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Toward Personalized Surgery in Advanced Prostate Cancer: Stratification by PTEN, AR-V7, TP53, TMPRSS2-ERG, and ERBB2 Genetic Alterations

Cristina Anita Ionescu (Mitu)[1,2‡], Georgeta Camelia Cozaru[3,4,5], Mariana Aşchie[4,5], Nicoleta Leopa[6*], Bogdan Cîmpineanu[2‡], Felix Voinea[2,7‡], Elena Matei[3], Anca Mitroi[3,4], Mariana Deacu[2,4], Ionuț Iorga[2,7], Mihaela Pundiche[2,6]

[1]Department of Genetics, Prof. Dr. Alexandru Trestioreanu Institute of Oncology, Bucharest, Romania
[2]Ovidius University, Faculty of Medicine, Constanta, Romania
[3]Center for Research and Development of the Morphological and Genetic Studies of Malignant Pathology, Ovidius University, Constanta, Romania
[4]Department of General Surgery, Cai Ferate Clinical Hospital Constanta, Romania
[5]Academy of Romanian Scientists, Bucharest, Romania
[6]Department of General Surgery, Emergency County Hospital, Constanta, Romania
[7]Department of Urology, Emergency County Hospital, Constanta, Romania

***Corresponding author:**
Nicoleta Leopa, MD
Ovidius University,
Faculty of Medicine and Pharmacy
General Surgery Department
Emergency Hospital of Constanta
No 145 Tomis Boulevard, 900591
Constanta, Romania
E-mail: gherghe_nicoleta02@yahoo.com

‡ These authors contributed equally to this work.

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Rezumat

Chirurgia personalizată în cancerul de prostată avansat: rolul alterărilor genetice PTEN, AR-V7, TP53, TMPRSS2-ERG și ERBB2 în orientarea deciziilor terapeutice

Introducere: Cancerul de prostată avansat este o afecțiune biologic heterogenă, marcată frecvent de multiple alterări genetice şi epigenetice care influențează progresia tumorală, rezistența la tratament şi prognosticul. Printre genele cel mai frecvent afectate se numără PTEN, AR-V7, TP53, TMPRSS2-ERG şi ERBB2, fiecare cu un potențial rol important în stratificarea riscului şi ghidarea terapiei țintite.
Metode: Acest studiu retrospectiv a inclus 43 de pacienți cu cancer de prostată avansat care au suferit prostatectomie radicală. Probele tumorale au fost analizate prin tehnica FISH (fluorescență in situ) pentru a evalua statusul mutațional al celor cinci markeri. Parametrii clinicopatologici, precum nivelul PSA, scorul Gleason, stadiul tumoral şi invazia locală, au fost corelați cu alterările moleculare utilizând modele de regresie logistică multinomială.
Rezultate: Cea mai frecventă alterare genetică izolată a fost pierderea expresiei PTEN (20,9%), urmată de amplificarea TP53 (16,3%), fuziunea TMPRSS2-ERG (13,9%), expresia AR-V7 (11,6%) şi amplificarea ERBB2 (7%). De asemenea, au fost observate combinații de doi sau trei markeri în cazuri cu profil agresiv. PTEN- şi AR-V7+ s-au asociat cu valori scăzute ale PSA, în ciuda agresivității tumorale, în timp ce ERBB2+ s-a corelat cu valori crescute ale PSA şi scor Gleason înalt. TP53+ şi ERBB2+ au fost semnificativ asociate cu forme tumorale de grad înalt (Gleason >7), iar AR-V7+ a fost singurul marker asociat cu invazia veziculelor seminale. Vârsta tânără a fost slab corelată cu AR-V7+ şi TP53+.
Concluzii: Profilul molecular a tumorilor prostatice avansate prin evaluarea

markerilor PTEN, AR-V7, TP53 şi ERBB2 identifică subtipuri distincte cu semnificație clinică şi terapeutică. Integrarea acestor markeri în practica clinică poate îmbunătăți stratificarea pacienților şi ghidarea terapiei personalizate. Sunt necesare studii suplimentare pe cohorte mai mari pentru validarea acestor asocieri şi dezvoltarea unor modele biomoleculare de risc.

Cuvinte cheie: cancerul de prostată, profil genetic, chirurgie personalizată, marker biologici, prognostic

Abstract

Background: Advanced prostate cancer is a biologically heterogeneous disease often marked by multiple genetic and epigenetic alterations that influence tumor progression, treatment resistance, and prognosis. Among the most frequently altered genes are PTEN, AR-V7, TP53, TMPRSS2-ERG, and ERBB2, each with potential relevance for stratifying risk and guiding targeted therapy.
Methods: This retrospective study included 43 patients with advanced prostate cancer who underwent radical prostatectomy. Tumor specimens were analyzed using fluorescence in situ hybridization (FISH) to assess the mutational status of the five markers. Clinicopathological parameters, including PSA levels, Gleason score, tumor stage, and invasion status, were correlated with molecular alterations using multinomial logistic regression.
Results: The most common isolated alteration was PTEN loss (20.9%), followed by TP53 amplification (16.3%), TMPRSS2-ERG fusion (13.9%), AR-V7 expression (11.6%), and ERBB2 amplification (7%). Combined alterations were also observed, with dual or triple marker expression in select aggressive cases. PTEN- and AR-V7+ were associated with low PSA values despite aggressive pathology, while ERBB2+ correlated with high PSA levels and high Gleason scores. TP53+ and ERBB2+ were also significantly associated with high-grade tumors (Gleason >7). AR-V7+ was the only marker significantly associated with seminal vesicle invasion. Younger age was weakly correlated with AR-V7+ and TP53+ status.
Conclusions: The molecular profile defined by PTEN, AR-V7, TP53, and ERBB2 identifies distinct biological subtypes in advanced prostate cancer, each with specific prognostic and therapeutic implications. Integration of these biomarkers into routine clinical assessment may improve treatment personalization and risk stratification. Validation in larger, prospective cohorts is warranted.

Keywords: prostate cancer, genetic profile, personalized surgery, biomarkers, prognosis

Introduction

Advanced prostate cancer is a complex clinical entity characterized by a series of cellular and molecular mechanisms that contribute to disease progression, resistance to treatments, and the development of metastases (1,2). These processes involve significant genetic and epigenetic alterations, which determine heterogeneous tumor phenotypes and varied clinical outcomes. At the molecular level, prostate cancer is often characterized by DNA alterations, including deletions, amplifications, and genetic rearrangements (3). Among the most common are MYC gene amplification and the TMPRSS2-ERG gene fusion, present in approximately 50% of cases, which activate oncogenic signaling pathways, such as the ERG-mediated one. In addition, recurrent mutations in genes such as TP53, PTEN, RB1, BRCA1/2, and PIK3CA contribute to genomic instability and disease progression (4,5).

In addition to genetic alterations, epigenetic alterations, such as aberrant DNA methylation and post-translational modifications of histones, can influence gene expression without altering the genetic sequence itself (6,7). These alterations can lead to the repression of tumor suppressor genes or the activation of oncogenic genes, playing a critical role in tumor initiation and progression. In this context, targeted epigenetic therapies, such as HDAC, EZH2, or DNMT inhibitors, are being investigated for their potential to reverse these aberrant alterations (8,9).

The androgen receptor (AR) remains a central element in the pathophysiology of prostate cancer. In advanced forms, persistent activation of the AR can occur even in the absence of circulating androgens, due to mutations, amplifications, or activation by alternative ligands (10). Furthermore, splicing isoforms of AR, such as AR-V7, are constitutively active and associated with resistance to androgen deprivation therapies (ADT), representing impor-

tant markers for prognostic assessment and choice of therapies (11,12). The understanding of these molecular mechanisms has been facilitated by recent genomic and epigenetic studies, which have highlighted tumor diversity and allowed the identification of relevant biomolecular markers (13). These can be used not only for diagnosis and monitoring, but especially for risk stratification and prognosis estimation of patients with advanced prostate cancer. There is no single biomarker capable of completely characterizing the evolution of the disease, but the combination of several markers – genetic, epigenetic, and protein – can provide valuable information for personalizing treatment and improving clinical outcomes (14). The identification and validation of these markers represents an essential step towards precision medicine in urologic oncology. Although each molecular marker has been analyzed individually in various cohorts, few studies have investigated their combined and differential impact on the clinicopathological characteristics of advanced prostate cancer, in a comparative and integrative manner.

In this context, the present study aims to explore the associations between the expression of PTEN, AR-V7, TMPRSS2-ERG, TP53, and ERBB2 markers and relevant clinicopathological variables, using multinomial logistic regression models. The main objective is to evaluate the potential of these biomarkers as risk stratification factors and as possible therapeutic targets, thus contributing to the personalization of therapeutic strategies in an oncological pathology marked by biological and evolutionary heterogeneity.

Material and Methods

Study Design and Population

The study included a sample of 43 patients diagnosed with advanced prostate cancer, who underwent radical prostatectomy within the Urology Department of Sf. Apostol Andrei County Emergency Hospital in Constanța, between 2020-2024. Advanced prostate cancer represents a progressive stage of the disease, in which the malignant process has extended beyond the anatomical boundaries of the prostate gland, invading adjacent structures or spreading to distant sites (1,2). Thus, from an a pathological and oncological perspective, prostate cancer is considered advanced in the presence of significant local extension (T3-T4), regional lymph node involvement (N1) or distant metastases (M1), frequently supported by biological markers of tumor aggressiveness such as a PSA >20 ng/mL, a Gleason score ≥8 or a Grade Group ≥ IV according to the ISUP classification (International Society of Urological Pathology) (15).

The inclusion criteria were: confirmed histopathological diagnosis, availability of tumor tissue for molecular analysis and complete existence of relevant clinicopathological data, including preoperative PSA level, Gleason score, tumor extension and lymph node status. Patients with a history of other malignant neoplasms or who underwent hormonal treatment before surgery were excluded. During the study period, a total of 440 malignant prostate tumors were diagnosed, of which 43 cases met the predefined inclusion criteria.

The biological material used was represented by tumor tissue fragments obtained from radical prostatectomy pieces. These were fixed in 10% buffered formalin and processed using the standard paraffin embedding technique. Sections of 3-4 µm thickness were stained with Hematoxylin-Eosin (HE) and histopathologically evaluated in the Clinical Pathology Department of the same hospital. Tumor classification was performed according to the Gleason system, in line with the updated recommendations of the international guidelines for reporting prostate cancer.

Evaluation of Genetic Markers

To assess the expression and mutational status of the PTEN, AR-V7, TMPRSS2-ERG, TP53, and ERBB2 markers, the FISH (Fluorescence In Situ Hybridization) technique was used, applied on paraffin-embedded tumor tissue sections. This method allows the direct detection of gene rearrangements, deletions, amplifications or translocations at the individual cell level, providing a precise profile of genetic alterations.

For each gene analyzed, specific fluorescently labeled samples were used, in accordance with the manufacturer's recommended protocols and international standards in the field of molecular pathology. To determine the genetic status of the genes analyzed in the study, specific commercial kits were used: PTEN/CEN10p (Abnova) – for PTEN gene deletions; CytoCell P53 Deletion (CytoCell) – for TP53 deletions; CytoCell TMPRSS2/ERG Breakapart (CytoCell) – for TMPRSS2-ERG fusion; mutaFISH™ ARwt/AR-V7 RNA Probes (Abnova) – for identification of the AR-V7 isoform; ERBB2 FISH Probe Kit (Vysis, Abbott) – for ERBB2

amplifications. Histological sections were processed according to standard protocols, and FISH signal evaluation was performed on a Zeiss Axioscop fluorescence microscope, using specific filters. For each case, 200 tumor nuclei were analyzed, excluding overlapping or artifactual nuclei. Genetic aberrations were defined as follows:

- *Normal status:* 1–2 gene signals and 1–2 centromeric signals per nucleus.
- *Moderate amplification:* ≥30% nuclei with ≥6 gene copies/nucleus and a gene/centromere ratio ≥2 in at least 15% of them.
- *High-grade amplification:* >10 gene copies/nucleus or gene/centromere ratio >5, or the presence of "clusters" of fluorescent signals.
- *Deletions or losses:* defined if ≥30% of nuclei showed losses of the signal specific to the gene analyzed.

Split signals were considered as a single signal, and the interpretation of the results was performed independently by two evaluators. In addition to the molecular analysis, relevant clinicobiological data were collected and noted for each patient, including: age at diagnosis, preoperative PSA value, Gleason score (according to ISUP classification), local tumor extension stage (pT), lymph node invasion status (pN), presence of positive margin (R1), relevant medical history.

Study Endpoints

The primary endpoint to evaluate the association between the mutational status of PTEN, AR-V7, TP53, TMPRSS2-ERG, and ERBB2 and key clinicopathological variables (e.g., PSA level, Gleason score, tumor stage), in order to identify their potential as prognostic and stratification biomarkers in advanced prostate cancer.

The secondary endpoints:

- To assess whether specific combinations of genetic alterations are associated with more aggressive tumor features or local extension (e.g., extraprostatic invasion, seminal vesicle involvement).
- To determine the prevalence and distribution of individual and combined genetic alterations in the studied population.
- To explore the potential utility of molecular markers in identifying tumor subtypes that may benefit from targeted therapies.

Ethical Approval

All patients included in the study signed an informed consent regarding the use of biological material for research purposes, and the study protocol was approved by the Ethics Committee for the Approval of Clinical and Research Developmental Studies (No 14/29.12.2023) in compliance with the Declaration of Helsinki on human testing.

Statistical Analysis

Associations between molecular marker status and clinicopathological characteristics were assessed using multivariate multinomial logistic regression. The significance of differences between groups was tested with analysis of variance (ANOVA). Results were expressed as odds ratios (OR) and 95% confidence intervals (CI), with a statistical significance threshold set at $p < 0.05$.

Results

The study cohort included 43 patients diagnosed with advanced prostate cancer, with a mean age of 63 years (range 47–76). Most of the patients (74.4%) underwent radical prostatectomy. Preoperative PSA levels were above 20 ng/mL in over half of the cases (53.5%), suggesting a biologically aggressive disease. Histopathological evaluation revealed that 54% of tumors had a Gleason score of 7, while 16% had scores of 8 or higher. Locally advanced tumor stages (pT3 or higher) were observed in 63% of patients, 38% had regional lymph node invasion (N1), and 28% presented with distant metastases (M1) (*Table 1*).

Table 1. Clinicopathological characteristics analyzed in patients in the study group

Variable	No. cases (%)	p
Average age (range, years)	63 (47–76)	0.837
Gleason Score		0.168
≤6	13 (30%)	
7	23 (54%)	
≥8	7 (16%)	
Preoperative PSA (ng/ml)(median)	17.8	0.177
< 10	4 (9.3%)	
10–20	16 (37.2%)	
> 20	23 (53.48%)	
Radical prostatectomy		0.308
Yes	32 (74.41)	
No	11 (25.59)	
Tumor stage		0.982
≤T2	16 (37%)	
≥T3	27 (63%)	
N stage		0.490
N0	27 (62.79%)	
N1	16 (37.21%)	
M stage		0.751
M0	31 (72%)	
M1	12 (28%)	

PSA: prostate-specific antigen; T: Tumor; N: Node; M: Metastasis.

Table 2. Relationship between the architectural pattern of prostate adenocarcinomas and tumor aggressiveness, measured by the Gleason score

Variable	Patterns 1		Patterns 2		Patterns 3		Patterns 4		ANOVA
	OR (95% CI)	*p-value	OR (95% CI)	*p- value	OR (95% CI)	*p- value	OR (95% CI)	*p- value	p-value
Gleason Score <7	0.38 (0.29–0.6)	<0.001	0.56 (0.41–0.75)	<0.001	1.31 (0.78–2.21)	0.392	0.93 (0.41–2.13)	0.961	<0.001
Gleason Score >7	1.27 (0.77–2.11)	0.421	0.75 (0.45–1.26)	0.464	2.12 (1.47–3.06)	<0.001	0.79 (0.38–2.14)	0.693	<0.001

ANOVA - analysis of variance; OR - odds ratio; CI - confidence interval; *MVA - multivariate analysis; Pattern 1 - adenocarcinomas with atrophic patterns; Pattern 2 - adenocarcinomas with pseudohyperplastic and microcystic patterns; Pattern 3 - adenocarcinomas with glomeruloid, cribriform, comedonecrotic and signet ring cell components; Pattern 4 - conventional and foam cell tumors. Values in italics indicate statistical significance (p <0.050).

A detailed assessment of architectural patterns revealed important correlations with tumor aggressiveness. Tumors exhibiting atrophic or pseudo-hyperplastic/microcystic features (Patterns 1 and 2) were significantly associated with lower Gleason scores (<7), indicating a more indolent behavior. In contrast, tumors with glomeruloid, cribriform, comedonecrotic, or signet-ring cell features (Pattern 3) were strongly correlated with higher Gleason scores (>7), consistent with a more aggressive histological profile (OR = 2.12, p < 0.001). Conventional and foamy cell patterns (Pattern 4) did not demonstrate a statistically significant association with tumor grade, suggesting a more heterogeneous biological behavior (*Table 2*; *Figs. 1, 2*).

From a molecular perspective, at least one genetic alteration was detected in 95.3% of patients. The most frequent alteration was PTEN loss, found in 9 cases (20.9%), followed by TP53 amplification (16.3%), TMPRSS2-ERG gene fusion (13.9%), AR-V7 splice variant expression (11.6%), and ERBB2 amplification (7%). While single mutations were predominant, nine patients exhibited co-alterations of two markers, and two cases harbored three simultaneous genetic changes. The most common combination was PTEN loss and TP53 amplification, a pattern associated with genomic instability and a high-risk phenotype. Other relevant combinations included PTEN loss together with AR-V7 or TMPRSS2-ERG positivity, as well as triple profiles such as PTEN-/AR-V7+/TP53+ or PTEN-/TP53+/ERBB2+, which may indicate rare but highly aggressive molecular subtypes (*Table 3, Figs. 3–6*).

Multinomial logistic regression analysis revealed several clinically relevant associations. Notably, PTEN loss and AR-V7 expression were both associated with low preoperative PSA levels (OR = 0.47 and OR = 0.48, respectively), despite their known link to tumor aggressiveness. This finding suggests that some biologically aggressive tumors may elude PSA-based monitoring, high-

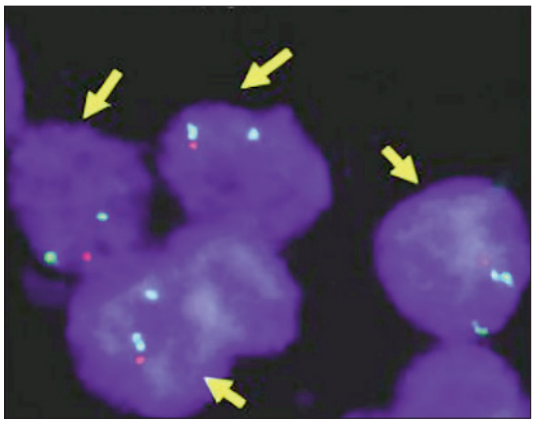

Figure 1. Prostate adenocarcinoma. FISH image for PTEN status -1 red signal (locus 10q23.3) and two green signals (centromere 10) per nucleus indicating hemizygous PTEN deletion.

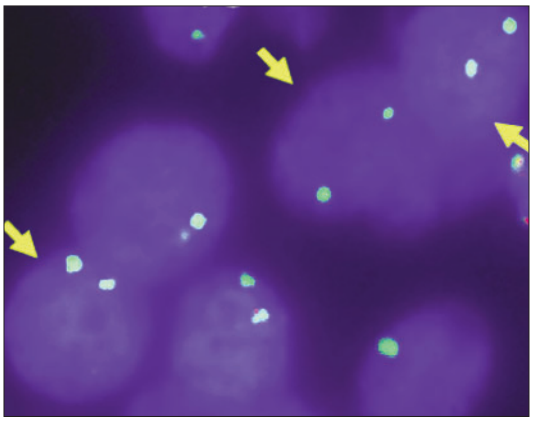

Figure 2. Prostate adenocarcinoma. FISH image for PTEN status - no red signals (locus 10q23.3) and two green signals (centromere 10) per nucleus indicating homozygous PTEN deletion.

Table 3. Incidence of genetic alterations in patients in the study group.

No. of markers identified	Mutational status	No. cases	Frequency (%)
A single marker modified (n = 30 cases)	PTEN-	9	20.90%
	AR-V7+	5	11.60%
	TMPRSS2-ERG+	6	13.90%
	TP53+	7	16.30%
	ERBB2+	3	7.00%
Two markers modified simultaneously (n = 9 cases)	PTEN-/AR-V7+	2	4.70%
	PTEN-/TMPRSS2-ERG+	2	4.70%
	PTEN-/TP53+	3	7.00%
	AR-V7+/TP53+	1	2.30%
	TP53+/ERBB2+	1	2.30%
Three markers modified simultaneously (n = 2 cases)	PTEN-/AR-V7+/TP53+	1	2.30%
	PTEN-/ERBB2+/TP53+	1	2.30%

Note: "-" = gene deletion (loss); "+" = gene amplification.

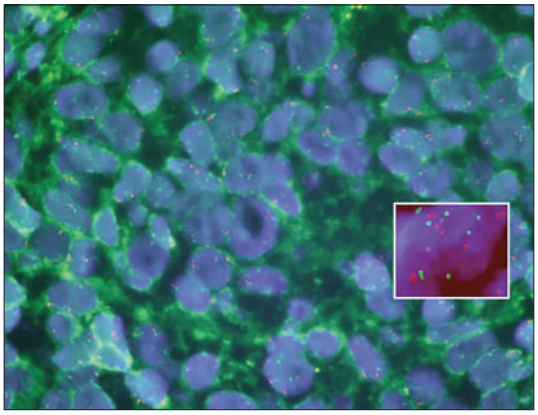

Figure 3. Prostate adenocarcinoma. FISH image suggestive of TP53 marker (P53, 17p13.1, Red; D17Z1, 17p11.1-q11.1, Green), moderate gene amplification.

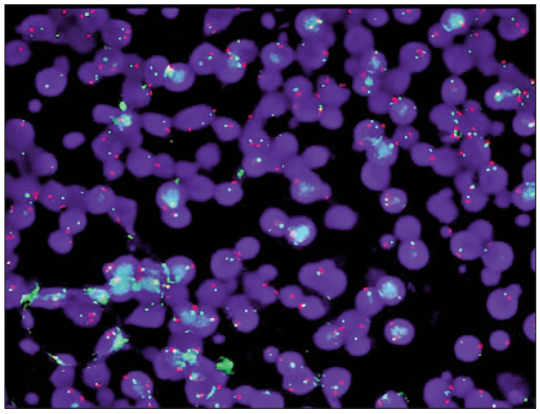

Figure 4. Prostate adenocarcinoma. FISH image for ERBB2 status - moderate amplification of the ERBB2 gene.

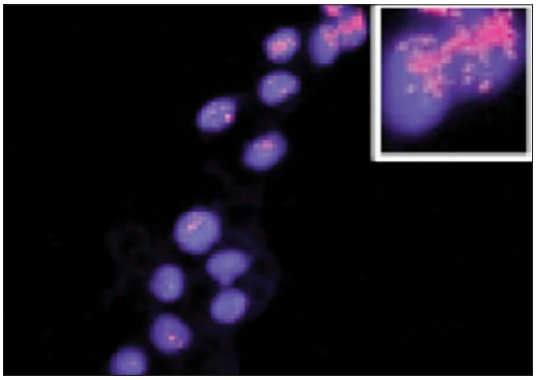

Figure 5. Prostate adenocarcinoma. FISH image suggestive of nuclei with negative AR-V7 marker (no red signals) and positive AR-V7 (areas with red signals).

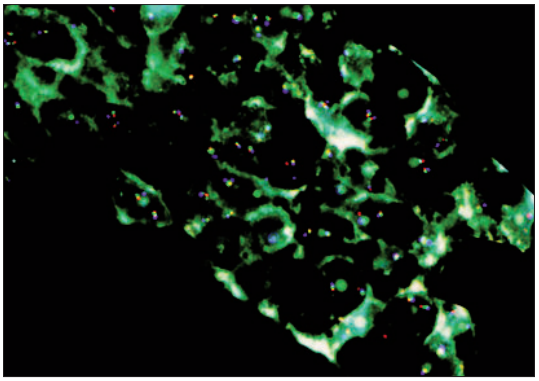

Figure 6. Prostate adenocarcinoma. FISH image suggestive of positive TMPRSS2-ERG marker (gene fusion bydeletion) (TMPRSS2, 21q22.2-q22.3, Red; TMPRSS2, 21q22.2-q22.3, Green; ERG, 21q22.13-q22.2, Blue)

lighting the importance of molecular profiling in patient evaluation. On the other hand, ERBB2 amplification was significantly correlated with high PSA levels (OR=2.12, p<0.001), possibly reflecting intense proliferative activity.

The presence of PTEN loss was also associated with extraprostatic extension (OR=1.80, p<0.001), underscoring its role in promoting local invasiveness. Interestingly, TP53 and ERBB2 alterations were negatively associated with extraprostatic extension, indicating that these alterations might be more linked to distant dissemination or systemic progression rather than local invasion. AR-V7 expression emerged as the only genetic marker significantly associated with seminal vesicle invasion (OR=2.27, p=0.004), suggesting its value as a marker of local aggressiveness and therapeutic resistance (*Table 4*).

Additional associations were found between molecular markers and tumor grade. PTEN loss was paradoxically associated with lower Gleason scores (<7), a finding that may reflect morphological underestimation of biologically aggressive disease. Conversely, TP53 amplification and ERBB2 overexpression were both significantly associated with high-grade tumors (Gleason >7), reinforcing their role as indicators of poor prognosis.

A weak but statistically significant association was observed between younger age and AR-V7 positivity (OR=0.97, p=0.045), with a similar trend for TP53 amplification (p=0.08). These findings suggest that younger patients may harbor more aggressive molecular phenotypes, requiring earlier intervention or intensified therapeutic strategies.

Interestingly, none of the investigated genetic markers showed a significant correlation with lymph node status, indicating that nodal involvement may be influenced by factors beyond the five analyzed alterations. Altogether, these results emphasize the biological complexity of advanced prostate cancer and support the role of molecular profiling in risk stratification and personalized therapeutic planning.

Discussions

Isolated expression of altered genetic markers was observed in 30 cases. Loss of PTEN (9 cases; 20.9%) was the most common single genetic alteration observed. This is consistent with the literature, which indicates PTEN as an essential tumor suppressor. Its absence favors activation of the PI3K/AKT pathway, with effects on tumor

Table 4. Multivariate multinomial logistic regression analysis between clinicopathological parameters and mutational status

Variable	PTEN-ANOVA		AR-V7+			TMPRSS2-ERG+			TP53+		
ERBB2+	OR (95% CI)	*p-value	OR (95% CI)	*p-value	OR (95% CI)	*p-value	OR (95% CI)	*p-value	OR (95% CI)	*p-value	p-value
Preoperative PSA	0.47 (0.33–0.68)	<0.001	0.48 (0.26–0.88)	0.021	0.81 (0.44–1.51)	0.42	1.73 (0.91–3.27)	0.05	2.12 (1.47–3.06)	<0.001	<0.001
Extraprostatic extension	1.80 (1.34–2.41)	<0.001	1.23 (0.75–2.01)	0.34	0.76 (0.46–1.26)	0.37	0.42 (0.25–0.7)	0.001	0.56 (0.41–0.75)	<0.001	<0.001
Invasion of the seminal vesicles	1.16 (0.83–1.62)	0.24	2.27 (1.35–3.82)	0.004	0.84 (0.47–1.53)	0.51	0.73 (0.4–1.32)	0.37	0.86 (0.62–1.2)	0.24	0.01
Gleason Score <7	0.96 (0.61–1.51)	0.93	0.75 (0.31–1.81)	0.58	0.89 (0.39–2.04)	0.79	0.93 (0.41–2.13)	0.96	1.04 (0.66–1.64)	0.93	<0.001
Gleason Score >7	0.43 (0.32–0.6)	<0.001	0.75 (0.45–1.26)	0.46	1.31 (0.78–2.21)	0.39	1.74 (1.05–2.88)	0.05	2.30 (1.68–3.15)	<0.001	<0.001
Nodule involvement	1.27 (0.77–2.11)	0.42	1.27 (0.79–2.04)	0.53	1.6 (0.78–3.27)	0.22	1.16 (0.49–2.76)	0.73	1.18 (0.89–1.56)	0.29	0.60
Age	1.00 (0.98–1.02)	0.71	0.97 (0.94–1.00)	0.045	1.01 (0.97–1.05)	0.62	0.97 (0.94–1.00)	0.08	1.01 (0.98–1.05)	0.71	0.24

ANOVA = analysis of variance; OR = odds ratio; CI = confidence interval; *MVA = multivariate analysis; PSA = prostate-specific antigen.

growth and aggressiveness of the disease. In this group, (PTEN-) was frequently associated with Gleason scores >7. AR-V7 expression (5 cases; 11.6%) suggests the presence of a splice variant of the androgen receptor, associated with resistance to hormonal therapy. According to the data published to date, these patients may present a refractory tumor form, requiring alternative therapeutic approaches (16).

TMPRSS2-ERG fusion (6 cases; 13.9%), although relatively rare in this group, remains a relevant marker for activation of the androgen pathway. Generally, this alteration is associated with better differentiated subtypes, but may contribute to progression when it coexists with other mutations. TP53 amplification (7 cases; 16.3%) reflects increased genomic instability. Studies published to date have shown that (TP53+) mutations are usually associated with poor prognosis, resistance to treatments and accelerated disease progression. Although rare, (ERBB2+) amplification (only 3 cases; 7.0%) may indicate a tumor subgroup susceptible to targeted therapies (anti-HER2). This finding supports the idea of a personalized approach in the treatment of advanced prostate cancer.

In 9 cases of advanced prostate cancer tumors, simultaneous expression of two markers was observed simultaneously, such as (PTEN-)&(AR-V7+) or (PTEN-)&(TMPRSS2-ERG+). The associations between PTEN loss and androgen pathway activation suggest a cooperation between tumor proliferation and therapeutic resistance mechanisms. These combinations may characterize particularly aggressive tumor forms. In three cases, tumor heterogeneity consisted of the presence of PTEN gene deletion and amplified TP53 gene (PTEN-&TP53+).

This was the most frequent dual combination observed. According to other studies, the coexistence of PTEN loss and TP53 amplification increases the risk of rapid progression and unfavorable prognosis. The mutational status (AR-V7+)&(TP53+) and (TP53+)&(ERBB2+), was identified in isolation, in a single case each. These combinations indicate the simultaneous activation of multiple oncogenic pathways, which can lead to cancers refractory to conventional therapies.

Another situation recorded was increased heterogeneity with the simultaneous expression of three genetic markers. One case identified with mutational status (PTEN-)&(AR-V7+)&(TP53+) (suggesting an extremely aggressive molecular profile, characterized by genomic instability and potential resistance to multiple therapeutic lines) and one case with (PTEN-) &(ERBB2+)&(TP53+) (this combination could describe a rare subgroup of patients who would benefit from targeted therapies, especially HER2 inhibitors or combination therapies). To assess the clinical relevance of the identified molecular markers (PTEN, AR-V7, TMPRSS2-ERG, TP53 and ERBB2), a multivariate multinomial logistic regression analysis was performed. The results obtained indicate significant associations between certain genetic alterations and key clinicopathological variables, relevant for risk stratification in advanced prostate cancer.

(PTEN-) and (AR-V7+) were associated with low PSA levels (PTEN: OR=0.47, $p<0.001$; AR-V7: OR=0.48, $p=0.021$), suggesting that these tumors, although they may have an aggressive biological potential, do not proportionally express PSA, which may lead to an underestimation of the severity of the disease. (ERBB2+) was correlated with an elevated PSA (OR=2.12, $p<0.001$), indicating intense tumor activity in these cases. We consider that the low PSA level in (PTEN-) and (AR-V7+) tumors may mask the real aggressiveness of the disease. Conversely, (ERBB2+) may constitute a marker of increased tumor activity and a possible criterion for the selection of targeted therapies.

(PTEN-) was also positively associated with extraprostatic extension (OR=1.80, $p<0.001$), reflecting the increased invasiveness of these tumors. Unexpectedly, (TP53+) (OR=0.42, $p=0.001$) and (ERBB2+) (OR=0.56, $p<0.001$) were negatively associated with extraprostatic extension, which may indicate a different pattern of progression. These results may reflect that (PTEN-) tumors are at increased risk of local invasion, while the presence of (TP53+) or (ERBB2+) may signal a different pathway of tumor progression, less dependent on local invasion and perhaps more focused on metastasis or genomic instability.

Only (AR-V7+) showed a significant association with seminal vesicle invasion (OR=2.27, $p=0.004$), suggesting a tumor with locally advanced potential and resistance to conventional hormonal therapies. Thus, (AR-V7+) is confirmed as a marker of tumor aggressiveness and loco-regional extension, potentially useful for the selection of patients requiring early systemic treatments.

We also note that (PTEN-) was associated with Gleason scores <7 (OR=0.43, $p < 0.001$), which may seem contradictory in the context of aggressive behavior. This discrepancy can be explained by the

biological heterogeneity of these tumors, which may be morphologically underestimated. In contrast, (TP53+) (OR=1.74, p=0.05) and (ERBB2+) (OR= 2.30, p<0.001) were significantly associated with Gleason scores >7, indicating a more aggressive tumor phenotype. TP53 and ERBB2 markers are correlated with high-grade tumor forms, with potential for rapid evolution, justifying more aggressive therapeutic strategies and careful monitoring.

A weak but statistically significant association was observed between younger age and expression of (AR-V7+) (OR=0.97, p=0.045), and a similar trend was observed for (TP53+) (p=0.08). These results may suggest that younger patients present with more aggressive molecular subtypes (AR-V7+, TP53+), which may require an early personalized approach, given the risk of hormonal resistance and rapid progression. No molecular marker demonstrated a significant association with lymph node involvement in this analysis. Although nodal invasion is an important prognostic indicator, in this study, no significant genetic associations were identified, suggesting that nodal involvement may be influenced by other factors independent of the molecular profile analyzed (17).

The results obtained highlight the importance of evaluating the molecular profile in advanced prostate cancer (14). The loss of PTEN expression and the presence of AR-V7 are associated in the literature with a more aggressive tumor phenotype, but with relatively low PSA levels, which may make it difficult to effectively monitor the disease. (18). A preclinical study in animal models demonstrated that the concomitant loss of PTEN and TP53 accelerates tumor progression and confers resistance to standard therapies (18). At the same time, AR-V7 has been repeatedly associated with resistance to second-line hormonal therapies, such as enzalutamide and abiraterone, especially in castration-resistant prostate cancer (19). For example, Antonarakis et al. (2014) reported that AR-V7-positive patients had a PSA response of 0% to enzalutamide, compared with 53% in AR-V7-negative patients (P=0.004), with a significant decrease in overall survival (20,21).

A recent meta-analysis confirms these observations, showing a 3.4-fold increased risk of mortality and shortened progression-free survival in AR-V7-positive patients (22).

The importance of AR-V7 testing before second-line hormonal treatments is also supported by data in the literature, which highlight that the presence of AR-V7 is an independent predictor of poor response and reduced survival - essential information for targeting patients to chemotherapy or PARP inhibitors (23).

Regarding TP53, mutations are recognized as predictors of reduced overall survival and associated with poor response to conventional therapies (24). Zhu KL et al. (2024) showed that TP53 dysfunction facilitates immunological escape and remodeling of the tumor microenvironment, disturbing the immune-suppressive balance — our results confirm the associations of TP53+ with high Gleason scores and more frequent occurrence in young patients.

ERBB2 (HER2) amplification in prostate cancer is relatively rare, but increasingly relevant due to emerging targeted therapies (e.g. trastuzumab derivatives), evaluated in phase II and III trials (25). In available clinical studies, ERBB2+ has been associated with elevated PSA and high Gleason score - signals of a biologically active and potentially aggressive subtype. Furthermore, the combination of ERBB2 amplification with PTEN loss appears to be a promising target for combination therapies with PI3K AKT inhibitors (26).

This study has several limitations. First, the relatively small sample size (n=43) may limit the statistical power and generalizability of the findings. Second, the retrospective and single-center design may introduce selection bias and limit external validity. Third, the absence of follow-up data precluded correlation with long-term oncological outcomes such as biochemical recurrence or survival. Fourth, only five molecular markers were analyzed, and the potential contribution of other relevant genetic or epigenetic alterations was not explored. Finally, FISH analysis, although precise, may not detect subclonal or low-level alterations present in heterogeneous tumor populations.

Conclusions

The molecular characterization of advanced prostate cancer using PTEN, AR-V7, TP53, TMPRSS2-ERG, and ERBB2 reveals distinct tumor subtypes with prognostic and therapeutic significance. PTEN loss and AR-V7 expression are linked to aggressive behavior with low PSA levels, while TP53 and ERBB2 alterations are associated with high-grade tumors. These findings support the integration of molecular markers into clinical practice to improve patient stratification and guide personalized treatment. Further studies on larger cohorts are needed to validate these associations and develop biomolecular risk models.

Author's Contributions

Conceptualization: Cristina Anita Ionescu (Mitu), Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea, Mihaela Pundiche; methodology: Cristina Anita Ionescu (Mitu), Georgeta Camelia Cozaru, Mariana Aşchie, Anca Mitroi, Mariana Deacu; software, Georgeta Camelia Cozaru; validation: Cristina Anita Ionescu (Mitu), Georgeta Camelia Cozaru, Mariana Aşchie, Bogdan Cîmpineanu, Felix Voinea, Elena Matei, Anca Mitroi, Mariana Deacu; investigation: Cristina Anita Ionescu (Mitu), Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea, Mihaela Pundiche; resources: Georgeta Camelia Cozaru, Mariana Aşchie, Anca Mitroi, Mariana Deacu, Elena Matei, Felix Voinea, Ionuț Iorga; data curation: Cristina Anita Ionescu (Mitu), Georgeta Camelia Cozaru, Mariana Aşchie, Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea; writing - original draft preparation, Cristina Anita Ionescu (Mitu), Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea, Mihaela Pundiche; writing - review and editing: Cristina Anita Ionescu (Mitu), Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea, Mihaela Pundiche; visualization: Cristina Anita Ionescu (Mitu), Georgeta Camelia Cozaru, Mariana Aşchie, Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea, Elena Matei, Anca Mitroi, Mariana Deacu, Ionuț Iorga, Mihaela Pundiche; supervision: Cristina Anita Ionescu (Mitu), Georgeta Camelia Cozaru, Mariana Aşchie, Nicoleta Leopa, Bogdan Cîmpineanu, Felix Voinea, Elena Matei, Anca Mitroi, Mariana Deacu, Ionuț Iorga, Mihaela Pundiche; administration: Cristina Anita Ionescu (Mitu), Georgeta Camelia Cozaru, Mariana Aşchie, Anca Mitroi, Mariana Deacu, Leopa Nicoleta, Mihaela Pundiche. All authors have read and agreed to the published version of the manuscript.

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