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Peripheral node addressin, a ligand for L-selectin is found in tumor cells and in high endothelial venules in endometrial cancer

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Abstract

Background: High endothelial venules (HEVs) are vessels specialized in the transport of lymphocytes shown to **be** implicated in various forms of cancer. They express peripheral node addressin (specifically recognized by the MECA-79 antibody). MECA-79 is also implicated in pregnancy through its expression by epithelial cells of the endometrium. However, the expression of MECA-79 by endothelial or epithelial (cancer) cells has never been studied in endometrial cancer.

Material and Methods: In this retrospective study, we investigated the immunohistochemical expression of MECA-79 in 40 endometrioid adenocarcinoma hysterectomy specimens and **compared** it with its expression in 30 non-cancer hysterectomies.

Results: HEVs were found in 22% of tumor specimens and in none of the non-cancer hysterectomies ($p=0.005$) and were positively associated with higher grade tumors ($p=0.04$). MECA-79 was expressed in tumor cells of 70% of carcinomas and in epithelial cells of 46.6% of normal endometria ($p=0.04$). It was inversely associated with parametrial invasion ($p=0.03$) and larger tumors (statistical trend of $p=0.07$). MECA-79 expression was not associated with overall or progression-free survival.

Conclusion: MECA-79 is found in HEVs and tumor cells in endometrial endometrioid adenocarcinoma.

1. Introduction

An important aspect of the tumor immune microenvironment is the transport of leucocytes across the tissues; the adhesion and transendothelial migration of lymphocytes into their “normal home”, the lymph node, is mediated by specialized postcapillary venules, called high endothelial venules (HEVs) [1,2]. HEVs express peripheral node addressin (PNAd, specifically recognized by the MECA-79 antibody), a ligand for L-selectin, leading to the capture and rolling of L-selectin+ lymphocytes [1,3]. Interestingly, this kind of vasculature has been also found in various tumor types [4], and is considered to play an important role in lymphocytic tumor response, thus, impacting on prognostic features [5]. Despite HEVs presence has been reported in various tumor types [3–12], it has never been studied in the endometrial cancer, to the best of our knowledge.

Furthermore, in the endometrium, PNAd (MECA-79) has been reported to be expressed by the epithelial cells themselves; thus, it is implicated in the attachment of the embryo to the endometrium by binding to the L-selectin+ trophoblast [13,14]. Indeed, its expression increases after ovulation and until the midsecretory phase [14]. In line with this observation, MECA-79 expression in the secretory phase is higher in fertile than in infertile women [15], and lower in post-menopausal women [16]. Moreover, a novel role of MECA-79 in pregnancy has been recently suggested since HEVs have been found in the decidua parietalis and their density was positively correlated with lymphocytic infiltration [17]; furthermore, recurrent pregnancy loss tissues showed lower HEVs density than normal controls [17].

Despite this evidence that MECA-79 as L-selectin ligand is implicated in the pathophysiology of the normal endometrium, but also in the progression of various non-endometrial tumors, its expression has been never studied in the endometrial cancer. Thus, we aimed to investigate if MECA-79 either in the form of an epithelial or an endothelial molecule is expressed in a series of endometrial carcinomas.

2. Material and Methods

The study included 40 patients treated with hysterectomy for endometrial cancer; 30 patients treated with hysterectomy for benign conditions, namely leiomyomas, adenomyosis, and prolapsus were used as controls. Since the possible presence of HEVs in normal uteri is unknown - these vessels are not distinguishable without immunohistochemistry, and no study exists reporting in their normal presence in whole tissue sections from uteri- we studied all control specimens in two separate sections of full thickness from the endometrium to the serosa from both the uterine isthmus and the fundus. Regarding cancer specimens, in terms of homogeneity, we studied only endometrioid adenocarcinomas. We included 23 cases with microsatellite instability (immunohistochemical analysis of MMR proteins: MLH1 (*ES05, Leica*), PMS2 (*EP-51, Dako*), MSH2 (*FE11, Dako*) and MSH6 (*EP-49, Dako*) antibodies, and microsatellite instability (MSI) analysis: OncoMate™ MSI Dx Analysis System, CE-IVD, Promega, for the 5 markers BAT25, BAT26, NR21, NR24 et mono 27). and 17 microsatellite-stable tumors, after immunohistochemical screening. The Local Ethics Committee approved the study (IRBN552021/CHUSTE).

Formalin-fixed paraffine-embedded 4- μ m thick full tumor sections were used for immunohistochemistry in an automated staining system (OMNIS, Dako-Agilent, Santa Clara, CA, USA). Primary antibody used was MECA-79 (Rat monoclonal, Clone MECA-79, Santa Cruz Biotechnology, sc-19602) in a dilution of 1/750, for 20 minutes. Normal lymph node was used as positive control and omission of the primary antibody as negative control. Positive immunoreactions were visualized using 3,3'-diaminobenzidine as the chromogenic substrate. Any expression, either in the normal endometrium or the endometrial cancer, as well as in the form of HEVs was recorded.

Data were analyzed using the StatView software (Abacus Concepts, Berkley, California). We used the chi-square test to explore any relationship between binary parameters and confirmed with Fisher's exact test for selected values, factorial analysis of variances and Mann-Whitney *U* test to consider the effect of at least one factor on a continuous parameter studied, and simple regression analysis to explore a possible relationship between two continuous parameters. Survival probability was estimated by Kaplan-Meier analysis. For all analyses, statistical significance was indicated at a *p* value of <0.05.

3. Results

Patients' characteristics are shown in Table 1. HEVs were absent from all normal uteri, while they were found in 9 (22.5%) cancer specimens ($p=0.005$, $\chi^2=7.7$ with Fisher's exact test $p=0.008$, **Figure 1**). Their presence was marginally associated with higher grade ($p=0.06$, Fisher's exact test), but with

no other tumor characteristics, such as tumor necrosis, lymphovascular invasion, tumor size or T status.

MECA-79 expression by epithelial cells (being often luminal, **Figures 2 and 3**) was found in 14 (46.6%) of normal hysterectomies and in 28 (70%) of cancer specimens ($p=0.04$, χ^2 test and $p=0.08$ for the Fisher's exact test). Its expression by tumors cells ranged from 0-90%, mean 18.8%, median 5%. Mann-Whitney U test revealed a statistically significant association with invasion of the parameters, since tumors with parametrial invasion showed lesser MECA-79 expression (mean of MECA-79 for absence of invasion 22% vs 11.8% for infiltrated parameters, $p=0.04$). Confirming by the χ^2 test and the Fisher's exact test when considering the MECA-79 expression as a binary parameter (presence or absence), p was 0.03 and 0.05, respectively. No other associations were found for the rest of the factors studied (Table 2). However, regression analysis (**Figure 4**) showed a trend for larger tumors to harbor less MECA-79 expression ($p=0.07$). The presence of HEVs was not associated with the presence of MECA-79 expression by tumor cells ($p=0.2$, $\chi^2=1.1$)

Overall survival was marginally and positively associated with the percentage of tumor cell MECA-79 expression ($p=0.07$ in regression analysis, **Figure 4**), but not in Kaplan Meier analysis. Patients whose tumors harbored HEVs showed worse progression-free survival, but this did not reach statistical significance ($p=0.1$ for the Mantel Cox log rank test, and 0.08 for the Breslow Gehan Wilcoxon rank test). Progression-free survival was not associated with the presence of MECA-79+ tumor cells (log-rank $p=0.5$).

4. Discussion

MECA-79+ HEVs are found in 55%-80% [3,6,18] of melanomas, in about 70% of breast carcinomas [8] and in almost 80% of colorectal carcinomas [9]. Almost 90% of oral carcinomas were been found to have high HEVs density [10], while in pharyngeal and laryngeal tumors there are found in 34% of cases [5]. We found in the current series that HEVs are present in 22% of endometrioid adenocarcinomas, lower than the rest of malignancies, but they were generally absent from normal uteri, implying an active role in the tumoral setting. Furthermore, they were rare in grade 1 adenocarcinomas, suggesting that they probably represent a structure activated with the aggressiveness of the tumors, thus, studies in higher grade non-endometrioid carcinomas are needed. Despite HEV higher density was associated with microsatellite-unstable tumors in colorectal tumors [7], there were not associated with microsatellite instability in the current series, probably explained by the different microenvironment between the two organs, which is known to impact on HEVs density: HEV density is associated with lymphocytic response in colorectal [9], head and neck [5] and breast cancer [8]. Another explanation could also be the differences in microsatellite instability, characterized by only minimal microsatellite shift in uterine tumors compared to colorectal ones [19].

In breast cancer, higher HEVs density was associated with better prognosis [4] and complete response after neoadjuvant treatment [8]. In oral carcinomas, HEVs were associated with longer survival [12]. No association between HEV density and stage of disease or survival was noted in colorectal cancer [9]. In the current series HEVs presence showed a trend to worse

PFS, but this was not statistically significant, and could also be associated with the higher grade, or even the limited follow up/number of recurrences, thus, larger studies are needed to better characterize this role.

MECA-79 expression by tumor cells themselves has been more rarely reported. This has been noted in 27% of gastric carcinomas [11], in some cases (number not specified) of colorectal carcinomas [9], and in 20% of laryngopharyngeal carcinomas [5]. In gastric cancer, MECA-79 expression by tumor cells was a negative prognostic factor [11], while in head and neck cancer there was no prognostic significance of MECA-79 expression by tumor cells [5]. In the current series, we found that MECA-79 expression was more frequent in the tumoral setting, and that tumors with less MECA-79 expression were larger and more aggressive showing parametrial invasion. However, given the short follow up (median 2 years) in the current series, more studies will be needed to reveal the prognostic significance of this molecule. It is unclear the underlying mechanism of this ectopic MECA-79 expression by tumor cells and of its possible significance, but it has been suggested that L-selectin+ leucocytes can cover tumor cells emboli and thus facilitate their extravasation in metastatic sites [9], and this definitely merits further **investigation.**

To conclude, this is the first report **in** investigating HEVs and PNA⁺ tumor cells in endometrial cancer, showing that HEVs are a feature mainly of the tumoral setting and that they are associated with higher tumor grade, while PNA⁺ tumor cells are associated with parametrial invasion. Further studies are necessary to better delineate the prognostic role of this molecule in endometrial cancer.

Compliance with Ethical Standards

Local Ethics Committee approved the study (IRBN552021/CHUSTE); the acquisition of written informed consent was waived by the institutional review board given the retrospective nature of the study and the anonymization of all data. The study was performed according to the Declaration of Helsinki.

Funding

No funding has been obtained.

Conflict of Interest

The authors have no conflict of interest to declare.

Contributions

GK and MP conceived the idea and designed the study, all authors participated in data acquisition and interpretation, GK drafted the manuscript, all authors approved the final form.

Data availability

Data are available upon reasonable request.

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Tables

Table 1.

Diagnosis (n=70)	
Malignant disease	40, 57.1%
Benign disease	30, 42.9%
Age (n=70)	
Range	33-90
Median	62.5
Mean± SD	60.5 13.5
MSI status (n=40)	
MSI High	23, 57.5%
MSI Stable	17, 42.5%
T status (n=40)	
T1 (T1b)	29, 72.5% (13, 32.5%)
T2	3, 7.5%
T3	8, 20%
N status (n=40)	
N0	39, 97.5%
N1	1, 2.5%
Tumor size (n=40)	
Range	1-18
Median	4.05
Mean ± SD	4.4 2.6
Tumor grade (n=40)	
1	16, 40%
2	17, 42.5%
3	7, 17.5%
Tumor necrosis (n=40)	
Yes	11, 27.5%
No	29, 72.5%
LVI (n=40)	
Yes	16 (40%)
No	24 (60%)
Follow-up (n=40)	
Range	24-168
Median	24
Mean ± SD	36 25
Recurrence (n=40)*	
Yes	6, 15%
No	34, 85%

*: 4 lymph nodes, 1 urethral, 1 brain.

Table 2. Correlation of immunohistochemical expression with clinicopathological characteristics

	Presence of HEVs	Absence of HEVs	p, χ^2	Presence of MECA-79 expression in epithelial cells	Absence of MECA-79 expression in epithelial cells	p, χ^2
Disease						
Malignant	9	31	0.005**, 7.7	28	12	0.04, 3.8
Benign	0	30		14	16	
Tumor grade						
1	1	15	0.1*, 4.1	12	4	0.6, 0.7
2	6	11		12	5	
3	2	5		4	3	
MSI status						
Unstable	5	18	0.8, 0.01	17	6	0.5, 0.3
Stable	4	13		11	6	
T status						
T1	5	24	0.4, 1.6	13	16	0.8, 0.2
T2	1	2		1	2	
T3	3	5		3	5	
Age (n=70)	71.8±10.5	59.4±13.3	0.03	60.5±15.1	60.5±12.3	0.3
Tumor size	4.2±0.8	4.4±2.8	0.9	4.1±1.3	4.5±3.2	0.09
Tumor necrosis						
Yes	3	8	0.6, 0.1	6	5	0.1, 1.7
No	6	23		22	7	
LVI						
Yes	5	11	0.2, 1.1	11	5	0.8, 0.02
No	4	20		17	7	
Recurrence						
Yes	3	3	0.08, 3	1	5	0.4 0.5
No	6	28		23	11	

*For tumors grade 1 vs grades 2+3, the two-tailed p value with the Fisher's exact test is 0.06 and $p=0.04$ with $\chi^2=4$ for the χ^2 test. ** Fisher's $p=0.008$

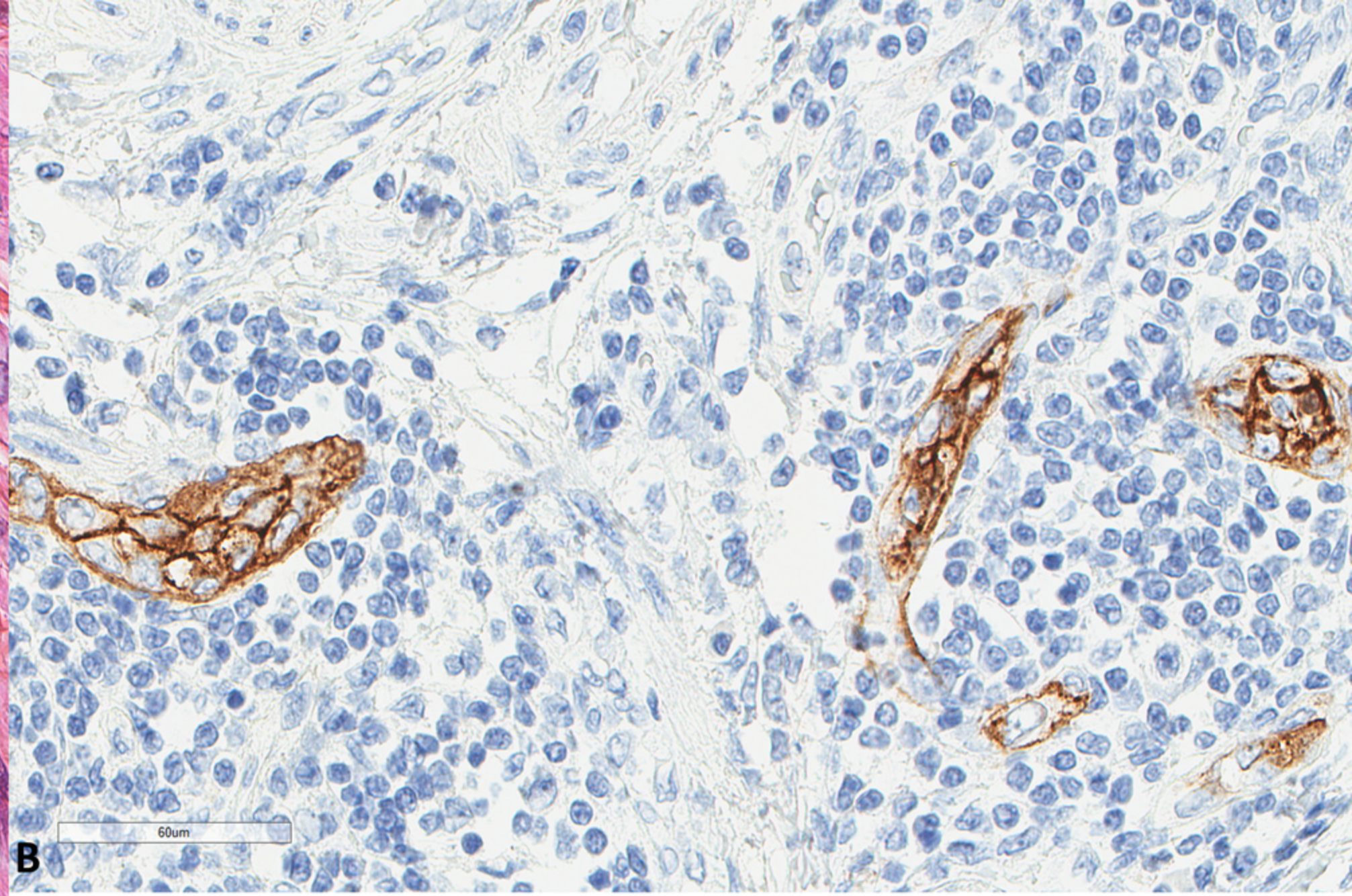
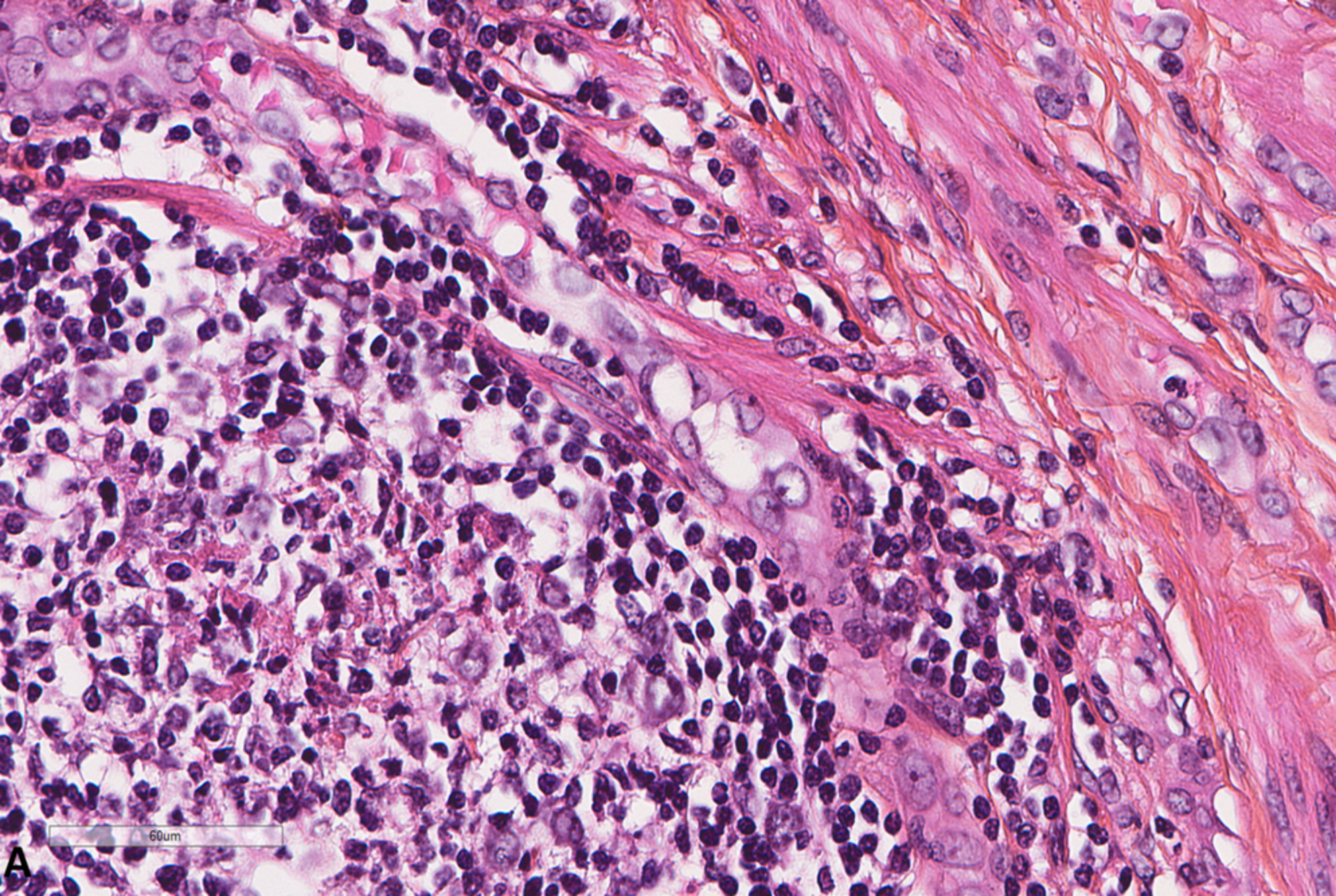
Figures

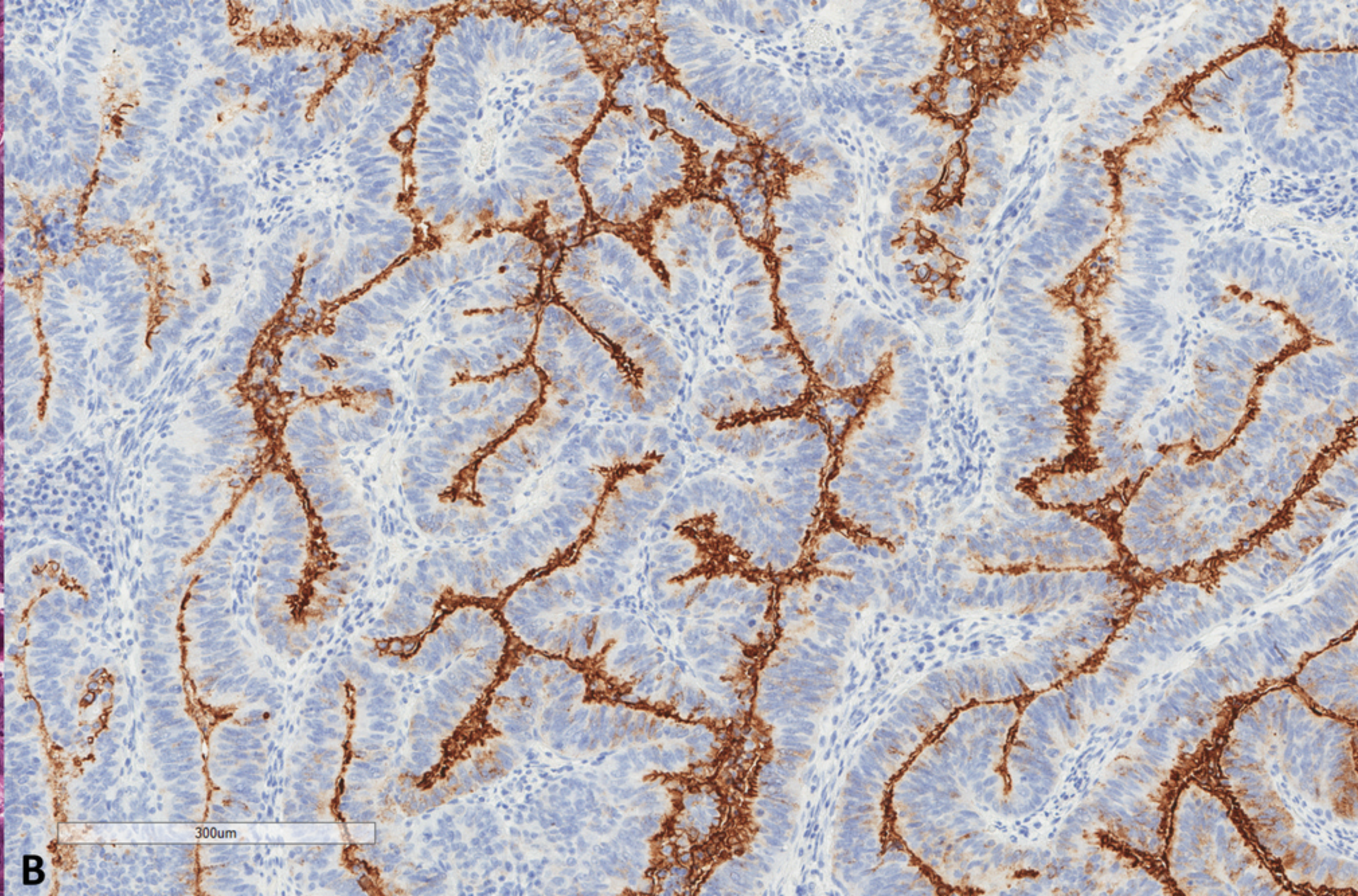
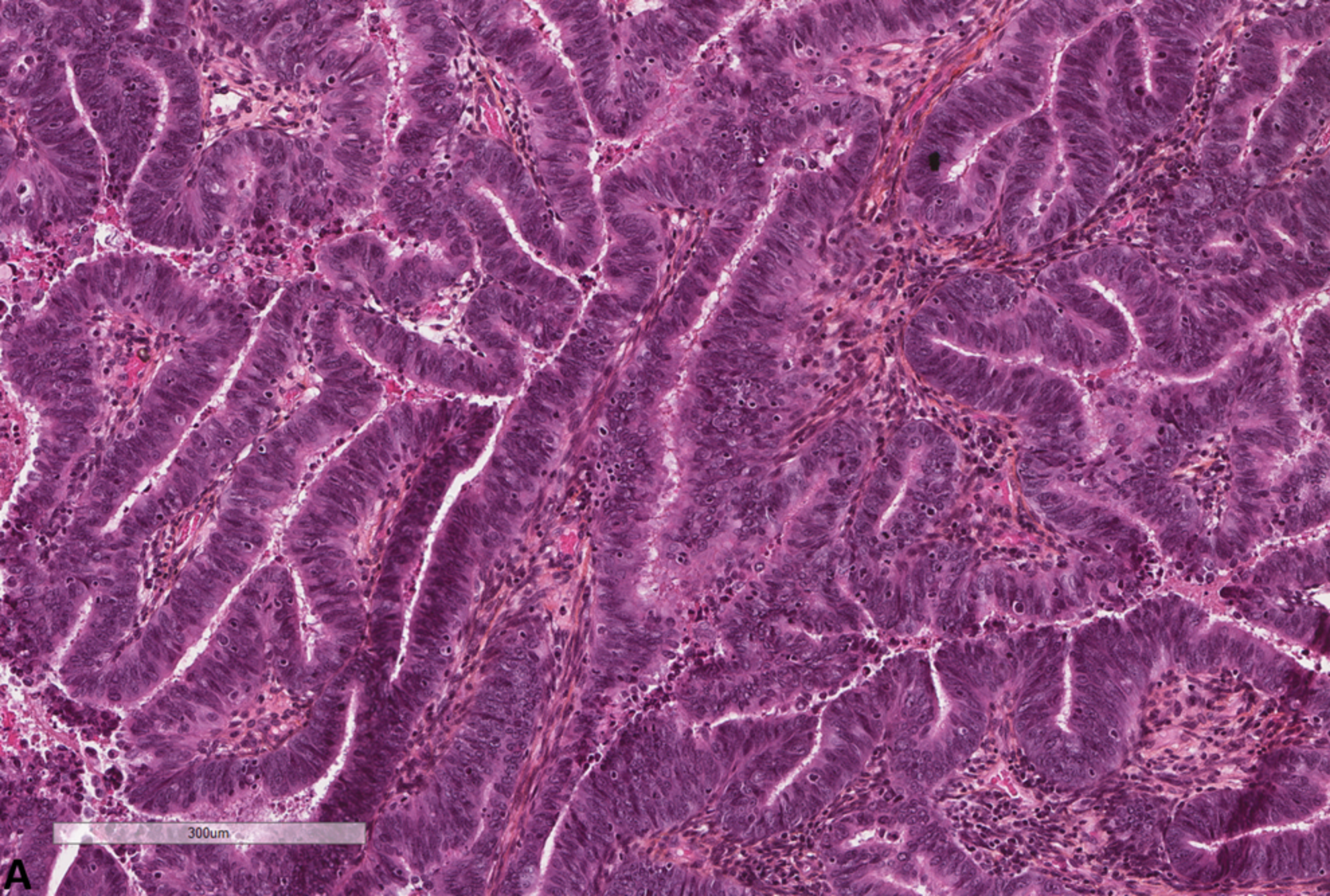
Figure 1. A high endothelial venule showing high columnar endothelial cells is surrounded by lymphocytes (HES x400). B. This endothelium expresses MECA-79 (x400).

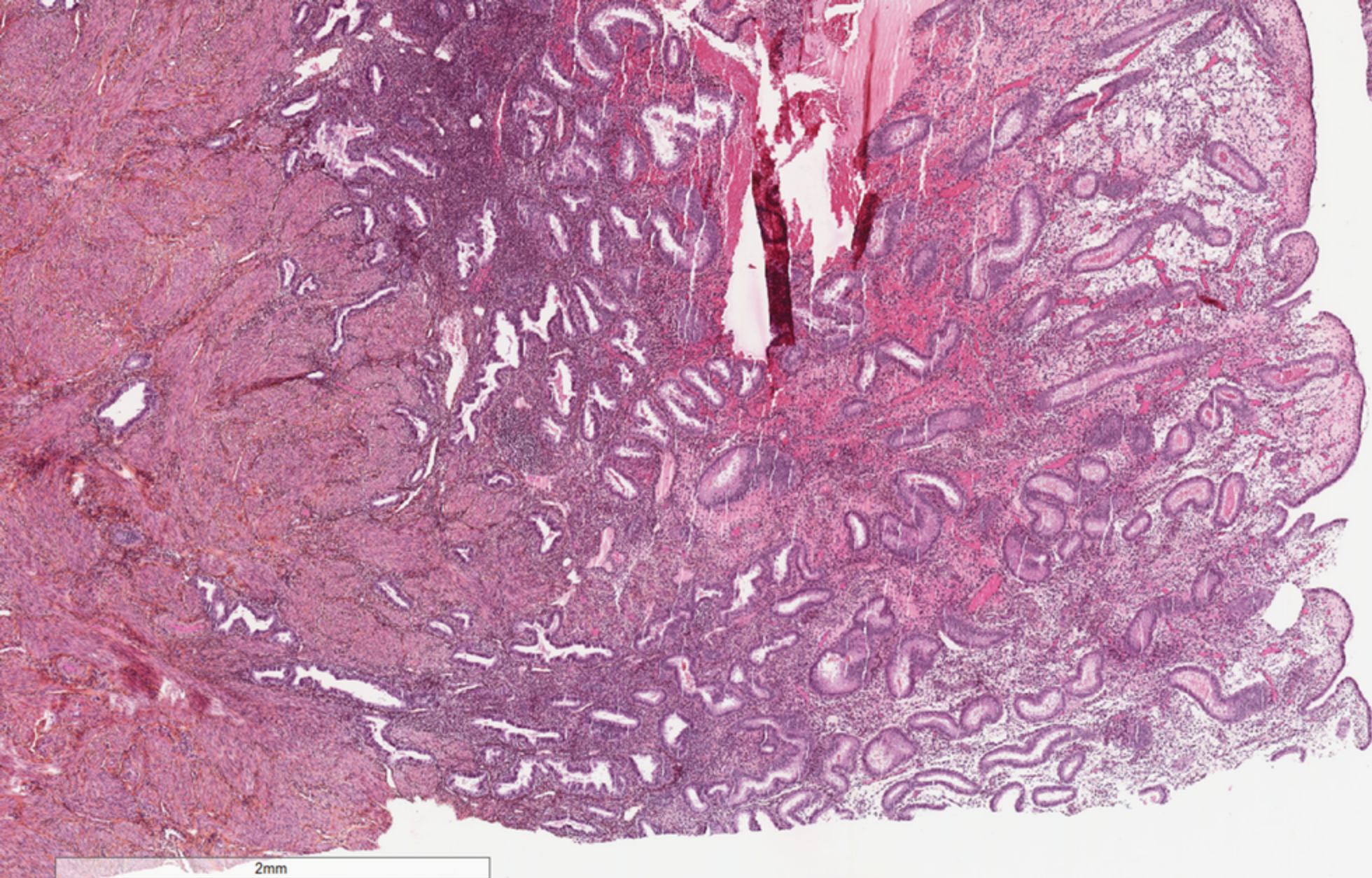
Figure 2. A. An endometrioid adenocarcinoma (HES x100) showing (B) luminal MECA-79 expression (x100).

Figure 3. Full thickness endo-myometrial junction (HESx20) showing MECA-79 expression (Bx20) by epithelial cells of the endometrium. Inset: Higher magnification showing MECA-79 luminal expression (x400).

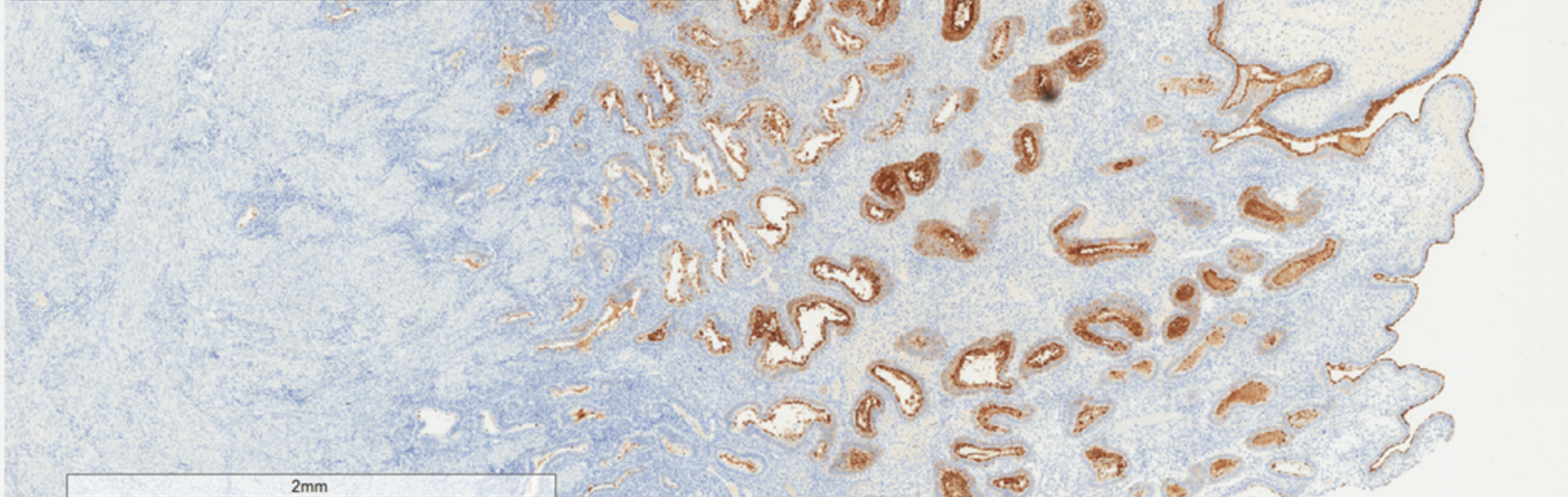
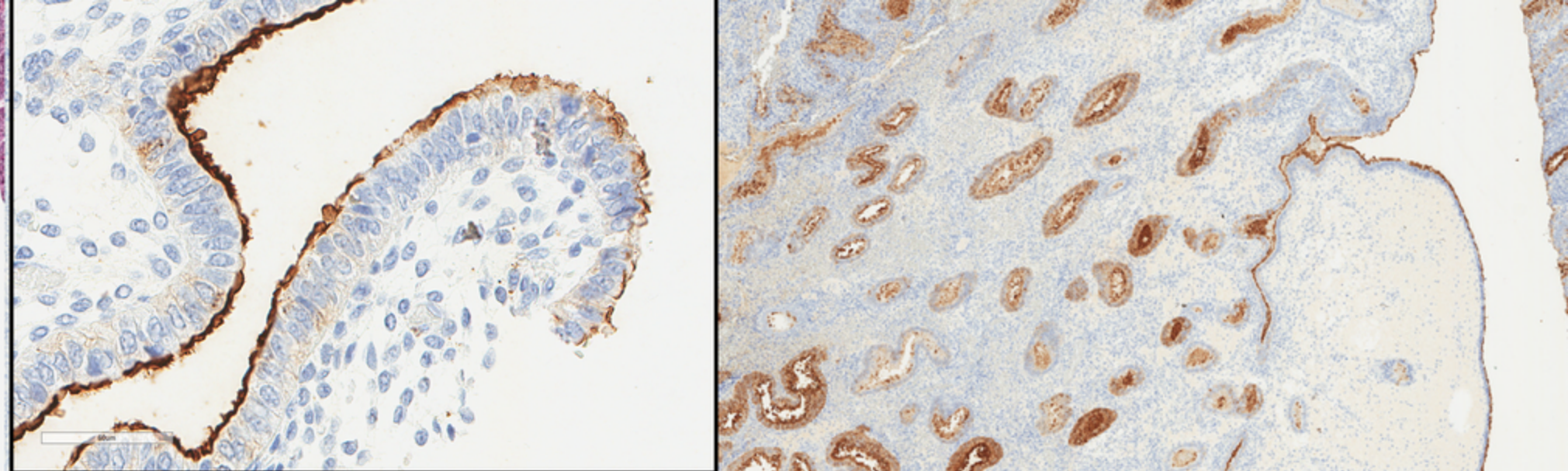
Figure 4. Regression analysis of follow up (A) and tumor size (B) in comparison to MECA-79 tumor cell expression.





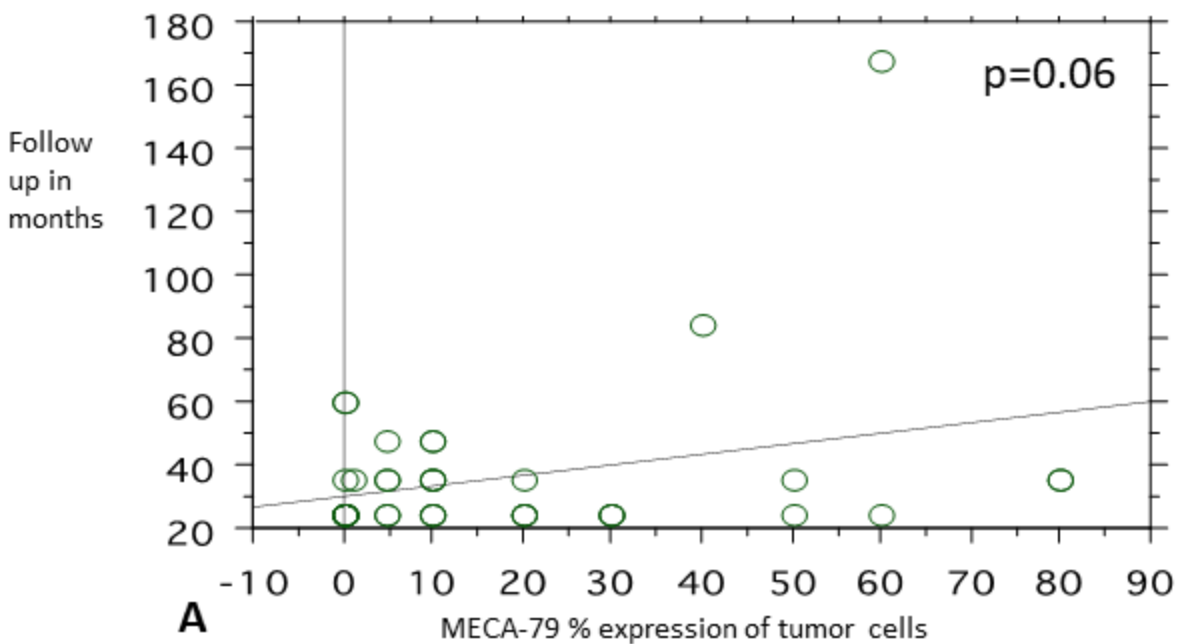


A



B

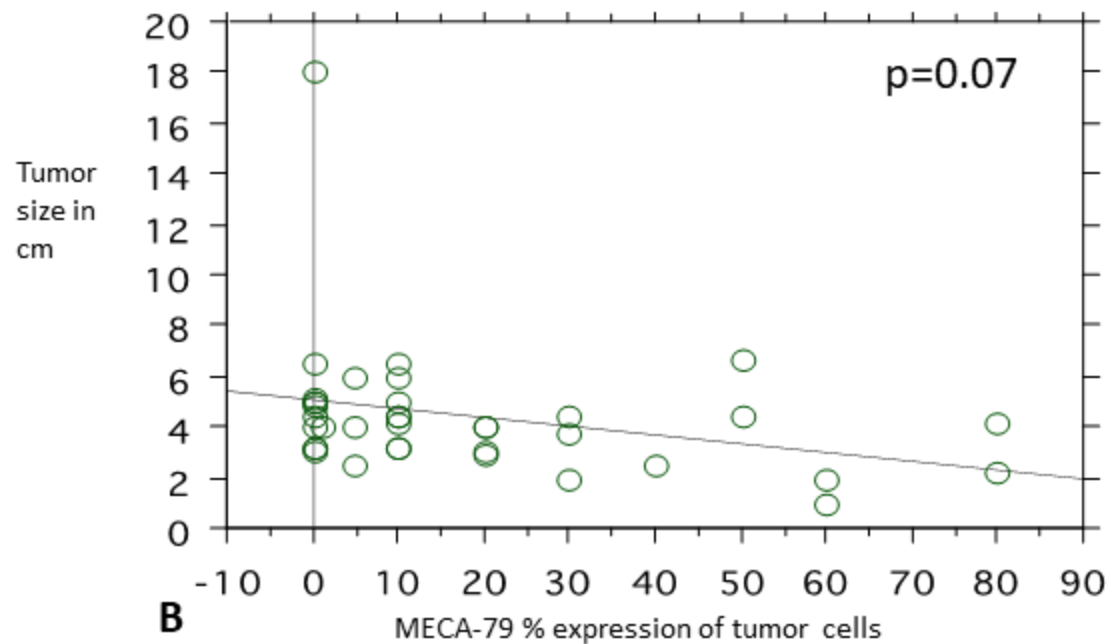
Regression Plot



A

$$Y = 30.228 + .332 * X; R^2 = .089$$

Regression Plot



B

$$Y = 5.043 - .034 * X; R^2 = .087$$