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Original article

The Diagnostic and Prognostic Significance of ARID1A and CD8+ Expression in Endometrial Hyperplasia and Endometrial Endometrioid Adenocarcinoma (Immunohistochemical Study).

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Abstract

Background: Epigenetic driver mutations and tumor microenvironment could play an important and promising role in diagnosis, prognosis, and prediction of different endometrial lesions. **Aim:** To determine their putative diagnostic and prognostic value, this work analyzes the immunohistochemical (IHC) expression of ARID1A and CD8+ in endometrial hyperplasia (EH) and endometrioid endometrial adenocarcinoma (EEA). **Material and methods:** The 80 distinct endometrial lesions included in this retrospective study included (20) EH without atypia, (30) atypical EH (AEH), and (30) EEA. Clinicopathological characteristics and survival of examined cases were correlated with the IHC expression of ARID1A and CD8+. **Results:** ARID1A negative expression was significantly associated with EEA (46.7%) and AEH (30%), while its positivity was associated with EH without atypia cases (100%) ($P<0.01$). ARID1A showed 100% specificity and 38.3% sensitivity for premalignant (AEH) and malignant lesions. When compared to normal endometrium and EH without atypia, the amount of intraepithelial and stromal CD8+ expression revealed a significant difference between the examined cases ($P<0.01$ and $P<0.05$, respectively). ARID1A loss and high CD8+ expression was shown to be significantly correlated ($P<0.01$). Longer disease-free and recurrence-free survival were linked to ARID1A loss and high CD8+ expression. There was no discernible relationship between tumor grade, histologic type, lympho-vascular invasion, lymph node metastasis, or FIGO stage and either ARID1A or CD8+. **Conclusion:** ARID1A can be a reliable diagnostic indicator for premalignant lesions. ARID1A and CD8+ can each independently indicate a patient's increased chance of living longer and serve as biomarkers for the effectiveness of immunotherapy.

Key words: *ARID1A, CD8+, Endometrial hyperplasia, Endometrioid adenocarcinoma*.

Introduction:

According to the World Health Organization classification 2020, endometrial hyperplasia (EH), the proposed precursor lesion for endometrioid endometrial adenocarcinoma (EEA), can be either atypical EH or EH without atypia [(1)]. The diagnosis and distinction between both types has been shown to be of great clinical importance in terms of proper treatment and risk of progression to EEA [(2)].

Endometrial cancer (EC) is the sixth most diagnosed cancer in women worldwide [(3)]. The prevalence of EC has increased over the past ten years, and it now poses a serious threat to public health [(4)]. In Egypt, with 1,694 new cases and 350 fatalities in 2020, EC occupies the 15th spot among all malignancies. Among women it is sixth most frequent cancer and the second most prevalent gynecological cancer after ovarian cancer [(3)].

According to the biological behavior and prognosis, EC was separated into two histological categories [(5)]: Type I are low-grade, endometrioid carcinomas that are estrogen-related, and often clinically indolent. Serous and clear cell carcinomas are examples of type II carcinomas which are non-endometrioid, clinically aggressive and unconnected to estrogen stimulation. However, the molecular, biological, and pathological heterogeneity within each category is not considered by this dualistic approach [(1)].

Molecular classification of EC generated four genetically defined subgroups [(6)]: Polymerase-Ɛ exonuclease (POLE) ultra-mutated, hypermutated microsatellite instable (MSI), copy number low (endometrioid), and copy number high cases ("serous-like"), with significantly different clinical outcome between them. Carcinomas with POLE-mutations carry an extremely favorable prognosis whereas copy number high cases show an unfavorable outcome necessitating aggressive treatment. Copy-number low and MSI cases have an overall intermediate prognosis [(7)].

Age, histological grade, myometrial invasion, lympho-vascular space invasion, and stage all have an enormous impact on EC prognosis and treatment. However, these clinicopathological traits are insufficient to predict how a disease would progress [(8)]. An amazing possibility for prevention and improved patient management of this

frequently occurring malignancy is an earlier and more precise diagnosis of EC, particularly its histologic antecedents. Effective biomarkers and therapeutic targets are essential to enhance the diagnosis and prognoses for EC patients [(9)].

A component of the chromatin-remodeling complex SWItch/Sucrose Non-Fermentable (SWI/SNF), the AT-rich interaction domain 1A (ARID1A) binds transcription factors and other complexes necessary for DNA replication, cell cycle progression, and cellular proliferation in an ATP-dependent manner [(10)].

The SWI/SNF complex's most frequently mutated member, ARID1A, has been found in a variety of human cancers, including those of the gastrointestinal system, ovary, lung, and breast [(11-14)]. A putative role for ARID1A in avoiding preneoplastic transformation has been shown by the finding of the ARID1A mutation in a variety of preneoplastic lesions, including disorders linked to endometriosis [(15)]. The bulk of the ARID1A mutations are nonsense or frameshift, which cause protein truncation and functional loss [(16)].

For cancer therapy to be effective, a thorough understanding of host immunity and its role in cancer is essential. Considering the enormous importance of the immune system in cancer therapy, several immunomodulatory drugs and immunotherapeutic agents have been used to treat various malignancies. CD8+ T cells are one of the primary tumor-infiltrating immune cells that deliver antitumor responses [(17)]. A positive association between infiltrating CD8+ T cells and prognosis has been reported in various solid cancers such as colorectal and ovarian cancer [(18, 19)]. However, there is limited data regarding the prognostic significance of tumor infiltrating lymphocytes (TILs) in endometrial carcinoma**.**

Even though the SWI/SNF complex has a well-established involvement in malignant transformation, the significance of the ARID1A mutation and its connection to immune infiltration in endometrioid cancer is not fully known [(20)]**.**

This study intends to assess the expression of ARID1A and CD8+ in EH without atypia, AEH, and EEA and correlate their expression with relevant clinicopathological characteristics and patient survival to clarify their probable diagnostic and prognostic importance. To investigate any potential connections between ARID1A and CD8+

expression, we sought to correlate their expression with each other.

Materials and Methods

Patients Selection

This is a retrospective study conducted on eighty cases with different endometrial lesions, twenty cases of EH without atypia, thirty cases of atypical EH, and thirty cases of EEC. Ten cases of normal (proliferative) endometrium were studied as a control group. The cases were obtained from the archive of the Department of Pathology and Early Cancer Detection Unit, Faculty of Medicine, Benha University, and from Pathology Department, Faculty of Medicine, Mansoura University, processed between March 2010 and November 2021.

Inclusion criteria:

- Cases with available clinicopathological data regarding age, tumor size, grade, lymph node status, cervical involvement, adnexal involvement, and stage.
- Follow-up data and survival outcomes for carcinoma cases.

Exclusion Criteria:

- Cases with other histology such as mixed carcinomas.
- Patients whose clinical data were not available
- Carcinoma cases without available follow-up data.

The Ethics Committee of Faculty of Medicine, Benha University, Egypt approved this study code *(MD 1-4-2020).*

Histopathological Analysis

For each block, we stained 3-4 mm thick section with Hematoxylin and Eosin (H&E) and carefully re-examined by two pathologists to confirm pathological diagnosis of cases and assess other variables. We classified endometrial hyperplasia cases according to WHO classification 2020 [(1)]. According to latest WHO classification 2020 [(1)], we classified endometrioid carcinoma cases and graded them using standard FIGO criteria [(21)]. We recorded depth of myometrial invasion in all cases and divided into invasion of <50% or ≥50% of myometrial thickness [(8)]. The myometrium was assessed for myoinvasive pattern either being non-myoinvasive, diffusely infiltrative, pushing/broad front, microcystic elongated and fragmented (MELF), adenomyosis-like pattern, or adenoma malignum-like pattern [(22)]. Lympho-vascular space invasion was evaluated as positive or negative [(8)]. Presence or absence of adenomyosis, cervical involvement, adnexal involvement, lymph node involvement was reported. The

cases were staged according to FIGO stage 2018 [21].

Immunohistochemical staining:

Anti-ARID1A antibody ***(Rabbit monoclonal antibody, 0.1mg/ml concentration, Chongqing Biospes company, Cat. # YMA1234, conc. China)*** and anti-CD8 antibody ***(Rabbit polyclonal antibody, 0.1mg/ml concentration, Chongqing Biospes company, Cat. # BPA1021, conc. China)*** were used in an immunohistochemical (IHC) process as directed by the manufacturer, at a dilution of 1:50, at room temperature overnight for both morkers**.** A conventional labelled streptavidin-biotin system was used for immunodetection ***(Genemed, CA 94080, USA, South San Francisco)*.** Using 10 mmol/L citrate monohydrate buffer (pH 6.0) and heating for 15 minutes in the microwave, antigen retrieval was carried out for both markers. The chromogen diaminobenzene ***(DAB, Envision ™ Flex /HRP-Dako, REF K 8000)*** used was freshly prepared. The counter satin was Mayer's hematoxylin.

Positive and negative controls:

We used normal renal tissue as positive control for ARID1A [23]. For CD8+, we used normal human tonsil tissue as a positive control [24]. For negative control, primary antibody was omitted from the staining process.

Immunohistochemical interpretation:

Interpretation of ARID1A expression:

Positive ARID1A expression was detected as nuclear brown coloration in tumor cells. Stromal cells were used as an internal positive control. ARID1A expression was assessed, and the results were categorized into 3 classes [25]: Retained expression/positive (positive nuclear staining in almost all tumor cells or >90% of tumor cells), Homogenous/complete loss (negative nuclear staining in almost all or >90% of tumor cells), and Heterogeneous/clonal loss (regional negativity with 10–90% of ARID1A-negative tumor cells). Both complete and clonal loss were regarded as ARID1A loss/negative expression.

Interpretation of CD8+ expression:

Positive CD8+ expression was detected as membranous brown coloration of T lymphocytes. CD8+ expression was assessed separately intraepithelial and stromal (mainly at invasive border), selecting hotspot areas for manual counting the number of CD8+ T lymphocytes CD8+ at 5 high power fields (x400). To determine

high CD8+ expression, the median number of intraepithelial and stromal cells was estimated independently. Cases with an expression number equal to or higher than the median were regarded as having high expression [(24)].

Statistical analysis:

Using a personal computer and the SPSS (version 22) statistical package for Microsoft Windows (SPSS Inc., Chicago, IL, USA), data were gathered, tabulated, and statistically analysed. P value was statistically significant when ≤0.05 and highly significant when ≤0.01. Receiver-operating characteristic (ROC) curve was used to estimate sensitivity and specificity. The log-rank test was used to establish the statistical significance of the survival data, which were presented as Kaplan-Meier curves. Both disease-free and recurrence-free survival were calculated as the number of months since the diagnosis. Data were censored if patient was disease-free, recurrence-free, or alive at last follow-up.

Results

Clinicopathological results:

The age of eighty studied cases ranged between 38 & 79ys, with mean age52.64±10.067. The mean age for EH without atypia was 46.45±6.54ys (range 39-68ys), 50.17±7.139ys. for AEH (range 38-70ys), and 59.23±10.925ys for EEA (range 42-79ys). The studied EEA cases were separated into 2 groups <60ys and ≥60ys according to mean age. The mean size of EEA cases was 3.53± 1.542 cm (range 1-6 cm). The follow-up time ranged from 8 to 75 months, the median follow-up time was 45 months. The clinicopathological features of studied EEA cases were shown in **(Table 1).**

Table 1. Clinicopathological characteristics of studied EEA cases (n=30).

Parameter		Frequency	Percentage
Age	<60 years	15	50%
	≥60 years	15	50%
Size	< 3.5 cm	15	50%
	≥ 3.5 cm	15	50%
Histologic type	Conventional EEA	17	56.7%
	Villoglandular EEA	6	20%
	EEA with squamous differentiation	6	20%
	EEA with mucinous differentiation	1	3.3%
FIGO grade	Grade 1	11	36.7%
	Grade 2	11	36.7%
	Grade 3	8	26.7%
Depth of MI	< 50%	17	56.7%
	≥ 50%	13	43.3%
Myo-invasive pattern	Non-invasive	2	6.7%
	Diffusely infiltrative	9	30%
	Broad front/pushing	16	53.3%
	MELF	3	10%
Adenomyosis	Present	6	20%
	Absent	24	80%
Cervical involvement	Positive	12	40%
	Negative	18	60%
Adnexal involvement	Positive	3	10%
	Negative	27	90%
LVSI	Positive	13	43.3%
	Negative	17	56.7%
Lymph node	Positive	8	26.7%
	Negative	22	73.3%
FIGO stage	Stage I	12	40%
	Stage II	9	30%
	Stage III	9	30%
Disease-free survival	Free	14	46.7%
	diseased/died	16	53.3%
Recurrence	Yes	10	33.3%
	No	20	67.7%

N: number; *EEA*: endometrioid endometrial adenocarcinoma; *LVSI:* lympho-vascular space invasion; *MELF*; microcystic elongated and fragmented.

Immunohistochemical results:

ARID1A expression:

All the control group cases and EH without atypia cases showed retained/positive ARID1A expression. In atypical EH group, 21 (70%) cases showed retained ARID1A expression, and 9 (30%) cases showed loss of ARID1A nuclear staining [8 (26.7%) cases showed clonal loss and one (3.3%) case showed complete loss]. In EEC group, 16 (53.3%) cases showed retained ARID1A expression, and 14 (46.7%) cases showed loss of ARID1A nuclear staining [6 (20%)

cases showed clonal loss and 8 (26.7%) cases showed complete loss] **(Figure 1a-d).** A statistically highly significant correlation was found between ARID1A loss and studied groups (P <0.01).

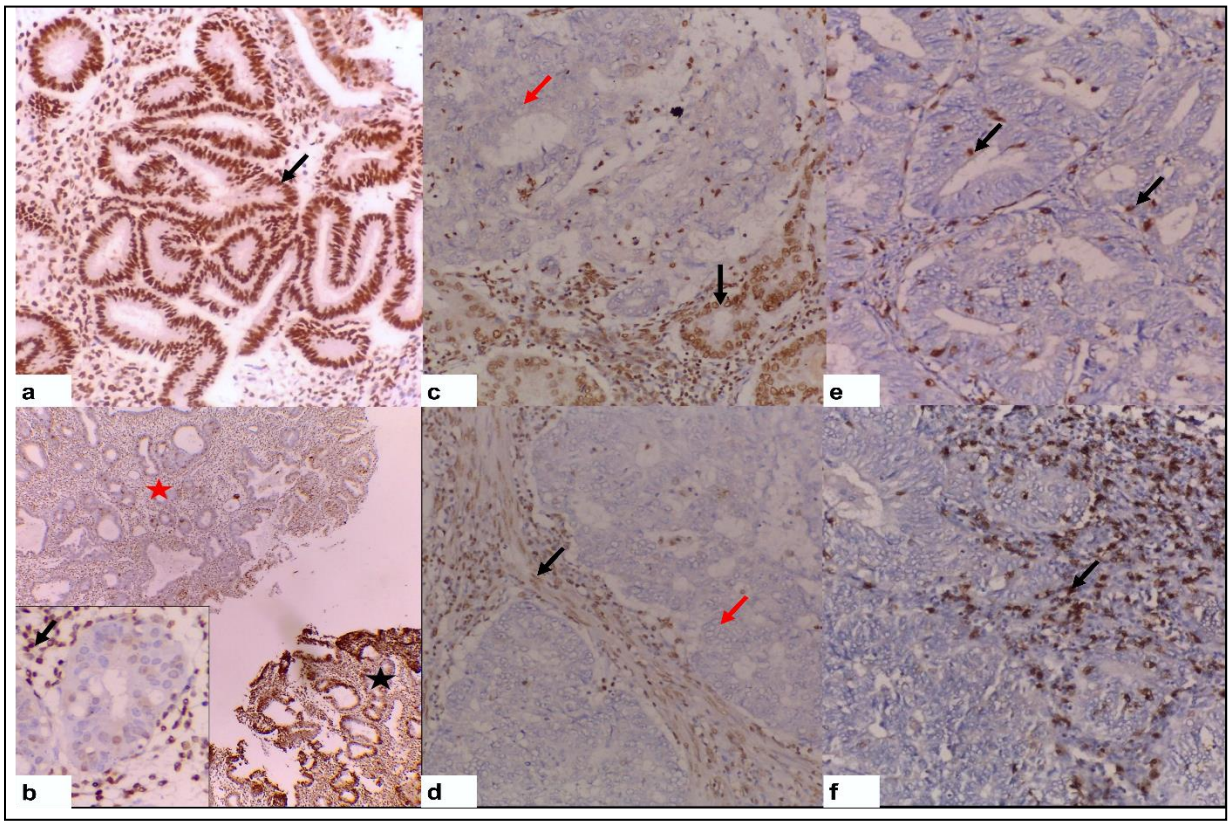

Figure 1. Representative micrographs of immunohistochemical staining results in studied cases: A) Endometrial hyperplasia without atypia showing positive/retained ARID1A nuclear expression in endometrial glands (*black arrow*) (ABC, x200), B) Atypical endometrial hyperplasia showing clonal ARID1A loss (red star: area of ARID1A loss of nuclear expression with positive internal control "*black arrow*", *black star*: area of retained ARID1A nuclear expression) (ABC, X40, inset x400), C) Endometrioid adenocarcinoma, grade 1, showing clonal ARID1A nuclear loss (*red arrow*: area of negative ARID1A staining, *black arrow*: area of positive ARID1A nuclear staining (ABC, x200), D) Endometrioid adenocarcinoma, grade 3 showing complete ARID1A loss of nuclear expression (*red arrow*) with internal positive stromal control (*black arrow*) (ABC, x200), D) Endometrioid adenocarcinoma, grade 1, showing high intraepithelial CD8+ number expression *(black arrow)* (ABC, x200), B) Endometrioid adenocarcinoma, grade 2, showing high stromal CD8+ number expression *(black arrow)* (ABC, x200). *ABC*; Avidin biotin complex. *ABC*; Avidin biotin complex.

Correlations of ARID1A expression with the clinicopathological features of EEA cases were shown in **(Table 2).**

Table 2. Correlation between ARID1A expression and clinicopathological parameters in studied EEA cases (n=30).

Parameter		ARID1A expression		*P* value
		Retained (n=16)	Lost (n=14)	
Histologic type	Conventional	11(64.7%)	6(35.3%)	>0.05
	Villoglandular	3(50%)	3(50%)	
	with squamous differentiation	2(33.3%)	4(66.7%)	
	with mucinous differentiation	0	1(100%)	
FIGO grade	Grade 1	6(54.5%)	5(45.5%)	>0.05
	Grade 2	6(54.5%)	5(45.5%)	
	Grade 3	4(50%)	4(50%)	
Myometrial invasion	< 50%	9(52.9%)	8(47.1%)	>0.05
	≥ 50%	7(53.8%)	6(46.2%)	
Myoinvasive pattern	Non-invasive	2(100%)	0	**<0.05***
	Diffusely infiltrative	6(66.7%)	3(33.3%)	
	Broad front/pushing	8(50%)	8(50%)	
	MELF	0	3(100%)	
Adenomyosis	Present	6(100%)	0	**<0.01****
	Absent	10(41.7%)	14(58.3%)	
Cervical involvement	Positive	8(66.7%)	4(33.3%)	>0.05
	Negative	8(44.4%)	10(55.6%)	
LVSI	Positive	5(38.5%)	8(61.5%)	>0.05
	Negative	11(64.7%)	6(35.3%)	
Lymph nodes	Positive	4(50%)	4(50%)	>0.05
	Negative	12(54.5%)	11(45.5%)	
FIGO stage	I	6(50%)	6(50%)	>0.05
	II	6(66.7%)	3(33.3%)	
	III	4(44.4%)	5(55.6%)	
Disease-free survival	Free	4 (28.6%)	10 (71.4%)	**<0.01****
	Diseased/ died	12 (75%)	4 (25%)	
Recurrence-free survival	Yes	8 (80%)	2 (20%)	**<0.05***
	No	8 (40%)	12 (60%)	

N: number; *EEA*: endometrioid endometrial adenocarcinoma; *LVSI:* lympho-vascular space invasion; *: significant; **: highly significant; *MELF*; microcystic elongated and fragmented.

The diagnostic accuracy of ARID1A expression was determined by using ROC Curve**,** these curves show the specificity (true negative fraction) and sensitivity (true positive fraction) of the test for all probable thresholds. The area under the curve indicates the test's accuracy (AUC). **Table 3** and **Figure 2** below display the diagnostic performance of ARID1A in various endometrial lesions.

Table 3. Statistical analysis of ARID1A as a marker for malignancy using ROC curve.

	Sensitivity (%)	Specificity (%)	AUC	*P* value
EEA *vs* EH	46.7	84	0.653	**<0.05***
EEA *vs* AEH	46.7	70	0.583	0.268
EEA *vs* EH without atypia	46.7	100	0.733	**<0.01****
AEH *vs* EH without atypia	30	100	0.650	0.075
AEH & EEA *vs* EH without atypia	38.3	100	0.692	**<0.01****

ROC: Receiver operating characteristic, *EEC:* Endometrioid endometrial carcinoma, *EH:* Endometrial hyperplasia, *AEH:* Atypical endometrial hyperplasia, *AUC* Area under the curve, *****: Significant, ******: Highly significant.

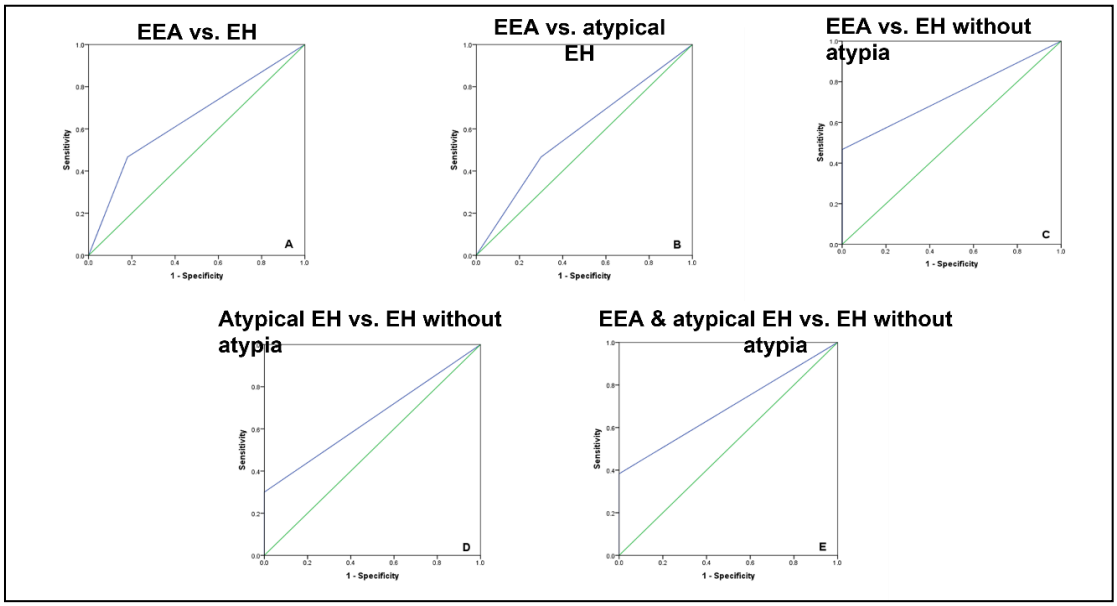

Figure 2. Diagnostic performance for ARID1A, using ROC curve analysis. *EEA:* Endometrioid endometrial adenocarcinoma, *EH:* Endometrial hyperplasia

Kaplan-Meier analysis showed that ARID1A loss is associated with prolonged disease-free survival (57 vs. 31.37 months, P <0.05), and prolonged recurrence-free survival and less occurrence of recurrence than cases with retained ARID1A (65.83 vs. 39.89 months, P <0.05) **(Figure 3a, b).**

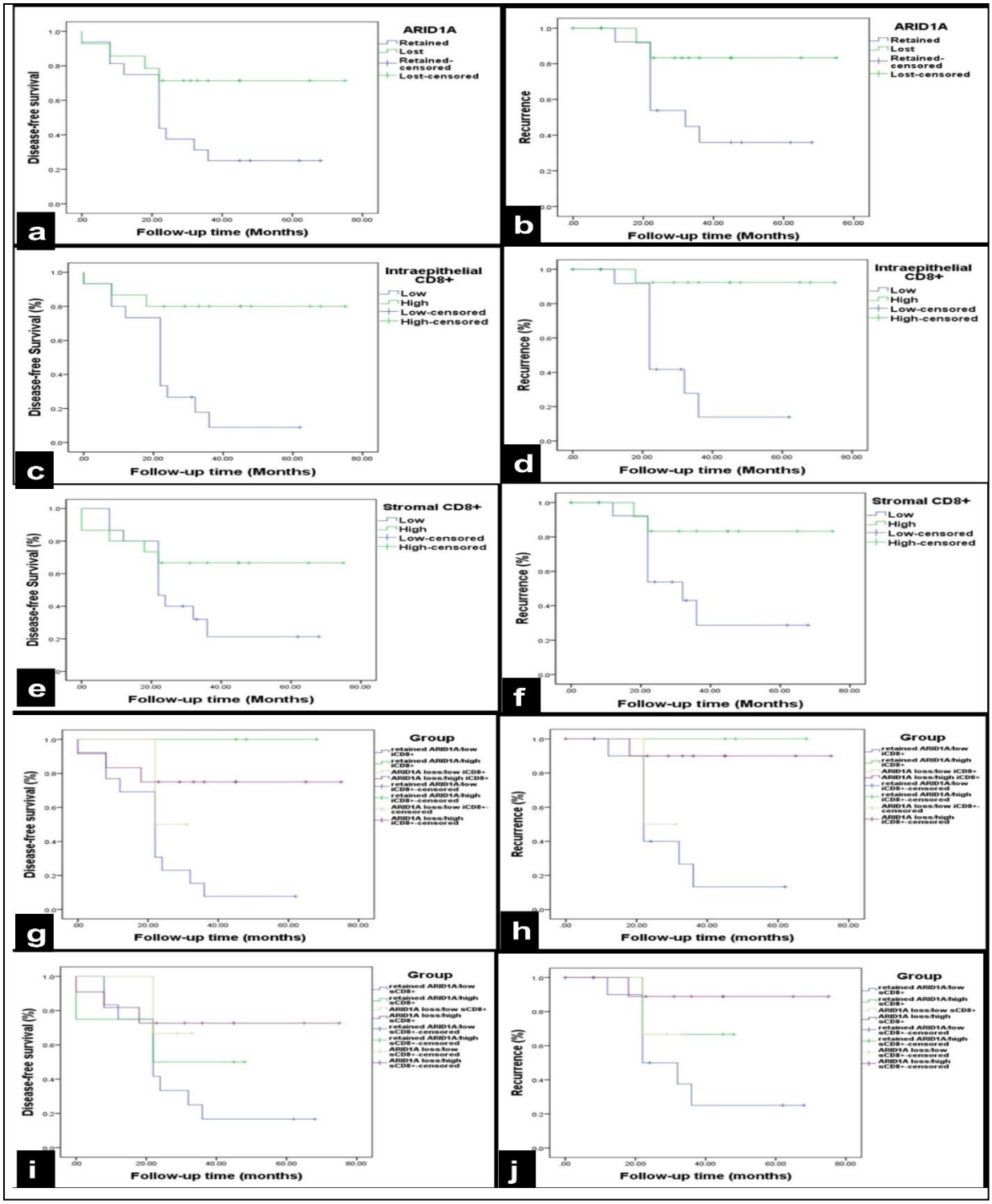

Figure 3. Kaplan–Meier estimates of disease-free survival and recurrence-free survival: a**, b)** ARID1A survival curves, **c, d)** intraepithelial CD8+ survival curves, **e, f)** stromal CD8+ survival curves, **g, h)** combined ARID1A and intraepithelial CD8+ survival curves, and **i, j)** Combined ARID1A and stromal CD8+ survival curves. i*CD8+*; Intraepithelial CD8+, *sCD8+*: Stromal CD8+.

CD8+ expression:

The mean number of intraepithelial CD8+ in control group was 1.9±1.449 (range 1-5 cell/5 HPFs), in EH without atypia was 3±1.414 (range 2-7 cell/5 HPFs), in atypical EH was 4.067±4.050 (range 1-20 cell/5 HPFs), and in EEA was 19.27±18.54 (range 0-70 cell/5 HPFs), that was statistically highly significant (P <0.01). The mean number of stromal CD8+ in control group was 13.50±4.116 (range 10-20 cell/5 HPFs), in EH without atypia was 30.75±22.84 (range 10-95 cell/5 HPFs), in atypical EH was 38.83±29.26 (range 5-160 cell/5 HPFs), and in EEA was 57±48.824 (range 0-200 cell/5 HPFs), that was statistically significant (P <0.05) **(Figure 1e, f).**

Cases with intraepithelial CD8+ number ≥10 cells/5HPFs were considered to have high intraepithelial CD8+ expression, while cases with stromal CD8+ number ≥60 cell/5HPFs were considered to have high stromal CD8+ expression. A statistically highly significant difference was found between studied groups and each of intraepithelial and stromal CD8+ expression (P<0.01). In EH without atypia group: all cases (100%) showed low intraepithelial CD8+. Eighteen cases (90%) showed low stromal CD8+, and 2 cases (10%) showed high stromal CD8+. In atypical EH group: 27 cases (90%) showed low intraepithelial CD8+, and 3 cases (10%) showed high intraepithelial CD8+. Twenty-six cases (86.7%) low stromal CD8+ and 4 cases (13.3%) showed high stromal CD8+. In EEA group: 15 cases (50%) showed low intraepithelial CD8+, and 15 cases (50%) showed high iCD8+. Fifteen cases (50%) showed low stromal CD8+, and 15 cases (50%) showed high sCD8+. Correlations of CD8+ expression with the clinicopathological features of EEA cases were shown in **(Table 4).**

Table 4. Correlation between CD8+ expression and clinicopathological parameters in studied EEA (n=30).

Parameter	Total (n=30)	Intraepithelial CD8+ Low (n=15) N (%)	Intraepithelial CD8+ High (n=15) N (%)	*P* value	Stromal CD8+ Low (n=15) N (%)	Stromal CD8+ High (n=15) N (%)	*P* value
Histologic type							
Conventional EEA	17	8 (47.1%)	9 (52.9%)	>0.05	10 (58.8%)	7 (41.2%)	>0.05
Villoglandular EEA	6	5 (83.3%)	1 (16.7%)		4 (66.7%)	2 (33.3%)	
with squamous differentiation	6	2 (33.3%)	4 (66.7%)		1 (16.7%)	5 (83.3%)	
with mucinous differentiation	1	0	1 (100%)		0	1 (100%)	
FIGO grade							
Grade 1	11	6 (54.5%)	5 (45.5%)	>0.05	7 (63.6%)	4 (36.4%)	>0.05
Grade 2	11	5 (45.5%)	6 (54.5%)		5 (45.5%)	6 (54.5%)	
Grade 3	8	4 (50%)	4 (50%)		3 (37.5%)	5 (62.5%)	
Myometrial invasion							
<50%	17	8 (58.8%)	9 (41.2%)	>0.05	9 (52.9%)	8 (47.1%)	>0.05
≥50%	13	5 (38.5%)	8 (61.5%)		6 (46.2%)	7 (53.8%)	
Adenomyosis							
Present	6	4 (66.7%)	2 (33.3%)	>0.05	3 (50%)	3 (50%)	>0.05
Absent	24	11 (45.8%)	13 (54.2%)		13 (54.2%)	11 (45.8%)	
Cervical involvement							
Positive	12	8 (66.7%)	4 (33.3%)	>0.05	9 (75%)	3 (25%)	**<0.05***
Negative	18	7 (38.9%)	11 (61.1%)		6 (33.3%)	12 (66.7%)	
LVSI							
Positive	13	4 (30.8%)	9 (69.2%)	>0.05	4 (30.8%)	9 (69.2%)	>0.05
Negative	17	11 (64.7%)	6 (35.3%)		11 (64.7%)	6 (35.3%)	
LN involvement							
Positive	8	3 (37.5%)	5 (62.5%)	>0.05	2 (25%)	6 (75%)	>0.05
Negative	22	12 (54.5%)	10 (45.5%)		13 (59.1%)	9 (40.9%)	
FIGO stage							
I	12	6 (50%)	6 (50%)	>0.05	6 (50%)	6 (50%)	>0.05
II	9	6 (66.7%)	3 (33.3%)		7 (77.8%)	2 (22.2%)	
III	9	3 (33.3%)	6 (66.7%)		2 (22.2%)	7 (77.8%)	
Disease-free survival							
Free	14	2 (14.3%)	12 (85.7%)	**<0.01****	4 (28.6%)	10 (71.4%)	**<0.05***
Diseased/ died	16	13 (81.3%)	3 (18.7%)		11 (68.8%)	5 (31.2%)	
Recurrence-free survival							
Yes	10	9 (90%)	1 (10%)	**<0.01****	8 (80%)	2 (20%)	**<0.05***
No	20	6 (30%)	14 (70%)		7 (35%)	13 (65%)	

N: Number, *****: Significant, ******: Highly significant, *EEA:* endometrioid endometrial adenocarcinoma, *LVSI*: lympho-vascular space invasion, *LN:* lymph node

Kaplan-Meier analysis showed that high intraepithelial CD8+ is significantly positively associated with prolonged disease-free survival (61.733 vs. 23.822 months, P <0.01), and prolonged recurrence-free survival (70.615 vs. 30.056 months, P <0.01) **(Figure 3c, d)**. For stromal CD8+, Kaplan-Meier analysis showed that high stromal

CD8+ is associated with prolonged disease-free survival but this didn't reach a statistical significance (53.200 vs. 31.707 months, P >0.05), while it was significantly positively correlated with prolonged recurrence-free survival (65.833 vs. 37.528 months, P <0.05) **(Figure 3e, f)**. This indicate that intraepithelial CD8+ is more favorable prognostic indicator than stromal CD8+.

Relation between ARID1A and CD8+ immunohistochemical expression:

We found a statistically highly significant inverse correlation between ARID1A loss and CD8+ expression (P <0.01) in EEA cases, 12 (85.7%) and 11 (78.6%) of EEA cases with ARID1A loss showed respectively high intraepithelial CD8+ and stromal CD8+expression, while 3 (18.8%) and 4 (25%) of EEA cases with retained ARID1A showed respectively high intraepithelial CD8+ and stromal CD8+expression **(Figure 4).** Loss of ARID1A was associated with high CD8+ expression both intraepithelial and stromal in EEA cases, indicating a possible benefit of cases with ARID1A loss from targeted immunotherapy and give an insight into the consequences of ARID1A loss that may create therapeutic vulnerabilities in ARID1A mutant tumors.

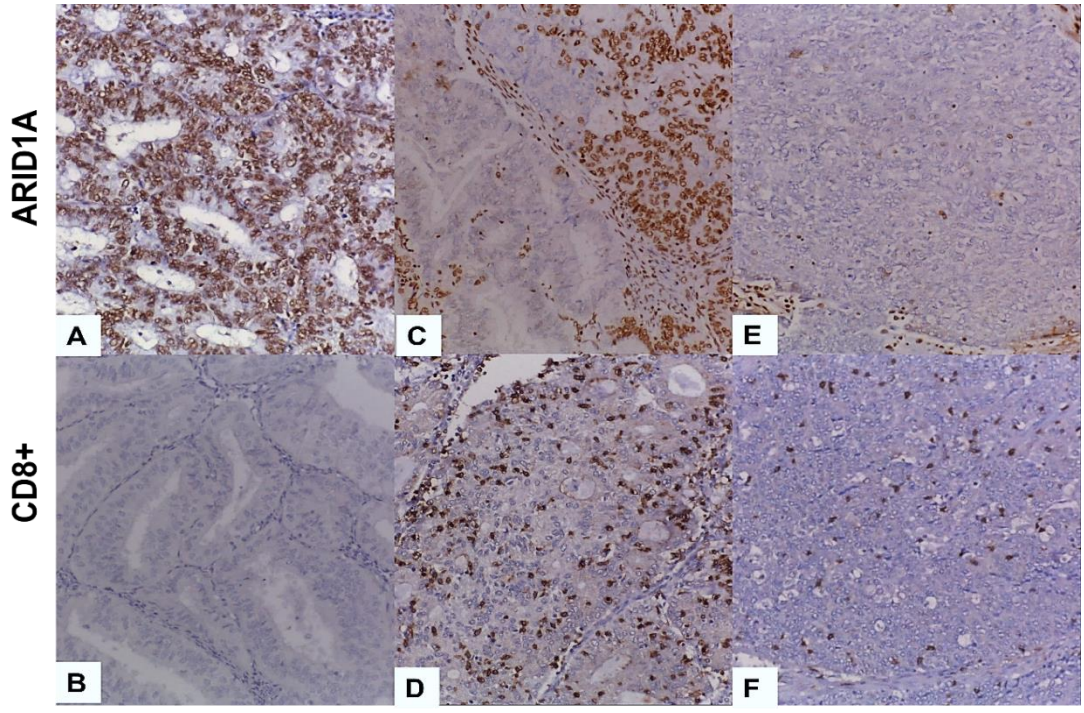

Figure 4. Representative micrographs demonstrating the inverse relation between ARID1A expression and CD8+ expression in endometrioid endometrial adenocarcinoma (EEA), **A)** EEA case positive for ARID1A expression showed **B)** negative/low CD8+ number expression, **C)** EEA case with clonal ARID1A loss showed **D)** high CD8+ number expression, and **E)** EEA case with complete ARID1A loss showed **F)** high CD8+ number expression. (ABC, x200). *ABC*; Avidin biotin complex.

Combined ARID1A and CD8+ expression in relation to patient survival:

To reveal the role of combined ARID1A and CD8+ expression on patient survival in EEA cases and which combination is associated with more favorable survival, EEA were divided into 4 groups as follow: **Group 1:** Retained ARID1A/Low CD8+ expression, **Group 2:** Retained ARID1A/High CD8+ expression, **Group 3:** ARID1A loss/Low CD8+ expression, and **Group 4:** ARID1A loss/ High CD8+ expression.

Kaplan-Meier survival analysis revealed that combined ARID1A loss with high CD8+ number expression was associated with the most favorable prognosis and was significantly associated with the most prolonged disease-free survival and recurrence free survival. Whilst the correlation was highly significant for combined ARID1A and intraepithelial CD8+ ($P<0.01$), it was non-significant for combined ARID1A and stromal CD8+ expression ($P>0.05$) **(Figure 3 g-j).**

Discussion:

Endometrioid endometrial adenocarcinoma (EEA) is the most prevalent histological type of endometrial cancer with excellent clinical outcomes in the earlier stage diseases, but the outcomes get worse in more advanced stages [(26)]. Endometrial hyperplasia is proposed to be a precursor to endometrioid carcinoma, and if detected early ad treated appropriately, prevention to progression to cancer can be performed [(3)].

Given the important functional roles of ARID1A in cell cycle regulation, DNA repair, and apoptosis, better understanding of how ARID1A inactivation contributes to tumor development and progression is critical to improve prognosis and treatment in ARID1A mutated cancers.

In this work we observed loss of ARID1A nuclear expression in only AEH (30%) and EEA (46.7%), while was positively expressed in all EH without atypia and normal endometrium cases (100%), with a statistically highly significant difference ($P<0.01$).

Our findings were compatible with previous studies evaluating ARID1A expression in AEH with reports of ARID1A loss in 16%, 22%, and 20% of AEH cases [(27-29)]. In consistency with our study, no loss of ARID1A IHC nuclear expression in normal endometrium and endometrial hyperplasia without atypia was reported in these studied.

On the other hand, retained ARID1A nuclear expression in all atypical EH cases were reported in other studies [30, 31].

In line with current work results, one study reported ARID1A loss in 44% of EEA cases [29], while other studies reported ARID1A loss in lower percentage of EEA cases 19%, 16% and 12.5% [28, 31, 32]. This can be explained by different number of cases studied, different histologic types, variability in antibodies, protocols and scoring methods used.

As atypical EH contains many of the genetic changes seen in EEA such as microsatellite instability, PAX inactivation; and PTEN, KRAS, and CTNNB1 mutations, this can explain the occurrence of ARID1A loss in AEH rather than EH without atypia and confirm the clonal nature of AEH [1].

The mechanism of negative ARID1A expression on endometrial tumorigenesis has been found to be closely related with the alterations of PI3K/AKT pathway which is closely related with the carcinogenesis of the endometrium related cancers [33].

The significant increase of ARID1A loss in atypical EH and EEA groups while its positive expression in normal and benign hyperplasia may support the tumor suppressor role of ARID1A and makes think that ARID1A loss could play a role in transition from hyperplasia to EEA and early phases of EEA tumorigenesis.

To explore the clinical importance of ARID1A, correlation of its expression with prognostic pathological parameters of EEA was done. A significant correlation between ARID1A loss and MELF pattern of myometrial invasion ($P<0.05$). This was concomitant with the results of previous works [9, 34]. Although we found ARID1A in two non-myoinvasive EEAs cases and its loss in myoinvasive EEA, we didn't find a statistical significance ($P>0.05$), that may be owed to small number of cases studied.

ARID1A normally maintains endometrial epithelial cell identity by repressing mesenchymal cell fates. Downregulation of ARID1A is associated with repression of E-cadherin in diverse types of cancers which promotes epithelial-to-mesenchymal transition (EMT) and hence invasion [35-37]. The MELF pattern of invasion may represent a type of EMT evidenced by down regulation of E-cadherin in neoplastic epithelium in MELF areas which is a sign of EMT [38]. This may support the association between ARID1A loss and MELF pattern by the role of ARID1A in EMT.

This work revealed non-statistically significant correlation between ARID1A expression and histologic type, tumor grade, LVSI, LN involvement and FIGO stage (P >0.05), which was matched with the results of previous studies on ARID1A [29, 30, 39-42].

Loss of ARID1A expression is an early occurrence in the formation of cancer, and it has been suggested that ARID1A loss may not be as important to tumor progression as it is to tumor initiation, which would explain why there is no link between ARID1A loss and pathological markers [30, 37].

Using ROC curve analysis, the specificity of ARID1A for premalignant lesions was 100%, while its sensitivity was 38.3%. A previous study compatible with our results found that ARID1A has low sensitivity 12% for premalignant EH and almost perfect specificity 99% [43]. This indicates that ARID1A loss is highly specific for premalignant lesion and may be a potentially useful diagnostic marker. Owing to its low sensitivity, ARID1A does not appear adequate as a stand-alone diagnostic marker of premalignant EH, but it may be useful as a 'rule-in' test for diagnosis of precancer, due to its excellent specificity.

Concerning patients' survival, Kaplan-Meier survival analysis revealed that patients with ARID1A loss tumors have a significantly longer disease-free survival and recurrence-free survival than those with retained ARID1A tumors (57 vs. 31.37 months, P <0.05, and 65.83 vs. 39.89 months, P<0.05, respectively). These findings indicate that ARID1A loss is in EEA associated with a favorable prognosis and prolonged patients' survival.

In agreement with our findings, ARID1A loss was reported to be associated with longer progression-free survival (P= 0.04) and that 7/9 of cases with recurrence in their study showed retained ARID1A expression [25]. Also, it was demonstrated that ARID1A mutations correlate with favorable survival in EEA using data from The Cancer Genome Atlas [44]. A possible explanation for favorable prognosis of ARID1A loss in EEA is the relation of ARID1A loss with certain molecular subtypes of endometrial carcinoma those with MMR deficiency and POLE mutations that are considered a favorable prognostic factor in EEA [25].

In contrast, negative expression of ARID1A was reported to be an independent negative prognosticator in endometrioid carcinoma [42]. This can be owed to their findings of highly significant association between

ARID1A loss and high FIGO stage which is associated with poor prognosis.

Relevant studies have reported adverse, beneficial, or absolutely no effect of ARID1A protein loss on the biological behavior, tumor recurrence, progression-free survival, and overall survival of cancer patients, indicating that the abnormal expression of ARID1A affects the prognosis in different ways, mainly depending on the type, stage, grade of the tumor and the existence of concomitant mutations [45, 46].

A considerable progress has been made in understanding the role of the immune system in the progression and prognosis of malignant tumors. The presence of tumor-infiltrating mononuclear cells indicates the existence of active immune response of the host that may be directed against the tumor cells. CD8+ T cells are the key immune cells for recognizing and killing cancer cells presenting major histocompatibility complex class I molecules [47].

In the present work, a statistically significant positive correlation was found between intraepithelial CD8+ and stromal CD8+ number expression and studied groups (P <0.01 and P<0.05, respectively). The number of both intraepithelial and CD8+ was higher in AEH and EEA than EH without atypia and normal endometrium.

Our results were compatible with a study performed using flow cytometry that the percentage of CD8+ T lymphocytes was significantly higher in the tumor compared with non-neoplastic endometrium [48]. Also, it was found that CD8+ T cell content_ using RT-PCR_ was significantly modified, as it was slightly and strongly increased in the hyperplasia and tumor grade 1 groups, respectively than control group (normal endometrium) (P<0.0083) [49].

High expression of CD8+ in EEA can be contributed to certain molecular subtype of endometrial carcinoma particularly, POLE-mutant and dMMR tumors that harbor many neoantigens and show high number of tumors infiltrating lymphocytes [50].

This work revealed no statistically significant correlation between CD8+ expression and the following parameters: histologic type, tumor grade, depth of myometrial invasion, pattern of myometrial invasion, LVSI, LN involvement, and FIGO stage (P >0.05). This agreed with the findings in the studies performed in previous studies [24, 48, 51,52].

Concerning patients' survival, Kaplan-Meier analysis showed that high intraepithelial CD8+ expression was associated with prolonged disease-free survival and recurrence-free survival (61.733 vs 23.822, P <0.01, and 70.615 vs 30.056, P <0.01, respectively). Although high stromal CD8+ was associated with longer disease-free survival and recurrence-free survival than low stromal CD8+, this was statistically non-significant (53.200 vs. 31.707, P >0.05, and 65.833 vs 37.528, P <0.05, respectively)**.**

Concomitant with these findings, association of higher CD8+ T cell counts with prolonged time to recurrence, improved overall survival and better progression-free survival was reported in previous research **(24, 53-55)**.
In contrast, other performed studies did not find significant relation between CD8+ expression and patients' survival **(50, 56)**. This can be explained by their work on both type 1 and type 2 endometrial carcinomas and different methodology used in their works.

In conclusion in this work, we found a good association between high CD8+ number expression and improved patient survival and that the association was better for intraepithelial CD8+ than stromal CD8+ expression owing to the direct contact of intraepithelial CD8+ with tumor cells and their antitumor specificity that offers more perfect protection against tumor cells, conferring long-lasting protection and prevent from cancer recurrence **(57)**.

Given the role of key genetic mutations in cancer initiation and progression, it has long been argued that driver mutation(s) may drive the cancer immune phenotype and immune tolerance in patients with cancer. Cancer epigenetic driver mutations such as ARID1A mutations can shape tumor immune phenotype, T cell immunity, and the efficacy of cancer immunotherapy **(58)**.

This work showed a statistically highly significant inverse correlation between ARID1A expression and CD8+ expression (P <0.01). Loss of ARID1A expression was associated with higher CD8+ number expression both intraepithelial and stromal.

This finding was consistent with the findings of previous studies that reported ARID1A mutations to be related to the high immune infiltrates in endometrial cancer and that their results revealed ARID1A-deficient tumors to display increased CD8+ TILs **(44, 59, 60)**. In another work, ARID1A loss was found to be associated with MMRd (p<0.001) and found highly significant association between

MMRd and increasing CD8+ TIL density [61].

In contrast, ARID1A-mutant renal clear cell carcinoma were found to have dramatically lower CD8+ T cell infiltrations than those without [59], indicating the association between ARID1A alterations and immune infiltrates was cancer-dependent.

Association between ARID1A loss and increased CD8+ expression can be explained by the role of ARID1A in DNA repair. Loss of ARID1A results in impaired DNA damage repair, MMR which correlates with MSI, and increased tumor mutation burden (TMB) which enhance immunogenic activity in cancer including CD8-positive T lymphocytes [62]. This is supported by the observation that ARID1A deficiency is more common in the MSI-H phenotype of endometrial cancer and gastric cancer [36, 44]. This has very important clinical significance because higher TMB and more neoantigens are important factors that associate with tumor immunogenicity to render tumors susceptible to immune checkpoint blockade therapy [36, 62, 63].

This work revealed a better and prolonged disease-free survival and recurrence-free survival in ARID1A loss/high intraepithelial CD8+ combined group (P<0.01). Although combining ARID1A with stromal CD8+ was statistically non-significant (P>0.05), the ARID1A loss/high stromal CD8+ group showed the most prolonged disease-free survival and recurrence-free survival.

These observations support the view that ARID1A deficiency better prognosis is linked to the increase of TILs, especially CD8+ T lymphocytes, suggesting the vulnerability of tumors harboring ARID1A loss to immunotherapy and improved survival. So, it is of clinical importance to identify molecular consequences of ARID1A deficiency that create therapeutic vulnerabilities in ARIDIA-mutant tumors.

The current study adds to a growing body of evidence indicating that the immune system plays a key role in endometrial cancer progression and prognosis. ARID1A and CD8+ can be a reliable biomarker for immunotherapy in EEA patients and can be used as a good prognostic and predictive biomarkers especially in advanced stage EEA and early-stage high grade cases.

Conclusions:

ARID1A may be a helpful diagnostic marker for distinguishing benign and premalignant and malignant endometrial lesions. ARID1A loss may be a favorable prognostic factor associated with prolonged disease-free and

recurrence-free survival. High CD8+ expression in endometrioid carcinoma may be a reliable independent prognostic factor of improved patient survival. Intraepithelial CD8+ lymphocytes could be more reliable independent prognostic factor of survival than stromal CD8+. Association of ARID1A loss with high CD8+ expression could be regarded a potential predictive for immune therapy and target therapy for endometrioid carcinoma.

References

1. Kim KR, Lax SF, Lazar AJ, Longacre TA, Malpica A, Matias-Guiu X, et al. Tumors of the uterine corpus. IN: WHO Classification of Female Genital Tumors, 5th ed. Lyon (France): International Agency for Research on Cancer (IARC) Press. 2020; Vol 4. Ch. 6: 245-308.

2. Doherty MT, Sanni OB, Coleman HG, Cardwell CR, McCluggage WG, Quinn D, et al. Concurrent and future risk of endometrial cancer in women with endometrial hyperplasia: A systematic review and meta-analysis. PLoS One. 2020;15(4): e0232231. doi: 10.1371/journal.pone.0232231.

3. 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(3):209-249. doi: 10.3322/caac.21660.

4. Constantine GD, Kessler G, Graham S, Goldstein SR. Increased Incidence of Endometrial Cancer Following the Women's Health Initiative: An Assessment of Risk Factors. J Womens Health (Larchmt). 2019;28(2):237-243. doi: 10.1089/jwh.2018.6956.

5. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10-7. doi: 10.1016/0090-8258(83)90111-7.

6. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67-73. doi: 10.1038/nature12113.

7. Willvonseder B, Stögbauer F, Steiger K, Jesinghaus M, Kuhn PH, Brambs C, et al. The immunologic tumor microenvironment in endometrioid endometrial cancer in the morphomolecular context: mutual correlations and prognostic impact depending on molecular alterations. Cancer Immunol Immunother. 2021;70(6):1679-89. doi: 10.1007/s00262-020-02813-3.

8. Singh N, Hirschowitz L, Zaino R, Alvarado-Cabrero I, Duggan MA, Ali-Fehmi R, et al. Pathologic Prognostic Factors in Endometrial Carcinoma (Other Than Tumor Type and Grade). Int J Gynecol Pathol. 2019;38 Suppl 1(Iss 1 Suppl 1): S93-S113. doi: 10.1097/PGP.0000000000000524.

9. Kir G, Soylemez T, Olgun ZC, Aydin A, & McCluggage WG. Correlation of PD-L1 expression with immunohistochemically determined molecular profile in endometrial carcinomas. Virchows Arch. 2020;477(6):845-856. doi: 10.1007/s00428-020-02867-9.

10. Wang R, Chen M, Ye X, & Poon K. Role and potential clinical utility of ARID1A in

gastrointestinal malignancy. Mutat Res Rev Mutat Res. 2021; 787:108360. doi: 10.1016/j.mrrev.2020.108360.

11. Heinze K, Nazeran TM, Lee S, Krämer P, Cairns ES, Chiu DS, et al. Validated biomarker assays confirm that ARID1A loss is confounded with MMR deficiency, $CD8^+$ TIL infiltration, and provides no independent prognostic value in endometriosis-associated ovarian carcinomas. J Pathol. 2022;256(4):388-401. doi: 10.1002/path.5849.

12. Jang SH, Lee JH, Lee HJ, Cho H, Ahn H, Song IH, et al. Loss of ARID1A expression is associated with poor prognosis in non-small cell lung cancer. Pathol Res Pract. 202;216(11):153156. doi: 10.1016/j.prp.2020.153156.

13. Li Y, Tang J, Li J, Du Y, Bai F, Yang L, et al. ARID3A promotes the chemosensitivity of colon cancer by inhibiting AKR1C3. Cell Biol Int. 2022;46(6):965-975. doi: 10.1002/cbin.11789.

14. Cui Y, Bai X, Niu M, Qin Y, Zhang X, & Pang D. Upregulated expression of AT-rich interactive domain-containing protein 1B predicts poor prognosis in patients with triple-negative breast cancer. Oncol Lett. 2019;17(3):3289-3295. doi: 10.3892/ol.2019.9961.

15. Er TK, Su YF, Wu CC, Chen CC, Wang J, Hsieh TH, et al. Targeted next-generation sequencing for molecular diagnosis of endometriosis-associated ovarian cancer. J Mol Med (Berl). 2016;94(7):835-47. doi: 10.1007/s00109-016-1395-2.

16. Mullen J, Kato S, Sicklick JK, & Kurzrock R. Targeting ARID1A mutations in cancer. Cancer Treat Rev. 2021 Nov; 100:102287. doi: 10.1016/j.ctrv.2021.102287.

17. Kumar S, Singh SK, Rana B, & Rana A. Tumor-infiltrating $CD8^+$ T cell antitumor efficacy and exhaustion: molecular insights. Drug Discov Today. 2021;26(4):951-967. doi: 10.1016/j.drudis.2021.01.002.

18. Kim KH, Choi KU, Kim A, Lee SJ, Lee JH, Suh DS, et al. PD-L1 expression on stromal tumor-infiltrating lymphocytes is a favorable prognostic factor in ovarian serous carcinoma. J Ovarian Res. 2019;12(1):56. doi: 10.1186/s13048-019-0526-0.

19. Kong JC, Guerra GR, Pham T, Mitchell C, Lynch AC, Warrier SK, et al. Prognostic Impact of Tumor-Infiltrating Lymphocytes in Primary and Metastatic Colorectal Cancer: A Systematic Review and Meta-analysis. Dis Colon Rectum. 2019;62(4):498-508. doi: 10.1097/DCR.0000000000001332.

20. Mehrvarz Sarshekeh A, Alshenaifi J, Roszik J, Manyam GC, Advani SM, Katkhuda R, et al. ARID1A Mutation May Define an Immunologically Active Subgroup in Patients with Microsatellite Stable Colorectal Cancer. Clin Cancer Res. 2021;27(6):1663-1670. doi: 10.1158/1078-0432.CCR-20-2404.

21. Amant F, Mirza MR, Koskas M, & Creutzberg CL. Cancer of the corpus uteri. Int J Gynaecol Obstet. 2018;143 Suppl 2:37-50. doi: 10.1002/ijgo.12612.

22. Cole AJ & Quick CM. Patterns of myoinvasion in endometrial adenocarcinoma: recognition and implications. Adv Anat Pathol. 2013;20(3):141-7. doi: 10.1097/PAP.0b013e31828d17cc.

23. Khalique S, Naidoo K, Attygalle AD, Kriplani D, Daley F, Lowe A, et al. Optimised ARID1A immunohistochemistry is an accurate predictor of ARID1A mutational status in gynaecological

cancers. J Pathol Clin Res. 2018;4(3):154-166. doi: 10.1002/cjp2.103.

24. Vagios S, Yiannou P, Giannikaki E, Doulgeraki T, Papadimitriou C, Rodolakis A, et al. The impact of programmed cell death-ligand 1 (PD-L1) and CD8 expression in grade 3 endometrial carcinomas. Int J Clin Oncol. 2019;24(11):1419-1428. doi: 10.1007/s10147-019-014840.

25. Kato MK, Yoshida H, Tanase Y, Uno M, Ishikawa M, & Kato T. Loss of ARID1A Expression as a Favorable Prognostic Factor in Early-Stage Grade 3 Endometrioid Endometrial Carcinoma Patients. Pathol Oncol Res. 2021; 27:598550. doi: 10.3389/pore.2021.598550.

26. Sahin EA, Toprak S, Sayal HB, Ekinci T, Yılmaz E, Bakay K, et al. Analysis of prognostic factors in Grade 3 endometrioid type endometrial carcinoma. Int J Gynaecol Obstet. 2022: 22. doi: 10.1002/ijgo.14277.

27. Mao TL, Ardighieri L, Ayhan A, Kuo KT, Wu CH, Wang TL, et al. Loss of ARID1A expression correlates with stages of tumor progression in uterine endometrioid carcinoma. Am J Surg Pathol. 2013;37(9):1342-8. doi: 10.1097/PAS.0b013e3182889dc3.

28. Hijazi N, Almadani N, Gilks CB, & Huntsman DG. ARID1A Loss of Expression in Complex Atypical Hyperplasia and Grade 1 Endometrioid Carcinoma of the Endometrium. Canadian Journal of Pathology. 2015; 7(3):23

29. Gamal Y & Mostafa MG. PTEN and ARID1A expression in a spectrum of endometrial lesions from proliferative endometrium and hyperplasia without atypia to atypical hyperplasia and endometrioid carcinoma. Egyptian Journal of Pathology. 2017; 37:221-228.

30. Zhang ZM, Xiao S, Sun GY, Liu YP, Zhang FH, Yang HF, et al. The clinicopathologic significance of the loss of BAF250a (ARID1A) expression in endometrial carcinoma. Int J Gynecol Cancer. 2014;24(3):534-40. doi: 10.1097/IGC.0000000000000092.

31. Vierkoetter K, Wong J, Ahn HJ, Shimizu D, Kagami L, & Terada K. Using gene expression in patients with endometrial intraepithelial neoplasia to assess the risk of cancer. Gynecol Oncol Rep. 2018; 24:24-26. doi: 10.1016/j.gore.2018.02.006.

32. Werner HM, Berg A, Wik E, Birkeland E, Krakstad C, Kusonmano K, et al. ARID1A loss is prevalent in endometrial hyperplasia with atypia and low-grade endometrioid carcinomas. Mod Pathol. 2013;26(3):428-34. doi: 10.1038/modpathol.2012.174.

33. Suryo Rahmanto Y, Shen W, Shi X, Chen X, Yu Y, Yu ZC, et al. Inactivation of Arid1a in the endometrium is associated with endometrioid tumorigenesis through transcriptional reprogramming. Nat Commun. 2020;11(1):2717. doi: 10.1038/s41467-020-16416-0.

34. De Leo A, de Biase D, Lenzi J, Barbero G, Turchetti D, Grillini M, et al. ARID1A and CTNNB1/β-Catenin Molecular Status Affects the Clinicopathologic Features and Prognosis of Endometrial Carcinoma: Implications for an Improved Surrogate Molecular Classification. Cancers (Basel). 2021;13(5):950. doi: 10.3390/cancers13050950.

35. Somsuan K, Peerapen P, Boonmark W, Plumworasawat S, Samol R, Sakulsak N, et al. ARID1A knockdown triggers epithelial-mesenchymal transition and carcinogenesis features of renal cells: role in renal cell

carcinoma. FASEB J. 2019;33(11):12226-12239. doi: 10.1096/fj.201802720RR.

36. Wang T, Gao X, Zhou K, Jiang T, Gao S, Liu P, et al. Role of ARID1A in epithelial-mesenchymal transition in breast cancer and its effect on cell sensitivity to 5-FU. Int J Mol Med. 2020;46(5):1683-1694. doi: 10.3892/ijmm.2020.4727.

37. Erfani M, Zamani M, Hosseini SY, Mostafavi-Pour Z, Shafiee SM, Saeidnia M, et al. ARID1A regulates E-cadherin expression in colorectal cancer cells: a promising candidate therapeutic target. Mol Biol Rep. 2021;48(10):6749-6756. doi: 10.1007/s11033-021-06671-9.

38. Mateva S, Nikolova M, & Yordanov A. Patterns of Myometrial Invasion in Endometrial Adenocarcinoma with Emphasizing on Microcystic, Elongated and Fragmented (MELF) Glands Pattern: A Narrative Review of the Literature. Diagnostics (Basel). 2021;11(9):1707. doi: 10.3390/diagnostics11091707.

39. Rahman M, Nakayama K, Rahman MT, Katagiri H, Katagiri A, Ishibashi T, et al. Clinicopathologic analysis of loss of AT-rich interactive domain 1A expression in endometrial cancer. Hum Pathol. 2013;44(1):103-9. doi: 10.1016/j.humpath.2012.04.021.

40. Liu G, Xu P, Fu Z, Hua X, Liu X, Li W, et al. Prognostic and Clinicopathological Significance of ARID1A in Endometrium-Related Gynecological Cancers: A Meta-Analysis. J Cell Biochem. 2017;118(12):4517-4525. doi: 10.1002/jcb.26109.

41. Döver DK, Aydın A, Senol S, Bostancı E, & KILICCI C. Utility of PTEN, MLH1, ARID1A and β-catenin, biomarkers in endometrial hyperplasia and endometrioid endometrial carcinoma. Zeynep Kamil Tıp Bülteni. 2018; 49(1):65-71.

42. Heckl M, Schmoeckel E, Hertlein L, Rottmann M, Jeschke U, & Mayr D. The ARID1A, p53 and ß-Catenin statuses are strong prognosticators in clear cell and endometrioid carcinoma of the ovary and the endometrium. PLoS One. 2018;13(2): e0192881. doi: 10.1371/journal.pone.0192881.

43. Raffone A, Travaglino A, Saccone G, Cieri M, Mascolo M, Mollo A, et al. Diagnostic and prognostic value of ARID1A in endometrial hyperplasia: a novel marker of occult cancer. APMIS. 2019;127(9):597-606. doi: 10.1111/apm.12977.

44. Shen J, Ju Z, Zhao W, Wang L, Peng Y, Ge Z, et al. ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade. Nat Med. 2018;24(5):556-562. doi: 10.1038/s41591-018-0012-z.

45. Choi JY, Han HH, Kim YT, Lee JH, Kim BG, Kang S, Cho NH. Ovarian Clear Cell Carcinoma Sub-Typing by ARID1A Expression. Yonsei Med J. 2017;58(1):59-66. doi: 10.3349/ymj.2017.58.1.59. PMID: 27873496; PMCID: PMC5122653.

46. Pavlidou EN & Balis V (2020). Diagnostic significance and prognostic role of the ARID1A gene in cancer outcomes. World Academy of Sciences Journal, 2(2):49-64.

47. Varadé J, Magadán S, & González-Fernández Á. Human immunology and immunotherapy: main achievements and challenges. Cell Mol Immunol. 2021;18(4):805-828. doi: 10.1038/s41423-020-00530-6.

48. Pascual-García M, Bértolo C, Nieto JC, Serrat N, Espinosa Í, D'Angelo E, et al. CD8 down-regulation on cytotoxic T lymphocytes of patients with endometrioid endometrial carcinomas. Hum Pathol. 2016; 56:180-8. doi: 10.1016/j.humpath.2016.05.025.

49. Antomarchi J, Ambrosetti D, Cohen C, Delotte J, Chevallier A, Karimdjee-Soilihi B, et al. Immunosuppressive Tumor Microenvironment Status and Histological Grading of Endometrial Carcinoma. Cancer Microenviron. 2019;12(2-3):169-179. doi: 10.1007/s12307-019-00225-1.

50. Workel HH, Komdeur FL, Wouters MC, Plat A, Klip HG, Eggink FA, et al. CD103 defines intraepithelial CD8+ PD1+ tumour-infiltrating lymphocytes of prognostic significance in endometrial adenocarcinoma. Eur J Cancer. 2016; 60:1-11. doi: 10.1016/j.ejca.2016.02.026.

51. Crumley S, Kurnit K, Hudgens C, Fellman B, Tetzlaff MT, & Broaddus R. Identification of a subset of microsatellite-stable endometrial carcinoma with high PD-L1 and CD8+ lymphocytes. Mod Pathol. 2019;32(3):396-404. doi: 10.1038/s41379-018-0148-x.

52. Nobari N, Niknejad N, Poorolajal J, Vaezi M, Nili F, & Jahanzad I. Clinicopathologic features and prognostic role of CD3, CD8, and PD-1 positive tumor-infiltrating lymphocytes and the association with COX-2 overexpression in endometrial carcinoma. International Journal of Cancer Management. 2020; 13(4): e99210.

53. Guo F, Dong Y, Tan Q, Kong J, & Yu B. Tissue Infiltrating Immune Cells as Prognostic Biomarkers in Endometrial Cancer: A Meta-Analysis. Dis Markers. 2020; 2020:1805764. doi: 10.1155/2020/1805764.

54. Miyamoto M, Takano M, Tsuda H, Matuura H, Aoyama T, Soyama H, et al. Zone Formation of Lymphocyte Infiltration at Invasive Front as a Biomarker of Prognosis in Endometrial Carcinomas. Oncology. 2019;96(2):93-100. doi: 10.1159/000493010.

55. Liu W, Sun L, Zhang J, Song W, Li M, & Wang H. The landscape and prognostic value of immune characteristics in uterine corpus endometrial cancer. Biosci Rep. 2021;41(4): BSR20202321. doi: 10.1042/BSR20202321.

56. Rashid T, Young-Pierce JL, Garrett-Mayer E, Graybill W, Neal S, & Spruill LS. CD8, FoxP3, and CD45RO+ Lymphocytic Infiltrates in Type I and Type II Endometrial Cancers in African American and European American Females. Int J Gynecol Pathol. 2017;36(6):540-549. doi: 10.1097/PGP.0000000000000359.

57. Galon J & Bruni D. Tumor Immunology and Tumor Evolution: Intertwined Histories. Immunity. 2020;52(1):55-81. doi: 10.1016/j.immuni.2019.12.018.

58. Li J, Wang W, Zhang Y, Cieślik M, Guo J, Tan M, et al. Epigenetic driver mutations in ARID1A shape cancer immune phenotype and immunotherapy. J Clin Invest. 2020;130(5):2712-2726. doi: 10.1172/JCI134402.

59. Jiang T, Chen X, Su C, Ren S, & Zhou C. Pan-cancer analysis of ARID1A Alterations as Biomarkers for Immunotherapy Outcomes. J Cancer. 2020 Jan 1;11(4):776-780. doi: 10.7150/jca.41296.

60. Li Z, Lin J, Zhang L, Li J, Zhang Y, Zhao C, et al. Comprehensive analysis of multiple parameters associated with tumor immune microenvironment in ARID1A mutant cancers.

Future Oncol. 2020;16(29):2295-2306. doi: 10.2217/fon-2020-0243.

61. Heinze K, Nazeran TM, Lee S, Kraemer P, Cairns ES, Chiu DS, et al. Prognostic and Immunological Significance of ARID1A Status in Endometriosis-Associated Ovarian Carcinoma. medRxiv, 2021.

62. Li L, Li M, Jiang Z, & Wang X. ARID1A Mutations Are Associated with Increased Immune Activity in Gastrointestinal Cancer. Cells. 2019;8(7):678. doi: 10.3390/cells8070678.

63. Kamori T, Oki E, Shimada Y, Hu Q, Hisamatsu Y, Ando K, et al. The effects of ARID1A mutations on colorectal cancer and associations with PD-L1 expression by stromal cells. Cancer Rep (Hoboken). 2022;5(1): e1420. doi: 10.1002/cnr2.1420.

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