Research Square

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L1CAM Expression in Recurrent Estrogen Positive/HER2 Negative Breast Cancer

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Abstract

Background: We investigate L1CAM expression in ER positive/HER2 negative breast carcinomas. The finding of a potential correlation between high L1CAM expression and recurrent/metastatic disease in luminal A and B breast carcinomas may be helpful for risk stratification and open opportunities for targeted therapies.

Methods: 304 cases comprising 152 cases of ER positive, PR positive/negative and HER2 negative recurrent/metastatic breast carcinomas and 152 non-recurrent controls were included. ER, PR, HER-2, Ki-67 status, Nottingham grade, tumor size, tumor stage, number of foci, lymph node status, lymphovascular invasion, phenotype, laterality, age at diagnosis and first distant or local recurrence were recorded.

Results: L1CAM positive cases showed increased specificity for recurrence and these patients were significantly younger than L1CAM negative ones. Compared to L1CAM negative recurrent cases, L1CAM positive ones had a noticeably higher Ki-67, tended to be larger and recurred sooner. All L1CAM positive recurrent/metastatic cases were of the luminal B subtype compared to 67.3% of the L1CAM negative cases.

Conclusions: L1CAM is highly specific for recurrence in a subset of breast cancer patients and may be associated with more aggressive behavior, particularly in luminal B breast cancers with higher Ki-67 expression. Further investigation about the prognostic value of L1CAM is warranted.

Background

Breast cancer is the most common cancer in women worldwide and the second leading cause of cancer death, surpassed only by lung cancer. The American Cancer Society estimates that there will be approximately 276,480 new cases of breast cancer in women and about 2,620 new cases in men in 2020 and although the death rates have been decreasing since 1989 – especially in women over 50 years of age – they still remain steady in those under 50 and roughly 42,170 women in the United States are expected to die from breast cancer in 2020 alone [1].

Classification of breast carcinoma is based on expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2) and is pivotal in helping determine therapy and predict survival. Using RNA expression profiling, four significant groups of breast cancer have been reported, based on the expression ER, PR and HER2 [2, 3]. The ER positive group is the most diverse subtype with the best prognosis, comprising two categories: luminal A breast carcinomas – ER positive and/or PR positive with a Ki-67 < 14% and luminal B type – ER positive and/or PR positive with a Ki-67 ≥ 14% or ER positive and/or PR positive with positive HER2 expression. The ER negative groups include the HER2-enriched – ER/PR negative and HER2 positive and basal- breast carcinomas – typically (but not always) negative for ER, PR, and HER2, the so-called “triple negative breast carcinomas” (TNBC), EGFR or CK5/6 positive. like In addition, TNBC that are negative for both EGFR and CK5/6 are defined as

TNBC-non basal [4]. These four breast cancer subtypes have significantly different treatment options and differences between subtypes have been shown to have relevance for locoregional relapse [4]. HER2-enriched tumors have a 5-year-locoregional recurrence rate of 15% compared to 1% for luminal A ones [5], while HER2-enriched and TNBC are both associated with higher risk of local recurrence than the luminal A subtype [6]. Local and distant recurrence for luminal A tumors at 10-years was 8% and 3%, respectively, after partial or total mastectomy, results that confirm the excellent survival outcome for this subtype of tumors [7]. Compared to the luminal A subtype, women with HER-2 enriched breast carcinoma have roughly a two-fold increased adjusted risk of breast cancer mortality [8] and patients with TNBC have a poorer short-term prognosis than all the other subtypes [9, 10].

Although expected to have similar outcomes due to the similar hormonal profile, luminal A and B type tumors can paradoxically have a significantly different prognosis. The latter group accounts for approximately 35% of ER/PR positive HER2 negative cases and have a higher risk of locoregional recurrences, which is in part attributable to a higher proliferation index, as a Ki-67 ≥ 14% has been shown to predict recurrence in small node-negative hormone positive tumors [11]. This difference is also reflected in relapses at 15 years (27.8% of luminal A tumors versus 42.9% for luminal B), in median survival from the time of first distant metastasis (2.2 years for luminal A versus 1.6 years for luminal B subtype), as well as in the overall survival (OS) at 10 years (70% luminal A versus 54.4% for luminal B tumors) [12].

Hormonal therapy only is the primary established treatment for patients with luminal A phenotype, while hormonal plus anti-HER2 target therapies is the strategy appropriate for treatment of luminal B HER2-enriched subtype [13]. Challenges remain in accurately identifying which subsets of luminal subtype patients will benefit from systemic chemotherapy, as 20–30% of patients with early stage luminal subtype breast carcinoma will relapse and have an unexpectedly poor outcome. These challenges have led to the proliferation of multigene assays to aid in clinical decision making around the suitability of chemotherapy as part of adjuvant treatment regimens [14].

L1CAM (L1 cell adhesion molecule) is a 200–220 kDa transmembrane glycoprotein of the immunoglobulin (Ig) superfamily, involved in neurogenesis, cell-cell interaction, synaptogenesis, myelination and neuron survival. The molecule is composed of six Ig-like domains and five fibronectin type III, followed by a transmembrane region that connects them to a highly conserved cytoplasmic tail [15]. A large multicenter evaluation of L1CAM expression in early-stage type I endometrial carcinoma – expected to have excellent prognosis, with more than 80% 10-year OS – showed that despite appropriate treatment, a number of patients exhibited recurrence and poor survival and this trend was associated with L1CAM expression [16, 17]. L1CAM has subsequently been proposed to be a strong prognostic factor in endometrial carcinoma and it has been suggested that the classical “low-risk” disease should potentially be further risk stratified [18].

Despite increasing evidence showing involvement of L1CAM in multiple different types of neoplasia [19–22], little is known about its expression and prognostic value in breast cancer. To-date, only two studies

have provided most of the information, both with findings associated primarily with hormone negative breast carcinomas [23, 24]. L1CAM is overexpressed in TNBC and is correlated with high tumor grade, propensity for lymph node involvement, negative ER status, HER2 overexpression, and most concerning, with shorter disease-free and OS; it has only been sporadically detected in other non-TNBC (HER2 positive tumors). This prompted our efforts to investigate L1CAM in ER positive/HER2 negative breast carcinomas. The invasive potential of a tumor is mediated by cell adhesion molecules which break and form cell-cell bonds. Since L1CAM promotes adhesion to the endothelium – the first step toward extravasation – the hypothesis that recurrent ER positive HER2 negative breast cancers might be L1CAM positive seems feasible. The finding of a potential correlation between high L1CAM expression and recurrent/metastatic disease in luminal A and B subtypes may be helpful for risk stratification and open new opportunities for targeted therapies.

Methods

Patients and data retrieval

A retrospective search of the pathology information system database between January 2008 and December 2015 after Institutional Review Board (IRB) approval was performed to identify patients with recurrent or metastatic ER positive, PR positive/negative and HER2 negative breast carcinomas. A total of 304 cases (303 patients) were included in this study (Table 1). A total of 165 cases of recurrent or metastatic breast carcinomas were identified and hematoxylin-eosin (H&E) slides and paraffin blocks for 152 cases (151 patients) were available and retrieved. One patient had bilateral disease and accounted for two separate cases. 152 cases (152 patients) of non-recurrent/non-metastatic ER positive, PR positive/negative and HER2 negative breast carcinomas from the same time period were identified and included for comparison analysis (Table 1).

Table 1
Population clinicopathologic characteristics

	Total population (n = 304)	Recurrent (n = 152)	Non-Recurrent (n = 152)	p-value
Years of age (mean/range)	60.0 (21–92)	61.2 (21–90)	58.7 (32–92)	NS
PR positive	255	122	133	NS
PR negative	49	30	19	
Nottingham grade				
1	87	24	64	< 0.0001
2	141	81	59	
3	76	47	29	
Ki-67 (mean/range)	21.9 (1–95)	27.8 (1–85)	17.4 (1–95)	< 0.0001
Ki-67 < 14	121	35	85	< 0.0001
Ki- 67 ≥ 14	142	79	64	
Excision and biopsy	289	137	152	NA
Biopsy only	15	15	0	
Tumor size (mean/range)	3.1 (0.4–22)	3.8 (0.6–22)	2.4 (0.4–15)	< 0.001
Stage				
pT1a	2	0	2	
pT1b	35	15	20	< 0.001
pT1c	101	29	72	
pT2	106	60	46	
pT3	38	26	12	
pT4	5	5	0	
Not evaluated	17	17	0	NA
Lymphovascular invasion				
Positive	86	61	25	< 0.001
Negative	201	74	127	
Unknown	17	17	0	NA

	Total population (n = 304)	Recurrent (n = 152)	Non-Recurrent (n = 152)	p-value
Lymph node involvement				
Positive	170	52	118	< 0.001
Negative	88	60	28	
Unknown	46	40	6	NA
Histological type				
Ductal	247	123	124	NS
Lobular	45	24	21	
Mixed (ductal and lobular)	11	4	7	
Other	1	1	0	
Years to recurrence (mean/range)		4 (0–21)	NA	NA
Metastatic disease at time of diagnosis	26	26	NA	NA
L1CAM positive	7	7	0	0.015
L1CAM negative	297	145	152	

Information on ER, PR, HER2, Ki-67, Nottingham grade, tumor size, tumor stage, number of foci, lymph node status, lymphovascular invasion, phenotype, and laterality were extracted from the pathology report. The Nottingham grade was calculated using the Nottingham modification of the Bloom-Richardson system [25, 26]. Information on age at diagnosis and first distant or local recurrence were extracted from the medical record.

Immunohistochemistry (IHC)

All tumor H&E and IHC slides were reviewed by at least two board-certified breast pathologists, with manual interpretation of ER, PR, HER2, and Ki-67, using standard histological criteria [27]. IHC was performed on whole sections from the surgical excision if available; otherwise, IHC was performed on the biopsy specimen. For cases with multiple foci of tumor, IHC was performed on the largest focus and for recurrent/metastatic cases positive for L1CAM, IHC was also performed on the metastatic focus.

ER, PR, and HER2 were evaluated using FDA-approved test kits - [DAKO] - ERα [clones ID5 and ER-2-123], PR [clone PgR1294] pharmDxTM), and HER2 IHC scores (Rabbit anti-human HER-2 HercepTestTM). HER2 FISH was performed (FDA-approved test kit [DAKO] - HER2 IQFISH pharmDxTM) on all equivocal HER2 IHC results. Ki-67 was evaluated by calculating the percentage of positive staining tumor cells on a single slide (Monoclonal mouse anti-human Ki-67 antigens [clone MIB-1, code M7240]). L1CAM analysis was performed using the L1 mAb clone 14.10 (BioLegend, San Diego, CA). Formalin-fixed paraffin-embedded

tissues were sectioned at 4 microns, placed on glass slides, and baked for 60 minutes at 60 °C. The slides were placed on the Dako Omnis Automated Staining Platform. The protocol uses Envision Flex TRS Low pH retrieval buffer for 30 minutes at 95 °C in which the antibody is diluted to 1:400 in Dako Antibody Diluent and incubated for 20 minutes, then visualized by Envision Flex HRP using DAB as the Chromogen. The slides were counterstained with hematoxylin, dehydrated through graded alcohols and cover slipped. High L1CAM-expressing normal kidney and brain tissue was used as positive control. L1CAM expression of ≥ 10% is considered positive [17].

Statistical evaluation

Available clinical and pathologic data were summarized using percentages, descriptive statistics (mean, range) and inferential statistics (chi-square (X^2) test of independence, relative-risk, and t-test). All data analyses were performed using the statistical Analysis ToolPak (Microsoft Excel Office 2010 version 14.0.7015.100) except for the inferential statistics, which were performed using JavaStat 2-way Contingency Table Analysis (revised version 7/23/2013 http://statpages.org/ctab2x2.html). For all results, a p-value of < 0.05 was considered significant. This study received IRB approval from the University of Rochester (IRB# 00003173).

Results

A summary of clinicopathologic features in the patient population is detailed in Tables 1 and 2.

Table 2
Clinicopathologic characteristics of L1CAM positive and L1CAM negative recurrent cases

	L1CAM positive (n = 7)	L1CAM negative (n = 145)	p-value
Years of age (mean/range)	41.0 (21–59)	62.2 (29–90)	< 0.001
PR positive	6	116	NS
PR negative	1	29	
Nottingham grade			
1	1	23	NS
2	3	78	
3	3	44	
Ki-67 (mean/range)	41.4 (20–60)	26.9 (1–85)	NS
Ki-67 < 14	0	35	NS
Ki- 67 ≥ 14	6	72	
Excision and biopsy	5	132	NS
Biopsy only	2	13	
Tumor size (mean/range)	4.9 (1.4–7)	3.7 (0.6–22)	NS
Stage			
pT1a	0	0	NS
pT1b	0	15	
pT1c	1	28	
pT2	1	59	
pT3	3	23	
pT4	0	5	
Not evaluated (biopsy only)	2	15	NA
Lymphovascular invasion			
Positive	3	58	NS
Negative	3	72	
Unknown	1	15	NA
Lymph node involvement			

	L1CAM positive (n = 7)	L1CAM negative (n = 145)	p-value
Positive	3	49	NS
Negative	2	59	
Unknown	2	37	
Histological type			
Ductal	6	117	NS
Lobular	0	24	
Mixed (ductal and lobular)	1	3	
Other	0	1	
Years to recurrence (mean/range)	3.2 (1–6)	4.0 (0–21)	NS
Metastatic disease at diagnosis	2	24	NS

The vast majority of cases were ductal carcinomas (n = 247), 216 ductal carcinoma not otherwise specified (NOS), 16 with micropapillary features, 9 with mucinous features, 3 with tubular features, 2 with papillary features, and one with cribriform features. 45 lobular carcinomas were identified (41 lobular carcinoma NOS, 3 pleomorphic subtypes, and 1 signet ring cell subtype). 11 cases with mixed ductal and lobular features and one with neuroendocrine features were identified. Ki-67 proliferation marker was available in 263 of the 304 cases. Time of recurrence/metastasis ranged from 1–21 years, with the predominant sites including ipsilateral or contralateral breast, chest wall, liver, lung, and bone, with sporadic involvement of orbital soft tissue, colon, and stomach; two cases presented as angiosarcoma at 6 and 8-years post initial diagnosis. Compared to patients with non-recurrent/non-metastatic tumors, patients with recurrences showed similar age, PR expression, and histological types. As would be expected, recurrent cases had significantly higher grade, higher Ki-67, higher percentage of luminal B type tumors, larger tumor size, higher tumor stage, more frequent lymphovascular invasion and lymph node involvement (Table 1).

Patients with recurrent/metastatic breast cancer were significantly associated with positive L1CAM staining compared to patients without recurrences (Table 1, Fig. 1: L1CAM pattern of staining: (a) L1CAM negative tumor; (b) L1CAM positive tumor). L1CAM positive cases showed increased specificity for recurrence ($p = 0.015$, RR = 2.048, Table 1 and Table 3) and L1CAM recurrent/metastatic patients were significantly younger than L1CAM negative recurrent ones ($p < 0.001$, Table 2). Although not quite reaching statistical significance ($p = 0.08$), L1CAM positive recurrent cases had a trend towards a noticeably higher Ki-67 than L1CAM negative ones and 100% of the L1CAM positive recurrent cases were of the luminal B subtype versus 67.3% L1CAM negative recurrences. Compared to L1CAM negative recurrent/metastatic tumors, L1CAM positive cases tended to be larger and recur sooner (Table 2). Interestingly, metastatic tumors in four of the seven L1CAM positive recurrent cases were also positive,

while two were negative and one case – a patient with lung metastasis for which endoscopic ultrasound-guided fine needle aspiration was performed resulting in a cytology specimen – could not be processed due to exhaustion of the cell block.

Table 3
Recurrent and non-recurrent case and L1CAM expression

	L1CAM positive	L1CAM negative
Recurrent	7	145
Non-recurrent	0	152
Sensitivity: 0.046		
Specificity: 1.000		
Positive Predictive Value: 1.000		
Negative Predictive Value: 0.512		
Relative Risk: 2.048 (p = 0.015)		

Discussion

L1CAM has lately garnered considerable interest due to its association with several malignancies portending poor prognosis – most notably ovarian and endometrial carcinoma and malignant melanoma, but also colon and pancreatic carcinoma [16, 17, 19, 20, 21], as well as its role in cell motility, invasion, chemoresistance and metastatic potential [28–30]. These findings have led to attempts to develop new targeted therapies, such as knockdown of L1CAM, which significantly reduces metastasis in a xenograft model of melanoma [31] and the use of circulating autoantibodies to anti-L1CAM that can be used as a potential diagnostic biomarker in esophageal squamous cell carcinoma [22]. A large international study focused on endometrial carcinoma FIGO grade 1 stage 1 - expected to have more than 80% 10-year OS – revealed that L1CAM-positive tumors are associated with a considerable increase in the likelihood of recurrence and unexpectedly poor overall survival. This finding raised questions regarding the particular management for these patients – until recently considered being “low-risk” – and adjuvant chemotherapy was proposed as a treatment option for patients with L1CAM-positive FIGO grade 1 stage 1 endometrial carcinoma [17]. Ongoing trials have established L1CAM as being important in tumor invasion as well as tumor recurrence and old paradigms regarding treatment of “low-risk” endometrial carcinoma patients are being questioned [18, 21, 32, 33]. In addition, L1CAM has also been detected in benign conditions, such as inflammatory bowel disease and endometriosis [34, 35].

Given these findings, examination of expression and prognostic value of L1CAM in breast carcinoma was expected. Upon evaluation of 15 benign and 25 malignant breast tumors (fibroadenoma and invasive ductal carcinoma, respectively) by one of the early studies, no expression of L1CAM was detected [36]. A

subsequent study using a larger number of cases, different IHC protocols and microarray analysis identified L1CAM staining and overexpression in 15% of breast tumors [23], associated with shortened disease free survival (DFS). These emerging data regarding the role of L1CAM in breast carcinoma subsequently led to an extensive analysis of TNBC, a particularly aggressive breast cancer subtype [24]. L1CAM expression was found to be more abundant in TNBC and expression of L1CAM was inversely correlated with androgen receptor (AR) expression, suggesting a possible correlation between L1CAM expression and an unfavorable prognosis in both OS and DFS in this patient population. This is of particular significance, as AR is a prognostic marker in TNBC. Expression of AR is associated with improved OS and DFS regardless of ER status, while loss of AR expression is associated with early recurrence in basal-like breast cancer and TNBC [37].

Overall, the literature to date suggests that L1CAM expression is associated with a particularly aggressive subtype of breast carcinomas. Our study results support this conclusion and suggest that L1CAM expression in a subset of ER-positive HER2 negative breast carcinomas is associated with a more aggressive clinical course and a significantly increased likelihood of recurrence, compared to L1CAM negative ER-positive HER2 negative cases. Recurrent tumors with L1CAM expression occurred in significantly younger women with luminal B phenotype (100% of cases) compared to L1CAM negative recurrent tumors (67.3%) and had a trend toward higher Ki-67 expression. These patients also tended to have larger tumors and earlier recurrences compared to patients whose tumors showed no evidence of L1CAM expression. We believe that, with a larger patient population, these findings would also be statistically significant.

L1CAM expression and recurrence was independent of age, phenotype, PR expression, tumor stage, and Nottingham grade. Unlike prior publications, we did not find an association with lymph node status or pathologic stage, at least not in this particular subset of breast cancer patients. Our study is limited because of its retrospective nature, the lack of uniform treatment as well as the fact that we only identified seven cases of L1CAM positive carcinomas; however, all of these patients had recurrent/metastatic breast cancer, while none of the 152 patients without recurrence demonstrated L1CAM expression. Given these limitations, we feel that our results are intriguing, hypothesis generating and warrant further examination of the role of L1CAM expression and its biologic significance in the luminal-B subtype of ER-positive breast cancers.

While it is generally accepted that expression of hormone receptors and absence of HER2 amplification is associated with better outcome in women with breast cancer, recurrence and/or metastases still occur in this patient population. This study, although limited in numbers, is an indication that L1CAM may play a deleterious role for DFS not only in patients with TNBC, but also in patients with ER-positive HER2 negative breast tumors. Additional investigations in larger populations are needed to further evaluate these findings, as well as the mechanisms of action and prognostic value of L1CAM. Potential therapies focused on down-regulation of this molecule may be warranted in breast cancers that show evidence of L1CAM expression.

Conclusion

Our findings suggest that L1CAM expression may be associated with more aggressive behavior, particularly in luminal B subtype breast carcinomas with higher Ki-67 expression. Additional studies with larger populations are needed to further validate these results.

List Of Abbreviations

Estrogen receptor (ER)

Progesterone receptor (PR)

Human epidermal growth factor receptor (HER2)

Triple negative breast carcinomas (TNBC)

Overall survival (OS)

L1CAM (L1 cell adhesion molecule)

Immunoglobulin (Ig)

Hematoxylin-eosin (H&E)

Immunohistochemistry (IHC)

Institutional Review Board (IRB)

Chi-square (X2)

Not otherwise specified (NOS)

Disease free survival (DFS)

Androgen receptor (AR)

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent for this research was waived according to Institutional Review Board (IRB) approval from the University of Rochester Medical Center (IRB# 00003173).

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: All authors declare that they have no conflict of interest.

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Authors' contributions: Dr. IM and HZ conducted the project in its entirety, analyzed the data and drew conclusions. Mr. MDA and Drs. IM and HZ pulled all the data. Dr. DH provided input on data analysis and edited the manuscript. Dr. BT analyzed the data along with Drs. IM and HZ and had a pivotal contribution to statistics and manuscript editing. All authors have read and approved the manuscript.

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Authors' information: Drs. Moisini and Zhang contributed equally to this work.

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Figures

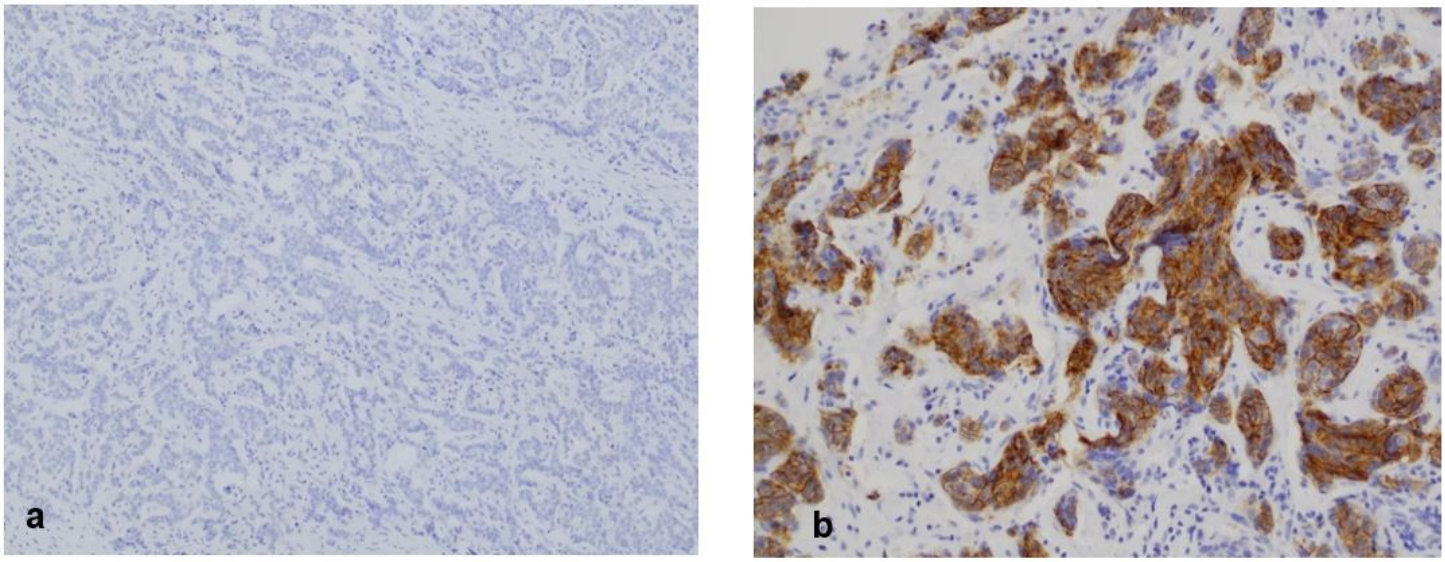

Figure 1

Figure 1