ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN

ONCOLOGIA, EMATOLOGIA E PATOLOGIA

Ciclo 36

Settore Concorsuale: Area 06 - Scienze mediche > MED/06 Oncologia medica

Settore Scientifico Disciplinare: 06/D3 Malattie del sangue, Oncologia e reumatologia

Combining molecular alterations and functional imaging in metastatic castration resistant prostate cancer treated with taxanes

Presentata da: Cristian Lolli

Coordinatore Dottorato

Manuela Ferracin

Supervisore

Ugo De Giorgi

Co-supervisore

Maria A Pantaleo

Esame finale anno 2024

Summary

Abstract

The treatment of metastatic castration-resistant prostate cancer (mCRPC) is currently characterized by several drugs with different mechanisms of action, such as new generation hormonal agents (abiraterone, enzalutamide), chemotherapy (docetaxel, cabazitaxel), PARP inhibitors (olaparib) and radiometabolic therapies (radium-223, LuPSMA). There is an urgent need to identify biomarkers to guide personalized therapy in mCRPC. In recent years, the status of androgen receptor (AR) gene detected in liquid biopsy has been associated with outcomes in patients treated with abiraterone or enzalutamide. More recently, plasma tumor DNA (ptDNA) and its changes during treatment have been identified as early indicators of response to anticancer treatments. Recent works also suggested a potential role of tumor-related metabolic parameters of ^{18}Fluoro-Choline Positron Emission Tomography (F^{18}CH-PET)-computed tomography (CT) as a prognostic tool in mCRCP. Other clinical features, such as the presence of visceral metastases, have been correlated with outcome in mCRPC patients.

Recent studies conducted by our research group have designed and validated a prognostic model based on the combination of molecular characteristics (ptDNA levels), metabolic features found in basal FCH PET scans (metabolic tumor volume values, MTV), clinical parameters (absence or presence of visceral metastases), and laboratory tests (serum lactate dehydrogenase levels, LDH).

Within this PhD project, 30 patients affected by mCRPC, pre-treated with abiraterone or enzalutamide, candidate for taxane-based treatments (docetaxel or cabazitaxel), have been prospectively evaluated. The prognostic model previously described was applied to this population, to interrogate its prognostic power in a more advanced cohort of patients, resulting in a further external validation of the tool.

1. Introduction

1.1 Epidemiology of prostate cancer

Prostate cancer (PCa) is the most frequent neoplasm among men in the majority of countries worldwide, with 1.4 million new cases estimated in 2020. The highest incidence rates are seen in Northern and Western Europe, the Caribbean, Australia/New Zealand, North and South America, and Southern Africa as reported in **figure 1**.

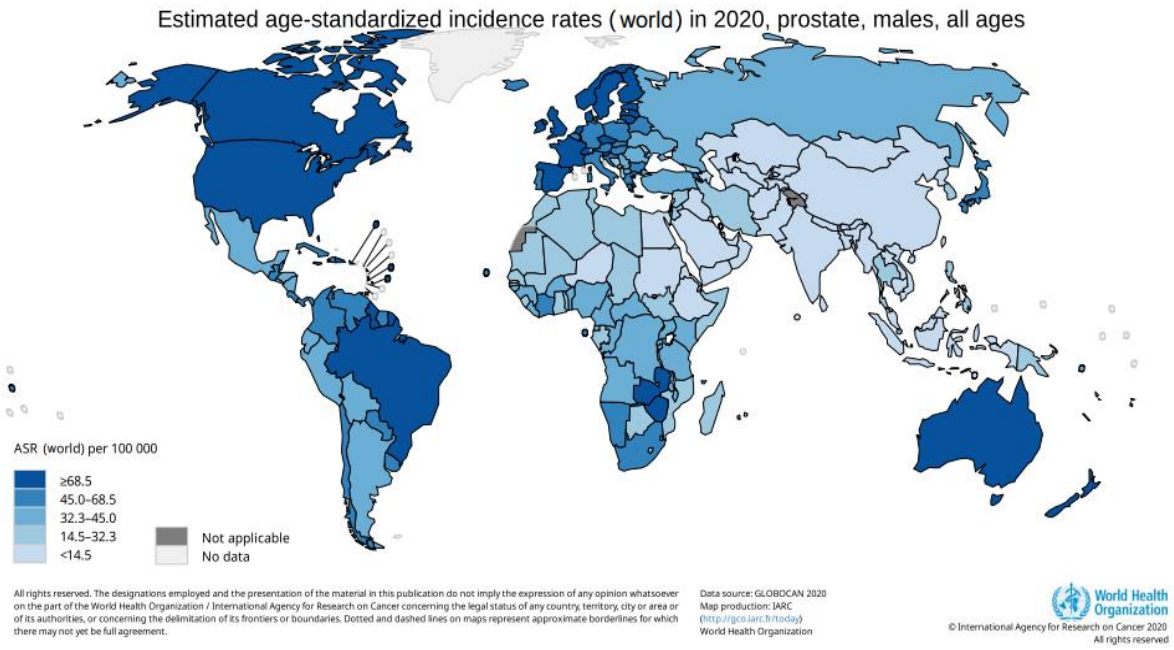

Figure 1. stimated age-standardized prostate cancer incidence (world)

PCa is the main cause of cancer death among men in 48 out of 185 countries, with 375000 deaths estimated in 2020.

Global differences in PCa incidence and mortality can be attributed to differences in screening, imaging, access to care, and availability of healthcare infrastructures **(figure 2).** In addition, emerging data suggest that differences in germline genetic factors, as well as lifestyle factors across populations, may enhance geographic differences. When

diagnosed and treated at localized stages, PCa is associated with a 97% 5-year cancer-specific survival compared with 30% in the metastatic setting [1-3].

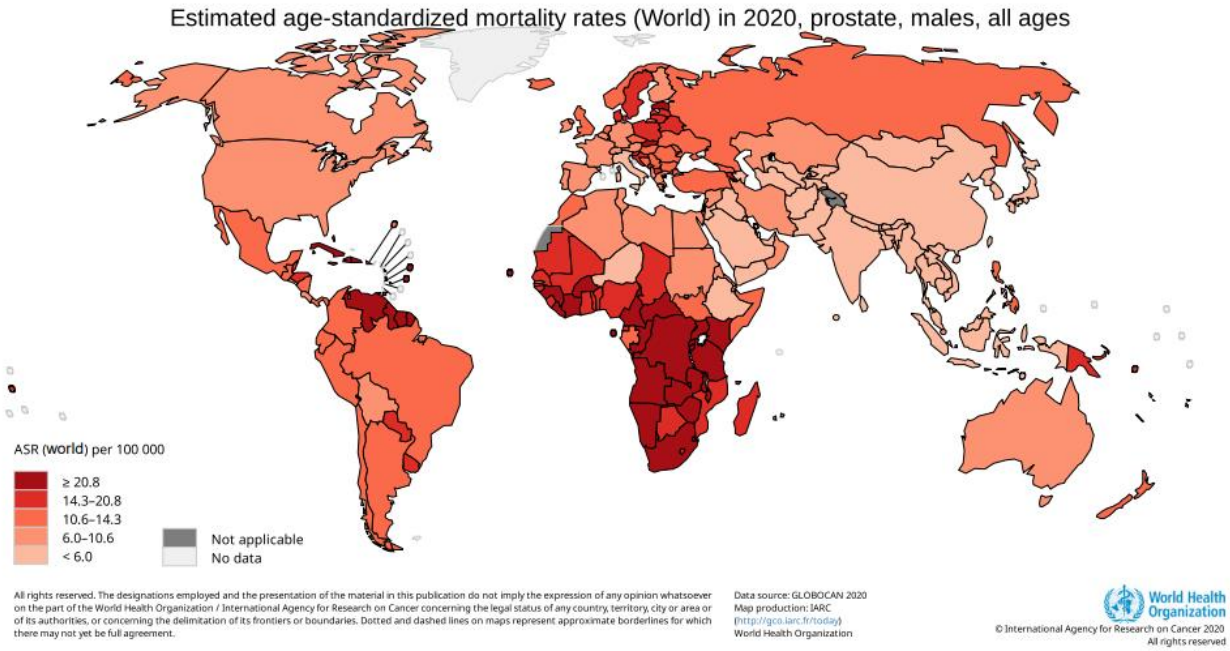

Figure 2. Estimated age-standardized prostate cancer mortality rate (worldwide).

1.2 Systemic treatments for prostate cancer

1.2.1 Hormone sensitive prostate cancer

In the 1940s it was discovered that patients with metastatic PCa responded to androgen deprivation therapy (ADT), thus becoming the standard treatment for metastatic PCa **[4].** Androgen deprivation therapy consists generally of intramuscular therapies consisting in luteinizing hormone-releasing hormone (LHRH) agonists (leuprolide and goserelin) or antagonists (degarelix), receptor antagonists. Nearly all patients initially respond to ADT; however, the duration of response may vary from months to years. Until PCa remains in a phase sensitive to hormonal treatments with ADT it is defined hormone sensitive prostate cancer (HSPC).

The consequent progression to a castration resistant phase leads patients to a lethal stage of disease.

The treatment landscape of metastatic prostate cancer (mCRPC) in the initial phase of hormone sensitivity has dramatically changed in the last years.

Whilst in the last decades the standard treatment for mHSPC has been monotherapy with ADT, recently, the addition of docetaxel chemotherapy and second-generation antiandrogens to ADT has demonstrated to improve OS and has become the new standard of care.

Combination therapy with ADT and docetaxel has become a standard of care for mHSPC since 2015, based on the results of three phase III trials. In the CHAARTED trial, a stratification by metastatic disease volume was performed, defining as high-volume patients those with visceral metastases or four or more bone metastases (at least one bone other than vertebral or pelvic bones) [5].

In the CHAARTED trial, 790 mHSPC patients were randomized to receive six cycles of docetaxel plus ADT or ADT alone. The results showed an overall survival (OS) improvement of 13.6 months in the combinatorial arm. Long-term survival analysis of the CHAARTED trial confirmed the OS improvement for high volume patients treated

with docetaxel. Compared to high-volume patients, no significant difference in OS was observed in low-volume patients.

In the STAMPEDE trial (arm C), ADT associated to six cycles of docetaxel prolonged OS by 10 months compared with ADT alone [6]. Additional analysis for patients with mHSPC showed that docetaxel in mHSPC setting prolonged OS by 16 months compared with ADT alone. There were no significant benefit differences between high- and low-volume patients.

In the first reported GETUG-AFU 15 trials, 385 mHSPC patients were randomized to receive ADT with or without docetaxel for up to nine cycles. No significant difference in OS was found [7]. Long term survival analysis of the GETUG-AFU trial showed that docetaxel did not improve OS in patients with de novo metastases, high-volume or low-volume [8]. A subgroup analysis of the CHAARTED and GETUG-AFU 15 trials showed that high-volume patients had more benefit than low-volume patients from docetaxel treatment [9]. Based on these results, docetaxel became the preferred first-line treatment in high-volume patients.

Two clinical trials, showing the efficacy of the androgen receptor signaling inhibitor (ARSI) abiraterone for mHSPC, were reported in 2018. In the LATITUDE trial, high risk was defined as the presence of two of three high-risk prognostic factors (Gleason score ≥8, three or more bone lesions, or visceral metastases) [10]. These are currently called LATITUDE criteria. A total of 1199 patients with high-risk mHSPC were randomized to receive ADT plus abiraterone or ADT alone. The results showed a significant improvement of OS in the abiraterone group. Long-term survival analysis showed that the median OS was improved by 13.6 months in the abiraterone group [11].

Results from the Arm G of the STAMPEDE trial, with a similar design to LATITUDE trial, showed prolonged OS in 1002 mHSPC patients treated with ADT with abiraterone compared to ADT alone [12]. Efficacy was independent from disease volume or risk [12].

Two clinical trials evaluating enzalutamide treatment in mHSPC were reported in 2019 (ARCHES and ENZAMET trials). In the ARCHES trial, 1150 patients with mHSPC were randomized to receive ADT with or without enzalutamide. In the enzalutamide arm an improved progression free survival (PFS) was reported, but no significant differences in OS were observed at a median follow-up of 1.2 years [13]. The final survival analysis showed improved OS in the enzalutamide group [14]. Furthermore, enzalutamide showed efficacy regardless of prior docetaxel treatment or disease volume.

In the ENZAMET trial, 1125 mHSPC patients were randomized to receive enzalutamide plus ADT or ADT alone [15]. It has been demonstrated that enzalutamide plus ADT improves OS. Subgroup analysis showed that the OS improvement of OS was smaller in the enzalutamide group in patients with high disease volume and in those previously treated with docetaxel [15].

The role of apalutamide in mHSPC has been evaluated in TITAN trial, reported in 2019 [16,17]. In this trial, 1052 patients with mHSPC were randomized to receive ADT with or without apalutamide. Upfront apalutamide improved OS both at a median follow-up of 2 years and in the final survival analysis. Efficacy of apalutamide was demonstrated regardless of metastatic disease volume. Patients pre-treated with docetaxel did not show improved OS.

In the PEACE-1 trial evaluating triple therapy for mHSPC, a total of 1173 patients were randomized to standard therapy (ADT −/+ docetaxel), standard therapy plus abiraterone, standard therapy plus radiation therapy (RT), or standard therapy plus abiraterone plus RT. Among the 710 patients treated with docetaxel, 355 received ADT+docetaxel (with or without RT) and 355 received ADT+docetaxel+ abiraterone (with or without RT). OS was improved in the abiraterone group as compared with the no-abiraterone group. Among patients treated with docetaxel, OS was improved in the abiraterone combination group, demonstrating a positive role of triple therapy. Overall survival was not affected by prostate radiotherapy [18].

In the ARASENS trial, 1306 patients with mHSPC were randomized to receive darolutamide+ADT+docetaxel or ADT+docetaxel. In the darolutamide group a significant improvement in OS compared with the ADT plus docetaxel group was reported [19].

The results of the clinical trials above reported have dramatically changed the therapeutic landscape of prostate tumors in hormone-sensitive setting.

1.2.2 Castration resistant prostate cancer (CRPC)

After a variable period of hormone sensitivity, PCa becomes resistant to ADT, entering the castration resistant prostate cancer (CRPC) stage.

CRPC status is defined as the presence of castrate serum testosterone levels (<50 ng/dL) plus either biochemical progression (three consecutive rises in prostate-specific antigen (PSA), resulting in two 50% increases over the nadir, and PSA >2 ng/mL) or radiological progression, consisting of the appearance of new lesions, such as two or more new bone lesions on bone scan or a soft tissue lesion using the Response Evaluation Criteria in Solid Tumors (RECIST) [20].

In Italy several drugs are currently approved for treatment of CRPC.

The sequence in which these drugs can be administered depends on numerous variables, such as previous treatments in the hormone sensitivity setting, patient's clinical conditions, comorbidity, etc.

A particular setting consists of non-metastatic CRPC (nmCRPC), a condition of resistance to ADT without distant metastases at conventional imaging. Recently, three phase III trials showed that ARSI (apalutamide, darolutamide, and enzalutamide) plus ADT significantly improved the metastatic free survival (MFS), which was the primary endpoint, in high-risk nmCRPC [21-23]. In the phase III SPARTAN trial, 1207 patients were randomized with a 2:1 design to receive apalutamide+ADT or placebo+ADT. The median MFS and OS were 40.5 and 73.9 months in the apalutamide group versus 16.2 and 52.8 months in the placebo group, respectively [21]. In the ARAMIS trial, 1509 (955/554) patients in total received ADT combined to darolutamide or placebo. The

authors reported a median MFS of 40.4 months in the darolutamide arm versus 18.4 months with placebo, and a 3-year OS rate of 83% in the darolutamide group versus 77% in the placebo group [23]. In the phase III PROSPER trial, a total of 1401 nmCRPC patients were randomly assigned (2:1 ratio) to receive enzalutamide or placebo, + ADT. Enzalutamide significantly decreased the risk of metastases or death (27% lower) compared to placebo [22].

All drugs are approved in Italy for nmCRPC patients.

Abiraterone acetate blocks CYP17, a critical enzyme in testosterone synthesis, thereby interrupting androgen synthesis by the adrenal glands, testis, and within the prostate tumor. The drug is administered with low-dose prednisone to prevent mineralocorticoid-related adverse events, including fluid retention, hypertension, and hypokalemia. In the COU-AA-301 trial, patients with mCRPC who had previously been treated with docetaxel were randomly assigned to receive abiraterone or placebo. After a median follow-up of 12.8 months, the OS was longer in the abiraterone group compared to the placebo group. Abiraterone also improved all secondary endpoints, including time to PSA progression, PFS, and PSA 50% response [24]. In the final analysis of the COU AA-301 trial, median OS in the abiraterone group was longer than that of the placebo group [25]. In the COU-AA-302 trial, abiraterone was evaluated in chemotherapy-naive mCRPC patients. Median radiographic PFS (rPFS) was 16.5 months with abiraterone and 8.3 months with placebo. In the final analysis of the COU-AA-302 trial, median OS was significantly longer in abiraterone group than that in placebo group (34.7 versus 30.3 months) [26]. The two COU-AA trials demonstrated therefore that abiraterone improved OS in both chemotherapy-pretreated and chemotherapy-naïve mCRPC patients.

Enzalutamide is a second-generation, nonsteroidal AR inhibitor that competitively binds the ligand-binding domain of the AR, inhibiting AR translocation to the cell nucleus and AR binding to DNA. In the AFFIRM trial, patients with mCRPC pre-treated with chemotherapy were randomly assigned to receive enzalutamide or placebo. Median OS was 18.4 months in the enzalutamide group versus 13.6 months

in the placebo group. The superiority of enzalutamide over placebo was demonstrated also for secondary endpoints, including time to first skeletal-related event (SRE), pain control, and patient-reported quality of life [27-28].

In the PREVAIL trial, enzalutamide was evaluated in mCRPC patients in a chemo-naïve setting. The 12-months PFS rate was 65% in patients treated with enzalutamide and 14% in patients receiving placebo. At the final analysis in the PREVAIL trial, median PFS was 20.0 months and 5.4 months in the enzalutamide and placebo arm respectively, while median OS was 35.3 months in the enzalutamide group and 31.3 months in the placebo group [29-30].

Similarly to abiraterone, enzalutamide has demonstrated excellent efficacy in both chemotherapy-pretreated and chemotherapy-naïve mCRPC patients.

Docetaxel, a taxane-based anticancer drug, has been widely used as a standard treatment for mCRPC since 2004 when it demonstrated a benefit in OS in mCRPC patients. In the TAX-327 study, patients with mCRPC were randomly assigned to receive mitoxantrone, docetaxel every 3 weeks (75 mg/m^2), or weekly docetaxel (30 mg/m^2). Docetaxel every 3 weeks had a better OS compared to mitoxantrone [31].

Cabazitaxel is a next-generation taxane approved for treatment of mCRPC patients in a post docetaxel setting. The phase 3 TROPIC trial compared cabazitaxel (25 mg/m^2) with mitoxantrone in patients with mCRPC pre-treated with docetaxel. Median OS was 15.1 months in the cabazitaxel group and 12.7 months in the mitoxantrone group (HR 0.70) [32].

Alteration in DNA repair genes are observed in up to 30% of PCa, and the most commonly mutated genes are BRCA1, BRCA2, and ATM [33]. These gene alterations can occur at a somatic or germline level. The germline mutations in BRCA1, BRCA2, and ATM are associated with PCa risk and aggressive phenotypes [34].

Tumors with gene alterations that affect homologous recombination repair (HRR) are sensitive to poly (adenosine diphosphate-ribose) polymerase inhibitors (PARPi) in prostate and other cancers [35-36].

In the TOPARP-B trial, Mateo et al. demonstrated the antitumor activity of olaparib in mCRPC with specific damage response and repair (DDR) gene aberrations [37]. The high and often durable responses rates observed in mCRPC patients with germline or somatic BRCAl/2 alterations support the use of olaparib in this subpopulation [37]. In the phase 2 TRITON2 study, Abida et al. found that rucaparib had antitumor activity in mCRPC patients with BRCA alterations [38].

The phase 3 PROfound trial enrolled mCRPC patientsprogressing during an ARSI (enzalutamide or abiraterone) treatment. Patients harbouring alterations in genes involved in HRR were randomly assigned to receive olaparib (a PARP inhibitor), or either enzalutamide or abiraterone. The primary outcome was efficacy, which was assessed evaluating PFS in patients with alterations in BRCAl, BRCA2, or ATM (cohort A). In this cohort, PFS was significantly longer in the olaparib group than that in the control group. Significant differences were also observed in objective response rate (ORR) and time to pain progression.

Median OS in cohort A was 19.1 months in the olaparib group versus 14.7 months in the control group. Despite crossover from control to olaparib, patients allocated in the olaparib arm had significantly longer OS than those assigned to receive enzalutamide or abiraterone (control arm) [39].

Preclinical studies hypothesize a synergy between PARP inhibitors (PARP-i) and ARSI. This synergy may depend on the involvement of PARP in the positive co-regulation of AR signaling, which leads to enhanced AR target gene suppression when PARP-AR signaling is corepressed [40]. ARSI seem to inhibit the transcription of some HRR genes, leading to a kind of “HRR deficiency” and an increased sensitivity to PARP inhibitors through nongenetic mechanisms [41]. These preclinical findings were at the base of three phase 3 trial: PROpel, MAGNITUDE and TALAPRO2.

The PROPEL trial randomised 796 unselected mCRPC patients to receive abiraterone plus olaparib or placebo [42]. Prior treatment with docetaxel in the hormone-sensitive setting was allowed. Combination treatment prolonged PFS irrespective of HRR status (median rPFS: 25 versus 16 months). In the HRR+ subgroup, median rPFS was not

reached for the combination arm and 14 months for the abiraterone+placebo control arm [42]. The MAGNITUDE trial evaluated abirateone plus niraparib or placebo in 423 patients with HRR gene mutations and in 247 HRR-proficient patients [43]. Prior docetaxel for HSPC and up to 4 months of abiraterone for mCRPC before random were allowed. In the HRR+ cohort, PFS was significantly better in the combination arm (median PFS: 17 versus 14 months) [43].

TALAPRO-2 evaluated the efficacy of enzalutamide plus talazoparib or placebo. The study enrolled 805 patients irrespective of HRR mutational status (cohort 1). The HRR-mutant cohort was then prospectively extended, with the recruitment of further 230 HRR+ patients. Median PFS was not reached for the combination arm and was 22 months for the control group of cohort 1 (HR 0.63). In the HRR+ subgroup analysis, median PFS was 28 months for the combination arm and 16 months for the control arm (HR 0.46) [44].

To date, in Italy, the available combinations of ARSI and PARPi are abiraterone + olaparib, that can be used for first line mCRPC irrespective of HRR mutational status, and abiraterone + niraparib, for BRCA1/2 mutated patients. Currently these combinations are available upon personal requests or compassionate use programs.

Prostate-specific membrane antigen (PSMA) is a transmembrane glutamate carboxypeptidase highly expressed in mCRPC cells [45]. 177Lu-PSMA-617 is a radiometabolic treatment that delivers beta-particle radiations to PSMA-expressing cells and surrounding microenvironment. In the phase 3 VISION trial, 68Ga-PSMA-positive mCRPC patients previously treated with ARSI and taxanes, were randomly assigned to receive either 177Lu-PSMA-617 plus standard care or standard care alone. Compared with the control arm, radioligand therapy plus standard care significantly prolonged PFS (median 8.7 versus 3.4 months; HR 0.40) and OS (median 15.3 versus 11.3 months; HR 0.62) [46].

Currently in Italy 177Lu-PSMA-617 is not reimbursed by the regulatory authorities in the indication of the VISION study yet.

In conclusion, to date abiraterone, enzalutamide, apalutamide, and docetaxel are approved and widely used to treat mHSPC in combination with ADT. Furthermore, the triplet therapy comprising docetaxel, ARSI, and ADT has recently emerged for the treatment of mHSPC. This intensification of treatment in the disease management represented an important step forward. However, cross-resistance between drugs may reduce the effectiveness of downstream therapies for mCRPC, promoting the development of more aggressive, treatment-resistant PCa phenotypes. The sequential administration of ARSI, such as abiraterone and enzalutamide, is associated with limited efficacy. For mCRPC patients previously treated with docetaxel progressing to an ARSI, treatment with cabazitaxel is recommended and should be considered if the patient is still eligible for chemotherapy. This assumption is suggested by the CARD trial where cabazitaxel was compared to an ARSI (abiraterone or enzalutamide) in mCRPC patientspreviously treated with docetaxel and progressing within 12 months while receiving an alternative ARSI (abiraterone or enzalutamide). Treatment with cabazitaxel led to better PFS [47]. In addition, several novel agents have been introduced in clinical practice for the treatment of mCRPC. In particular, Lu-PSMA and PARPi are emerging as effective therapeutic options. PSMA-PET is used to determine the eligibility for Lu-PSMA therapy, although this treatment is not reimbursed by the regulatory authorities in Italy yet. Evaluation of BRCA1/2 status is currently mandatory, to identify mCRPC patients eligible for treatment with PARPi.

1.3 Biomarkers in prostate cancer

Prostate cancer is a heterogeneous disease, characterised by high variability in clinical outcomes. There is an urgent clinical need to identify novel tools to improve risk stratification at clinical decision points and select the most effective treatment that could maximise cure efficacy and extend life expectancy. Molecular profiling of solid cancers has been used across multiple cancer types to identify poorer prognosis cancers and guide treatment selection. Most studies have evaluated nucleic acids (DNA and/or

RNA) and/or protein on primary tumor biopsies or, less commonly, on metastatic lesions.

This approach has some significant limitations. First, there are often practical and clinical difficulties to obtain tissue from poorly accessible metastases. This is especially true for prostate cancer as up to 90% of patients have bone metastases only [48]. Secondly, intrapatient and tumour heterogeneity may cause an incorrect classification of cancer, due to spatial or temporal differences. Third, repeated tumor biopsies to monitor tumor evolution and treatment response is not ethically acceptable.

Due to these limitations, there has been an increasing interest in blood-based biomarkers, through the so-called liquid biopsies, to better characterise tumor molecular drivers and response to treatments. Nucleic acids, proteins, cells and vesicles, circulate in human blood and can be isolated using various molecular techniques.

The portion of circulating cell free DNA (cfDNA) derived by tumor is named circulating tumor DNA (ctDNA) or plasma tumor DNA (ptDNA) [49]. The ptDNA fraction depends on disease setting and tumor spread, and can range from 1% or below at the initial stages of the disease, to 90% in patients with high-volume progressing CRPC metastases [50-52].

The analysis of ctDNA in mCRPC patients allows to identify PCa genomic features. When ptDNA is sufficiently high, there is a strong concordance with tissue findings for the detection of genomic alterations present in concurrently collected metastases biopsies [53].

The presence of a low tumor fraction could be a technical limitation for plasma DNA analyses. However, the introduction of new genomic technologies with high sensitivity and specificity, including next-generation sequencing (NGS), has positively contributed to the study of ptDNA [54].

NGS is a powerful DNA sequencing technology that allows for the rapid and high-throughput sequencing of millions to billions of DNA fragments simultaneously. It has revolutionized genetic analysis and has many advantages over traditional sequencing,

including lower sample input requirements, higher accuracy, and the ability to detect variants at lower allele frequencies [55].

Numerous biomarker studies have been developed starting from liquid biopsies and the evaluation of circulating tumor genetic material. The most common genomic alterations studied as prognostic or predictive biomarkers for PCa have been explored further below.

Androgen Receptor

Androgen receptor is a steroid and nuclear receptor, acting as an intracellular transcriptional factor, and it is highly expressed in prostate cells [56]. Its ligands are testosterone and 5α-dihydrotestosterone (5α-DHT). The binding between those ligands and AR determines intracellular receptor activation, consisting of homodimerization, autophosphorylation, and translocation to the nucleus [57].

AR plays a key role in PCa development by promoting cell survival and proliferation but also migration and invasion. [58]. Indeed, therapeutic approaches to suppress AR signaling in PCa cells through inhibition of androgen biosynthesis by luteinizing hormone-releasing hormone (LHRH) agonist or antagonist, or through the use of receptor antagonists (antiandrogen drugs) have been the main available therapies against metastatic PCafor decades [59].

Disruptions of the AR pathway consist of AR point mutations, truncated variants, and gene amplifications, and all of these confer selective advantage to PCa cells [60].

AR point mutations are responsible for acquired resistance through alterations in the ligand affinity. These mutations act their role in several ways: by reducing affinity to antiandrogenic drugs, such as flutamide and bicalutamide or enzalutamide and apalutamide, but also by modifying affinity for other ligands such as a higher affinity for progesterone or prednisone [60-61].

AR gene amplifications, which have been detected in up to 60% of pretreated CRPC patients [62], are also responsible for tumor progression despite optimal ADT, by determining higher expression of AR in PCa tissue with consequent cell proliferation

despite low androgen levels [63]. Gene amplifications are rare in treatment-naïve patients, suggesting their role in adaptive response to anticancer therapies [64].

AR variants consist in altered protein transcriptions characterized by ligand binding domain loss, determining constitutively activated truncated AR that translocate to the nucleus [65]. AR-V7 is the most frequent alteration, detected in up to 75% of CRPC on ADT [66].

As already stated, AR alterations are rare in treatment-naïve metastatic PCa. This could be relevant in the current treatment scenario for mHSPC where prospective studies are evaluating the efficacy of standard chemotherapy and new generation hormonal treatment in combination with LHRH analogues [67]. Most of these aberrations have been detected in patients who progressed on ADT, and their clinical significance is both prognostic and predictive. Their prognostic role depends on their association to poor survival [68], and the predictive role on the lower probability of response to other hormonal agents due to a constitutively activation of the mutated AR [69].

To date, the use AR gene alterations as biomarkers is not recommended in clinical practice because their role in therapy selection has not been prospectively validated[70]. Nevertheless, the detection of AR gain in plasma samples has been proven to be associated with resistance to enzalutamide/abiraterone in both chemotherapy-naïve and post-docetaxel CRPC settings, with worse OS and PFS and reduced PSA responses [71]. Patients harboring these gains seem to obtain more benefit from taxane-based therapies for mCRPC compared to hormonal agents [72-74]. Therefore, cell-free AR gains could represent a predictive biomarker in patients previously exposed to AR pathway-targeting agents [75]. Similarly, expression of AR-V7 is associated with resistance to AR-targeted therapies [66, 76]

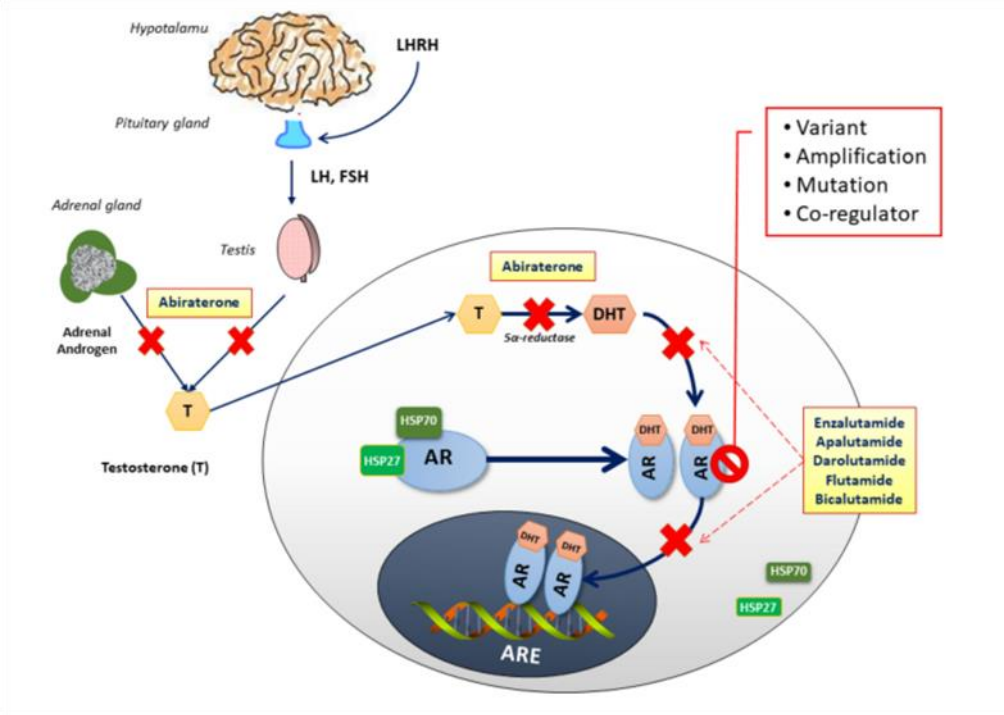

Figure 3. Androgen-dependent signaling through the androgen receptor (AR). [62]

PTEN

The phosphatase and TENsin homolog (PTEN) gene encodes for the homonym tumor suppressor protein, which plays a fundamental role in physiological functions as embryonic development, stem cell growth and differentiation, cell adhesion, and migration [77]. It is mainly involved in the phosphatidylinositol (PIP) metabolism. PTEN loss causes accumulation of phosphatidylinositol 3,4,5-trisphosphate (PIP3) with an increase in phosphorylation of AKT and activation of its signaling pathway, causing unregulated cellular growth [78].

PTEN loss has also been hugely investigated as a prognostic biomarker, and it has been associated with poor survival in metastatic patients [79-81]. PTEN loss evaluated by immunohistochemistry (IHC) has been thoroughfully studied as predictive biomarker of response to hormonal and chemotherapeutic agents. Two large retrospective studies showed that PTEN loss (defined as <10% of cancer cells presenting positive staining) was associated with low response to abiraterone [79]. However, PTEN loss cancers had the same sensitivity to docetaxel as PTEN normal tumors [81]. PTEN expression was also prospectively studied as a predictive biomarker in mCRPC in a phase II

randomized trial, which showed a longer PFS in PTEN-loss tumors treated with ipatasertib, a small tyrosine-kinase inhibitor (TKI) that inhibits AKT [82]. A larger phase III trial with ipatasertib plus abiraterone versus placebo plus abiraterone has confirmed these results [82]. Despite the above-mentioned advantage in PFS, no positive data on OS are available in favor of ipatasertib to support its use in clinical practice.

Homologous recombination deficiency (HRD)

Homologous recombination deficiency (HRD) consists of the loss of ability of normal and tumor cells to repair double strand breaks that occur into DNA.

In PCa, genes encoding for these proteins have been found to be mutated in different percentages. In recent works, BRCA2 was described as the most commonly mutated HRD gene (13.3%), followed by ATM (7.3%), CDK12 (4.7%), and BRCA1 (0.7%) [83].

The predictive role of mutations affecting HRD genes has been investigated in several clinical trials with PARPi in mCRPC patients. To date, among PARPi tested in PCa, the main clinical data derive from the use of olaparib, niraparib, and rucaparib as above reported [37-39; 42-44].

Plasma tumor DNA (ptDNA)

In prostate cancer, ptDNA itself has been studied as a biomarker. Detection of ptDNA is prognostic and a change in ptDNA levels during anticancer treatments is associated with differential outcome with anticancer treatments.

Recent studies have shown an association between pre-treatment ptDNA fraction, assessed by NGS, and clinical outcome [51-52, 72, 84-85] in PCa. A recent randomised phase 2 study evaluating 202 mCRPC patients treated with first line abiraterone or enzalutamide showed that low pre-treatment ptDNA fraction was correlated with a good prognosis [86].

The role of ptDNA changes in response to treatment, also termed plasma DNA dynamics, has also been evaluated as an early assessment of therapy efficacy for mCRPC. A recent study observed that patients with an increase in ptDNA fraction had a significantly increased risk of progression at 3-month radiographic assessment. Conversely, patients with a decrease in ptDNA fraction had a significantly higher chance of having a response to anticancer treatment [87].

A recent poster by our working group at ESMO 2023 congress, evaluated ptDNA to identify biomarkers of resistance to cabazitaxel in metastatic castration-resistant prostate cancer (mCRPC) patients (pts). This work described that ptDNA changes from baseline to cycle 3, were strongly associated with outcomes (OS e PFS).

These results highlighted the potential of adding ptDNA assessment to routine monitoring of mCRPC patients.

Not-molecular features related to prognosis

The study of prognostic biomarkers in mCRPC patients is not limited to molecular features but includes also numerous clinical, laboratory and metabolic imaging-related elements that have been correlated with outcomes.

Caroli et al. evaluated the role of F^{18}CH-PET-derived parameters to predict the clinical outcome of mCRPC patients treated with abiraterone or enzalutamide. In particular, whole-body tumor burden indices based on metabolic tumour volume (MTV) and total lesion activity (TLA) measured by FCH-PET/CT were found to be prognostic of OS [88].

Many clinical aspects of prostate cancer patients have been corretated with prognosis. Among these, the presence of visceral metastases appears to be an important predictor of clinical outcome in CRPC patients treated with both hormonal agents and taxanes but also with radiometabolic therapies such as LuPSMA [89-91].

Lactate dehydrogenasis (LDH) is a key enzyme in the last step of the glycolysis pathway and is related to the glycolysis level of the tumor. It has been demonstrated

that there is a linear correlation between LDH levels and the progression of PCa; higher LDH levels are associated with higher risk of tumor progression [92].

1.3.1 A novel prognostic model

In a previous work by our group (Conteduca et al. 2022, we evaluated the prognostic role of the combination between molecular, clinical, and radiological features of patients affected by mCRPC. The aim of this study was to desing a prognostic score to classify these patients and help clinicians in daily practice [93].

This study considered 102 patients affected by CRPC receiving abiraterone or enzalutamide. In the training set, multivariable analyses showed that ptDNA, MTV and serum lactate dehydrogenase together with visceral metastasis were independent predictors of both OS and PFS (**table 1**).

	N. patients	**N. events**	**Median OS (months) (95% CI)**	***P***	**HR (95% CI)**	***P***
Overall	65	63	17.6 (11.1-23.1)	-	-	-
Age, years						
≤74	37	35	17.6 (9.2-25.9)		1.00	
>74	28	28	17.9 (11.1-22.9)	0.988	1.00 (0.60-1.66)	0.988
Prostatectomy						
No	38	36	13.4 (9.9-23.1)		1.00	
Yes	27	27	21.8 (11.21-26.5)	0.321	0.77 (0.46-1.29)	0.322
Radical radiotherapy						
No	40	39	18.6 (11.0-25.3)		1.00	
Yes	25	24	17.6 (8.7-22.5)	0.517	1.18 (0.71-1.98)	0.518
Gleason score						
6-7	28	27	22.7 (11.0-26.5)		1.00	
8-10	30	29	11.2 (7.4-21.4)	0.681	1.12 (0.65-1.92)	0.681
Site of metastasis						
No bone	5	5	9.8 (2.1-nr)		1.00	
Bone	60	58	17.9 (11.1-23.1)	0.708	1.20 (0.47-3.06)	0.709
No lymph nodes	30	29	18.2 (11.0-27.1)		1.00	
Lymph nodes	35	34	17.6 (9.2-23.7)	0.642	1.13 (0.68-1.86)	0.643
No visceral	55	53	21.8 (11.9-25.3)		1.00	
Visceral	10	10	10.2 (6.3-17.4)	0.005	2.77 (1.33-5.81)	0.007
ECOG PS						
0-1	63	61	18.3 (11.0-23.1)		1.00	
≥2	2	2	14.4 (11.4-nr)	0.439	1.75 (0.42-7.33)	0.444
Presence of pain						

No	59	57	20.7 (11.1-24.0)		1.00	
Yes	6	6	11.8 (2.1-nr)	0.013	2.92 (1.20-7.11)	0.018
Chemotherapy-naïve						
No	17	15	20.7 (7.4-25.3)		1.00	
Yes	48	48	15.6 (11.0-23.7)	0.615	1.16 (0.65-2.09)	0.615
Prior therapeutic lines						
1-2	43	41	18.3 (10.6-23.1)		1.00	
>2	22	22	15.6 (9.4-29.9)	0.520	0.84 (0.49-1.43)	0.521
Serum LDH, U/l						
<225	49	47	21.4 (13.7-24.0)		1.00	
≥225	16	16	9.3 (5.6-17.6)	0.003	2.40 (1.32-4.35)	0.004
ALP, U/l						
<129	51	49	19.0 (11.4-24.0)		1.00	
≥129	14	14	14.5 (2.9-22.5)	0.062	1.76 (0.96-3.23)	0.066
NLR						
<3	34	33	15.6 (10.6-22.5)		1.00	
≥3	31	30	18.3 (9.4-25.9)	0.453	0.82 (0.50-1.36)	0.454
Serum CGA, ng/mL						
<120	27	26	18.3 (10.6-23.1)		1.00	
≥120	38	37	17.5 (9.9-25.3)	0.405	0.80 (0.48-1.35)	0.407
Hemoglobin, g/dl						
>12.5	25	25	8.4 (4.4-11.4)		1.00	
≤12.5	40	38	7.3 (5.0-9.0)	0.826	0.94 (0.57-1.57)	0.825
Serum albumin, g/dl						
>4	30	29	8.5 (5.6-13.9)		1.00	
≤4	30	29	7.3 (4.4-9.5)	0.275	1.33 (0.79-2.24)	0.277
Serum PSA, ng/dl						
<32.20	32	31	8.2 (4.4-11.7)		1.00	
≥32.20	33	32	7.2 (4.6-9.0)	0.287	1.31 (0.79-2.16)	0.289
N. of lesions						
<12	33	31	9.2 (7.4-11.7)		1.00	
≥12	32	32	5.8 (3.6-8.4)	0.009	1.95 (1.17-3.26)	0.011
SUV max						
<93.48	35	33	9.2 (5.0-11.7)		1.00	
≥93.48	29	29	6.5 (4.4-8.6)	0.022	1.82 (1.08-3.07)	0.024
MTV						
<102.79	35	33	9.2 (6.8-11.7)		1.00	
≥102.79	30	30	6.2 (4.4-8.6)	0.032	1.74 (1.04-2.91)	0.034
TLA						
<235455	28	26	8.9 (5.0-13.3)		1.00	
≥235455	37	37	7.2 (4.6-9.0)	0.046	1.70 (1.01-2.88)	0.048
ptDNA						
≤0.201	35	34	9.3 (7.4-11.7)		1.00	
>0.201	30	29	4.1 (3.0-8.4)	0.037	1.69 (1.02-2.80)	0.040
***AR* copy number**						
Normal	50	48	8.6 (6.0-10.4)		1.00	
Gain	15	15	5.6 (1.6-7.5)	0.009	2.22 (1.20-4.09)	0.011

Table 1 Univariate analysis of Overall Survival in the training cohort

Abbreviations: ECOG: Eastern Cooperative Oncology Group. **PS:** performance status. **AR**: androgen recaptor. **ALP**: Alcaline phosphatasis. **LDH**: lactate dehydrogenasis. **NLR**: neutrofil to lhymphocites ratio. **CgA**: Chromogranine. **PSA**: prostatic specific antigen**. SUV**: standardized uptake value. **MTV:** metabolic tumour volume. **TLA**: total lesion activity. **ptDNA**: plasma tumor DNA.

Considering the presence or absence of visceral metastases and the presence of values (MTV value on choline PET scans, plasma tumor DNA levels, serum LDH levels) higher or lower than a statistically defined median, a risk score was obtained to allocate each patient in pre-defined risk classes (I-III). Prognostic scores were generated, with the identification of three groups of patients with significantly different median OS (29.2, 15.9 and 8.7 months) and PFS (13.3, 7.7 and 3.2 months).

From a statystical point of view, categorical variables were summarized using frequency whereas continuous variables were described using median value and interquartile range.

Median fraction of ptDNA before starting treatment was 0.188 (0.014–0.96). The association between categorical variables was determined using the chi-squared or Fisher's exact test, as appropriate. Spearman correlation was used to assess the association between continuous variables.

Univariable and multivariable Cox regression models were used to explore potential factors able to predict PFS and OS and to estimate hazard ratios (HR) and their 95% confidence interval (CI).

A Weibull multiple regression model to assess the matched impact of molecular, laboratory and imaging characteristics on outcome was used. From a full model including these factors, a final parsimonious model by using a backward selection procedure was achieved. The prognostic score was built on the final model consisting of the four previously cited factors. Partial scores were procured by splitting the value of each regression coefficient by the smallest regression coefficient. The total score for each patient resulted from a sum of appropriate partial scores, and three patient groups with different median survival probabilities were recognized. For OS, if the total score was 1 or below, between 1.1 and 2.5, and > 2.5, patients were classified as group I, group II and group III, respectively. For PFS, if the total score was 1 or below, between

1.0 and 2.1, and > 2.1, patients were classified as group I, group II and group III (table 2 and 3).

	Factor estimate (standard error)	Standard error	*P*	HR (95% CI)	Partial score
MTV	0.599	0.268	0.026	1.82 (1.08–3.08)	1.00
ptDNA	0.848	0.289	0.003	2.34 (1.32–4.12)	1.40
Visceral metastasis	1.033	0.383	0.007	2.81 (1.33–5.95)	1.70
Serum LDH, $U{\cdot}L^{-1}$	1.239	0.331	0.0002	3.45 (1.81–6.60)	2.10

Risk groups	No. pts (%)	Total score
I	22 (33.9)	< 1.4
II	24 (36.9)	1.4–2.8
III	19 (29.2)	≥ 2.8

Table 2 Multivariable analysis of OS after backward stepwise procedure in the training cohort.

Abbreviations: MTV: metabolic tumour volume. **ptDNA**: plasma tumor DNA. **LDH**: lactate dehydrogenasis. **HR**: hazard ratio. **U-L**: upper-limit.

	Factor estimate (standard error)	Standard error	*P*	HR (95% CI)	Partial score
MTV	0.586	0.271	0.031	1.80 (1.06–3.06)	1.00
ptDNA	0.645	0.266	0.015	1.91 (1.13–3.21)	1.10
Visceral metastasis	0.997	0.424	0.019	2.71 (1.18–6.22)	1.70
Serum LDH, $U{\cdot}L^{-1}$	1.204	0.323	0.0002	3.33 (1.77–6.27)	2.05

Risk groups	No. pts (%)	Total score
I	15 (23.1)	< 1.0
II	34 (52.3)	1.0–2.1
III	16 (24.6)	> 2.1

Table 3 Multivariable analysis of PFS after backward stepwise procedure in the training cohort.

Abbreviations: MTV: metabolic tumour volume. **ptDNA**: plasma tumor DNA. **LDH**: lactate dehydrogenasis. **HR**: hazard ratio. **U-L**: upper-limit.

The differences in median survival between risk groups were confirmed in the validation cohort for both OS and PFS (**Figure 4 and 5**).

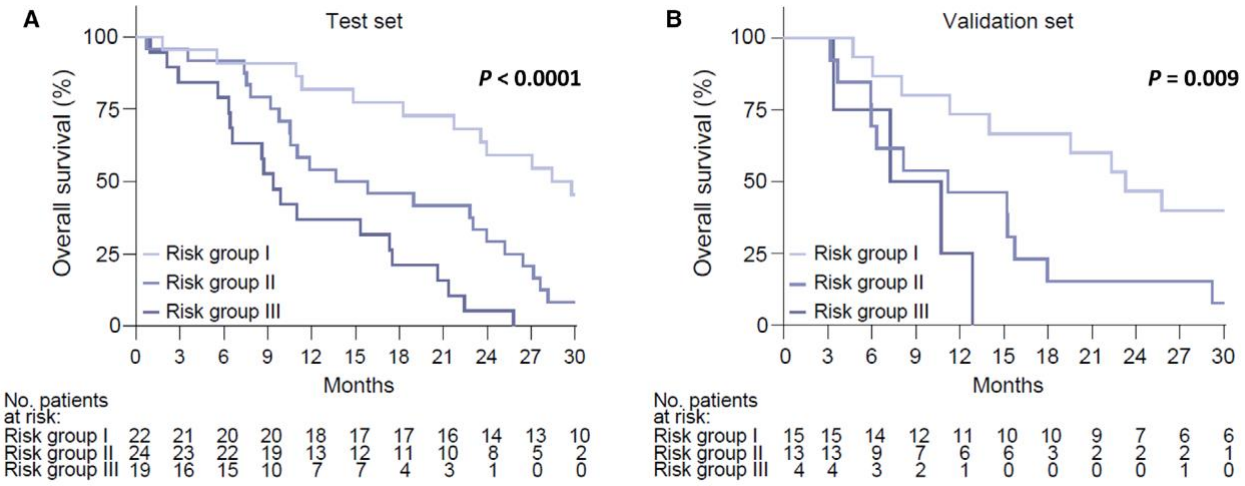

Figure 4 Risk group survival probabilities. Kaplan–Meier curves for OS by OS risk groups in the training set (A) and validation set (B)

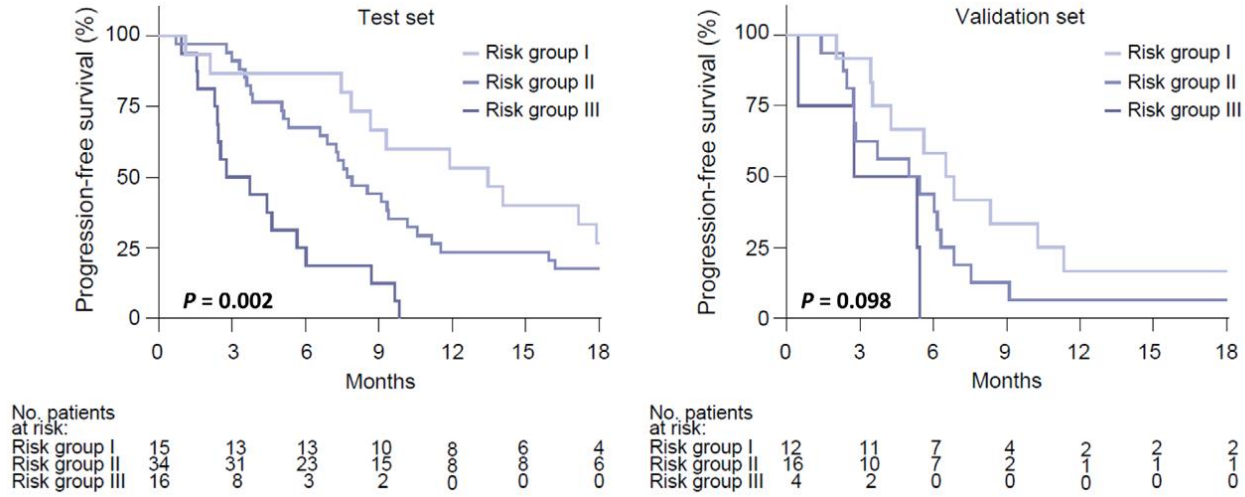

Figure 5 Risk group survival probabilities. Kaplan–Meier curves for PFS by PFS risk groups in the training set (A) and validation set (B)

We performed also an evaluation about the associations between ptDNA, clinical variables and functional imaging. We demonstrated a significant correlation between ptDNA and the number of tumoral lesions. (**Figure 6A**). However, ptDNA did not significantly associate with the number of different types of sites of metastasis (Figure 6B). Furthermore, we investigated the association between choline uptake measured as median SUVmax, MTV, TLA and ptDNA levels (**Figures 6 C, D, E**). It was reported a meaningful correlation between pIDNA fraction and choline uptake measured by

SUVmax, MTV, and TLA. A direct relationship between ptDNA and choline uptake on FCH-PET was showed in a post-docetaxel patient treated with abiraterone (**Figure 6F**).

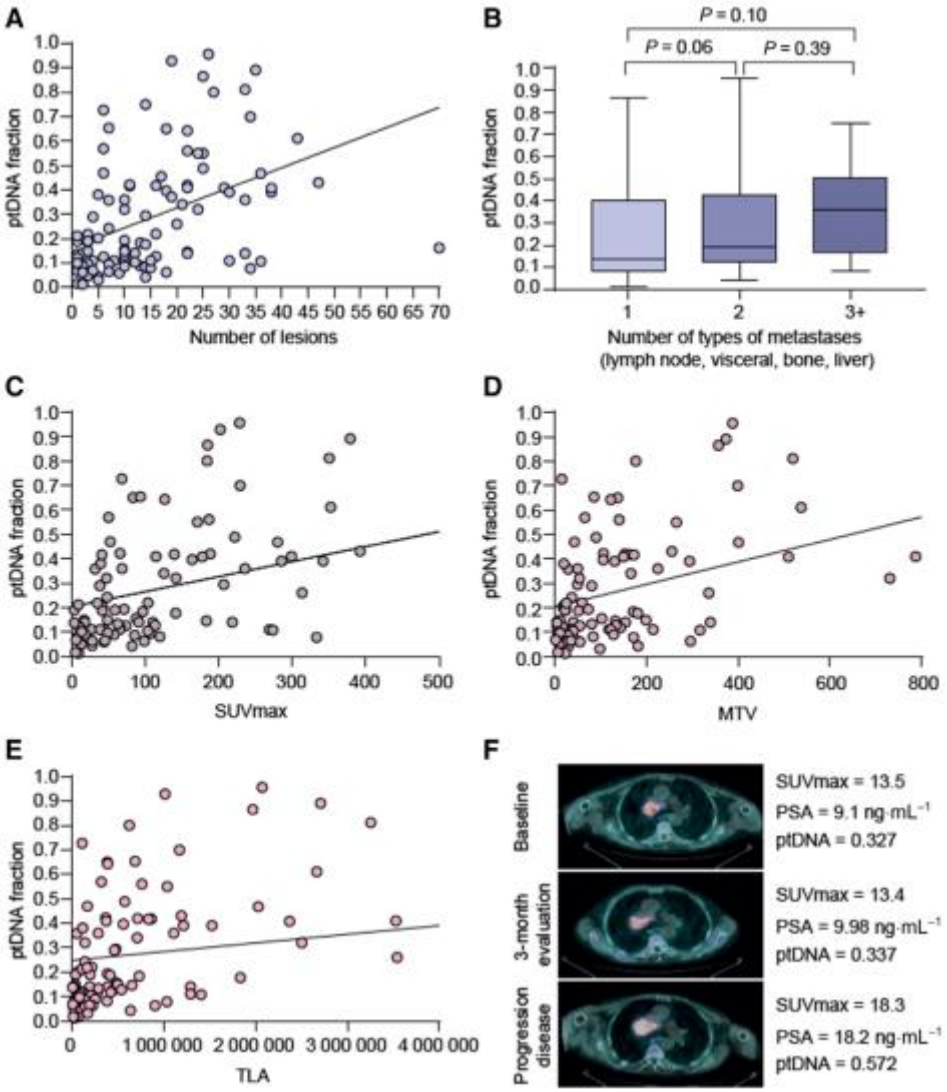

Figure 6. Correlation between ptDNA and number of tumoral lesions (A). Association of median ptDNA fraction and the number of types of metastases (B). Association of SUVmax (C), MTV (D) and TLA (E) with ptDNA fraction. Representative case of association of metabolic activity and ptDNA fraction (F).

2. Project objectives

We have validated a prognostic model for mCRPC, combining clinical, metabolic and laboratory features that showed a promising role in ARSI treated patients.
The aim of this PhD project is to interrogate this novel prognostic model, as a further external validation, in mCRPC patients treated with taxanes, a more advanced setting of PCa.

3. Materials and Methods

3.1 Ethics statement

The study was performed in accordance with the Good Clinical Practice and the Declaration of Helsinki. All the patients enrolled in the study have been followed up within a pre-existing prospective biological study (IRST-B073, approval nr. L3P1380), previously approved by the local ethics committee, active at Istituto Tumori della Romagna “Dino Amadori” (IRST) in Meldola, since 2017.

3.2 Patients and samples

Patients included in the analysis were treated at the Istituto Tumori della Romagna “Dino Amadori” (IRST) in Meldola.

Castration-resistant prostate cancer patients, with evidence of biochemical and radiological progression (according to PCWG3 criteria) to standard treatment with ADT have been included in the study before the beginning of a new line of anticancer treatment.

During the first two years of the PhD project, 55 patients have been included in the analysis. Treatment (abiraterone, enzalutamide, taxanes) was selected according to clinical practice. Blood samples collection has been conducted within a pre-existing prospective biological study (IRST-B073, approval nr. L3P1380), as above reported.

Patients underwent baseline blood sampling prior to treatment start,after 3 months, and at progression. Blood samples have been stored in the Bioscience laboratory of the Institute. Peripheral blood of patients was collected and stored at -80°C for the subsequent molecular analyses. Genomic DNA was extracted with QIAamp DNA mini kit (Qiagen) and quantified using Qubit dsDNA BR Assay kit (Thermo Fisher Scientific).

The following data from all consenting patients after registration were electronically collected:

- demographic data: birthday, weight and height at the time of treatment initiation, ECOG performance status;
- tumor information: date of diagnosis, type of primary treatment of prostate cancer, prostate cancer histology, gleason grade and stage,
- treatment information: date of start and end of ADT therapies, type of hormonal therapeutic regimen, informations about chemotherapies administered, number of cycles administered, date of progression (if any) for any treatment administered;
- Date of death or last follow-up (if still alive).

3.3 Patient evaluated and considerations

At the end of the second year of the PhD Project, we critically revised the cohort of 55 patients, and concluded that the most homogeneous population suitable for statistical analysis was the one including patients treated with taxanes (docetaxel and cabazitaxel) who underwent a Choline PET scan as baseline functional test.

The use of the PET scans with new tracers (e.g. PSMA), hypothesized in the initial project, was hindered by long waiting times for basal examinations, often not compatible with the need to promptly start anticancer treatments. For this reason, the population of patients with basal PSMA PET scans was numerically low and inhomogeneous. It was therefore decided to perform the overall analysis in patients treated with taxanes and who had performed choline PET scan as baseline functional test, as above mentioned.

This specific population reached the total number of 30 patients.

3.4 Positron emission tomography (PET) scans

Before starting the new treatment line, each patient underwent a PET/CT scan with F-choline for baseline tumor staging. PET scans have been performed at the Department of Nuclear Medicine at IRST, Meldola.

FCH-PET/CT scans were carried out on an integrated PET/CT system (Discovery LS camera; General Electric Medical Systems, Waukesha, WI, USA) in 2D acquisition mode for 3 min per bed position. The PET/CT scan takes 45 min after intravenous injection of 18F-methylcholine (3.7 MBq_kg _1 of body weight, AAA-Advanced Accelerator Applications, Meldola, Italy). The field of view included the skull to midfemurs. Low dose CT (120 kV, 80 mA) without contrast agents was made for attenuation correction and as an anatomical map. The emission data were adjusted for scatter, random coincidence events, and system dead time.

Semiquantitative criteria based on the maximum standardized uptake value (SUVmax) and the target-to-background ratio were utilized to aid the visual analysis. The metabolic tumour volume (MTV) parameter was obtained by adding each three-dimensional volume of interest, and for each lesion volume and SUV mean was multiplied and then summed to have the total lesion activity (TLA).

Metabolic features, such as SUVmax, TLA and MTV were evaluated analysing images of 18F-fluorocholine PET/CT with the envolvement of nuclear medicine specialists.

3.5 Plasma tumor DNA analysis

Plasma tumor DNA analysis has been performed in Biosciences Laboratory of IRST. Cell-free DNA was extracted from 1 to 2 mL of plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen, Santa Clarita, CA, USA) and quantified by spectrophotometric evaluation (NanoDrop_ ND-1000; Celbio, Milan, Italy) or Quant-iT High Sensitivity Pico- Green Double-Stranded DNA Assay Kit (Invitrogen, Carlsbad, CA, USA). In plasma and patient-matched germline DNA, targeted NGS was assessed by the PGM Ion Torrent using a 316 or 318 Chip aiming to reach 10009 coverage per target. The ptDNA fraction for each plasma sample has been estimated using an ad-hoc customized computational tool (CLONET). CLONET is a computational tool used to estimate the clonality of somatic genomic aberrations in tumors. It is designed to compute the clonality of somatic copy number changes, point

mutations, and rearrangements in a coherent mathematical model enabling the estimation of the clonal composition of a tumor sample, and allow to extimate the fraction of tumor DNA among all cfDNA [94].

3.6 Statistical analysis

Progression-free survival was considered as the time between the first day of taxane based therapy and the date of progression disease or death (whichever came first). Overall survival was considered as the time between the first day of taxanes treatment and the date of death from any cause or the date of the last follow-up visit.

For each patient we obtained and recorded in a specific spreadsheet the values relating to the 4 factors considered within the prognostic score described previously. Depending on the individual scores obtained in the 4 elements considered, the patients were distributed into the three risk classes (I-III).

Survival curves for each risk class were estimated by the Kaplan–Meier method, and comparisons were made using the logrank test. All P-values were two-sided, and a $P < 0.05$ was defined as statistically significant. Statistical analyses were done with SAS 9.4 software (SAS Institute, Cary, NC, USA).

4. Results

4.1 Study population

Between January 2019 and November 2022, 30 patients were treated with taxanes (docetaxel or cabazitaxel) for mCRPC. All patients had been previously treated with at least one ARSI (abiraterone or enzalutamide).

Eleven patients received a treatment with cabazitaxel and 19 patients were treated with docetaxel. Principal clinical characteristics of our study population are presented in **Table 4**.

	TAXANES SET (n=30)
	N. (%)
Age	
≤74 yrs	17 (56.7)
>74 yrs	13 (43.3)
ARCN	
Normal	21 (70.0)
Gain	9 (30.0)
Visceral metastasis	
No	26 (86.7)
Yes	4 (13.3)
Gleason score	
6-7	12 (42.9)
8-10	16 (57.1)
No. Previous lines	
1-2	19 (63.3)
>2	11 (36.7)
ECOG PS	
0-1	26 (86.7)
≥2	4 (13.3)
Site of disease	
Bone	30 (100)
Lymph nodes	9 (30.0)
Lung	1 (3.3)
ALP	
<129	21 (70.0)
≥129	9 (30.0)
LDH	
<225	18 (60.0)
≥225	12 (40.0)
NLR	
<3	16 (53.3)
≥3	14 (46.7)
CgA	
<120	16 (53.3)
≥120	14 (46.7)

Hb	
>12.5	15 (50.0)
≤12.5	15 (50.0)
Previous prostatectomy	
No	18 (60.0)
Yes	12 (40.0)
Previous radiotherapy	
No	20 (66.7)
Yes	10 (33.3)
PSA (median value)	
<23.24	10 (33.3)
≥23.24	20 (66.7)
MTV (median value)	
<102.79	15 (50.0)
≥102.79	15 (50.0)
SUV mean (median value)	
<53.60	10 (33.3)
≥53.60	20 (66.7)
Unknown/missing	
SUV max (median value)	
<83.60	10 (33.3)
≥83.60	20 (66.7)
Unknown/missing	
TLA (median value)	
<391343	30 (100)
≥391343	0
N. lesions (median value)	
<12	13 (43.3)
≥12	17 (56.7)
ptDNA (median value) o **TCF**	
≤0.188	10 (33.3)
>0.188	20 (66.7)

Table 4. Patients' charateristics

Legenda: ARCN: androgen recaptor copy number, **ECOG:** Eastern Cooperative Oncology Group **PS:** performance status, **ALP**: Alcaline phosphatasis, **LDH**: lactate dehydrogenasis, **NLR**: neutrofil to lhymphocites ratio, **CgA**: Chromogranine; **Hb**: Haemoglobin, **PSA**: prostatic specific antigen, **MTV:** metabolic tumour volume **SUV**: standardized uptake value, **TLA**: total lesion activity, **ptDNA**: plasma tumor DNA.

4.2 Risk classes

For each patient, the status of the 4 features considered in the prognostic model was assessed and partial scores were assigned generating a total score. Based on the results of **table 5**, each patient, depending on the total score obtained, was associated with a different risk class.

The four values evaluated in the prognostic model (with their relative scores) were:

- ptDNA: partial score of 1.4 if over the median value of 0.188
- MTV: partial score of 1 if over the median value of 102.79

- Visceral metastases: partial score of 1.7 if present
- LDH: partial score of 2.1 if above the upper limit value of the laboratory

Risk Group	No pts (%)	Total score
I	8 (28)	<1.4
II	11 (36)	1.4-2.8
III	11 (36)	≥2.8

Table 5 Distribution of patients in the 3 risk classes

4.3 Survival analyses

The survival probability of the three categories of patients was established by the progostic score. Survival probabilities were assessed by the exponential model and by the Kaplan–Meier method. For the 30 patients evaluated in this PhD project, with a median follow up of 15 months (range 3-48), we observed a different median OS among the three risk groups (risk group I, 18.1 months [95% CI, 15.2– 33.1 months]; risk group II, 12.7 months [95% CI, 4.9–18.6 months]; and risk group III, 10.1 months [95% CI, 3.4–15.4 months]; p= 0.012).

Results of the survival analysis are summarized in **table 6**.

Survival curve for OS is showed in **figure 7**.

Risk groups	N. pts / N. events	Median OS (months) (95% CI)	p
I	8/8	18.1 (15.2-33.1)	
II	11/11	12.7 (4.9-18.6)	
III	11/11	10.1 (3.4-15.4)	0.012

Table 6 Survival analysis for OS according to the three risk groups

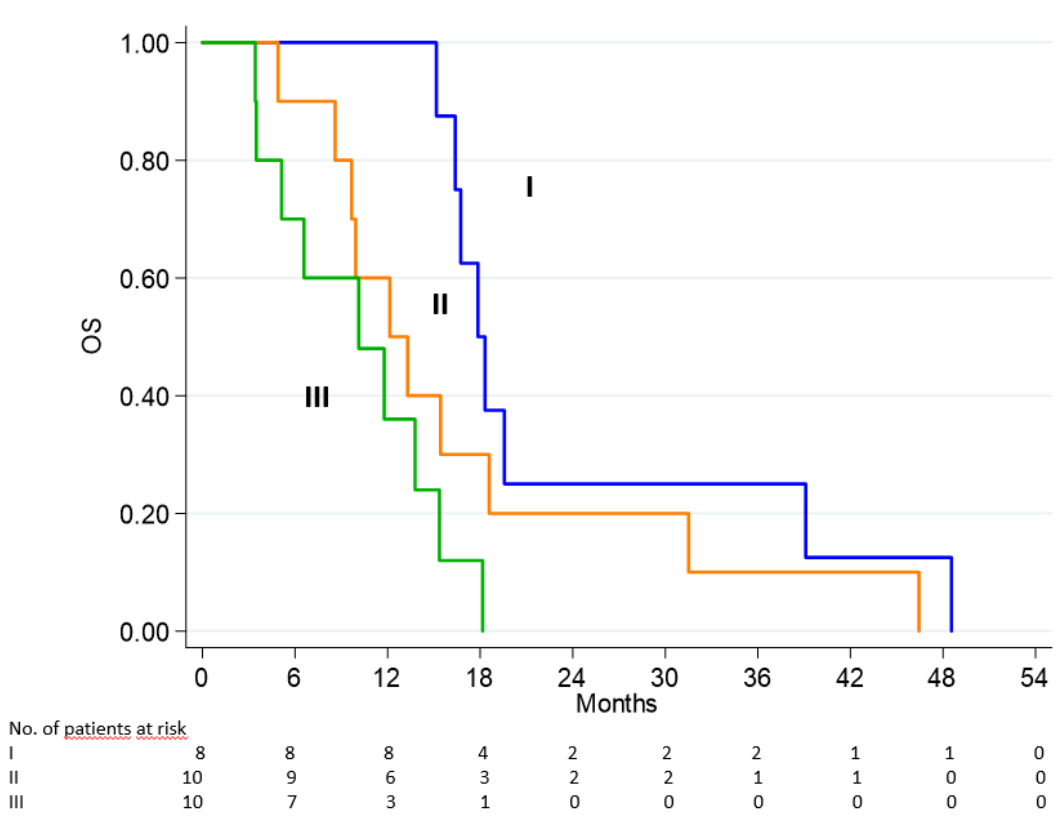

Figure 7 Risk group survival probabilities. Kaplan–Meier curve for OS by OS risk groups.

The taxane patients group was evaluated also for PFS. We decided to use the same prognostic partial scores evaluated for OS, since the very similar prognostic weight of the four variables included in the prognostic score.

We observed a different median PFS among the three risk groups (risk group I, 11.7 months [95% CI, 10.1– 13.6 months]; risk group II, 5.0 months [95% CI, 3.0–6.9 months]; and risk group III, 2.8 months [95% CI, 0.7–5.0 months]; p= 0.0006).

Results of the PFS according to risk groups are summarized in **table 7**.

Survival curve for OS is showed in **figure 8**.

Risk groups	N. pts / N. events	Median PFS (months) (95% CI)	p
I	8/8	11.7 (10.0-13.6)	
II	11/11	5.0 (3.0-6.9)	
III	11/11	2.8 (0.7-5.0)	0.0006

Table 7 Survival analysis for PFS according to the three risk groups

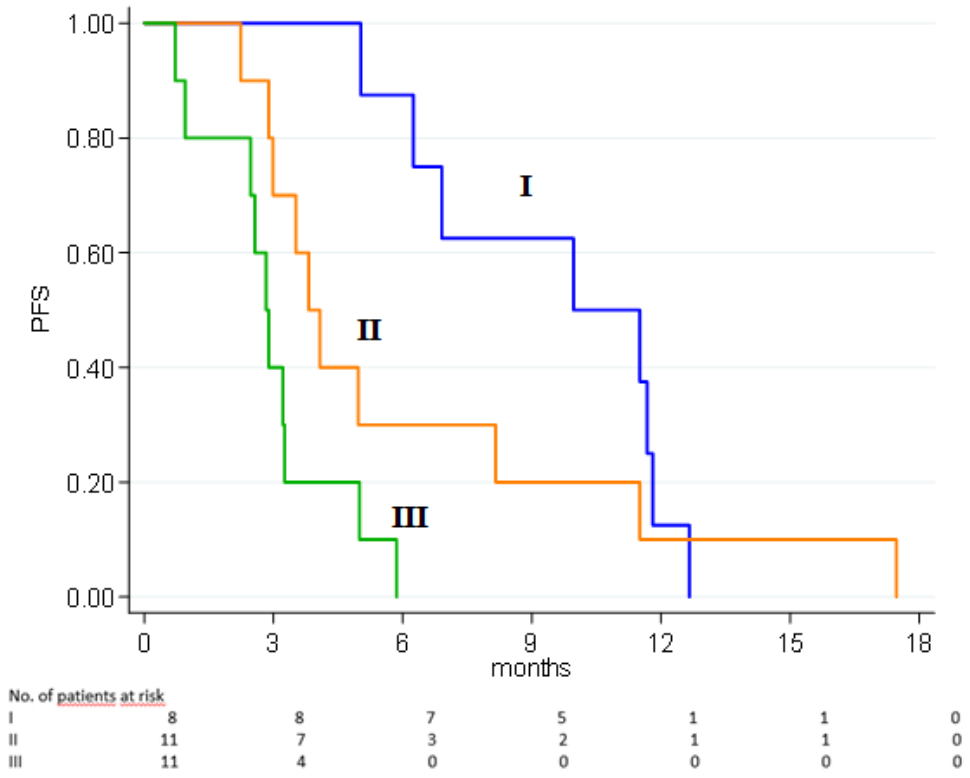

Figure 8 Kaplan–Meier curve for PFS by risk groups.

The area under the ROC curve (AUC) intended as the concordance index of the prognostic tool is 0.830 (95% CI 0.665-0.994) for OS.

5. Discussion

In recent years, several prognostic scores have been evaluated by integrating different clinical characteristics and associating them with prognosis in patients with mCRPC undergoing various anticancer treatments [95-102]. Most of these nomograms have had a limited role in clinical practice. The elements considered in the above-mentioned prognostic models included, among the others, the expression of AR-V7 in circulating tumor cells (CTCs), many genetic aberrations involving AR, TPS3, PTEN and the PI3K/AKT pathway and HRR.

A recent prospective study [103] showed the utility of integrating functional imaging using 'F-NaF PET/CT scan and CTC analysis in mCRPC patients treated with enzalutamide. The authors demonstrated a different expression of AR and AR-V7 in different metastatic sites and also the presence of neuroendocrine markers that may be responsible for a heterogeneous response to enzalutamide. This study, however, did not propose a real prognostic tool.

Furthermore, De Laere et al. [104] developed a risk stratification system, using both clinical features and TP53-alteration status in liquid biopsy, to stratify patients treated with ARSI in good or poor prognostic subgroups. No functional imaging data were used in this model.

The work by Conteduca et al. which has been previously described in detail and which represents the basis of development of the present project, tried to improve outcomes prediction in mCRPC patients, through the combination of ptDNA analysis and functional imaging. The novel prognostic score proposed and validated in patient treated with abiraterone and enzalutamide, obtained its prognostic power from the demonstration of the association between ptDNA fraction with metabolic tumor activity and the number of lesions, as similarly shown in previous NGS studies on plasma samples from mCRPC [105-106]. This assumption suggests that ptDNA fraction may provide interesting aspects of tumor biology and volume that may not be exhaustively described only by common clinical factors. The interesting observation

that both ptDNA and metabolic tumor activity were independent predictors of clinical outcomes in multivariate regression models promises to increase the accuracy of tumor response prediction and prognostication in mCRPC patients if these two elements are combined within a prognostic score.

The prognostic score described by our research group evaluated patients at baseline of treatment with abiraterone and enzalutamide both in pre- and post-docetaxel settings. In this PhD project, this prognostic model has been evaluated on further 30 patients. These patients have been treated with docetaxel or cabazitaxel (19 and 11 patients respectively) in a more advanced setting of the disease, considering that cabazitaxel is approved only after a previous treatment with docetaxel.

The present PhD project aimed to interrogate the novel prognostic model, already described and validated, in a more advanced cohort of patients to further confirm its actual prognostic power. The distribution of patients among the three risk classes is consistent with that reported in the initial work, with the difference of an increased percentage of patients in the highest risk class (36% versus 29%), compatible with the more advanced oncological setting of that patients.

This prognostic power was confirmed by positive results and clearly distinct survival curves in OS and PFS, according to risk categories.

There are some limitations in this study. First, the cohort is quite small. During the PhD project period a higher number of patients was evaluated, but the need to identify a homogeneous cohort, suitable for statistical evaluation, led to the identification of 30 patients. On the other hand, however, even with a limited number of patients, the prognostic power of the prognostic score was confirmed.

The original hypothesis of the PhD was to include patients with basal CT/PET performed using new tracers (e.g. PSMA), which are currently of routine use in clinical practice. Unfortunately, during the patients enrollment, there was no possibility to obtain novel tracers-PET scans for a sufficient number of patients, due to waiting lists often incompatible with the need to start systemic treatments for progressive disease. For this reason, the population of patients with basal PSMA PET scans was numerically

low and inhomogeneous. On the other hand, the use of choline PET provided a population more comparable to that evaluated in uor previous work, making the prognostic model generated with choline PET applicable.

A further limitation may be the inclusion in the same cohort of both patients treated with cabazitaxel and docetaxel, which are two different drugs. However, the mechanism of action of the two drugs is quite similar, making it possible to consider all patients as a single prognostic group.

The availability of validated prognostic scores has a potentially very useful impact in clinical practice. Oncologists have always faced the challenge of defining patients' prognosis with certainty, often causing issues among clinicians in the communications with patients and their families. The progostic evaluation performed both in the work of Conteduca et al. and in the present PhD project, may allow clinicians to have a better knowledge of the survival probability for different categories of patients, offering more precise data to consider when communicating patients' prognosis.

More precise prognostic data may also lead to more informed therapeutic choices. The identification of patients with particularly negative prognosis, for example, might allow clinicians to anticipate the discontinuation of potentially useless systemic treatments, avoiding episodes of therapeutic obstinacy and anticipating recourse to palliative and supportive care treatments.

The use of this novel prognostic score in daily routine may not be easy to apply. Plasma tumor DNA, among the four elements evaluated in the score, is certainly the most complex to obtain. On the other hand, the development of the technique and the increasingly frequent use of liquid biopsy also in PCa (e.g. evaluation of HRR), could make this technique routinely available, potentially creating standardized diagnostic paths which also may include the evaluation of plasma tumor DNA, if this information would be considered of primary importance for the best clinical management of the patient.

6. Conclusions and future perspectives

Researches about prognostic and predictive biomarkers in mCRPC are intense. The present PhD project has provided further confirmation about the prognostic power of a novel prognostic score proposed by our group.

It has been shown that the association between molecular, clinical, laboratory and metabolic features can contribute to define the prognosis of mCRPC patients treated with taxanes.

The prognostic score may not only be used as a static measure but also as a dynamic entity. The features evaluated in the prognostic score could change in response to anti-tumor treatments, creating a dynamic of the score with changes that may predict the responses to anticancer therapies. This approach recognizes that the tool's performance may vary across different treatment modalities, emphasizing the need for a comprehensive validation study that adapts to the dynamic nature of medical interventions.

Moreover, the current routinely use of PET scans with new tracers suggests to expand the research by first confirming the prognostic power of metabolic values (MTV, TLA, SUV), and then incorporating them into the prognostic model here reported.

Lastly, it would be of extreme clinical and scientific interest to expand the analysis also to patients with mHSPC, a setting characterized by patients with better prognosis and tumors with very different biologies.

7. References

1. Sung, H, Ferlay, J, Siegel, RL, Laversanne, M, Soerjomataram, I, Jemal, A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021: 71: 209-249.
2. Siegel, RL, Miller, KD, Fuchs, HE, Jemal, A. Cancer statistics, 2022. CA Cancer J Clin. 2022.
3. Bergengren O, Pekala KL, Matsoukas K, Fainberg J, Mungovan SF, Bratt O, et al. 2022 Update on Prostate Cancer Epidemiology and Risk Factors—A Systematic Review, European Urology, Volume 84, Issue 2, 2023, 191-20
4. Huggins C, Stevens RE Jr, Hodges CV (1941) Studies on prostatic cancer: II. The efects of castration on advanced carcinoma of the prostate gland. Arch Surg 43(2):209–223
5. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M et al. (2015) Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. N Engl J Med 373(8):737–746
6. Clarke NW, Ali A, Ingleby FC, Hoyle A, Amos CL, Attard G et al. (2019) Addition of docetaxel to hormonal therapy in lowand high-burden metastatic hormone sensitive prostate cancer: long-term survival results from the STAMPEDE trial. Ann Oncol 30(12):1992–2003
7. Gravis G, Fizazi K, Joly F, Oudard S, Priou F, Esterni B et al. (2013) Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol 14(2):149–158
8. Gravis G, Boher JM, Joly F, Soulie M, Albiges L, Priou F et al. (2016) Androgen deprivation therapy (ADT) plus docetaxel versus ADT alone in metastatic non castrate prostate cancer: impact of metastatic burden and long-term survival analysis of the randomized phase 3 GETUG-AFU15 trial. Eur Urol 70(2):256–262
9. Gravis G, Boher JM, Chen YH, Liu G, Fizazi K, Carducci MA et al. (2018) Burden of metastatic castrate naive prostate cancer patients, to identify men more likely to beneft from early docetaxel: further analyses of CHAARTED and GETUG-AFU15 studies. Eur Urol 73(6):847–855
10. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY et al. (2017) Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 377(4):352–360
11. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY et al. (2019) Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castrationsensitive prostate cancer (LATITUDE): fnal overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol 20(5):686–700

12. James ND, de Bono JS, Spears MR, Clarke NW, Mason MD, Dearnaley DP et al. (2017) Abiraterone for prostate cancer not previously treated with hormone therapy. N Engl J Med 377(4):338– 351.
13. Armstrong AJ, Szmulewitz RZ, Petrylak DP, Holzbeierlein J, Villers A, Azad A et al. (2019) ARCHES: a randomized, phase III study of androgen deprivation therapy with enzalutamide or placebo in men with metastatic hormone-sensitive prostate cancer. J Clin Oncol 37(32):2974–2986.
14. Armstrong AJ, Azad AA, Iguchi T, Szmulewitz RZ, Petrylak DP, Holzbeierlein J et al. (2022) Improved survival with enzalutamide in patients with metastatic hormone-sensitive prostate cancer. J Clin Oncol 40(15):1616–1622.
15. Davis ID, Martin AJ, Stockler MR, Begbie S, Chi KN, Chowdhury S et al. (2019) Enzalutamide with standard frst-line therapy in metastatic prostate cancer. N Engl J Med 381(2):121–131
16. Chi KN, Agarwal N, Bjartell A, Chung BH, de Santana P, Gomes AJ, et al. (2019) Apalutamide for metastatic, castration-sensitive prostate cancer. N Engl J Med 381(1):13–24
17. Chi KN, Chowdhury S, Bjartell A, Chung BH, de Santana P, Gomes AJ, et al. (2021) Apalutamide in patients with metastatic castration-sensitive prostate cancer: fnal survival analysis of the randomized, double-blind, phase III TITAN study. J Clin Oncol 39(20):2294–2303
18. Fizazi K, Foulon S, Carles J, Roubaud G, McDermott RS, Flechon A et al. (2022) Abiraterone plus prednisone added to androgen deprivation therapy and docetaxel in de novo metastatic castration-sensitive prostate cancer (PEACE-1): a multicentre, openlabel, randomised, phase 3 study with a 2 × 2 factorial design. Lancet S1040–6736(22):00367–00371.
19. Smith MR, Hussain M, Saad F, Fizazi K, Sternberg CN, Crawford ED et al. (2022) Darolutamide and survival in metastatic, hormone-sensitive prostate cancer. N Engl J Med 386(12):1132– 1142
20. Cornford P, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTROESUR-SIOG guidelines on prostate cancer. Part II-2020 update: treatment of relapsing and metastatic prostate cancer. Eur Urol 2021;79:263-82
21. Smith MR, Saad F, Chowdhury S, Oudard S, Hadaschik BA, Graff JN, et al.; SPARTAN Investigators. Apalutamide treatment and metastasis-free survival in prostate cancer. N Engl J Med 2018;378:1408-18
22. Hussain M, Fizazi K, Saad F, Rathenborg P, Shore N, Ferreira U, et al. Enzalutamide in men with nonmetastatic, castration resistant prostate cancer. N Engl J Med 2018;378:2465-74.
23. Fizazi K, Shore N, Tammela TL, Ulys A, Vjaters E, Polyakov S, et al.; ARAMIS Investigators. Darolutamide in nonmetastatic, castration-resistant prostate cancer. N Engl J Med 2019;380:1235-46. Erratum in: N Engl J Med 2022;387:860

24. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al.; COU-AA-301 Investigators. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995-2005
25. Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, et al.; COU-AA-301 Investigators. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2012;13:983-92
26. Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, et al.; COU-AA-302 Investigators. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2015;16:152-60
27. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al.; AFFIRM Investigators. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187-97
28. Fizazi K, Scher HI, Miller K, Basch E, Sternberg CN, Cella D, et al. Effect of enzalutamide on time to first skeletal related event, pain, and quality of life in men with castration resistant prostate cancer: results from the randomised, phase 3 AFFIRM trial. Lancet Oncol 2014;15:1147-56. Erratum in: Lancet Oncol 2014;15:e475
29. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al.; PREVAIL Investigators. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424-33.
30. Beer TM, Armstrong AJ, Rathkopf D, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in men with chemotherapy naïve metastatic castration-resistant prostate cancer: extended analysis of the phase 3 PREVAIL study. Eur Urol 2017;71:151- 4
31. Petrylak DP, Tangen CM, Hussain MH, Lara PN Jr, Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med 2004;351:1513-20
32. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al.; TROPIC Investigators. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet 2010;376:1147-54
33. Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. JCO Precis Oncol 2017;2017:PO.17.00029.
34. Hatano K, Nonomura N. Genomic profiling of prostate cancer: an updated review. World J Mens Health 2022;40:368-79.
35. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, PerezLopez R, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015;373:1697-708.

36. Jonsson P, Bandlamudi C, Cheng ML, Srinivasan P, Chavan SS, Friedman ND, et al. Tumour lineage shapes BRCA-mediated phenotypes. Nature 2019;571:576-9. Erratum in: Nature 2019;577:E1.
37. Mateo J, Porta N, Bianchini D, McGovern U, Elliott T, Jones R, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP B): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol 2020;21:162-74.
38. Abida W, Patnaik A, Campbell D, Shapiro J, Bryce AH, McDermott R, et al.; TRITON2 Investigators. Rucaparib in men with metastatic castration-resistant prostate cancer harboring a BRCA1 or BRCA2 gene alteration. J Clin Oncol 2020;38:3763-72.
39. Hussain M, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al.; PROfound Trial Investigators. Survival with olaparib in metastatic castration-resistant prostate cancer. N Engl J Med 2020;383:2345-57
40. Schiewer MJ, Goodwin JF, Han S, Brenner JC, Augello MA, Dean JL, et al. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov 2012;2:1134-49.
41. Li L, Karanika S, Yang G, Wang J, Park S, Broom BM, et al. Androgen receptor inhibitor-induced "BRCAness" and PARP inhibition are synthetically lethal for castration-resistant prostate cancer. Sci Signal 2017;10:eaam7479
42. Clarke NW, Armstrong AJ, Thiery-Vuillemin A, Oya M, Shore N, Loredo E, et al. Abiraterone and olaparib for metastatic castration-resistant prostate cancer. NEJM Evid 2022;1.
43. Chi KN, Rathkopf DE, Smith MR, et al. Niraparib and abiraterone acetate for metastatic castration-resistant prostate cancer. J Clin Oncol 2023;41:3339–51.
44. N. Agarwal, A. Azad, J. Carles, et al. TALAPRO-2: phase 3 study of talazoparib (TALA)enzalutamide (ENZA) versus placebo (PBO)ENZA as first-line (1L) treatment in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) J Clin Oncol, 41 (6 Suppl) (2023)
45. Paschalis A, Sheehan B, Riisnaes R, Rodrigues DN, Gurel B, Bertan C, et al. Prostate-specific membrane antigen heterogeneity and DNA repair defects in prostate cancer. Eur Urol 2019;76:469-78
46. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al.; VISION Investigators. Lutetium-177-PSMA-617 for metastatic castration-resistant prostate cancer. N Engl J Med 2021;385:1091-103
47. de Wit R, de Bono J, Sternberg CN, Fizazi K, Tombal B, Wülfing C, et al.; CARD Investigators. Cabazitaxel versus abiraterone or enzalutamide in metastatic prostate cancer. N Engl J Med 2019;381:2506-18
48. Lorente D, Omlin A, Zafeiriou Z, Nava-Rodrigues D, Perez-Lopez R, Pezaro C, et al. Castration-resistant prostate cancer tissue acquisition from bone metastases for molecular analyses. Clin Genitourin Cancer. 2016;14:485–93.
49. Trujillo B, Wu A, Wetterskog D, Attard G. Blood-based liquid biopsies for prostate cancer: clinical opportunities and challenges. Br J Cancer. 2022 Nov;127(8):1394-1402.

50. Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci USA. 2005;102:16368–73.
51. Carreira S, Romanel A, Goodall J, Grist E, Ferraldeschi R, Miranda S, et al. Tumor clone dynamics in lethal prostate cancer. Sci Transl Med. 2014;6:254ra125.
52. Romanel A, Gasi Tandefelt D, Conteduca V, Jayaram A, Casiraghi N, Wetterskog D, et al. Plasma AR and abiraterone-resistant prostate cancer. Sci Transl Med. 2015;7:312re10
53. Wyatt, A. W., Romanel, A., Conteduca, V., Casiraghi, N., Sigouros, M., Franceschini, G. M. et al. Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer. J. Natl Cancer Inst. 110, 78–86 (2018)
54. Murtaza, M., Dawson, S. J., Tsui, D. W., Gale, D., Forshew, T., Piskorz, A. M. et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 497, 108–112 (2013)
55. Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child Educ Pract Ed. 2013 Dec;98(6):236-8. doi: 10.1136/archdischild-2013-304340. Epub 2013 Aug 28
56. Gao, W.; Bohl, C.E.; Dalton, J.T. Chemistry and Structural Biology of Androgen Receptor. Chem. Rev. 2005, 105, 3352–3370.
57. Shang, Y.; Myers, M.; Brown, M. Formation of the androgen receptor transcription complex. Mol. Cell. 2002, 9, 601–610, Erratum in: Shang, Y.; Myers, M.; Brown, M. Formation of the androgen receptor transcription complex. Mol. Cell. 2003, 11, 1697
58. Culig, Z.; Santer, F. Androgen receptor signaling in prostate cancer. Cancer Metastasis Rev. 2014, 33, 413–427
59. Singer, E.; Golijanin, D.J.; Messing, E.M. Androgen deprivation therapy for advanced prostate cancer: Why does it fail and can its effects be prolonged? Can. J. Urol. 2008, 15, 4381–4387.
60. Jernberg, E.; Bergh, A.; Wikström, P. Clinical relevance of androgen receptor alterations in prostate cancer. Endocr. Connect. 2017, 6, R146–R161.
61. Hara, T.; Miyazaki, J.-I.; Araki, H.; Yamaoka, M.; Kanzaki, N.; Kusaka, M. et al. Novel mutations of androgen receptor: A possible mechanism of bicalutamide withdrawal syndrome. Cancer Res. 2003, 63, 149–153.
62. Conteduca, V.; Mosca, A.; Brighi, N.; de Giorgi, U.; Rescigno, P. New Prognostic Biomarkers in Metastatic Castration-Resistant Prostate Cancer. Cells 2021, 10, 193.
63. Coutinho, I.; Day, T.K.; Tilley, W.; Selth, L.A. Androgen receptor signaling in castration-resistant prostate cancer: A lesson in persistence. Endocr. Relat. Cancer 2016, 23, T179–T197.
64. Visakorpi, T.; Hyytinen, E.R.; Koivisto, P.; Tanner, M.; Keinänen, R.; Palmberg, C.; et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat. Genet. 1995, 9, 401–406.

65. Dehm, S.M.; Tindall, D.J. Alternatively spliced androgen receptor variants. Endocr.-Relat. Cancer 2011, 18, R183–R196.
66. Sharp, A.; Coleman, I.; Yuan, W.; Sprenger, C.; Dolling, D.; Rodrigues, D.N.; et al. Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer. J. Clin. Investig. 2018, 129, 192–208.
67. E Hall, M.; Huelster, H.L.; Luckenbaugh, A.N.; A Laviana, A.; A Keegan, K.; Klaassen, Z.; et al. Metastatic Hormone-sensitive Prostate Cancer: Current Perspective on the Evolving Therapeutic Landscape. OncoTargets Ther. 2020, 13, 3571–3581.
68. Wang, J.; Zhang, Y.; Wei, C.; Gao, X.; Yuan, P.; Gan, J.; et al. Prognostic Value of Androgen Receptor Splice Variant 7 in the Treatment of Metastatic Castration-Resistant Prostate Cancer: A Systematic Review and Meta Analysis. Front. Oncol. 2020, 10, 562504.
69. Steinestel, J.; Luedeke, M.; Arndt, A.; Schnoeller, T.J.; Lennerz, J.K.; Wurm, C.; et al. Detecting predictive androgen receptor modifications in circulating prostate cancer cells. Oncotarget 2015, 10, 4213–4223.
70. Parker, C.; Castro, E.; Fizazi, K.; Heidenreich, A.; Ost, P.; Procopio, G.; et al. Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 2020, 31, 1119–1134
71. Romanel, A.; Tandefelt, D.G.; Conteduca, V.; Jayaram, A.; Casiraghi, N.; Wetterskog, D.; et al. Plasma AR and abiraterone-resistant prostate cancer. Sci. Transl. Med. 2015, 7, 312re10
72. Conteduca, V.; Wetterskog, D.; Sharabiani, M.T.A.; Grande, E.; Pérez, M.P.F.; Jayaram, A.; et al. Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: A multi-institution correlative biomarker study. Ann. Oncol. 2017, 28, 1508–1516.
73. Conteduca, V.; Jayaram, A.; Romero-Laorden, N.; Wetterskog, D.; Salvi, S.; Gurioli, G.; et al. Plasma Androgen Receptor and Docetaxel for Metastatic Castration-resistant Prostate Cancer. Eur. Urol. 2018, 75, 368–373.
74. Conteduca, V.; Castro, E.; Wetterskog, D.; Scarpi, E.; Jayaram, A.; Romero-Laorden, N.; et al. Plasma AR status and cabazitaxel in heavily treated metastatic castration-resistant prostate cancer. Eur. J. Cancer 2019, 116, 158–168
75. Tolmeijer, S.; Boerrigter, E.; Schalken, J.A.; Geerlings, M.J.; van Oort, I.M.; van Erp, N.P.; et al. A Systematic Review and Meta-Analysis on the Predictive Value of Cell-Free DNA–Based Androgen Receptor Copy Number Gain in Patients With Castration-Resistant Prostate Cancer. JCO Precis. Oncol. 2020, 4, 714–729.
76. Yu, Z.; Chen, S.; Sowalsky, A.; Voznesensky, O.S.; Mostaghel, E.A.; Nelson, P.S.; et al. Rapid Induction of Androgen Receptor Splice Variants by Androgen Deprivation in Prostate Cancer. Clin. Cancer Res. 2014, 20, 1590–1600
77. Yamada, K.M.; Araki, M. Tumor suppressor PTEN: Modulator of cell signaling, growth, migration and apoptosis. J. Cell Sci. 2001, 114, 2375–2382.

78. Álvarez-Garcia, V.; Tawil, Y.; Wise, H.M.; Leslie, N.R. Mechanisms of PTEN loss in cancer: It's all about diversity. Semin. Cancer Biol. 2019, 59, 66–79.
79. Ferraldeschi, R.; Rodrigues, D.N.; Riisnaes, R.; Miranda, S.; Figueiredo, I.; Rescigno, P.; et al. PTEN Protein Loss and Clinical Outcome from Castration-resistant Prostate Cancer Treated with Abiraterone Acetate. Eur. Urol. 2014, 67, 795–802.
80. Gupta, S.; Abbass, I.M.; Craggs, C.; Satram, S.; To, T.M.; Mahrus, S.; et al. Overall survival of patients with metastatic castrate-resistant prostate cancer (mCRPC) who have PTEN tumor suppressor gene loss of function. J. Clin. Oncol. 2021, 39, 58.
81. Rescigno, P.; Lorente, D.; Dolling, D.; Ferraldeschi, R.; Rodrigues, D.N.; Riisnaes, et al. Docetaxel Treatment in PTEN- and ERG-aberrant Metastatic Prostate Cancers. Eur. Urol. Oncol. 2018, 1, 71–77.
82. De Bono, J.S.; De Giorgi, U.; Rodrigues, D.N.; Massard, C.; Bracarda, S.; Font, A.; et al. Randomized Phase II Study Evaluating Akt Blockade with Ipatasertib, in Combination with Abiraterone, in Patients with Metastatic Prostate Cancer with and without PTEN Loss. Clin. Cancer Res. 2018, 25, 928–936.
83. Robinson, D.; van Allen, E.M.; Wu, Y.-M.; Schultz, N.; Lonigro, R.J.; Mosquera, J.-M.; et al. Integrative Clinical Genomics of Advanced Prostate Cancer. Cell 2015, 161, 1215–1228.
84. Wu, A., Cremaschi, P., Wetterskog., D., Conteduca., V., Franceschini., G. M., Kleftogiannis., D. et al. The plasma methylome of metastatic prostate cancer. J. Clin. Invest. 130, 1991–2000
85. Wyatt, A. W., Azad, A. A., Volik, S. V., Annala, M., Beja, K., McConeghy, B. et al. Genomic alterations in cell-free DNA and enzalutamide resistance in castration resistant prostate cancer. JAMA Oncol. 2, 1598–1606 (2016).
86. Annala, M., Vandekerkhove, G., Khalaf, D., Taavitsainen, S., Beja, K., Warner, E. W. et al. Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. Cancer Discov. 8, 444–457 (2018).
87. Conteduca V, Wetterskog D, Scarpi E, Romanel A, Gurioli G, Jayaram A, et al. Plasma tumour DNA as an early indicator of treatment response in metastatic castration-resistant prostate cancer. Br J Cancer. 2020 Sep;123(6):982-987.
88. Caroli P, De Giorgi U, Scarpi E, Fantini L, Moretti A, Galassi R, at al. Prognostic value of 18F-choline PET/CT metabolic parameters in patients with metastatic castration-resistant prostate cancer treated with abiraterone or enzalutamide. Eur J Nucl Med Mol Imaging. 2018 Mar;45(3):348-354. doi: 10.1007/s00259-017-3866-2.
89. Conteduca V, Caffo O, Fratino L, Lo Re G, Basso U, D'Angelo A, et al. Impact of visceral metastases on outcome to abiraterone after docetaxel in castration-resistant prostate cancer patients. Future Oncol. 2015;11(21):2881-91.
90. Kessel K, Seifert R, Schäfers M, Weckesser M, Schlack K, Boegemann M, at al. Second line chemotherapy and visceral metastases are associated with poor

survival in patients with mCRPC receiving ^{177}Lu-PSMA-617. Theranostics. 2019 Jul 9;9(17):4841-4848.
91. Iwamoto H, Kano H, Shimada T, Naito R, Makino T, Kadomoto S, et al. Sarcopenia and Visceral Metastasis at Cabazitaxel Initiation Predict Prognosis in Patients With Castration-resistant Prostate Cancer Receiving Cabazitaxel Chemotherapy. In Vivo. 2021 May-Jun;35(3):1703-1709
92. Qiu R, Bu K, An H, Tao N. A retrospective study: analysis of the relationship between lactate dehydrogenase and castration-resistant prostate cancer based on restricted cubic spline model. PeerJ. 2023 Oct 6;11:e16158.
93. Conteduca V, Scarpi E, Caroli P, Lolli C, Gurioli G, Brighi N, et al. Combining liquid biopsy and functional imaging analysis in metastatic castration-resistant prostate cancer helps predict treatment outcome. Mol Oncol. 2022 Jan;16(2):538-548.
94. Prandi D, Demichelis F. Ploidy- and Purity-Adjusted Allele-Specific DNA Analysis Using CLONETv2. Curr Protoc Bioinformatics. 2019 Sep;67(1):e81
95. Armstrong AJ, Garrett-Mayer ES, Yang Y-CO, de Wit R, Tannock IF & Eisenberger M (2007) A contemporary prognostic nomogram for men with hormone-refractory metastatic prostate cancer: a TAX327 study analysis. Clin Cancer Res 13, 6396–6403.
96. Halabi S, Lin C-Y, Kelly WK, Fizazi K, Moul JW, Kaplan EB, et al. (2014) Updated prognostic model for predicting overall survival in firstline chemotherapy for patients with metastatic castration-resistant prostate cancer. J Clin Oncol 32, 671–677.
97. Chi KN, Kheoh T, Ryan CJ, Molina A, Bellmunt J, Vogelzang NJ, et al. (2016) A prognostic index model for predicting overall survival in patients with metastatic castration-resistant prostate cancer treated with abiraterone acetate after docetaxel. Ann Oncol 27, 454– 460.
98. Smaletz O, Scher HI, Small EJ, Verbel DA, McMillan A, Regan K, et al. (2002) Nomogram for overall survival of patients with progressive metastatic prostate cancer after castration. J Clin Oncol 20, 3972–3982.
99. Halabi S, Small EJ, Kantoff PW, Kattan MW, Kaplan EB, Dawson NA, et al. (2003) Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. J Clin Oncol 21, 1232–1237.
100. Stangl-Kremser J, Mari A, Suarez-Ibarrola R, D'Andrea D, Korn SM, Pones M, et al. (2020) Development of a prognostic model for survival time prediction in castration-resistant prostate cancer patients. Urol Oncol 38, 600.e9–600.e15
101. Armstrong AJ, Lin P, Higano CS, Sternberg CN, Sonpavde G, Tombal B, et al. (2018) Development and validation of a prognostic model for overall survival in chemotherapy-na€ıve men with metastatic castrationresistant prostate cancer. Ann Oncol 29, 2200–2007.
102. Ryan CJ, Kheoh T, Li J, Molina A, De Porre P, Carles J, et al. (2017) Prognostic index model for progression-free survival in chemotherapy-naıve metastatic castrationresistant prostate cancer treated with abiraterone acetate plus prednisone. Clin Genitourin Cancer S1558- 7673(17)30211-2.

103. Kyriakopoulos CE, Heath EI, Ferrari A, Sperger JM, Singh A, Perlman SB, et al. (2020) Exploring spatial-temporal changes in 18F-sodium fluoride PET/CT and circulating tumour cells in metastatic castration-resistant prostate cancer treated with enzalutamide. J Clin Oncol 38, 3662–3671.
104. De Laere B, Oeyen S, Mayrhofer M, Whitington T, van Dam PJ, Van Oyen P, et al. (2019) TP53 outperforms other androgen receptor biomarkers to predict abiraterone or enzalutamide outcome in metastatic castration resistant prostate cancer. Clin Cancer Res 25, 1766–1773.
105. Attard G, Gormley M, Urtishak K, Simon JS, Ricci DS, Parekh TV, et al. (2020) Association of detectable levels of circulating tumour DNA (ctDNA) with disease burden in prostate cancer (PC). J Clin Oncol 38 (15_suppl), 5562
106. Beltran H, Romanel A, Conteduca V, Casiraghi N, Sigouros M, Franceschini GM, et al. (2020) Circulating tumour DNA profile recognizes transformation to castration-resistant neuroendocrine prostate cancer. J Clin Invest 130, 1653–1668.
107. Brighi N, Wetterskog D., Gurioli G., Orlando F., Conteduca V., Casadei C.: et al. Dynamics of plasma tumour DNA and copy number alterations in advanced metastatic castration-resistant prostate cancer (mCRPC) patients treated with cabazitaxel: A prospective biomarker trial. ESMO 2023, poster 1809P.