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The role of the Androgen Receptor (AR) in endometrial cancer aggressiveness: Correlation with other prognostic markers and therapeutic implications. A retrospective observational study

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ABSTRACT

Endometrial carcinoma (EC) is the most common gynecological malignancy, with increasing incidence linked to rising risk factors. This retrospective observational study investigates the role of the Androgen Receptor (AR) in EC aggressiveness, its correlation with other prognostic markers, and its potential therapeutic implications. A total of 143 cases of EC treated with hysterectomy were analyzed for AR expression and its association with clinicopathological and molecular markers, including estrogen receptor (ER), progesterone receptor (PR), Ki-67, p53, β-catenin, E-cadherin, Bcl-2, Cyclin D1, and mismatch repair (MMR) status. AR expression was significantly higher in low-grade endometrioid carcinoma (LGEC) compared to high-grade endometrioid carcinoma (HGEC) and other high-risk histologies ($p = 0.015$), suggesting a role in less aggressive tumor phenotypes. AR strongly correlated with ER and PR ($p < 0.0001$), indicating shared regulatory pathways. A borderline association with tumor-infiltrating lymphocytes (TILs) suggests a potential role in immune response. However, AR expression did not significantly correlate with markers of proliferation (Ki-67) or tumor suppression (p53), nor with β-catenin, E-cadherin, Bcl-2, Cyclin D1, or MMR status. These findings support AR as a prognostic marker in hormone-responsive EC subtypes and suggest that AR-targeted therapies could be beneficial, particularly in ER/PR-negative tumors. The study highlights the potential integration of AR status into molecular profiling, aiding in personalized treatment strategies for improved patient outcomes in EC management.

List of abbreviations: AR, Androgen Receptor; EC, Endometrial Carcinoma; PCOS, Polycistic Ovaric Syndrome; ER, Estrogen Receptor; PR, Progesterone Receptor; MSI, Microsatellite Instability; SI, Staining Index; FIGO, International Federation of Gynecology and Obstetrics staging system; HGEC, High Grade Endometrioid Carcinoma; LGEC, Low Grade Endometrioid Carcinoma; OHEC, Other High Grage Endometrial Cancer; TILs, Tumor-Infiltrating Lymphocytes; LVSI, Lymph-Vascular Spaces Invasion; IHC, Immunohistochemistry; MSS, Microsatellite Stable; DHT, dihydrotestosterone.

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1. Introduction

Endometrial carcinoma (EC), the most common female reproductive malignancy in developed countries, predominantly affects postmenopausal women, particularly those aged 55–64. Its incidence and mortality have risen, linked to increased obesity, diabetes, and hormone replacement therapy use. In 2024, the American Cancer Society estimated 67,880 new EC cases and 13,250 deaths in the U.S. alone [1]. Globally, there were 417,000 new cases and 97,000 deaths in 2020 [2]. Major risk factors include obesity, diabetes, hypertension, and Polycistic Ovaric Syndrome (PCOS), while oral contraceptives and physical activity offer protection. Incidence varies geographically and is higher in North America and Europe [3].

EC is a heterogeneous group of tumors with diverse histopathological and molecular profiles, leading to varied clinical behaviors. Bokhman's dualistic model classifies EC into Type I (estrogen-dependent, low-grade) and Type II (non-estrogen-dependent, high-grade) [4,5]. However, overlaps in molecular features, especially in high-grade endometrioid carcinomas, complicate this classification and treatment strategies [6].

Lax (2004) proposed a molecular-based classification of endometrial carcinoma, distinguishing two major types based on their distinct genetic pathways and clinical behaviour. Type I carcinomas, typically endometrioid, are associated with estrogen exposure, frequent PTEN mutations, microsatellite instability, and K-ras mutations, whereas Type II carcinomas, often serous, are estrogen-independent and characterized by p53 mutations, p16 inactivation, and HER2/neu amplification. This dualistic model underscores the different molecular mechanisms underlying endometrial carcinogenesis, aiding in risk stratification and treatment approaches [7].

Recent studies highlight the importance of incorporating molecular profiling into EC management. The Cancer Genome Atlas (TCGA) classifies ECs into four molecular subgroups: POLE-ultramutated, microsatellite instability (MSI), copy-number low, and copy-number high [8]. This molecular classification provides valuable prognostic and predictive information, but implementing these approaches clinically is challenging, as not all centers can perform POLE sequencing, though guidelines still allow for stratification [9,10].

As previously reported by our group, we selected a small panel of immunohistochemical (IHC) markers commonly used in clinical practice for prognostic evaluation in EC, including estrogen receptor (ER), progesterone receptor (PR), Ki67, p53, E-cadherin, β-catenin, Bcl-2, and Cyclin D1 [11]. In type I endometrioid ECs, high ER and PR expression correlates with favorable prognosis and better hormone therapy response, typically seen in low-grade, early-stage tumors. The loss of ERα is linked to higher tumor grade and poorer prognosis. Zannoni et al. [12] showed that lower ERα/ERβ1 and ERα/ERβ2 ratios are associated with a higher mortality risk [12]. Ki67, a proliferation marker, indicates tumor aggressiveness and distinguishes high-grade from low-grade tumors. p53 mutations, frequent in type II serous ECs, are indicative of poor prognosis. Reduced E-cadherin and β-catenin correlate with increased invasiveness, while Bcl-2 overexpression is associated with better survival; its loss suggests aggressive behavior. Overexpression of Cyclin D1, crucial for cell cycle regulation, is observed in high-grade ECs, helping identify high-risk patients [11].

The role of androgen receptor (AR) in EC is an emerging research area, potentially enhancing our understanding of this complex disease [13]. AR is a nuclear receptor activated by androgens, which translocate to the nucleus and influence gene expression. In EC, AR expression is linked to tumor progression, aggressiveness, and therapeutic response. AR signaling intersects with pathways regulated by ER and PR, suggesting that hormonal interactions may influence EC cell behavior and inform combination therapies [14]. Higher AR expression is often found in well-differentiated endometrioid tumors, correlating with a better prognosis [15]. AR's presence suggests androgen may have antiproliferative effects similar to progesterone, offering a potential therapeutic target. While AR expression frequently coincides with ER and PR, it can also appear independently, especially in high-grade tumors like serous carcinomas, indicating potential for AR-targeted therapies where traditional hormone therapies fail. The therapeutic use of AR antagonists, effective in prostate and breast cancers, is under investigation for EC. However, due to the interplay between androgen and estrogen, AR-targeted therapies in AR-positive, ER-positive tumors must avoid enhancing estrogen-driven proliferation. Thus, while AR shows promise as a marker and therapeutic target in EC, further studies are necessary to refine treatment strategies [16,17]. This study aims to explore AR's role in EC aggressiveness, analyzing its expression and correlation with other prognostic markers to evaluate its potential as a prognostic biomarker and therapeutic target in personalized EC treatments.

2. Materials and methods

In this retrospective observational study, we reviewed the computerized archives of IRCCS Ospedale Policlinico San Martino, Genoa, Italy, to identify patients treated with radical hysterectomy for EC between 2013 and 2018. Only cases with tested AR expression were included. Exclusion criteria were neoadjuvant chemotherapy, prior hormonal therapy, or incomplete data.

Hysterectomy specimens were routinely fixed and processed into 3 μm-thick histological sections, stained with hematoxylin/eosin. Additional sections from the most representative paraffin block underwent IHC testing for markers listed in Table 1 following an institutional protocol.

Histological groups were categorized as low grade endometrioid carcinoma (LGEC; Endometrioid Carcinoma G1 and G2 FIGO), high grade endometrioid carcinoma (HGEC; Endometrioid Carcinoma G3 FIGO), and other high-grade EC (OHEC; aggressive histologies). FIGO staging was classified into FIGO I-II (local) and FIGO III-IV (advanced).

Clinicopathological features were dichotomized: invasion (expansive or infiltrative); desmoplasia, tumor-infiltrating lymphocytes (TILs), tumor necrosis, and lymph-vascular space invasion (LVSI) as absent/mild or moderate/severe.

For IHC, we used the Benchmark XT automatic immunostainer (Ventana Medical Systems SA, Strasbourg, France). Antigen retrieval employed citrate buffer (pH 6) at 90 °C for 30 minutes, and sections were incubated with primary antibodies for 1 hour at 37 °C. The Ventana Medical System Ultraview Universal DAB Detection Kit was used for detection, followed by counterstaining with modified Gill's hematoxylin and mounting in Eukitt.

The tested antibodies are described in Table 1.

2.1. Legend: SI: staining Index

The staining index (SI) for all proposed molecular markers, representing the percentage of positive tumor cells, was evaluated by two

Table 1
List of antibodies.

Marker	Clone	Manufacturer	Dilution	Low SI	High SI
ER	6F11	Ventana	pre-diluted	< 20 %	≥ 20 %
PR	100	Ventana	pre-diluted	< 20 %	≥ 20 %
AR	SP107	Ventana	pre-diluted	< 20 %	≥ 20 %
ki67	30–9	Ventana	pre-diluted	< 40 %	≥ 40 %
p53	DO−7	Ventana	pre-diluted	N/A	N/A
β-catenin	14	Ventana	pre-diluted	< 60 %	≥ 60 %
E-cadherin	36	Ventana	pre-diluted	< 60 %	≥ 60 %
Bcl−2	124	Ventana	pre-diluted	< 20 %	≥ 20 %
Cyclin D1	SP4-R	Ventana	pre-diluted	< 20 %	≥ 20 %
MLH1	M1	Ventana	pre-diluted	N/A	N/A
PMS2	EPR3947	Ventana	pre-diluted	N/A	N/A
MSH2	G219–1129	Ventana	pre-diluted	N/A	N/A
MSH6	44	Ventana	pre-diluted	N/A	N/A

expert pathologists (MP & VGV) independently and blinded. Discrepancies were resolved through discussion at a multiheaded microscope. SI values for each marker were dichotomized into low and high categories based on study population distribution, as detailed in Table 1. The staining index (SI) was defined as the percentage of positively stained tumor cells and is conceptually equivalent to the labelling index, particularly in the case of Ki67.

For p53, abnormal expression was defined as either strong nuclear positivity in at least 80 % of tumor cells or a complete absence of expression with an internal positive control. Variable nuclear staining intensity indicated wild-type expression. MMR status was assessed by immunohistochemistry (IHC) for MLH1, PMS2, MSH2, and MSH6. Tumors were classified as MMR-proficient (MMRp) if all proteins showed retained nuclear expression and MMR-deficient (MMRd) if there was a complete loss in one or more proteins, with intact internal controls. MLH1 loss was always paired with PMS2 loss, and MSH2 with MSH6 due to their dimeric interactions. Cases with dubious or inadequate staining were excluded from definitive classification.

Data were anonymized and entered a Microsoft Excel™ spreadsheet, with statistical analysis performed using MedCalc™ software. Discrete variables were compared using the $\chi 2$ test, and continuous variables using the Kruskal-Wallis test. Spearman rank correlation was used for correlations between continuous variables. A 95 % significance level was chosen. Continuous numeric variables are presented as mean ±standard deviation (SD), and discrete variables as the number of cases (percentage).

The study adhered to the Declaration of Helsinki's ethical principles and received institutional review board approval (CER Liguria 46/2020 DB id 10320). This work complies with STROBE guidelines.Fig. 1

3. Results

A total of 143 patients with EC were included. The mean age was 68.7 years (SD±10.4). The majority (58 %) were over 65 years old.

Most cases were classified as LGEC, accounting for 72 % of cases. Within this category, the distribution between Endometrioid G1 and G2 subtypes was almost equal, representing 36.40 % and 35.70 % of the total, respectively. HGEC was identified in 10.50 % of patients. The remaining 17.50 % of cases were categorized as OHEC, which included a mix of histological subtypes such as Mixed (6.30 %), Serous (4.90 %),

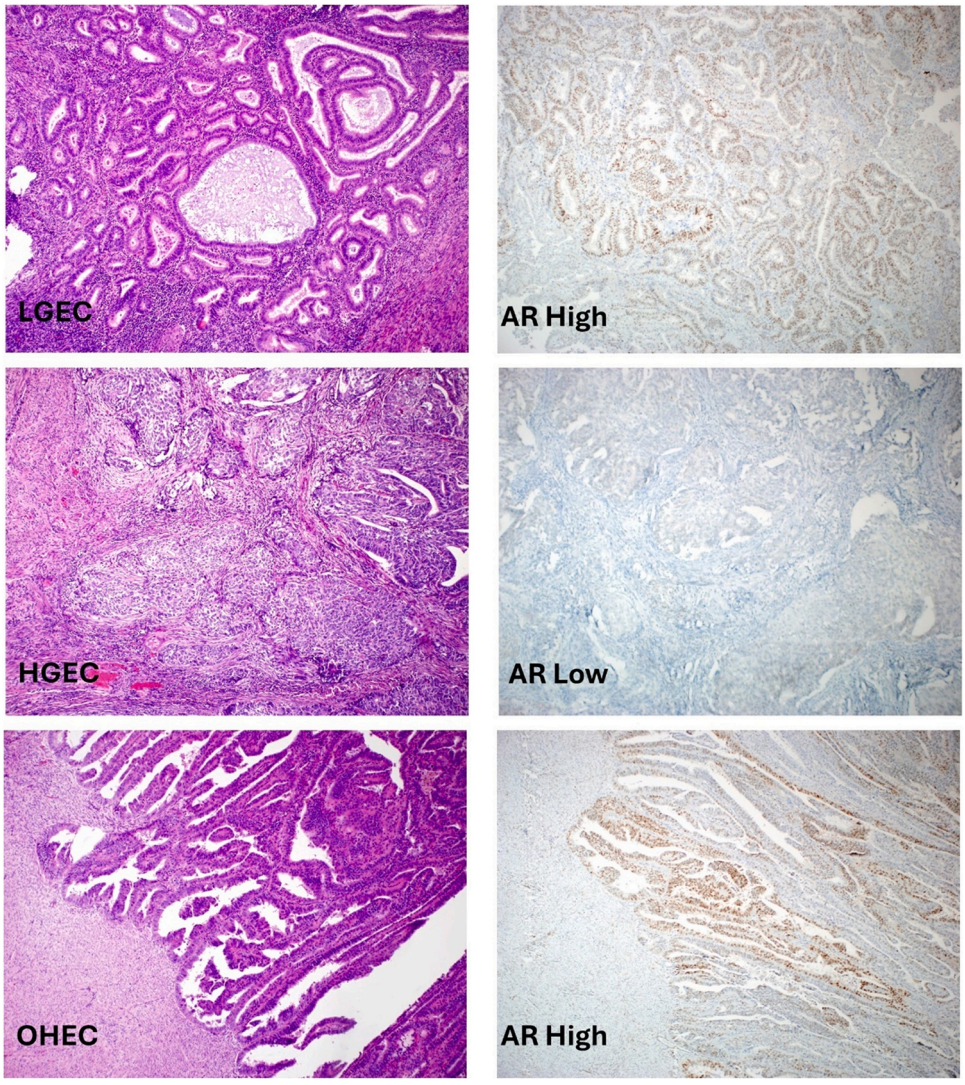


Fig. 1. (on the left column: Hematoxylin/Eosin. Original Magnification 100X; on the right column: AR Immunostaining. Original Magnification 100X): on the left side the three considered histological groups are shown while on the right side the most prevalent staining category is depicted. **Abbreviations:** LGEC: low grade endometrial carcinoma (FIGO G1-G2); HGEC high grade endometrioid carcinoma (FIGO G3), OHEC: other high-grade (aggressive histologies other than endometriod; serous carcinoma in the picture); AR High: Androgen Receptor SI≥ 20 %; AR Low: Androgen Receptor SI< 20 %. The AR-low category includes cases with minimal detectable staining.

Dedifferentiated/Undifferentiated (3.50 %), Carcinosarcoma (1.40 %), and Clear Cell (1.40 %). Immunostaining results showed that the percentage of positive staining for each molecular marker varied according to the histological type. The specific immunostaining percentages and their distributions across histological groups are summarized in Table 2.

Cyclin D1 (cell cycle regulator) all showed statistically significant differences in expression across the groups ($p < 0.05$), but the specific patterns varied.

A statistically significant positive correlation exists between AR expression and both ER and PR expression ($p < 0.0001$ for both). This

	LGEC		HGEC		OHEC		Total		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P-value
ER%	76.36	22.99	48.67	28.06	22.80	25.42	64.09	31.64	***< 0.000001***
PR%	66.35	29.23	35.00	31.62	11.44	18.21	53.50	35.16	***< 0.000001***
AR%	27.69	27.80	11.00	14.29	15.60	21.71	23.83	26.36	***0.015938***
ki67 %	38.03	20.82	55.67	16.68	58.04	19.76	43.32	21.86	***0.00002***
p53 %	4.45	11.45	17.07	31.61	52.80	43.32	14.30	29.09	***0.000326***
β-cat%	96.09	7.80	90.00	11.34	82.80	21.51	93.09	12.73	***0.000221***
E cadh.%	99.80	0.98	94.67	15.98	92.00	21.94	97.86	10.93	***0.000654***
Bcl−2 %	38.27	31.92	21.40	23.35	32.20	34.31	35.40	31.82	**0.171518**
CyclD1 %	20.76	23.47	19.33	20.25	15.68	21.32	19.71	22.72	**0.503817**

Legend: LGEC: low grade endometrioid carcinoma, HGEC: high grade endometrioid carcinoma, and OHEC: other high-grade EC.
ER and PR expression are significantly higher in LGEC compared to HGEC and OHEC ($p < 0.000001$ for both). This suggests a stronger hormonal influence in the lower-grade tumors.
AR expression was observed in an average of 23.8 % of cells. with a range from 0 % to 95 %. AR expression was statistically higher in LGEC compared to HGEC and OHEC ($p = 0.015$). although the overall expression levels were lower than ER and PR (Fig. 1).

Ki-67, a marker of cell proliferation, was significantly higher in HGEC and OHEC compared to LGEC ($p = 0.00002$). P53, a tumor suppressor, showed the opposite trend with significantly higher expression in HGEC and OHEC ($p = 0.000326$). These findings were consistent with the expected behavior of these markers in aggressive tumors.

Other Markers: β-catenin (involved in cell adhesion and signaling). E-cadherin (cell adhesion molecule). Bcl-2 (anti-apoptotic protein). and

suggests that tumors expressing higher levels of ER and PR are also more likely to express AR

No statistically significant correlation was found between AR expression and Ki-67 (cell proliferation marker), p53 (tumor suppressor), β-catenin (involved in cell adhesion and signaling), E-cadherin (cell adhesion molecule), Bcl-2 (apoptosis inhibitor), and Cyclin D1 (regulator of cell proliferation) (Table 3).

Table 2
Histological Group and immunostaining percentage.

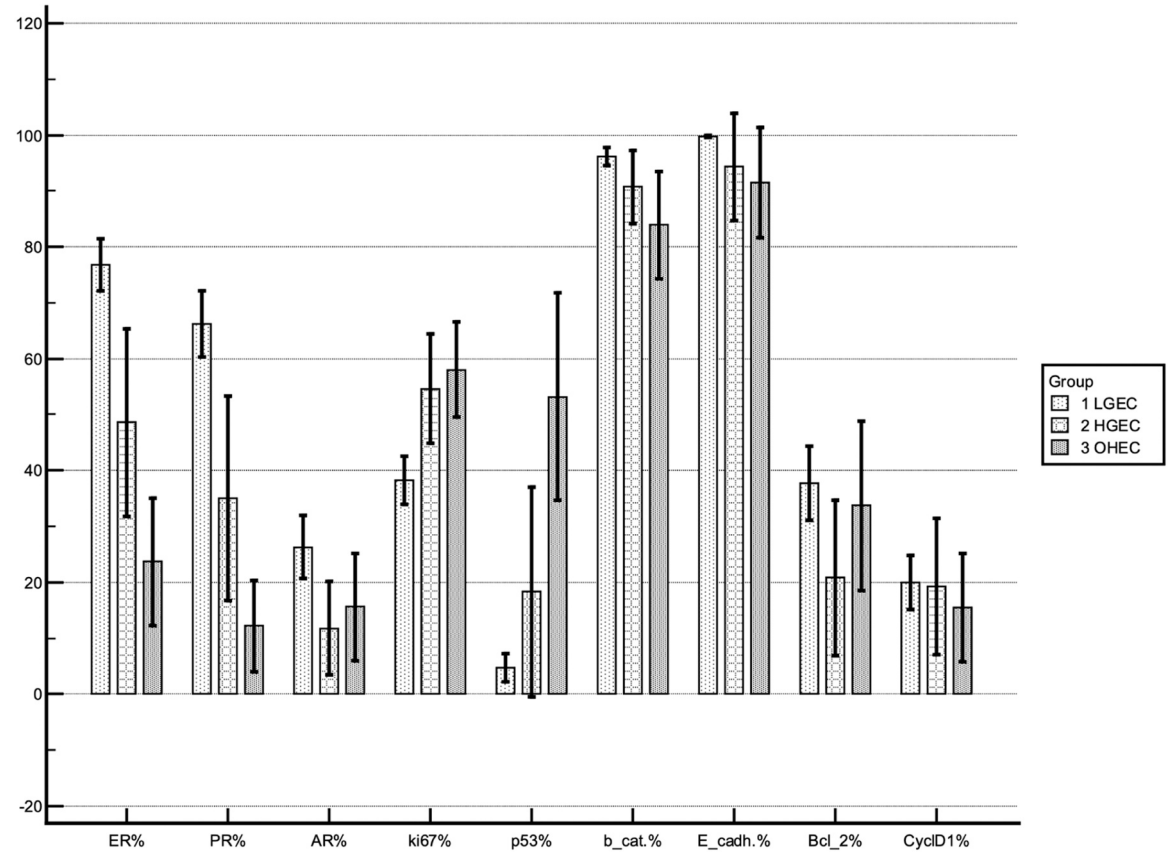
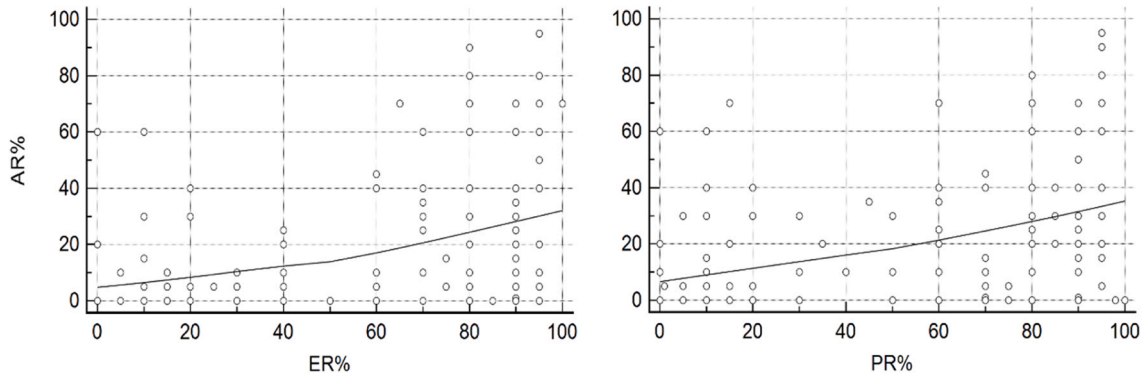


Table 3
Immunostaining percentage correlogram. AR/ER correlation diagram and AR/PR correlation diagram.

Correlation table

	ER%	PR%	AR%	ki67%	p53%	b.cat.%	E.cadh.%	Bcl 2%	CyclD1%
ER%	1								
PR%	0.791 P<0,0001	1							
AR%	0.405 P<0,0001	0.441 P<0,0001	1						
ki67%	-0.293 P0,0005	-0.295 P0,0005	-0.145 P0,0899	1					
p53%	-0.128 P0,1282	-0.157 P0,0635	-0.082 P0,3291	0.324 P0,0001	1				
b.cat.%	0.409 P<0,0001	0.457 P<0,0001	0.184 P0,0293	-0.08 P0,3593	-0.2 P0,0178	1			
E.cadh.%	0.306 P0,0002	0.292 P0,0005	0.146 P0,0860	-0.125 P0,1512	-0.217 P0,0102	0.332 P0,0001	1		
Bcl 2%	0.128 P0,1292	0.265 P0,0016	0.119 P0,1611	-0.178 P0,0382	0.029 P0,7319	0.212 P0,0120	0.218 P0,0101	1	
CyclD1%	-0.104 P0,2184	-0.09 P0,2940	0.08 P0,3470	0.163 P0,0592	0.066 P0,4404	0.073 P0,3886	0.149 P0,0791	-0.001 P0,9929	1

Spearman rank correlation coefficient



AR expression did not show a significant association with patient age, with a distribution of high AR expression observed similarly across age categories (>65 vs. ≤65 years. p = 0.1255). Additionally, there was no significant correlation between AR expression and tumor stage. as defined by FIGO classifications (I-II vs. III-IV; p = 0.3748).

A significant association was found between AR expression and histological type of EC (p = 0.0307). Higher AR expression was observed in low-grade endometrioid carcinomas (LGEC) compared to high-grade endometrioid carcinomas (HGEC) and other high-risk histologies (OHEC), suggesting a potential role for AR in less aggressive tumor phenotypes.

AR expression did not significantly correlate with the type of tumor invasion (expansive vs. infiltrative; p = 0.6457), the degree of desmoplasia (absent/mild vs. moderate/severe; p = 0.5157), or the presence of necrosis (absent vs. present; p = 0.2926). However, a borderline non-significant trend was noted between AR expression and the presence of tumor-infiltrating lymphocytes (TILs) (p = 0.0620).

Significant correlations were found between AR expression and the expression of ER and PR. Higher AR expression was strongly associated with higher ER (p = 0.0048) and PR (p = 0.0001) levels, indicating a potential co-regulatory relationship between these hormone receptors in endometrial cancer.

A borderline non-significant trend was observed between AR expression and tumor-infiltrating lymphocytes (TILs) (p = 0.062), suggesting a potential, though modest, association between AR presence and immune cell infiltration within the tumor microenvironment

AR expression did not show significant associations with Ki67, β-catenin, E-cadherin, Bcl-2 and Cyclin D1.

No significant associations were found between AR expression and MMR status (MMR-deficient or MMR-proficient), including the expression of MLH1, PMS2, MSH2, and MSH6, nor with microsatellite stability status (p > 0.5) (Table 4).

Table 4
clinicopathological and immunohistochemical markers.

	AR high n (%)		**AR low** n (%)		**Total** n (%)		**p**
Age							
>65	44	(30.77)	39	(27.27)	83	(58.04)	**0.1255**
≤ 65	24	(16.78)	36	(25.17)	60	(41.96)	
Stage							
FIGO I-II	55	(38.46)	56	(39.16)	111	(77.62)	**0.3748**
FIGO III-IV	13	(9.09)	19	(13.29)	32	(22.38)	
Histological Group							
LGEC	56	(39.16)	47	(32.87	103	(72.03)	***0.0307***
HGEC	4	(2.8)	11	(7.69)	15	(10.49)	
OHEC	8	(5.59)	17	(11.89)	25	(17.48)	
Invasion							
expansive	43	(30.07)	44	(30.77)	87	(60.84)	**0.6457**
infiltrative	25	(17.48)	30	(20.98)	55	(38.46)	
N/A					1	(0.7)	
Desmoplasia							
absent/mild	50	(34.97)	51	(35.66)	101	(70.63)	**0.5157**
moderate/severe	16	(11.19)	21	(14.69)	37	(25.87)	
N/A					5	(3.5)	
TILs							
absent/mild	52	(36.36)	47	(32.87)	99	(69.23)	**0.062**
moderate/severe	14	(9.79)	26	(18.18)	40	(27.97)	
N/A					4	(2.8)	
Necrosis							
absent/mild	38	(26.57)	36	(25.17)	74	(51.75)	**0.2926**
moderate/severe	28	(19.58)	38	(26.57)	66	(46.15)	
N/A					3	(2.1)	
LVSI							
absent/mild	51	(35.66)	47	(32.87)	98	(68.53)	***0.0772***
moderate/severe	15	(10.49)	27	(18.88)	42	(29.37)	
N/A					3	(2.1)	
ER							
high	64	(44.76)	58	(40.56)	122	(85.31)	***0.0048***
low	4	(2.8)	17	(11.89)	21	(14.69)	
PR							
high	59	(41.26)	42	(29.37)	101	(70.63)	***0.0001***
low	9	(6.29)	33	(23.08)	42	(29.37)	
Ki67							
high	37	(25.87)	45	(31.47)	82	(57.34)	**0.5076**
low	28	(19.58)	27	(18.88)	55	(38.46)	
N/A					6	(4.2)	
p53							
wild-type	54	(37.76)	54	(37.76)	108	(75.52)	**0.3049**
abnormal	14	(9.79)	21	(14.69)	35	(24.48)	
β-catenin							
high	64	(44.76)	73	(51.05)	137	(95.8)	**0.8971**
low	2	(1.4)	2	(1.4)	4	(2.8)	
N/A					2	(1.4)	
E-cadherin							
high	65	(45.45)	72	(50.35)	137	(95.8)	**0.6293**
low	1	(0.7)	2	(1.4)	3	(2.1)	
N/A					3	(2.1)	
Bcl−2							
high	45	(31.47)	42	(29.37)	87	(60.84)	**0.139**
low	21	(14.69)	33	(23.08)	54	(37.76)	
N/A					2	(1.4)	
Cyclin D1							
high	21	(14.69)	19	(13.29)	40	(27.97)	**0.3957**
low	45	(31.47)	56	(39.16)	101	(70.63)	
N/A					2	(1.4)	
MSI							
MMR-proficient	49	(34.27)	57	(39.86)	106	(74.13)	**0.5906**
MMR deficient							
MLH1/PMS2-	10	(6.99)	11	(7.69)	21	(14.69)	
MSH2 MSH6-	0	(0)	1	(0.7)	1	(0.7)	
Dubious/Not evaluated	9	(6.29)	6	(4.2)	15	(10.49)	

4. Discussion

4.1. Summary of main results

EC diagnosis is advancing with molecular profiling, offering valuable prognostic insights. However, high costs of advanced testing (e.g. whole-genome sequencing) limit accessibility. Researchers are developing cost-effective approaches, combining immunohistochemistry with targeted genetic analysis, to enhance cancer understanding while controlling costs. The aim is to personalize treatment based on molecular and traditional risk factors.

This study shows that AR expression is significantly linked to less

aggressive EC subtypes, especially LGEC, compared to HGEC and high-risk histologies. AR positively correlates with hormone receptors ER and PR, indicating a role in hormone-regulated pathways. No significant correlation was found between AR and markers of proliferation or aggressiveness, such as Ki-67 and p53. These results suggest AR as a prognostic marker for less aggressive, hormone-responsive EC and as a potential therapeutic target, supporting more personalized treatment approaches in endometrial cancer.

The nearly significant correlation between AR expression and TILs underscores a possible interaction where AR may play a role in modulating immune infiltration, warranting further investigation into AR's impact on the immune landscape in endometrial cancer.

4.2. Results in the context of published literature

While estrogens are well-known in EC progression, androgens, particularly dihydrotestosterone (DHT), also play a role, though less understood. DHT may have antiproliferative effects on EC cells via the AR pathway [17]. Our study showed AR expression averaging 23.8 % in EC tissues, with higher levels in LGEC compared to HGEC and OHEC. This suggests AR's involvement in differentiation and less aggressive forms of EC.

A significant positive correlation was observed between AR, ER, and PR expressions, suggesting these receptors may share common regulatory pathways, potentially influencing tumor behavior and response to hormone-based therapies [16,17]. AR expression did not correlate with aggressiveness markers like Ki-67, p53, β-catenin, or E-cadherin, indicating its role may be more related to differentiation rather than direct tumor proliferation [16,17].

AR's lack of association with patient age, tumor stage, or mismatch repair proteins suggests it could serve as an independent prognostic marker, particularly for less aggressive, hormone-responsive EC subtypes. The significant correlation with ER and PR further supports AR's role in hormone-regulated pathways and its potential as a therapeutic target, especially in ER/PR-positive tumors. The absence of AR in high-grade ECs and its loss, often linked to poorer survival, underscores its prognostic value [13].

This study aligns with prior research, including Ito et al. and Hashmi et al. [18,19], confirming higher AR expression in LGEC compared to more aggressive EC types. Additionally, Zadeh et al.'s findings of AR in high-grade tumors, often with ER and PR co-expression, suggest AR-targeted therapies might benefit specific subgroups [15–17]. Our study identified a unique subset of advanced-stage EC patients with high AR but low ER/PR, indicating a potential therapeutic target for AR antagonism [16].

Our findings align with and expand upon Lax's dualistic model of endometrial carcinoma by highlighting the role of the AR as a potential prognostic marker within hormone-driven pathways. Consistent with Lax's characterization of Type I endometrioid carcinomas as hormone-responsive tumors frequently exhibiting PTEN mutations and microsatellite instability, our study demonstrates that AR expression is significantly higher in low-grade endometrioid carcinomas and strongly correlates with ER and PR expression. This suggests that AR may serve as an additional marker of differentiation within these tumors, reinforcing their hormonal regulation. Conversely, the lack of significant AR expression in high-risk histologies such as serous carcinoma, characterized by p53 mutations and aggressive behavior as described by Lax, further supports the distinction between the two molecular pathways and highlights AR's limited role in Type II tumors. These findings suggest that incorporating AR into the molecular profiling of EC could enhance risk stratification and therapeutic strategies, particularly in hormone-responsive subtypes.[7].

Transitioning to a molecular classification of EC, as recommended by ESGO-ESTRO-ESP guidelines, could enhance precision medicine, particularly with AR-targeted therapies [8,20]. The presence of AR in metastatic EC and its association with poorer survival in cases of high AR to ER ratio further supports exploring AR-targeted therapies, similar to approaches used in prostate cancer [16,21]. These findings suggest AR's role not only as a biomarker but also as a target for developing personalized treatment strategies in endometrial cancer.

Recent research has further highlighted the potential tumor-suppressive role of androgens in endometrial carcinoma. A study by Kayahashi et al. (2024) demonstrated that dihydrotestosterone (DHT) suppresses the malignant behavior of endometrial carcinoma cells via the androgen receptor (AR) pathway and identified FOXP4 as a key downstream target of AR in this process. Their study, utilizing clinical samples, endometrial cancer cell lines, and murine models, revealed that AR expression and serum DHT levels are associated with lower disease-free survival (DFS). Functional experiments showed that DHT administration inhibited proliferation, migration, and tumor growth in AR-transfected endometrial cancer cells and PTEN-deficient murine models. Additionally, FOXP4 expression was inversely correlated with AR expression, and its overexpression counteracted the tumor-suppressive effects of DHT/AR signaling, suggesting that FOXP4 inhibition could be a potential therapeutic approach.

Our findings are consistent with these observations, as we demonstrated that AR expression is significantly associated with less aggressive tumor phenotypes, particularly low-grade endometrioid carcinoma (LGEC), and strongly correlates with ER and PR expression. However, our study did not include functional assays to directly assess the effects of AR activation, nor did we investigate FOXP4 expression or its regulatory role. While Kayahashi et al. provided compelling evidence for AR-mediated suppression of endometrial carcinoma progression, their results were largely derived from experimental models and transfected cell lines, whereas our study focused on clinically relevant prognostic associations in a well-characterized patient cohort. Furthermore, we did not observe a significant correlation between AR expression and survival outcomes, likely due to the retrospective nature of our study and the absence of follow-up survival data [22].

4.3. Strengths and weaknesses

The submitted paper thoroughly explores AR in EC, highlighting its correlations with prognostic markers and therapeutic potential. Strengths include a large sample size and robust statistical analysis, offering valuable insights into AR's role across histological subtypes. However, limitations such as its retrospective, single-center design, lack of functional mechanism studies, and absence of survival analysis affect generalizability. Acknowledging the lack of a universally accepted standard for staining index stratification, we adopted a classification approach based on the distribution of SI values within our study population to ensure data-driven and context-specific interpretation Nonetheless, the study lays a strong groundwork for future research on AR's role in endometrial cancer and its potential as a therapeutic target.

The lack of comprehensive molecular characterization, including POLE analysis, represents a further limitation of this retrospective study, as such analyses were not routinely performed during the study period and applying them retrospectively to archived specimens could pose methodological and practical challenges.

Recent studies have established p53 immunohistochemistry (IHC) as a reliable surrogate marker for TP53 mutation status in endometrial carcinoma, demonstrating both high sensitivity and specificity. These studies have identified four distinct p53 staining patterns—diffuse overexpression, complete absence (null), subclonal, and cytoplasmic—as key indicators for molecular classification. In our study, which primarily investigates the role of the Androgen Receptor (AR) in endometrial carcinoma, we applied an 80 % cut-off or a complete absence of staining (null) to define abnormal p53 expression. This approach, rooted in historical practices and influenced by the practical constraints of our retrospective design, effectively grouped cases with a predominant mutant component. Additionally, we observed only a single case of cytoplasmic staining, which was interpreted as abnormal,

with no evidence of subclonal loss of p53. While current literature recommends incorporating all four staining patterns to enhance diagnostic accuracy, our findings suggest that the 80 % cut-off remains a pragmatic and effective approach within the specific context of our study. Moving forward, standardized criteria for p53 IHC interpretation, alongside ER, PR and AR evaluation, will be crucial in refining prognostic and therapeutic strategies for endometrial carcinoma [23,24].

4.4. Implications for practice and future research

Future research should address the current study's limitations, including its retrospective and single-center design. A larger, multicenter study would improve generalizability and provide a broader understanding of AR expression in diverse populations. Functional studies in vitro and in vivo are needed to clarify AR's role in EC progression and explore its therapeutic potential.

This study, in line with the most recent literature, support a growing body of evidence suggesting that AR signaling plays a complex role in endometrial carcinoma, potentially acting as a tumor suppressor in specific molecular subgroups. Future investigations should explore the functional relationship between AR and FOXP4 in human EC tissues, as well as the therapeutic implications of modulating the AR pathway in hormone-responsive and hormone-refractory subtypes of endometrial carcinoma.

Additionally, investigating AR targeting in ER/PR-low tumors could lead to novel treatment strategies. Personalized treatment plans incorporating AR status into molecular profiling could enhance outcomes for EC patients. Although our findings suggest an association between AR expression and lower-grade histology, the integration of AR into molecular classification models necessitates further validation through prospective studies with more comprehensive genetic characterization.

5. Conclusions

In conclusion, our study highlights the complex role of AR in EC and underscores the need for further investigation into its potential as a prognostic marker and therapeutic target. The findings contribute to the growing body of evidence on the molecular underpinnings of EC and pave the way for future research aimed at optimizing patient care through personalized medicine.

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Author Statement

Michele Paudice: Conceptualization, Formal analysis, Methodology, Data Curation. **Luca Valle**: Formal analysis, Methodology. **Nataniele Piol**: Formal analysis, Methodology. **Fabio Barra**: Formal analysis, Methodology **Marco Greppi**: Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Serafina Mammoliti**: Resources, Visualization, Validation. **Simone Ferrero**: Resources, Visualization, Validation. ***Emanuela Marcenaro**: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. ***Valerio Gaetano Vellone**: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

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Vellone Valerio Gaetano: Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Marcenaro Emanuela:** Writing – original draft, Validation, Supervision, Project administration, Investigation, Funding acquisition. **Ferrero Simone:** Visualization, Validation, Resources. **Mammoliti Serafina:** Visualization, Validation, Resources. **Greppi Marco:** Validation, Supervision, Formal analysis, Data curation. **Barra Fabio:** Visualization, Validation, Resources. **Piol Nataniele:** Validation, Methodology, Formal analysis. **Valle Luca:** Validation, Methodology, Formal analysis. **Paudice Michele:** Validation, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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