GEORGIAN BIOMEDICAL NEWS

VOLUME 4 ISSUE 1. JAN-MAR 2026

Tumor-Associated Macrophages and Regulatory T Cells as Predictors of Recurrence in Endometrial Stromal Tumors

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DOI: 10.52340/GBMN.2026.01.01.159

ABSTRACT

Background: Endometrial stromal tumors (EST) comprise a rare and biologically heterogeneous group of uterine mesenchymal neoplasms. While histological classification remains the primary prognostic determinant, recurrence patterns are not fully explained by tumor grade alone. The prognostic significance of immunosuppressive components of the tumor microenvironment in EST remains insufficiently characterized.

Objectives: To evaluate the relationship between CD163-positive tumor-associated macrophages, FOXP3-positive regulatory T cells, and disease-free survival in patients with endometrial stromal tumors.

Methods: This retrospective multicenter study included 90 patients diagnosed with endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LG-ESS), or high-grade endometrial stromal sarcoma (HG-ESS) between 2017 and 2025. Immunohistochemical expression of CD163 (tumor-associated macrophages) and FOXP3 (regulatory T cells) was quantified using digital image analysis. Disease-free survival (DFS) was evaluated using Kaplan–Meier estimates and Cox proportional hazards modeling.

Results: Median follow-up was 55 months. Recurrence occurred in 34 patients (0 ESN, 12 LG-ESS, 22 HG-ESS). CD163 and FOXP3 densities increased significantly with histological grade ($p<0.01$). High CD163 and FOXP3 expression were independently associated with reduced DFS (Hazard Ratios 2.41 and 2.09, respectively; $p<0.01$). Tumors exhibiting concurrent high CD163 and FOXP3 infiltration demonstrated the poorest survival outcomes (HR 3.12, $p<0.001$), independent of grade.

Conclusions: Enrichment of M2-polarized macrophages and regulatory T cells significantly influences the risk of recurrence in endometrial stromal tumors. Immune microenvironment profiling provides prognostic information beyond histological classification and may support future immunomodulatory therapeutic strategies.

Keywords: CD163; disease-free survival; endometrial stromal sarcoma; FOXP3; regulatory T cells; tumor-associated macrophages; tumor microenvironment.

BACKGROUND

Endometrial stromal tumors (EST) constitute a rare and heterogeneous group of uterine mesenchymal neoplasms, accounting for less than 1% of all uterine malignancies.[1] According to the current World Health Organization classification, EST encompasses endometrial stromal nodules (ESN), low-grade endometrial stromal sarcomas (LG-ESS), and high-grade endometrial stromal sarcomas (HG-ESS).[2] These entities differ in their biological behavior, molecular background, and clinical course. ESNs are typically well-circumscribed lesions with benign clinical outcomes, whereas LG-ESS are characterized by infiltrative growth and a propensity for late recurrence. HG-ESS displays aggressive behavior with early metastasis and markedly reduced survival rates.[3]

Despite the central role of histological classification in risk stratification, recurrence patterns remain heterogeneous even within grade-defined subgroups. LG-ESS may recur decades after initial treatment, whereas a subset of HG-ESS demonstrates particularly rapid progression. Such variability suggests that additional biological factors beyond conventional morphology contribute to tumor behavior.

In recent years, attention has shifted toward the tumor microenvironment as a key regulator of oncologic progression.[4] Tumor behavior is increasingly recognized as the product not only of intrinsic genetic alterations but also of dynamic interactions between tumor cells and surrounding stromal, vascular, and immune components. Among immune populations, tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) have emerged as principal mediators of immune tolerance within the tumor niche.[5-7]

Macrophages display functional plasticity and can polarize toward either pro-inflammatory (M1) or immunosuppressive (M2) phenotypes. CD163-positive macrophages represent the M2-polarized subset, characterized by the secretion of anti-inflammatory cytokines, the promotion of angiogenesis, and the facilitation of extracellular matrix remodeling. High M2 macrophage density has been associated with poor prognosis in numerous solid malignancies, including breast carcinoma, endometrial carcinoma, and soft tissue sarcomas. In mesenchymal tumors specifically, macrophage-rich microenvironments have been linked to enhanced invasiveness and resistance to therapy.[8-10]

Regulatory T cells, identified by nuclear FOXP3 expression, suppress cytotoxic T-cell activation and contribute to tumor immune escape. Elevated Treg infiltration has been correlated with adverse outcomes in multiple gynecologic cancers, including ovarian and endometrial carcinomas. Experimental models suggest that macrophage-derived cytokines can recruit and expand Treg populations, thereby establishing a cooperative immunosuppressive axis.[11-13]

Although immune infiltration has been increasingly studied in epithelial gynecologic malignancies, data regarding the immune microenvironment of endometrial stromal tumors remain limited. Small series and isolated reports suggest that

higher-grade sarcomas may harbor enriched immunosuppressive infiltrates; however, systematic evaluation of tumor-associated macrophages and regulatory T cells in relation to disease-free survival has not been comprehensively addressed in EST.

Understanding the immune landscape of EST is particularly relevant to emerging immunotherapeutic strategies. While immune checkpoint inhibitors have shown limited and variable efficacy in uterine sarcomas, the biological determinants of response remain unclear. Characterization of macrophage and Treg infiltration may provide insight into immune evasion mechanisms and identify subgroups with distinct recurrence risk profiles.

The present study was therefore designed to evaluate the distribution and prognostic significance of CD163-positive tumor-associated macrophages and FOXP3-positive regulatory T cells in a retrospective cohort of endometrial stromal tumors. By integrating digital quantification of immune infiltration with long-term disease-free survival analysis, this investigation aims to clarify the contribution of immune microenvironment remodeling to the risk of recurrence across the EST spectrum.

METHODS

A retrospective multicenter analysis was conducted on 90 patients diagnosed with ESN (n=30), LG-ESS (n=30), and HG-ESS (n=30) between 2017 and 2025 at Tbilisi State Medical University Study-diagnostic-Research Lab. Diagnoses were confirmed according to the 2020 WHO classification of female genital tumors. Clinical and follow-up data were retrieved from institutional records.

Disease-free survival (DFS) was defined as the interval from definitive surgical treatment to the first documented recurrence or metastasis. Patients without recurrence were censored at the last follow-up.

Immunohistochemistry and quantification

Formalin-fixed paraffin-embedded tumor sections underwent immunohistochemical staining for CD163 and FOXP3 using standardized protocols. Digital image analysis (QuPath software) was employed to quantify positive cells in representative tumor regions. Results were expressed as cell density (cells/mm²).

Median expression values were used to stratify tumors into low- and high-infiltration groups. A combined immune phenotype was defined as concurrent high expression of CD163 and FOXP3.

Statistical analysis

Comparisons of immune cell densities across tumor grades were performed using non-parametric tests. The correlation between CD163 and FOXP3 densities was assessed using Spearman's rank correlation coefficient.

DFS was evaluated using Kaplan–Meier analysis with log-rank testing. Cox proportional hazards regression was applied to determine independent predictors of recurrence. Multivariable models were adjusted for histological grade. Hazard ratios (HR) with 95% confidence intervals (CI) were reported. A p-value <0.05 was considered statistically significant.

The tumor microenvironment has emerged as a critical regulator of cancer progression. Among its cellular components, tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) play central roles in immune modulation. CD163-positive macrophages represent an M2-polarized phenotype associated with immunosuppression, angiogenesis, and extracellular matrix remodeling. FOXP3-positive regulatory T cells suppress cytotoxic immune responses and contribute to tumor immune escape.

In soft tissue sarcomas, increased infiltration by TAMs and Tregs has been associated with adverse clinical outcomes. Nevertheless, the immune landscape of endometrial stromal tumors remains poorly defined, and its prognostic implications have not been sufficiently studied.

This study aimed to evaluate the relationship between CD163-positive macrophages, FOXP3-positive regulatory T cells, and disease-free survival in a retrospective cohort of endometrial stromal tumors.

RESULTS

Clinicopathological characteristics and survival

The median age at diagnosis was 52 years (range 31–72). Median follow-up was 55 months. Recurrence occurred in 34 patients (37.8%). No recurrences were observed among ESN cases. Twelve patients with LG-ESS (40%) and twenty-two patients with HG-ESS (73.3%) experienced recurrence. Kaplan–Meier analysis demonstrated significant differences in DFS across histological subtypes (log-rank $p < 0.001$), with HG-ESS exhibiting early relapse predominantly within the first 2 postoperative years. The comparative data for all three groups are detailed below (Tab.1).

TABLE 1. Clinicopathological characteristics

Variable	ESN (n=30)	LG-ESS (n=30)	HG-ESS (n=30)	p-value
Age (median)	48	50	62	0.01
Tumor size (cm)	3.5	5.8	7.9	<0.001
LVSI (%)	0	40	73	<0.001
Recurrence (%)	0	40	73	<0.001

Abbreviations: ESN, endometrial stromal nodule; HG-ESS, high-grade endometrial stromal sarcoma; LG-ESS, low-grade endometrial stromal sarcoma.

Distribution of immune infiltrates

CD163-positive macrophage density increased significantly from ESN to HG-ESS ($p<0.001$). FOXP3-positive regulatory T-cell density followed a similar pattern ($p=0.002$). A moderate positive correlation was observed between CD163 and FOXP3 densities (Spearman's $r=0.48$, $p<0.001$) (Tab.2).

CD163 and disease-free survival

High CD163 infiltration was associated with significantly reduced DFS (log-rank $p<0.001$). Median DFS was 18 months in

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

the high-expression group and was not reached in the low-expression group. In univariate Cox analysis, high CD163 density was associated with a hazard ratio of 2.75 (95% CI 1.62–4.67; p < 0.001). The data are shown in the table below (Tab.2).

TABLE 2. Cox regression analysis

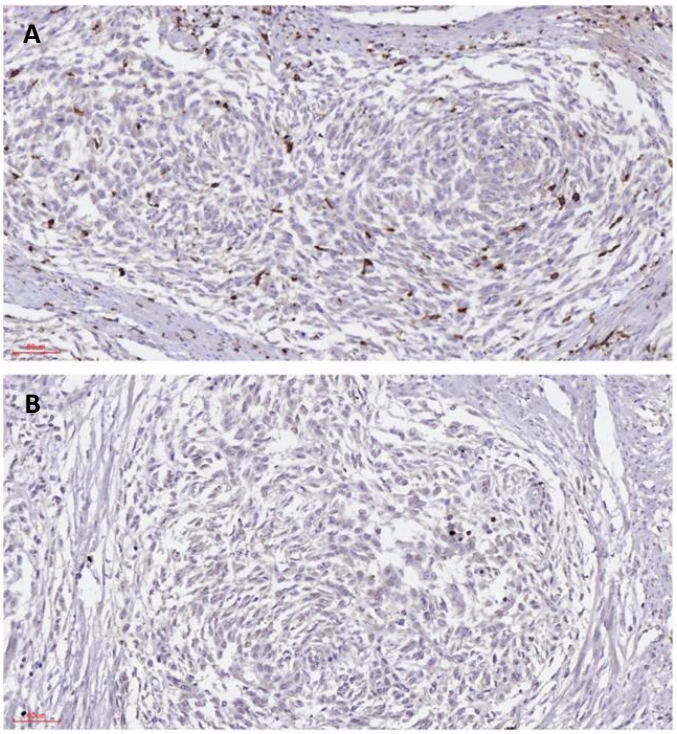
Variable	HR	95% CI	p-value
High CD163	2.41	1.48-3.92	0.001
High FOXP3	2.09	1.27-3.44	0.004
Immune-suppressive phenotype	3.12	1.84-5.29	<0.001
HG-ESS vs LG-ESS	3.8	2.1–7.4	<0.001

Abbreviations: CD163, tumor-associated macrophages; FOXP3, regulatory T-cells; HG-ESS, high-grade endometrial stromal sarcoma; LG-ESS, low-grade endometrial stromal sarcoma.

FOXP3 and disease-free survival

High FOXP3 expression was similarly associated with shortened DFS (log-rank p=0.002). Median DFS was 20 months in the high-expression group and not reached in the low-expression group. Univariate Cox analysis yielded an HR of 2.31 (95% CI 1.34–3.98, p=0.003) (Fig.1).

FIGURE 1. (A) CD163 expression in endometrial stromal sarcoma, high-grade, IHC, 400X; (B) FOXP3 expression in endometrial stromal sarcoma, high-grade, IHC, 400X



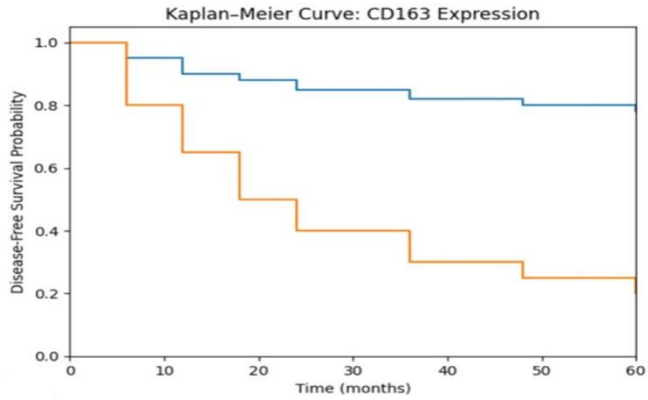
Combined immune-suppressive phenotype

Tumors exhibiting concurrent high CD163 and high FOXP3 expression demonstrated the poorest survival outcomes, with a 2-year DFS rate of 28% compared to 78% in immune-low tumors (log-rank p<0.0001). This combined phenotype provided greater prognostic separation than either marker alone (Fig.2).

Multivariable analysis

After adjustment for histological grade, CD163 remained independently associated with recurrence (HR 2.41, 95% CI 1.48–3.92, p=0.001), as did FOXP3 (HR 2.09, 95% CI 1.27–3.44, p=0.004). Inclusion of the combined immune phenotype demonstrated the strongest independent association with recurrence (HR 3.12, 95% CI 1.84–5.29, p<0.001), indicating additive prognostic value beyond histological classification.

FIGURE 2. (A) Statistical Analysis, Kaplan-Meier Test for DSF and CD163 expression; (B) Statistical Analysis, Kaplan-Meier Test for DSF and FOXP3 expression; (C) Statistical Analysis, Kaplan-Meier Test for DSF and Combined Immune Phenotype



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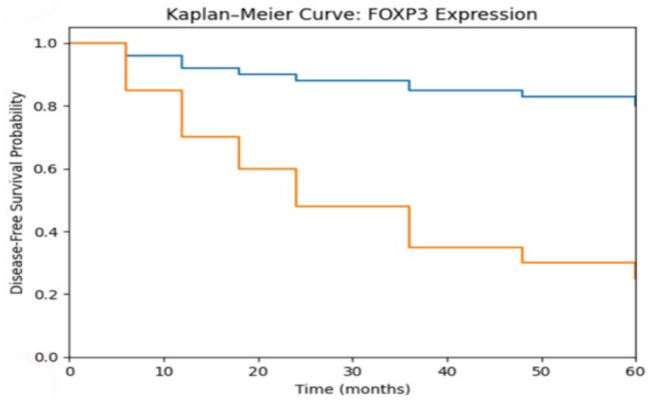

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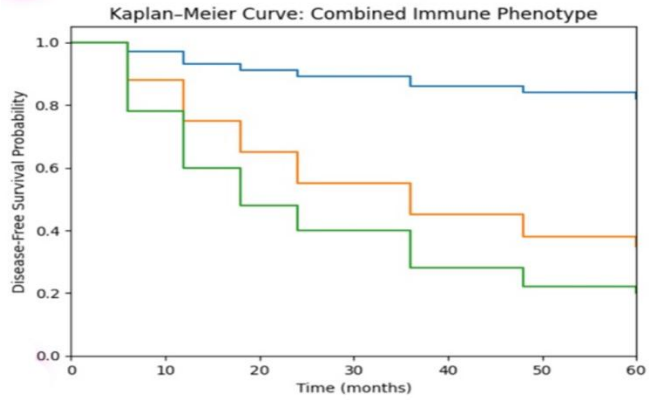

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ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

DISCUSSION

Our study demonstrates that immune-suppressive remodeling of the tumor microenvironment is closely linked to the risk of recurrence in endometrial stromal tumors. Specifically, enrichment of CD163-positive M2-polarized macrophages and FOXP3-positive regulatory T cells was associated with shortened disease-free survival, independent of histological classification.[14] These findings suggest that immune contexture contributes to clinical behavior beyond traditional morphological grading.

Although histological subtype remains the principal determinant of prognosis in endometrial stromal tumors, recurrence patterns often vary within grade-defined categories. Our data indicate that differences in immune infiltration may partly explain this variability.[15] The progressive increase in CD163 and FOXP3 densities from ESN to HG-ESS suggests that immune suppression is not merely a consequence of high-grade morphology, but rather a co-evolving biological feature of aggressive disease.

Tumor-associated macrophages have emerged as key orchestrators of tumor progression across solid malignancies. CD163-positive M2 macrophages promote angiogenesis, extracellular matrix remodeling, and suppression of cytotoxic T-cell responses through cytokine secretion, including IL-10 and TGF-β. In soft tissue sarcomas, elevated M2 macrophage infiltration has been associated with poor survival and increased metastatic potential.[16] Similar observations have been reported in uterine leiomyosarcoma and undifferentiated uterine sarcomas, where macrophage density correlated with adverse outcomes and chemoresistance. The present study extends these findings specifically to the spectrum of endometrial stromal tumors.

CONCLUSIONS

Despite the abovementioned limitations, this study provides novel evidence linking macrophage and regulatory T-cell infiltration to the risk of recurrence in endometrial stromal tumors. The uniform cohort distribution, digital quantification methodology, and survival-centered analysis strengthen the reliability of the findings.

Future investigations should explore whether immune infiltration correlates with specific molecular subtypes, such as YWHAE-rearranged or BCOR-altered tumors, and whether immune-targeted therapies could modify outcomes in high-risk cases. Integration of spatial immune profiling and multiplex immunohistochemistry may further elucidate the structural organization of the immunosuppressive niche.

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ACKNOWLEDGEMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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ISSN (Online): 2720-8796 ISSN (Print): 2720-7994