-	33	-	54

A total number of 54 mutations in DDR-related genes were found in liquid biopsy, compared to 34 mutations in tissue biopsy of primary tumor. 6 BRCA1/2 mutated patients have also other mutations in other DDR-related genes. We included in group A all patients with at least one or more pathogenic mutations in DDR-related genes. In patients with both tissue and liquid samples available, the level of agreement between tissue and liquid biopsy is represented in table 3.No statistically significant difference has been found in terms incidence of molecular DDR alterations between tissue and liquid biopsy.

Table 3. 61/150 patients enrolled performed both liquid and tissue NGS analyses: Concordance analysis between solid and liquid biopsy.

	Liquid biopsy			
	WT	**Mutated**	**Total**	**K value (95% CI)**
Tissue biopsy	**N (%)**	**N (%)**	**N (%)**	
BRCA2				
WT	52 (98.1)	1 (1.9)	53 (100)	
Mutated	0	8 (100)	8 (100)	0.94 (0.80 to 1.00)
Total	52	9	61	
DR (95% CI)	1.64% (0-4.83)			

ATM				
WT	51 (96.2)	2 (3.8)	53	
Mutated	1 (12.5)	7 (87.5)	8	0.80 (0.57 to 1.00)
Total	52	9	61	
DR (95% CI)	4.92% (0-10.34)			
FANCA				
WT	60 (100)	0	60 (100)	
Mutated	0	1 (100)	1 (100)	1.00 (1.00 to 1.00)
Total	60	1	61	
DR (95% CI)	0%			
RAD 51				
WT	60 (100)	0	60 (100)	
Mutated	1 (100)	0	1 (100)	-
Total	60	0	61	
DR (95% CI)	1.64% (0-4.83)			
BRCA1				
WT	56 (100)	0	56 (100)	
Mutated	2 (40.0)	3 (60.0)	5 (100)	0.73 (0.38 to 1.00)
Total	58	3	61	
DR (95% CI)	3.28% (0-7.97)			
CHECK 2				
WT	56 (96.7)	2 (3.3)	58 (100)	
Mutated	0	3 (100)	3 (100)	0.73 (0.38 to 1.00)
Total	56	5	61	
DR (95% CI)	3.28% (0-7.97)			
PALB2				
WT	60 (100)	0	60 (100)	
Mutated	0	1 (100)	1 (100)	-
Total	60	1	61	

DR (95% CI)	0%			
CDK12				
WT	56 (98.3)	1 (1.7)	57 (100)	
Mutated	1 (25.0)	3 (75.0)	4 (100)	-
Total	57	4	61	
DR (95% CI)	3.28% (0-7.97)			

Among 150 patients enrolled, 11 ones have not experienced mCRPC, remaining hormone-sensitive during the observation period.

Among 139 mCRPC patients enrolled, 18 did not develop bone metastases and, consequently, they have been excluded from the final analyses. We therefore included 121 mCRPC patients with bone metastases, divided into group A ("BRCA/DDR mutated", 38 patients, 31.4%) and group B (not mutated,83 patients, 68.6%) according to their molecular status. Clinical characteristics of these patients are resumed in table 3. There were no statistically significant differences between Group A and Group B patients in terms of clinical characteristics: age at diagnosis, Gleason score of the primary PCa, stage at diagnosis, type of presentation of mHSPC (high risk and/or high volume according to CHARTEED and LATITUDE criteria [50,51], and type of first-line treatment for mCRPC received.

Table 3.Clinical characteristics of mCRPC patients with bone metastasis

	TOTAL (No. 121)	**Group A (No. 38)**	**Group B (No. 83)**	
Variable	**No. (%)**	**No. (%)**	**No. (%)**	**P value**
Age at diagnosis (years): Median value (range)	65 (42-85)	65 (52-85)	65 (42-82)	n.s.
Gleason score				
<8	41 (33.9%)	14 (36.8%)	27 (32.5%)	n.s.
≥8	80 (66.1%)	24 (63.2%)	56 (67.5%)	
Stage at diagnosis				
LocalizedPCa	59 (48.8%)	19 (50%)	40 (48.2%)	n.s.
Mhspc	62 (51.2%)	19 (50%)	43 (51.8%)	
mHSPC type				
High risk and/or volume	59 (48.8%)	17 (44.7%)	42 (50.6%)	n.s.
No high risk and/or volume	62 (51.2%)	21 (55.3%)	41 (49.4%)	
Sites of metastases (mCRPC)				
Bone only	53	16 (42.1%)	37 (44.6%)	n.s.
Bone and visceral	11	4 (10.5%)	7 (8.4%)	
Bone and nodes	55	18 (47.4%)	37 (44.6%)	
Bone, visceral and nodes	2	0	2 (2.4%)	
Type of first line treatment mCRPC				
Chemotherapy	13	4 (10.5%)	9 (10.8%)	n.s.
ARSI	108	34 (89.5%)	74 (89.2%)	
Denosumab or bisphosphonates				
Yes	38	10 (26.3%)	28 (33.7%)	n.s
No	83	28 (73.7%)	55 (66.3%)	

Among the 121 mCRPC patients with bone metastases, divided into group A and group B, 45 patients, have both liquid and solid samples.
In this subgroup of patients, the level of agreement between tissue and liquid biopsy remained overall good, with different value according to gene analysed (table 4).

Table 4. Concordance analysis in 45 patients with mCRPC and bone metastases with both liquid and tissue samples.

	Liquid biopsy			
	WT	Mutated		
Tissue biopsy	N (%)	N (%)	Total	K value (95% CI)
BRCA2				
WT	37 (97.4)	1 (2.6)	38 (100)	
Mutated	0	7 (100)	7 (100)	0.92 (0.77 - 1.00)
Total	37	8	45	
DR (95% CI)	2.22% (0-6.52)			
ATM				
WT	39 (95.1)	2 (4.9)	41	
Mutated	1 (25.0)	3 (75.0)	4	0.63 (0.25 - 1.00)
Total	40	5	45	
DR (95% CI)	6.67% (0-13.94)			
FANCA				
WT	44 (100)	0	44 (100)	
Mutated	0	1 (100)	1 (100)	1.00 (1.00 - 1.00)
Total	44	1	45	
DR (95% CI)	0%			
RAD51C				
WT	44 (100)	0	44 (100)	
Mutated	1 (100)	0	1 (100)	-
Total	45	0	45	
DR (95% CI)	2.22% (0-6.52)			
BRCA1				
WT	43 (100)	0	43 (100)	
Mutated	2 (100)	0	2 (100)	-
Total	45	0	45	
DR (95% CI)	4.44% (0-10.46)			
CHECK2				
WT	40 (95.1)	2 (4.9)	41 (100)	
Mutated	0	3 (100)	3 (100)	0.72 (0.372 -1.000)
Total	40	5	45	
DR (95% CI)	4.44% (0-10.69)			
PALB2				
WT	44 (100)	0	44 (100)	
Mutated	0	1 (100)	1 (100)	-
Total	44	1	45	
DR (95% CI)	0%			
CDK12				
WT	42 (100)	0	42 (100)	
Mutated	0	3 (100)	3 (100)	-
Total	42	3	45	
DR (95% CI)	0%			

Every patient in group B has no mutation in both liquid and solid biopsy. The level of agreement between solid and liquid biopsy in Group A is resumed in table 5.

Table 5. Concordance analysis in 20 patients of group A with both liquid and tissue samples.

	Liquid biopsy			
	WT	**Mutated**		
Tissue biopsy	**N (%)**	**N (%)**	**total**	**K value (95% CI)**
BRCA2				
WT	12	1	13	
Mutated	0	7	7	0.89 (0.692 -1.000)
Total	12	8	20	
DR (95% CI)	5.55			
ATM				
WT	14	2	16	
Mutated	1	3	4	0.57 (0.142 -.000)
Total	15	5	20	
DR (95% CI)	11.5			
FANCA				
WT	20	0	19	
Mutated	0	1	1	1.00 (1.00-1.00)
Total	19	1	20	
DR (95% CI)	0%			
RAD51C				
WT	19	0	19	
Mutated	1 (100)	0	1	-
Total	20	0	20	
DR (95% CI)		5.55		
BRCA1				
WT	18	0	18	
Mutated	2	0	2	-
Total	20	0	20	
DR (95% CI)	4.44% (0-10.46)			
CHECK2				
WT	15	2	17	
Mutated	0	3	3	0.69 (0.30 - 1.000)
Total	15	5	20	
DR (95% CI)				
PALB2				
WT	19	0	19	
Mutated	0	1	1	1.00 (1.00-1.00)
Total	19	1	20	
DR (95% CI)	0%			
CDK12				
WT	17	0	17	
Mutated	0	3	3	1.00 (1.00-1.00)
Total	17	3	20	
DR (95% CI)	0%			

Then, we evaluated the impact of DDR gene mutations on bone-related efficacy endpoints at the time of mCRPC diagnosis, by dividing patients in two aforementioned molecular groups.
We investigated differences between the two groups in terms of time from bone metastases onset to death, skeletal metastatic tumor burden (sites and number of lesions), skeletal-related events (SREs) incidence, and time to first on-study SRE. The use of antiresorptive agents (bisphosphonates or denosumab) was similar between the two groups.
Concerning bone sites, we divided the patients in those with lesions localized in the axial skeleton only and those with at least one extra-axial lesion; we did not find any difference according to this parameter between group A and B.
SRE were similar in both groups, in terms of incidence but also of median onset time from the diagnosis of bone metastases. Bone pain did not differ between the two groups. (Table 6) No statistical difference have been found according to the first line treatment administred (chemotherapy or NHT).

Table 6.Variables in bone metastases positive mCRPC cohort

Variable	Group A (=38)	Group B (= 83)	P value
Age at mCRPC diagnosis (range)	71 (53-86)	69 (44-85)	0.196
Bone sites - **axial only** - **extra-axial**	 10 (26.3%) 28 (73.7%)	 33 (39.8%) 50 (60.2%)	 0.152
Number of bone metastases - **< 4** - **≥ 4**	 7 (18.4%) 31 (81.6%)	 29 (34.9%) 54 (65.1%)	 0.065
Number of bone metastases - **< 10** - **≥ 10**	 11 (28.9%) 27 (71.1%)	 47 (56.6%) 36 (43.4%)	 0.005
Incidence of SRE	16/38 (42.1%)	38/83 (45.7%)	0.706
Median time to SRE onset (mo.)	48 (13-not reached)	21 (11-not reached)	0.312
Median time from bone metastases onset to death (mo.)	Not reached	57.6 (44.6-not reached)	0.763
Bone pain - **No** - **Yes** - **Unknown/missing**	17 (46%) 20 (54%) 1	45 (57.7%) 33 (42.3%) 5	0.238

For the number of bone lesions, we adopted two different threshold values, 4 and 10 lesions; interestingly, we found a higher bone metastatic burden in group A than group B, being statistically significant only for the value of 10 lesions as threshold (p=0.005) (Figure 1).

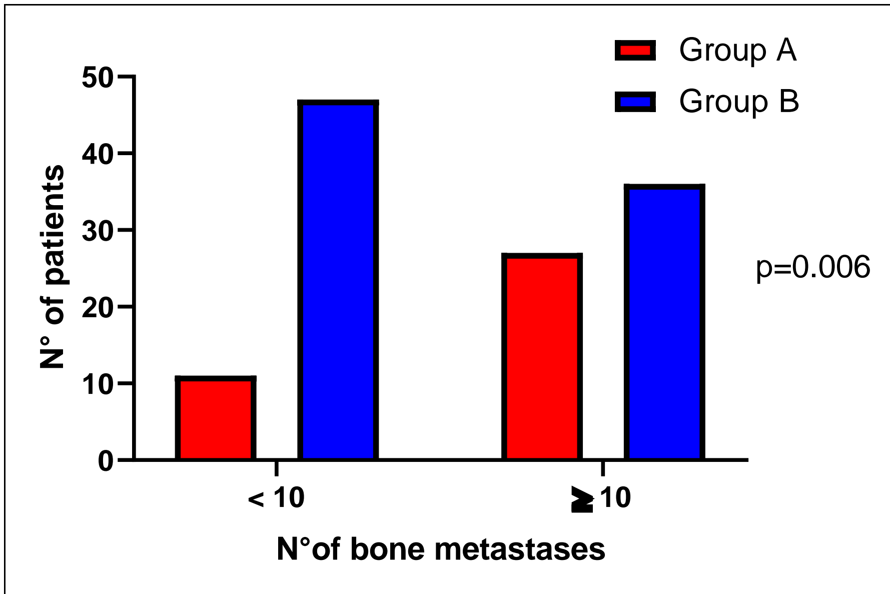

Figure 1: number of bone metastases in the two molecular groups, adopting the threshold of 10 lesions**.**

We also evaluated the median time from bone metastases onset to death, without finding any difference (Figure 2).

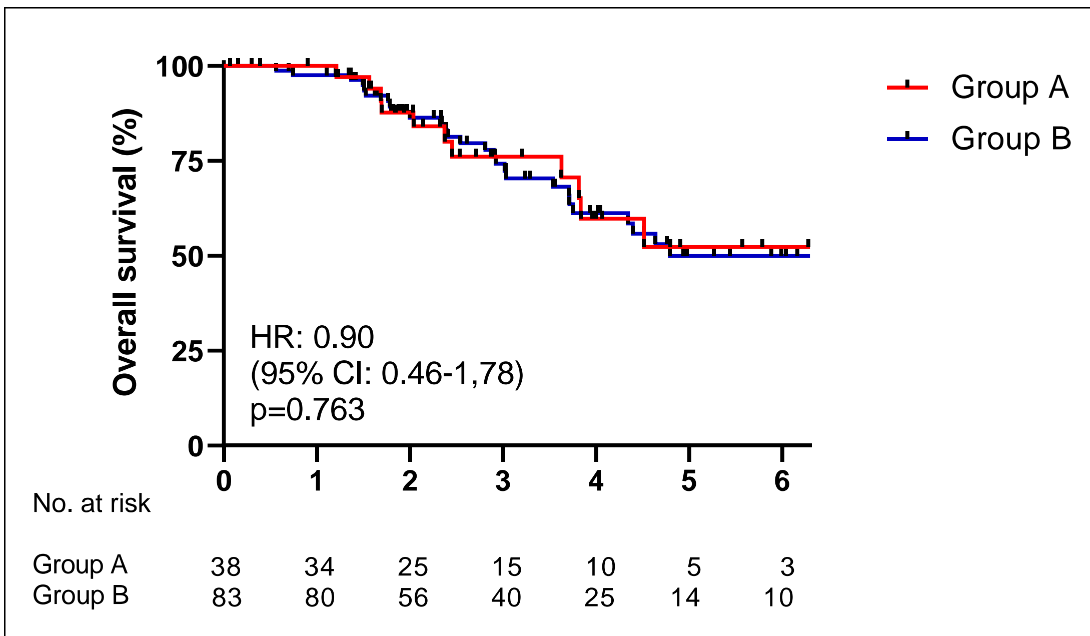

Figure 2. Time from bone metastases onset to death according to molecular status.

Discussion

DNA damage response and repair (DDR) genes are involved in the mechanisms of genetic instability, the repair of DNA aberrations during cell cycle, and the detection and repair of DNA damage, leading to apoptosis of dangerous mutated cells. Pathogenetic variants of DDR genes reduce the ability to effectively repair single- and double-strand breaks of DNA damage. The poly-ADP-ribose polymerase (PARP) system is involved in detecting and repairing primarily single-strand breaks, whereas the homologous recombination repair (HRR) pathway of DDR genes is primarily responsible for the repairing double-strand breaks DNA lesions. BRCA1-2 are the most famous genes involved in the HRR system, associated with an increased risk of developing breast, ovarian, prostatic, pancreatic, and colon cancers [52]. We propose testing all PCa patients for somatic DRR mutations at diagnosis of mCRPC. Somatic determination of molecular alterations on liquid biopsy and/or on the primary site on historic paraffin preparations (if available) was performed.

The incidence of mutations in DDR genes among men with mCRPC varied between 11% and 33% [53], which was significantly higher than in non-metastatic PCa, and BRCA2 mutations were more frequent when compared to other DDR genes (13%), followed by an ATM incidence of 7.3% [54]. The incidence of DDR mutations in this study is in line with literature data, 25.3% of all patients enrolled present at least a mutation in DDR related genes and BRCA2 was the more frequent one, followed by ATM.

BRCA alterations, in several neoplasms, have been associated with short metastatic-free survival, short cancer-specific survival (CSS) and are predictive of response to PARP inhibitors and to platinum salts

In PCa, Castro E. et al. demonstrated that BRCA1-2 mutations were more frequently associated with a Gleason score of ≥ 8 , T3/T4 stage , nodal involvement and metastases at diagnosis [55].In the PROREPAIR B trial, BRCA2 mutations alone resulted in an independent prognostic factor, negatively affecting CSS, and had deleterious impact on outcomes in mCRPC patients in relation to first-line therapy choice.

However, there are no literature data regarding the impact of DDR genes alterations on bone outcomes of mCRPC patients.

This study investigated differences between the DDR gene alterations-carriers and non-carriers in mCRCP with bone metastases outcomes in terms of time from bone metastases onset to death, skeletal metastatic tumor burden (sites and number of lesions), skeletal-related events (SREs) incidence, and time to first on-study SRE. We hypothesized that the DNA-repair defects in mCRPC may be associated to poor prognoses in terms of bone related outcomes.

Our study does not demonstrate any difference in bone-related outcomes in DDR genes mutations carriers compared to non-carriers: incidence of SRE, median onset time of the first SRE from the diagnosis of bone metastases as well as bone pain did not differ between the two groups. However, DDR mutated status showed an association with higher bone tumor burden in mCRPC: these patients had a superior count of bone metastases at the diagnoses of mCRPC compared to patients with normal DDR status. It should be noticed that the two groups of patients were balanced in terms of use of antiresorptive agents bisphosphonates or denosumab) and first line treatment performed for mCRPC.

In this study, patients were defined DDR-gene mutation carriers if one or more mutation in DDR-related genes was detected in solid and/or liquid biopsy. It is well- know that genomic alterations can be acquired during the progression of the disease as a consequence of the selective pressure of treatments and biological molecular changes occurring during disease progression itself [19]; consequently, a biopsy of the metastatic tumor represents the ideal approach to identify molecular alterations. The PROfound trial [6], which evaluated 2792 biopsies of mCRPC, showed that DDR genes alterations were present in 28% of all samples, with a similar incidence considering the primary tumor (27%) or metastatic sites (32%). However, somatic determination on a metastatic site, in particular, bone, may be associated with various biases, as well as possible side effects [46]; the PROfound study, for example, pointed out that 30% of biopsy samples may not be of sufficient quality for gene sequencing [56].

The analysis of free circulating DNA (ctDNA) is a promising approach as it may overcome the difficulties that are associated with obtaining tissue; currently, however, there are no solid data that currently allow the reliably use of this test. In our study, the incidence of mutations in DDR-related genes in liquid biopsy (performed at the diagnosis of mCRPC) is numerically higher as compared to solid biopsy (performed on tissue samples of primary tumor); therefore, there was a substantial agreement between solid and liquid biopsy for DDR gene alterations, with different value according to the gene analysed. For example, mutations of BRCA1/2, PALB 2, FANCA showed an almost perfect agreement, on the other hand ATM showed a moderate agreement between solid and liquid biopsy. According to these data, liquid biopsy could be a valid tool also in PCa with prevalent bone involvement, in which solid biopsy of the metastatic sites is difficult to performe and in case of an older primary tissues which are unlikely to be adequate for molecular analysis [56].

Among limitations of our study, the main is the retrospective nature of the data analyzed; another limitation is that patients did not perform a solid biopsy of the metastatic site at the time of diagnosis of mCRPC which may be more comparable to liquid biopsy, reflecting the selective pressure of treatment received from the diagnosis of PCa to the diagnosis of mCRPC.

These results should be considered preliminary and further work is needed to determine the relevance of these findings.

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