

ORIGINAL ARTICLE

# L1CAM/CD171 expression in human tumors and its association with tumor phenotype

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## ABSTRACT

**Background and purpose:** L1CAM (CD171) is suggested to play a critical role in cancer. Because of its expression in only few normal tissues and its membranous nature, L1CAM is a promising drug target.

**Patient/material and methods:** To clarify the role of L1CAM expression in different cancer types, a tissue microarray containing 20,079 samples from 135 different tumor entities and 608 samples of 76 different normal tissue types was analyzed by immunohistochemistry.

**Results:** Membranous L1CAM staining was found in 1,175 (9.1%) of 12,888 interpretable tumor samples, including 301 (2.3%) with weak, 569 (4.4%) with moderate, and 305 (2.4%) with strong positivity. 74 of 135 tumor entities showed L1CAM staining, and 36 tumor categories included at least one case with strong L1CAM staining. The frequency of L1CAM positivity was high in subtypes of neural and neuroendocrine neoplasms (up to 100%), endometrium carcinoma (24.1–31.3%), ovarian cancer (10.0–33.1%), cervical adenocarcinoma (9.1%), malignant melanoma (24.1–31.3%), malignant mesothelioma (16.7–20.8%), adenocarcinomas of the gastrointestinal and biliopancreatic tract (4.9–14.1%), and in urothelial tumors (up to 10.3%). High L1CAM expression was associated with invasive tumor growth (pTa vs. pT2–4) in urothelial carcinoma of the bladder ( $p < 0.0001$ ) and with mismatch repair deficiency in colorectal adenocarcinoma ( $p = 0.0064$ ). However, L1CAM staining was unrelated to tumor phenotype in seven other tumor entities.

**Interpretation:** The results highlighted a small number of tumor entities that could be targeted by anti-L1CAM drugs, once these are proved to be sufficiently safe and efficient. L1CAM expression does not appear to confer an aggressive phenotype to affected cancer cells.

## ARTICLE HISTORY

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## Introduction

The L1 cell adhesion molecule (CD171; L1CAM) is a transmembrane glycoprotein of the immunoglobulin superfamily which was initially characterized as a cell surface antigen of the central nervous system [1, 2]. L1CAM plays an important role in the regulation of neuronal cell migration, adhesion, neuronal differentiation, myelination and axon growth (summarized in [3]). Germline L1CAM mutations cause severe disorders of brain development, including corpus callosum hypoplasia, retardation, aphasia, spastic paraplegia and hydrocephalus (CRASH syndrome) [4]. In normal tissues, L1CAM expression occurs in only a few non-neural cell types. However, L1CAM protein can be aberrantly expressed in various cancer types [5, 6].

Data from various cancer studies have linked L1CAM protein expression to tumor progression [7], metastasis [8], therapy resistance [9], stemness [10], and dismal prognosis [7, 11]

(summarized in [12]). Evidence for a prognostic role of aberrant L1CAM expression has been found in endometrial cancer [13], oral squamous cell carcinoma [14], carcinosarcoma of the uterus [15], cervical cancer [16], serous carcinoma of the ovary [17], adenocarcinoma of the lung [18], ductal adenocarcinoma of the pancreas [19], esophageal squamous cell carcinoma [20], colorectal cancer [21] and neuroblastoma [22]. The potential role of L1CAM in tumor progression, in combination with its membranous location and its expression in only a few normal cell types, makes L1CAM an attractive candidate drug target. Targeting L1CAM protein in cancer cells is currently being evaluated by various approaches, including neutralizing antibodies [23], bispecific antibodies [24], radioimmunoconjugates [25] and chimeric antigen receptor-redirected T (CAR-T) cells [26]. More than 200 studies have so far analyzed L1CAM expression in cancer by immunohistochemistry (IHC). Although many of these studies have suggested associations

between L1CAM expression and increased cancer aggressiveness, the available data on the prevalence of L1CAM expression are remarkably discrepant. For example, L1CAM positivity ranged from 2 to 100% in neurofibroma, 24–95% in serous carcinoma of the ovary, 24–96% in malignant melanoma, 0–56% in colorectal adenocarcinoma, 3–100% in endometrial cancer, 21–100% in papillary renal cell carcinoma (RCC), 2–93% in ductal adenocarcinoma of the pancreas, 11–63% in hepatocellular carcinoma and 2–74% in gastrointestinal stromal tumor (GIST) (Table 3). These conflicting data may be caused by the use of different antibodies, immunostaining protocols and criteria to define L1CAM positivity in these studies.

To better understand the prevalence and clinical significance of L1CAM expression in cancer, a comprehensive study analyzing a large number of neoplastic and non-neoplastic tissues under highly standardized conditions is needed. Therefore, L1CAM expression was analyzed in more than 20,000 tumor tissue samples from 135 different tumor types and subtypes, as well as 76 non-neoplastic tissue categories, using IHC in a tissue microarray (TMA) format in this study.

## Patients/material and methods

### *Tissue microarrays*

Our normal TMA was composed of samples from 8 different donors from each of 76 different normal tissue categories (608 samples on one slide). The cancer TMAs included a total of 20,079 primary tumors from 135 different tumor types and subtypes. Detailed histopathological and molecular data were available for cancers of the bladder ( $n = 2,434$ ), colon ( $n = 2,351$ ), kidney ( $n = 1,757$ ), ovary (serous high-grade;  $n = 369$ ), endometrium (endometrioid;  $n = 182$ ), stomach ( $n = 327$ ), and the pancreas ( $n = 598$ ). The composition of normal and cancer TMAs is described in the results section. All samples were obtained from the archives of the Institute of Pathology, University Medical Center Hamburg-Eppendorf, Germany, the Institute of Pathology, Clinical Center Osnabrueck, Germany and the Department of Pathology, Academic Hospital Fuerth, Germany. Tissues were fixed in 4% buffered formalin and then embedded in paraffin. The TMA manufacturing process has been described earlier in detail [27, 28]. In brief, one tissue spot (diameter: 0.6 mm) per patient was used. The use of archived remnants of diagnostic tissues for TMA manufacturing, their analysis for research purposes, and the use of patient data were according to local laws (HmbKHG, §12) and data analysis had been approved by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

### *Immunohistochemistry*

Freshly cut TMA sections were stained on one day and in one experiment. Slides were deparaffinized with xylol, rehydrated through a graded alcohol series and exposed to heat-induced antigen retrieval for 5 minutes in an autoclave at 121°C in pH 7.8

Tris-EDTA-Citrat (TEC) buffer. Endogenous peroxidase activity was blocked with Dako REAL Peroxidase-Blocking Solution (Agilent Technologies, Santa Clara, CA, USA; #S2023) for 10 minutes. The primary antibody specific for L1CAM (recombinant rabbit monoclonal, MSVA-171R, MS Validated Antibodies GmbH, Hamburg, Germany, #6263-171R) was applied at 37°C for 60 minutes at a dilution of 1:100. For the purpose of antibody validation, the normal tissue TMA was also analyzed by the mouse monoclonal L1CAM antibody 14.10 (BioLegend, San Diego, CA, USA; #826701) at a dilution of 1:50 and an otherwise identical protocol. The bound antibody was then visualized using the Dako REAL EnVision Detection System Peroxidase/DAB+, Rabbit/Mouse kit (Agilent Technologies, Santa Clara, CA, USA; #K5007) according to the manufacturer's instructions. The sections were counterstained with hemalaun. For tumor tissues, the percentage of positive neoplastic cells was estimated, and the staining intensity was semi-quantitatively recorded (0, 1+, 2+, 3+). For statistical analyses, the staining results were categorized into four groups. Tumors without any staining and positive staining in less than 10% of tumor cells were considered negative. Tumors with 1+ staining intensity in  $\leq 70\%$  of tumor cells or 2+ intensity in  $\leq 30\%$  of tumor cells were considered weakly positive. Tumors with 1+ staining intensity in  $> 70\%$  of tumor cells, 2+ intensity in 31–70%, or 3+ intensity in  $\leq 30\%$  of tumor cells were considered moderately positive. Tumors with 2+ intensity in  $> 70\%$  or 3+ intensity in  $> 30\%$  of tumor cells were considered strongly positive.

### *Statistics*

Statistical calculations were performed with JMP17® software (SAS®, Cary, NC, USA). Contingency tables and the  $\chi^2$ -test were performed to search for associations between L1CAM immunostaining and tumor phenotype.

## Results

### *Technical issues*

A total of 12,888 (64.2%) of 20,079 tumor samples were interpretable in our TMA analysis. Non-interpretable samples demonstrated lack of unequivocal tumor cells or lack of entire tissue spots. A sufficient number of samples ( $\geq 4$ ) of each normal tissue type was evaluable.

### *L1CAM in normal tissues*

L1CAM staining was most intense in nerve fibers, especially in the grey matter of the cerebrum, the molecular layer of the cerebellum and the neurohypophysis, but also in many other organs, such as for example the muscular wall and the mucosa of the gastrointestinal tract, as well as in the stroma of the prostate and the seminal vesicles. A strong membranous L1CAM staining also occurred in medullary cells of the adrenal gland, a fraction of collecting ducts of the kidney, a small fraction of epithelial cells of the fallopian tube and – less intense – in chorion

cells of the placenta and a subset of epithelial cells in the adenohypophysis. A weak to moderate L1CAM staining was also seen in subsets of B-cells and monocytic cells, mainly in the germinal centers of lymphatic tissues. Very rarely, L1CAM staining was also observed in endothelial cells. Representative images are shown in Figure 1. All these cell types identified by MSVA-171R were confirmed by clone 14.10 on consecutive tissue sections (Supplementary Figure 1). Except for nerve fibers, L1CAM staining was absent in thyroid, parathyroid gland, respiratory epithelium, lung, salivary glands, esophagus, liver, gallbladder, pancreas, urothelium, testis, epididymis, breast, myometrium of the uterus, ectocervix, endocervix, and endometrium of the uterus, ovary, first trimester and mature placenta, amnion, epidermis, sebaceous glands, skeletal heart, and smooth muscle, vessel walls, fat, bone marrow, spleen and the thymus.

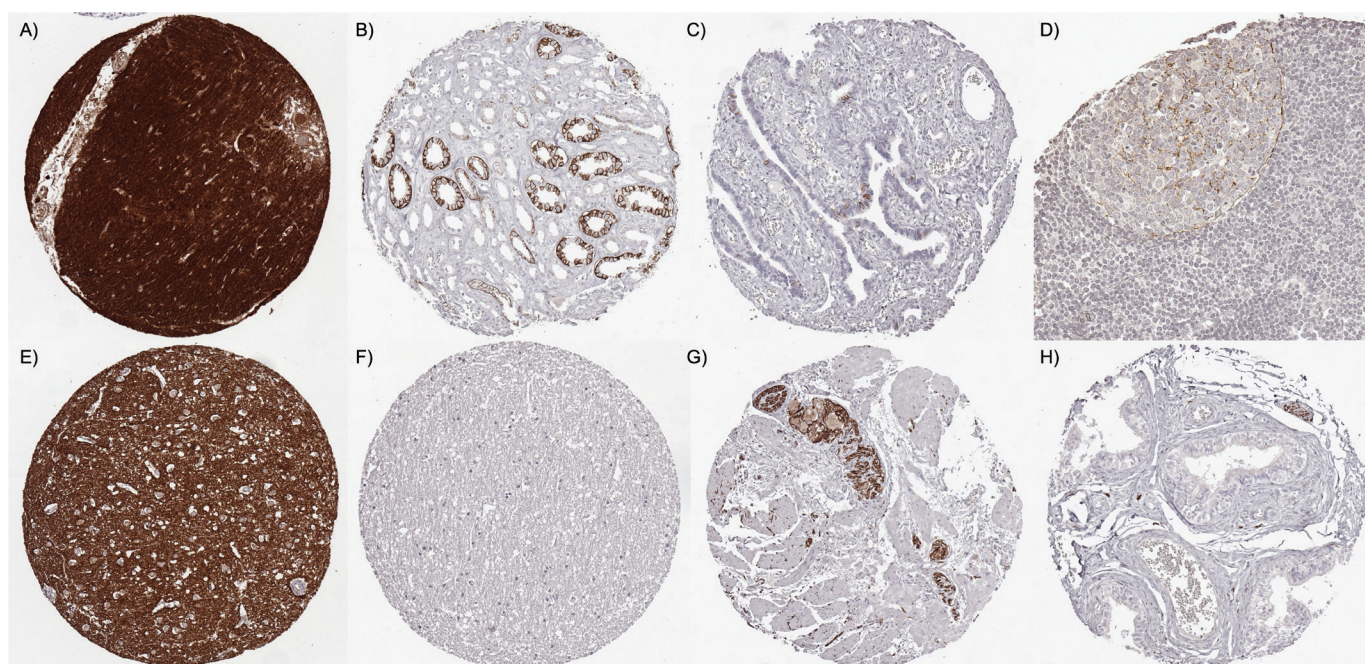
### L1CAM in tumor tissues

A predominantly membranous L1CAM staining was found in 1,175 (9.1%) of the 12,888 interpretable tumor samples, including 301 (2.3%) with weak, 569 (4.4%) with moderate, and 305 (2.4%) with strong positivity. A total of 74 of 135 tumor entities (54.8%) showed L1CAM staining in at least one case, and 36 tumor categories (26.7%) included at least one case with strong L1CAM staining (Table 1). The frequency of L1CAM positivity was particularly high in several subtypes of neural and neuroendocrine neoplasms (up to 100%), endometrium carcinoma (3.9–50.0%), ovarian cancer (10.0–33.1%), cervical adenocarcinoma (9.1%), malignant melanoma (24.1–31.3%), malignant mesothelioma (16.7–20.8%), adenocarcinomas of the gastrointestinal

and the biliopancreatic tract (4.9–14.1%) and in urothelial tumors (up to 10.3%). Representative images are shown in Figure 2. A graphical representation of a ranking order of L1CAM positive cancers and of strongly positive cancers is given in Figure 3. The relationship between L1CAM immunostaining and parameters of cancer aggressiveness is summarized in Table 2. High L1CAM expression was associated with invasive tumor growth (pTa vs. pT2–4) in urothelial carcinoma of the bladder ( $p < 0.0001$ ) and with mismatch repair protein deficiency ( $p = 0.0064$ ) and left-sided tumor location ( $p = 0.0104$ ) in colorectal adenocarcinoma. L1CAM staining was unrelated to tumor phenotype in muscle-invasive urothelial carcinoma, clear cell and papillary RCC, serous high-grade carcinoma of the ovary, endometrioid endometrium carcinoma, gastric adenocarcinoma and in pancreatic ductal adenocarcinoma.

### Discussion and conclusion

Our analysis of 12,888 tumors from 135 different tumor categories provides a comprehensive overview of L1CAM expression on tumor cells in different cancer types. Considering the heterogeneity of literature data (summarized in Figure 4), such information could hardly be compiled from published studies. That particularly high L1CAM positivity rates were observed on tumor cells in neural and neuroendocrine cancers fits with the expression of L1CAM in corresponding normal tissues and with data from the literature [5, 6]. Considering the absence of L1CAM expression in corresponding normal cell types, L1CAM tumor cell positivity of most other tumor types might reflect neo-expression of the protein. Despite conflicting literature data,



**Figure 1.** L1CAM immunostaining of normal tissues. The panel shows intense staining in the cerebellar cortex (A), strong predominantly membranous staining in a subset of collecting duct cells of the kidney medulla (B), strong staining of a small subset of epithelial cells of fallopian tube (C), weak to moderate staining of subsets of B-cells and monocytic cells, mainly of the germinal center of lymph node (D), intense staining in the grey matter of the cerebrum (E), lack of staining of the white matter of the cerebrum (F), strong staining of nerve fibers of the muscular wall of the esophagus (G) and lack of staining in the epididymis (staining only occurs in a nerve) (H). L1CAM: L1 cell adhesion molecule.

Table 1. L1CAM immunostaining in human tumors.

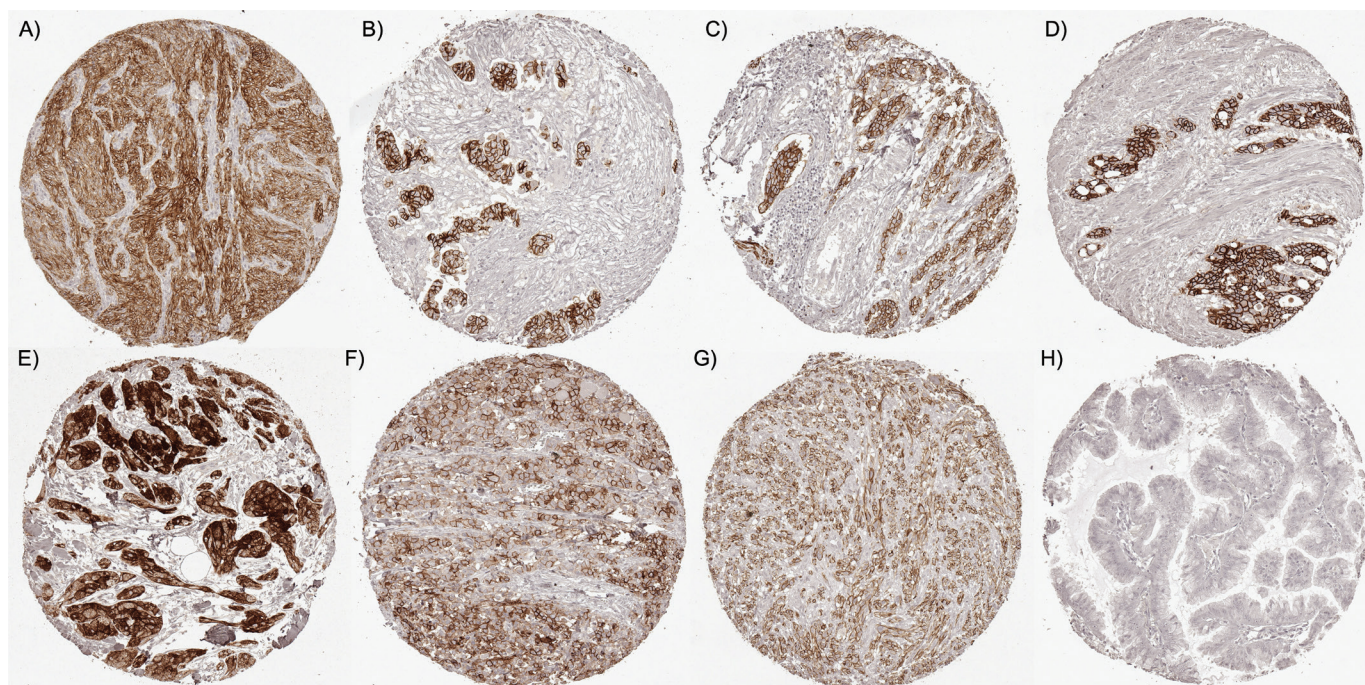
|                                       |                                                   | L1CAM immunostaining |                |              |          |              |            |
|---------------------------------------|---------------------------------------------------|----------------------|----------------|--------------|----------|--------------|------------|
| Tumor entity                          |                                                   | on TMA (n)           | analyzable (n) | negative (%) | weak (%) | moderate (%) | strong (%) |
| Tumors of the skin                    | Pilomatricoma                                     | 35                   | 35             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Basal cell carcinoma of the skin                  | 89                   | 35             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Squamous cell carcinoma of the skin               | 145                  | 87             | 96,6         | 0,0      | 3,4          | 0,0        |
|                                       | Malignant melanoma                                | 65                   | 16             | 68,8         | 0,0      | 18,8         | 12,5       |
|                                       | Malignant melanoma lymph node metastasis          | 86                   | 79             | 75,9         | 7,6      | 11,4         | 5,1        |
|                                       | Merkel cell carcinoma                             | 48                   | 1              | 0,0          | 0,0      | 100,0        | 0,0        |
| Tumors of the head and neck           | Squamous cell carcinoma of the larynx             | 109                  | 99             | 99,0         | 0,0      | 1,0          | 0,0        |
|                                       | Squamous cell carcinoma of the pharynx            | 60                   | 57             | 98,2         | 1,8      | 0,0          | 0,0        |
|                                       | Oral squamous cell carcinoma (floor of the mouth) | 130                  | 121            | 96,7         | 0,0      | 3,3          | 0,0        |
|                                       | Pleomorphic adenoma of the parotid gland          | 50                   | 45             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Warthin tumor of the parotid gland                | 104                  | 46             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Basal cell adenoma of the salivary gland          | 101                  | 15             | 100,0        | 0,0      | 0,0          | 0,0        |
| Tumors of the lung, pleura and thymus | Adenocarcinoma of the lung                        | 246                  | 179            | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Squamous cell carcinoma of the lung               | 130                  | 73             | 97,3         | 2,7      | 0,0          | 0,0        |
|                                       | Mesothelioma, epithelioid                         | 40                   | 30             | 83,3         | 3,3      | 6,7          | 6,7        |
|                                       | Mesothelioma, biphasic                            | 77                   | 24             | 79,2         | 0,0      | 12,5         | 8,3        |
|                                       | Thymoma                                           | 29                   | 27             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Lung, neuroendocrine tumor (NET)                  | 29                   | 29             | 72,4         | 6,9      | 17,2         | 3,4        |
| Tumors of the female genital tract    | Squamous cell carcinoma of the vagina             | 78                   | 29             | 93,1         | 3,4      | 3,4          | 0,0        |
|                                       | Squamous cell carcinoma of the vulva              | 157                  | 99             | 97,0         | 2,0      | 1,0          | 0,0        |
|                                       | Squamous cell carcinoma of the cervix             | 315                  | 87             | 95,4         | 2,3      | 2,3          | 0,0        |
|                                       | Adenocarcinoma of the cervix                      | 56                   | 22             | 90,9         | 4,5      | 4,5          | 0,0        |
|                                       | Endometrioid endometrial carcinoma                | 338                  | 255            | 96,1         | 0,0      | 3,1          | 0,8        |
|                                       | Endometrial serous carcinoma                      | 86                   | 30             | 50,0         | 0,0      | 36,7         | 13,3       |
|                                       | Carcinosarcoma of the uterus                      | 57                   | 55             | 72,7         | 5,5      | 9,1          | 12,7       |
|                                       | Endometrial carcinoma, high grade, G3             | 13                   | 13             | 92,3         | 7,7      | 0,0          | 0,0        |
|                                       | Endometrial clear cell carcinoma                  | 9                    | 8              | 50,0         | 0,0      | 25,0         | 25,0       |
|                                       | Endometrioid carcinoma of the ovary               | 130                  | 78             | 79,5         | 7,7      | 11,5         | 1,3        |
|                                       | Serous carcinoma of the ovary                     | 580                  | 459            | 66,9         | 9,2      | 19,0         | 5,0        |
|                                       | Mucinous carcinoma of the ovary                   | 101                  | 57             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Clear cell carcinoma of the ovary                 | 51                   | 47             | 85,1         | 8,5      | 4,3          | 2,1        |
|                                       | Carcinosarcoma of the ovary                       | 47                   | 40             | 90,0         | 5,0      | 5,0          | 0,0        |
|                                       | Granulosa cell tumor of the ovary                 | 44                   | 41             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Leydig cell tumor of the ovary                    | 4                    | 4              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Sertoli cell tumor of the ovary                   | 1                    | 1              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Sertoli Leydig cell tumor of the ovary            | 3                    | 3              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Steroid cell tumor of the ovary                   | 3                    | 3              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Brenner tumor                                     | 41                   | 28             | 100,0        | 0,0      | 0,0          | 0,0        |
| Tumors of the breast                  | Invasive breast carcinoma of no special type      | 1810                 | 475            | 97,3         | 1,3      | 0,8          | 0,6        |
|                                       | Lobular carcinoma of the breast                   | 364                  | 132            | 99,2         | 0,0      | 0,8          | 0,0        |
|                                       | Medullary carcinoma of the breast                 | 34                   | 8              | 75,0         | 0,0      | 12,5         | 12,5       |
|                                       | Tubular carcinoma of the breast                   | 29                   | 2              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Mucinous carcinoma of the breast                  | 65                   | 6              | 100,0        | 0,0      | 0,0          | 0,0        |
| Tumors of the digestive system        | Adenomatous polyp, low-grade dysplasia            | 50                   | 20             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Adenomatous polyp, high-grade dysplasia           | 50                   | 34             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Adenocarcinoma of the colon                       | 2533                 | 2177           | 87,0         | 2,3      | 8,7          | 1,9        |
|                                       | Gastric adenocarcinoma, diffuse type              | 265                  | 177            | 99,4         | 0,0      | 0,6          | 0,0        |
|                                       | Gastric adenocarcinoma, intestinal type           | 265                  | 176            | 89,2         | 1,7      | 9,1          | 0,0        |
|                                       | Gastric adenocarcinoma, mixed type                | 62                   | 48             | 93,8         | 0,0      | 4,2          | 2,1        |
|                                       | Adenocarcinoma of the esophagus                   | 133                  | 78             | 88,5         | 3,8      | 5,1          | 2,6        |
|                                       | Squamous cell carcinoma of the esophagus          | 125                  | 70             | 95,7         | 0,0      | 2,9          | 1,4        |
|                                       | Squamous cell carcinoma of the anal canal         | 91                   | 67             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Cholangiocarcinoma                                | 121                  | 38             | 97,4         | 0,0      | 2,6          | 0,0        |
|                                       | Gallbladder adenocarcinoma                        | 51                   | 42             | 92,9         | 0,0      | 4,8          | 2,4        |
|                                       | Gallbladder Klatskin tumor                        | 42                   | 29             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Hepatocellular carcinoma                          | 312                  | 259            | 97,3         | 1,2      | 1,2          | 0,4        |
|                                       | Ductal adenocarcinoma of the pancreas             | 709                  | 430            | 95,1         | 2,1      | 1,9          | 0,9        |
|                                       | Pancreatic/Ampullary adenocarcinoma               | 128                  | 64             | 85,9         | 3,1      | 10,9         | 0,0        |
|                                       | Acinar cell carcinoma of the pancreas             | 18                   | 17             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Gastrointestinal stromal tumor (GIST)             | 62                   | 55             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Appendix, neuroendocrine tumor (NET)              | 25                   | 16             | 81,3         | 12,5     | 6,3          | 0,0        |
|                                       | Colorectal, neuroendocrine tumor (NET)            | 12                   | 9              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Ileum, neuroendocrine tumor (NET)                 | 53                   | 49             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Pancreas, neuroendocrine tumor (NET)              | 101                  | 80             | 92,5         | 1,3      | 6,3          | 0,0        |
|                                       | Colorectal, neuroendocrine carcinoma (NEC)        | 14                   | 11             | 72,7         | 18,2     | 9,1          | 0,0        |
|                                       | Ileum, neuroendocrine carcinoma (NEC)             | 8                    | 7              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                       | Gallbladder, neuroendocrine carcinoma (NEC)       | 4                    | 4              | 50,0         | 0,0      | 50,0         | 0,0        |
|                                       | Pancreas, neuroendocrine carcinoma (NEC)          | 14                   | 14             | 78,6         | 0,0      | 21,4         | 0,0        |

Continued

Table 1. L1CAM immunostaining in human tumors. (continued)

|                                              |                                                                | L1CAM immunostaining |                |              |          |              |            |
|----------------------------------------------|----------------------------------------------------------------|----------------------|----------------|--------------|----------|--------------|------------|
| Tumor entity                                 |                                                                | on TMA (n)           | analyzable (n) | negative (%) | weak (%) | moderate (%) | strong (%) |
| Tumors of the urinary system                 | Non-invasive papillary urothelial carcinoma, pTa G2 low grade  | 264                  | 78             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Non-invasive papillary urothelial carcinoma, pTa G2 high grade | 221                  | 71             | 98,6         | 0,0      | 1,4          | 0,0        |
|                                              | Non-invasive papillary urothelial carcinoma, pTa G3            | 313                  | 112            | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Urothelial carcinoma, pT2-4 G3                                 | 1318                 | 514            | 89,7         | 2,5      | 6,2          | 1,6        |
|                                              | Squamous cell carcinoma of the bladder                         | 22                   | 20             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Small cell neuroendocrine carcinoma of the bladder             | 23                   | 5              | 60,0         | 0,0      | 40,0         | 0,0        |
|                                              | Sarcomatoid urothelial carcinoma                               | 25                   | 22             | 90,9         | 0,0      | 0,0          | 9,1        |
|                                              | Urothelial carcinoma of the kidney pelvis                      | 62                   | 54             | 96,3         | 0,0      | 3,7          | 0,0        |
|                                              | Clear cell renal cell carcinoma                                | 1655                 | 1086           | 91,8         | 6,7      | 0,9          | 0,6        |
|                                              | Papillary renal cell carcinoma                                 | 433                  | 290            | 91,7         | 0,3      | 5,2          | 2,8        |
|                                              | Clear cell (tubulo) papillary renal cell carcinoma             | 33                   | 18             | 88,9         | 5,6      | 5,6          | 0,0        |
|                                              | Chromophobe renal cell carcinoma                               | 190                  | 139            | 92,1         | 1,4      | 0,7          | 5,8        |
|                                              | Oncocytoma of the kidney                                       | 279                  | 209            | 93,8         | 0,0      | 2,9          | 3,3        |
| Tumors of the male genital organs            | Adenocarcinoma of the prostate, Gleason 3+3                    | 83                   | 82             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Adenocarcinoma of the prostate, Gleason 4+4                    | 80                   | 78             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Adenocarcinoma of the prostate, Gleason 5+5                    | 85                   | 85             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Adenocarcinoma of the prostate (recurrence)                    | 258                  | 227            | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Small cell neuroendocrine carcinoma of the prostate            | 19                   | 2              | 50,0         | 0,0      | 0,0          | 50,0       |
|                                              | Seminoma                                                       | 682                  | 625            | 98,2         | 1,6      | 0,2          | 0,0        |
|                                              | Embryonal carcinoma of the testis                              | 54                   | 39             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Leydig cell tumor of the testis                                | 31                   | 29             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Sertoli cell tumor of the testis                               | 2                    | 1              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Sex cord stromal tumor of the testis                           | 1                    | 1              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Spermatocytic tumor of the testis                              | 1                    | 1              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Yolk sac tumor                                                 | 53                   | 37             | 94,6         | 0,0      | 5,4          | 0,0        |
|                                              | Teratoma                                                       | 53                   | 24             | 91,7         | 0,0      | 8,3          | 0,0        |
|                                              | Squamous cell carcinoma of the penis                           | 92                   | 86             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Adenoma of the thyroid gland                                   | 113                  | 63             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Papillary thyroid carcinoma                                    | 391                  | 329            | 99,7         | 0,0      | 0,3          | 0,0        |
|                                              | Follicular thyroid carcinoma                                   | 154                  | 105            | 100,0        | 0,0      | 0,0          | 0,0        |
| Tumors of endocrine organs                   | Medullary thyroid carcinoma                                    | 111                  | 55             | 98,2         | 1,8      | 0,0          | 0,0        |
|                                              | Parathyroid gland adenoma                                      | 43                   | 39             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Anaplastic thyroid carcinoma                                   | 45                   | 19             | 94,7         | 0,0      | 5,3          | 0,0        |
|                                              | Adrenal cortical adenoma                                       | 48                   | 41             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Adrenal cortical carcinoma                                     | 27                   | 17             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Pheochromocytoma                                               | 51                   | 44             | 4,5          | 4,5      | 29,5         | 61,4       |
| Tumors of hematopoietic and lymphoid tissues | Hodgkin's lymphoma                                             | 103                  | 98             | 99,0         | 0,0      | 1,0          | 0,0        |
|                                              | Small lymphocytic lymphoma, B-cell type (B-SLL/B-CLL)          | 50                   | 49             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Diffuse large B cell lymphoma (DLBCL)                          | 113                  | 112            | 91,1         | 2,7      | 5,4          | 0,9        |
|                                              | Follicular lymphoma                                            | 88                   | 87             | 97,7         | 0,0      | 2,3          | 0,0        |
|                                              | T-cell non-Hodgkin's lymphoma                                  | 25                   | 25             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Mantle cell lymphoma                                           | 18                   | 18             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Marginal zone lymphoma                                         | 16                   | 16             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Diffuse large B-cell lymphoma (DLBCL) in the testis            | 16                   | 16             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Burkitt lymphoma                                               | 5                    | 5              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Granular cell tumor                                            | 53                   | 17             | 5,9          | 0,0      | 0,0          | 94,1       |
|                                              | Leiomyoma                                                      | 50                   | 48             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Leiomyosarcoma                                                 | 94                   | 90             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Liposarcoma                                                    | 145                  | 93             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Malignant peripheral nerve sheath tumor (MPNST)                | 15                   | 15             | 86,7         | 6,7      | 6,7          | 0,0        |
|                                              | Myofibrosarcoma                                                | 26                   | 26             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Angiosarcoma                                                   | 74                   | 36             | 97,2         | 0,0      | 2,8          | 0,0        |
|                                              | Angiomyolipoma                                                 | 91                   | 87             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Dermatofibrosarcoma protuberans                                | 21                   | 18             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Ganglioneuroma                                                 | 14                   | 14             | 7,1          | 0,0      | 21,4         | 71,4       |
|                                              | Kaposi sarcoma                                                 | 8                    | 5              | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Neurofibroma                                                   | 117                  | 106            | 59,4         | 25,5     | 12,3         | 2,8        |
|                                              | Sarcoma, not otherwise specified (NOS)                         | 74                   | 70             | 95,7         | 0,0      | 4,3          | 0,0        |
|                                              | Paraganglioma                                                  | 41                   | 39             | 0,0          | 7,7      | 25,6         | 66,7       |
|                                              | Ewing sarcoma                                                  | 23                   | 15             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Rhabdomyosarcoma                                               | 7                    | 7              | 85,7         | 0,0      | 0,0          | 14,3       |
|                                              | Schwannoma                                                     | 122                  | 116            | 11,2         | 5,2      | 19,8         | 63,8       |
|                                              | Synovial sarcoma                                               | 12                   | 11             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Osteosarcoma                                                   | 44                   | 17             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Chondrosarcoma                                                 | 40                   | 12             | 100,0        | 0,0      | 0,0          | 0,0        |
|                                              | Rhabdoid tumor                                                 | 5                    | 5              | 80,0         | 0,0      | 20,0         | 0,0        |
|                                              | Solitary fibrous tumor                                         | 17                   | 17             | 100,0        | 0,0      | 0,0          | 0,0        |

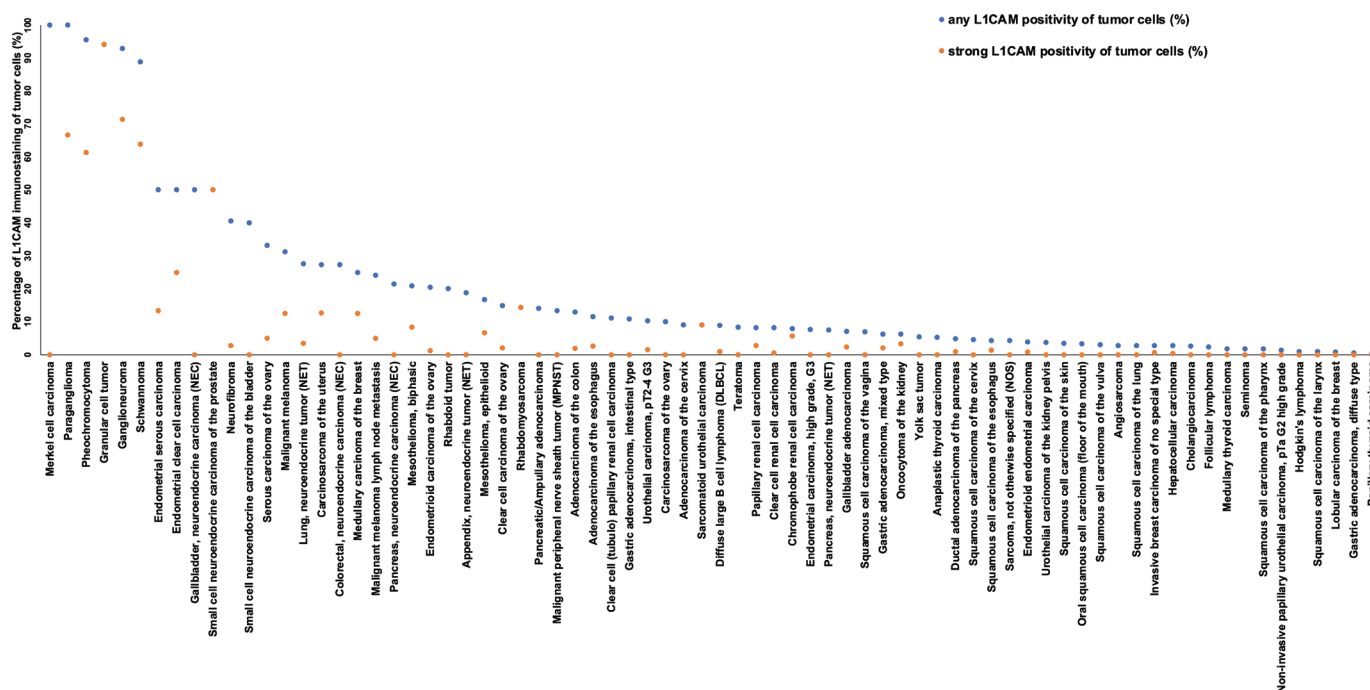
L1CAM: L1 cell adhesion molecule; TMA: tissue microarray.



**Figure 2.** L1CAM immunostaining in cancer. The panel shows an intense, predominantly membranous L1CAM immunostaining in a neuroendocrine tumor of esophagus (A), a serous high-grade ovarian carcinoma (B), an invasive urothelial carcinoma (C), a gastric adenocarcinoma (D), a granular cell tumor (E), a colorectal adenocarcinoma (F) and a melanoma (G). L1CAM staining is lacking in a pulmonary adenocarcinoma (H). L1CAM: L1 cell adhesion molecule.

particularly high rates of L1CAM tumor cell positivity in tumors arising from endometrium, ovary, endocervix, mesothelium, the gastrointestinal and the biliopancreatic tract, as well as in malignant melanoma are consistent with at least a fraction of previous studies (Table 3). The ranking order of tumor entities according to their rate of L1CAM tumor cell expression and the observation that high-level L1CAM expression on tumor cells

can occur in more than 40 different tumor categories, represent the key results of this study. L1CAM belongs to a group of potential new molecules in the field of targeted cancer therapy, and several approaches targeting L1CAM are currently being evaluated, including neutralizing antibodies [23], bispecific antibodies [24], radioimmunoconjugates [25], and CAR-T cells [26]. Our ranking of L1CAM expression prevalence highlights the most



**Figure 3.** Ranking order of L1CAM immunostaining in tumors. Both the percentage of positive cases (blue dots) and the percentage of strongly positive cases (orange dots) are shown. L1CAM: L1 cell adhesion molecule.

**Table 2.** L1CAM immunostaining and tumor phenotype.

|                                 |                | n   | L1CAM immunostaining |          |              |            | P       |
|---------------------------------|----------------|-----|----------------------|----------|--------------|------------|---------|
|                                 |                |     | negative (%)         | weak (%) | moderate (%) | strong (%) |         |
| Clear cell renal cell carcinoma | ISUP 1         | 243 | 85,6                 | 3,7      | 10,3         | 0,4        | 0,0014  |
|                                 | ISUP 2         | 364 | 93,1                 | 1,4      | 5,5          | 0,0        |         |
|                                 | ISUP 3         | 239 | 93,7                 | 1,7      | 3,3          | 1,3        |         |
|                                 | ISUP 4         | 74  | 90,5                 | 1,4      | 4,1          | 4,1        |         |
|                                 | Fuhrman 1      | 61  | 77,0                 | 6,6      | 14,8         | 1,6        | <0.0001 |
|                                 | Fuhrman 2      | 616 | 92,0                 | 1,9      | 6,0          | 0,0        |         |
|                                 | Fuhrman 3      | 266 | 93,6                 | 1,1      | 3,4          | 1,9        |         |
|                                 | Fuhrman 4      | 87  | 90,8                 | 2,3      | 3,4          | 3,4        |         |
|                                 | Thoenes 1      | 324 | 88,9                 | 3,4      | 7,4          | 0,3        | 0,0431  |
|                                 | Thoenes 2      | 437 | 92,7                 | 1,6      | 5,0          | 0,7        |         |
|                                 | Thoenes 3      | 97  | 89,7                 | 2,1      | 4,1          | 4,1        |         |
|                                 | UICC 1         | 310 | 88,4                 | 4,2      | 7,1          | 0,3        | 0,0976  |
|                                 | UICC 2         | 35  | 94,3                 | 0,0      | 2,9          | 2,9        |         |
|                                 | UICC 3         | 87  | 87,4                 | 1,1      | 9,2          | 2,3        |         |
|                                 | UICC 4         | 69  | 92,8                 | 0,0      | 5,8          | 1,4        |         |
|                                 | pT1            | 611 | 90,8                 | 2,9      | 5,9          | 0,3        | 0,0364  |
|                                 | pT2            | 120 | 95,0                 | 1,7      | 2,5          | 0,8        |         |
|                                 | pT3-4          | 306 | 91,5                 | 0,7      | 6,2          | 1,6        |         |
|                                 | pN0            | 152 | 90,1                 | 0,7      | 8,6          | 0,7        | 0,6900  |
|                                 | pN+            | 26  | 96,2                 | 0,0      | 3,8          | 0,0        |         |
|                                 | pM0            | 100 | 86,0                 | 2,0      | 10,0         | 2,0        | 0,4592  |
|                                 | pM+            | 87  | 90,8                 | 1,1      | 4,6          | 3,4        |         |
| Papillary renal cell carcinoma  | ISUP 1         | 37  | 97,3                 | 0,0      | 2,7          | 0,0        | 0,4662  |
|                                 | ISUP 2         | 119 | 92,4                 | 1,7      | 0,8          | 5,0        |         |
|                                 | ISUP 3         | 66  | 89,4                 | 3,0      | 4,5          | 3,0        |         |
|                                 | ISUP 4         | 5   | 100,0                | 0,0      | 0,0          | 0,0        |         |
|                                 | Fuhrman 1      | 3   | 100,0                | 0,0      | 0,0          | 0,0        | 0,8732  |
|                                 | Fuhrman 2      | 164 | 93,3                 | 0,6      | 2,4          | 3,7        |         |
|                                 | Fuhrman 3      | 68  | 91,2                 | 2,9      | 2,9          | 2,9        |         |
|                                 | Fuhrman 4      | 9   | 88,9                 | 0,0      | 11,1         | 0,0        |         |
|                                 | Thoenes 1      | 54  | 98,1                 | 0,0      | 1,9          | 0,0        | 0,0841  |
|                                 | Thoenes 2      | 137 | 92,7                 | 1,5      | 1,5          | 4,4        |         |
|                                 | Thoenes 3      | 14  | 85,7                 | 0,0      | 14,3         | 0,0        |         |
|                                 | UICC 1         | 91  | 95,6                 | 0,0      | 2,2          | 2,2        | 0,1880  |
|                                 | UICC 2         | 16  | 93,8                 | 0,0      | 6,3          | 0,0        |         |
|                                 | UICC 3         | 4   | 100,0                | 0,0      | 0,0          | 0,0        |         |
|                                 | UICC 4         | 9   | 66,7                 | 0,0      | 11,1         | 22,2       |         |
|                                 | pT1            | 179 | 93,9                 | 2,8      | 1,7          | 1,7        | 0,0940  |
|                                 | pT2            | 44  | 90,9                 | 0,0      | 4,5          | 4,5        |         |
|                                 | pT3-4          | 27  | 77,8                 | 3,7      | 7,4          | 11,1       |         |
|                                 | pN0            | 18  | 88,9                 | 0,0      | 5,6          | 5,6        | 0,1721  |
|                                 | pN+            | 13  | 69,2                 | 7,7      | 23,1         | 0,0        |         |
|                                 | pM0            | 23  | 95,7                 | 0,0      | 4,3          | 0,0        | 0,0464  |
|                                 | pM+            | 11  | 63,6                 | 9,1      | 9,1          | 18,2       |         |
| Urothelial bladder carcinoma    | pTa G2 low     | 353 | 100,0                | 0,0      | 0,0          | 0,0        | 0,0640  |
|                                 | pTa G2 high    | 153 | 98,7                 | 1,3      | 0,0          | 0,0        |         |
|                                 | pTa G3         | 96  | 100,0                | 0,0      | 0,0          | 0,0        |         |
|                                 | pT2            | 376 | 90,2                 | 6,4      | 0,8          | 2,7        | 0,9062* |
|                                 | pT3            | 484 | 88,6                 | 7,0      | 1,2          | 3,1        |         |
|                                 | pT4            | 241 | 88,4                 | 6,6      | 2,1          | 2,9        |         |
|                                 | G2             | 87  | 95,4                 | 2,3      | 0,0          | 2,3        | 0,9210* |
|                                 | G3             | 992 | 88,6                 | 7,1      | 1,4          | 2,9        |         |
|                                 | pN0            | 521 | 87,3                 | 8,4      | 1,3          | 2,9        | 0,1488* |
|                                 | pN+            | 359 | 91,4                 | 4,7      | 0,8          | 3,1        |         |
| Adenocarcinoma of the pancreas  | pT1            | 15  | 53,3                 | 33,3     | 13,3         | 0,0        | 0,1027  |
|                                 | pT2            | 72  | 41,7                 | 29,2     | 15,3         | 13,9       |         |
|                                 | pT3            | 321 | 61,1                 | 23,4     | 10,6         | 5,0        |         |
|                                 | pT4            | 34  | 52,9                 | 29,4     | 11,8         | 5,9        |         |
|                                 | G1             | 16  | 43,8                 | 25,0     | 18,8         | 12,5       | 0,5170  |
|                                 | G2             | 312 | 55,1                 | 26,0     | 12,5         | 6,4        |         |
|                                 | G3             | 93  | 64,5                 | 21,5     | 7,5          | 6,5        |         |
|                                 | pN0            | 96  | 59,4                 | 21,9     | 12,5         | 6,3        | 0,8568  |
|                                 | pN+            | 345 | 56,2                 | 26,1     | 11,3         | 6,4        |         |
|                                 | MMR proficient | 388 | 56,4                 | 25,5     | 11,9         | 6,2        | 0,3321  |
|                                 | MMR deficient  | 3   | 100,0                | 0,0      | 0,0          | 0,0        |         |

Continued

|                                    |                     |      |      |      |     |     |        |
|------------------------------------|---------------------|------|------|------|-----|-----|--------|
| Adenocarcinoma of the stomach      | pT1-2               | 42   | 88,1 | 9,5  | 2,4 | 0,0 | 0,1991 |
|                                    | pT3                 | 101  | 82,2 | 11,9 | 4,0 | 2,0 |        |
|                                    | pT4                 | 95   | 93,7 | 4,2  | 2,1 | 0,0 |        |
|                                    | pN0                 | 60   | 91,7 | 8,3  | 0,0 | 0,0 | 0,1437 |
|                                    | pN+                 | 178  | 86,0 | 9,0  | 3,9 | 1,1 |        |
|                                    | MMR proficient      | 207  | 85,5 | 10,1 | 2,9 | 1,4 | 0,9461 |
|                                    | MMR deficient       | 29   | 96,6 | 0,0  | 3,4 | 0,0 |        |
| Endometrioid endometrial carcinoma | pT1                 | 105  | 95,2 | 4,8  | 0,0 | 0,0 | 0,3796 |
|                                    | pT2                 | 24   | 95,8 | 4,2  | 0,0 | 0,0 |        |
|                                    | pT3-4               | 36   | 91,7 | 2,8  | 2,8 | 2,8 |        |
|                                    | pN0                 | 49   | 95,9 | 2,0  | 0,0 | 2,0 | 0,3879 |
|                                    | pN+                 | 30   | 93,3 | 3,3  | 3,3 | 0,0 |        |
| Serous carcinoma of the ovary      | pT1                 | 29   | 75,9 | 17,2 | 0,0 | 6,9 | 0,1105 |
|                                    | pT2                 | 41   | 58,5 | 29,3 | 2,4 | 9,8 |        |
|                                    | pT3                 | 246  | 54,5 | 32,5 | 7,3 | 5,7 |        |
|                                    | pN0                 | 81   | 65,4 | 23,5 | 6,2 | 4,9 | 0,3687 |
|                                    | pN1                 | 156  | 53,8 | 33,3 | 7,1 | 5,8 |        |
| Adenocarcinoma of the colon        | pT1                 | 77   | 81,8 | 13,0 | 5,2 | 0,0 | 0,1943 |
|                                    | pT2                 | 410  | 78,5 | 16,6 | 2,0 | 2,9 |        |
|                                    | pT3                 | 1162 | 81,0 | 14,6 | 2,6 | 1,8 |        |
|                                    | pT4                 | 419  | 84,5 | 11,7 | 1,9 | 1,9 |        |
|                                    | pN0                 | 1076 | 80,8 | 15,1 | 2,3 | 1,9 | 0,6921 |
|                                    | pN+                 | 985  | 82,1 | 13,3 | 2,4 | 2,1 |        |
|                                    | V0                  | 1503 | 81,2 | 14,4 | 2,3 | 2,1 | 0,9517 |
|                                    | V1                  | 529  | 81,7 | 13,8 | 2,6 | 1,9 |        |
|                                    | L0                  | 684  | 81,9 | 14,5 | 2,2 | 1,5 | 0,6334 |
|                                    | L1                  | 1357 | 81,4 | 14,0 | 2,3 | 2,3 |        |
|                                    | right side          | 442  | 85,1 | 12,0 | 1,1 | 1,8 | 0,0104 |
|                                    | left side           | 1175 | 78,7 | 15,9 | 3,1 | 2,2 |        |
|                                    | MMR proficient      | 1127 | 78,5 | 16,6 | 2,8 | 2,0 | 0,0064 |
|                                    | MMR deficient       | 83   | 92,8 | 4,8  | 1,2 | 1,2 |        |
|                                    | RAS wildtype        | 429  | 80,0 | 15,9 | 2,6 | 1,6 | 0,5177 |
|                                    | RAS mutation        | 340  | 78,2 | 16,5 | 3,2 | 2,1 |        |
|                                    | BRAF wildtype       | 128  | 82,0 | 14,1 | 2,3 | 1,6 | 0,5779 |
|                                    | BRAF V600E mutation | 21   | 90,5 | 9,5  | 0,0 | 0,0 |        |

\*only in pT2-4 urothelial bladder carcinomas, abbreviation: pT: pathological tumor stage, G: Grade, pN: pathological lymph node status, pM: pathological status of distant metastasis, MMR: mismatch repair, ISUP: International Society of Urological Pathology, UICC: Union for International Cancer Control, V: venous invasion, L: lymphatic invasion, BRAF: B-Raf Proto-Oncogene, RAS: RAS Oncogene family

promising tumor entities that could potentially benefit from new therapies targeting L1CAM.

The availability of several large cohorts of tumors of the same histological type enabled us to analyze the relationship between L1CAM expression on tumor cells and parameters of cancer aggressiveness in this study. However, the significantly higher rate of L1CAM positivity in muscle-invasive than in non-invasive urothelial carcinoma was the only association found between L1CAM tumor cell expression and aggressive tumor phenotype. The absence of significant associations with histological parameters of dismal prognosis in clear cell and papillary RCC, pT2–4 urothelial carcinoma of the bladder, adenocarcinomas of the pancreas, the colorectum and the stomach, endometrioid endometrial carcinoma and serous high-grade carcinoma of the ovary argues against a major cancer driving role of L1CAM. In correlation with our data, others have also failed to find associations with aggressive tumor phenotype or poor prognosis in cohorts of endometrial carcinoma [29], squamous cell carcinoma of the cervix [30] and the oral cavity [31], ductal adenocarcinoma of the pancreas [32], clear cell carcinoma of the ovary [33], adenocarcinoma and squamous cell carcinoma of the esophagus [34], GIST [35] and neuroblastoma [22].



**Figure 4.** Comparison of L1CAM immunostaining results with previous L1CAM studies. An 'X' indicates the fraction of L1CAM positive cancers in the present study, dots indicate the reported frequencies from the literature for comparison: red dots mark studies with  $\leq 10$  tumors analyzed, yellow dots mark studies with  $11 \leq 25$  tumors analyzed and green dots mark studies with  $> 25$  tumors analyzed. All studies are listed in Supplementary Table 1. L1CAM: L1 cell adhesion molecule.

However, it is of note that a significant number of other studies have highlighted a role of L1CAM in cancer progression and described associations between high L1CAM expression and unfavorable prognostic tumor features [36]. For example, L1CAM expression on tumor cells was linked to aggressive cancer features and poor patient outcome in endometrial carcinoma [13, 37–40], oral squamous cell carcinoma [14], uterine mesonephric-like adenocarcinoma [41], breast cancer [42], endometrioid carcinoma of the ovary [43], ductal adenocarcinoma of the pancreas [19], colorectal cancer [44], cholangiocarcinoma [45], hepatocellular carcinoma [46], carcinosarcoma of the uterus [15], serous carcinoma of the ovary [17], adenocarcinoma of the lung [18] and esophageal squamous cell carcinoma [20]. Several,

functional studies have linked high L1CAM expression to increased tumor growth in colorectal cancer cells [47], increased cell proliferation, cell migration, and cell invasion in squamous carcinoma [14] and gastric cancer cells [7], higher motility and proliferation in glioblastoma cells [48], and enhanced transendothelial migration (TEM) in a pancreatic cancer model [49]. Enforced downregulation of L1CAM was associated with reduced cell migration in melanoma cell lines [50], inhibition of cell proliferation, cell invasion, and induction of cell cycle arrest in pancreatic cancer cell lines [51] and decreased tumor growth as well as increased survival of glioma-bearing animals.

In view of these conflicting data, we are confident in our IHC data because our assay was extensively validated according to the recommendations of the International Working Group of Antibody Validation (IWGAV) [52] by comparing our IHC findings in normal tissues with data obtained using another independent anti-L1CAM antibody, as well as with published normal tissue RNA data from three different databases [53–56]. To test a broad range of proteins and their relevant posttranslational modifications for a possible cross-reactivity, 76 different normal tissue categories were included in this analysis. The validity of our IHC assay was supported by the detection of L1CAM positivity by IHC in all tissues with documented L1CAM RNA expression, including adrenal gland, pituitary gland, prostate, kidney, seminal vesicles, and the gastrointestinal tract. While L1CAM RNA expression was not detected in the fallopian tube, for which a distinct staining of a subset of cells was observed by our assay, fallopian tube staining was confirmed by the independent L1CAM antibody 14.10, which also confirmed all other normal cell staining of our assay. The critical impact of assays for IHC studies has also been illustrated in an earlier analysis of 5,833 tumors from 128 tumor entities where a low sensitivity assay using the L1CAM antibody UJ127 resulted in positive cases in only 28 of 128 tumor entities (our study: 74 of 135) and a L1CAM positivity in only 5% of 22 endometrial carcinomas (our study: 12.5%). It is of note that our IHC assay was designed to detect L1CAM protein on tumor cells. Cleaved or exosomal fragments of L1CAM protein, which are formed under certain conditions [57], cannot be detected by IHC and thus did not influence our data.

In summary, our data show that L1CAM can be expressed in a broad range of different tumor entities, while its expression level is largely unrelated to cancer aggressiveness. Our ranking list of tumor entities according to their rate of L1CAM positivity helps to define groups of cancer patients which could best benefit from anti-L1CAM therapies.

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## Disclosure statements

The rabbit recombinant L1CAM antibody, clone MSVA-171R was provided by MS Validated Antibodies GmbH, Hamburg, Germany (owned by a family member of GS).

## Data availability statement

All data generated or analyzed during this study are included in this published article.

## Ethics declarations & trial registry information

The use of archived remnants of diagnostic tissues for manufacturing of TMAs and their analysis for research purposes as well as patient data analysis has been approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

## Author contributions

SB, MLe, ML, RS, MK, and GS contributed to conception, design, data collection, data analysis and manuscript writing. ML, KM, FV, RS, AM, AML, VR, AH, FL, SW, FJ, DD, CB, PL, AHM, CF, NG, EB, SM, SS, TSC, TK, and VC participated in pathology data analysis, data interpretation, and collection of samples. RS, MK, and CHM were involved in data analysis. MLe, RS, and GS provided supervision for the study. All authors agreed to be accountable for the content of the work.

## References

- [1] Moos M, Tacke R, Scherer H, Teplow D, Fruh K, Schachner M. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. *Nature*. 1988;334(6184):701–3. <https://doi.org/10.1038/334701a0>
- [2] Rathjen FG, Schachner M. Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. *EMBO J*. 1984;3(1):1–10. <https://doi.org/10.1002/j.1460-2075.1984.tb01753.x>
- [3] Samatov TR, Wicklein D, Tonevitsky AG. L1CAM: cell adhesion and more. *Prog Histochem Cytochem*. 2016;51(2):25–32. <https://doi.org/10.1016/j.proghi.2016.05.001>
- [4] Yamasaki M, Thompson P, Lemmon V. CRASH syndrome: mutations in L1CAM correlate with severity of the disease. *Neuropediatrics*. 1997;28(3):175–8. <https://doi.org/10.1055/s-2007-973696>
- [5] The human protein atlas [Internet]. January, 2025. [accessed January, 2025] Available from: <https://www.proteinatlas.org/ENSG00000198910-L1CAM/summary/rna>
- [6] Sjostedt E, Zhong W, Fagerberg L, Karlsson M, Mitsios N, Adori C, et al. An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science*. 2020;367(6482):eaay5947. <https://doi.org/10.1126/science.aay5947>
- [7] Chen DL, Zeng ZL, Yang J, Ren C, Wang DS, Wu WJ, et al. L1cam promotes tumor progression and metastasis and is an independent unfavorable prognostic factor in gastric cancer. *J Hematol Oncol*. 2013;6:43. <https://doi.org/10.1186/1756-8722-6-43>
- [8] Ganesh K, Basnet H, Kaygusuz Y, Laughney AM, He L, Sharma R, et al. Author correction: L1CAM defines the regenerative origin of metastasis-initiating cells in colorectal cancer. *Nat Cancer*. 2020;1(11):1128. <https://doi.org/10.1038/s43018-020-00130-3>
- [9] Zhang H, Zheng Y, Wang Z, Dong L, Xue L, Tian X, et al. KLF12 interacts with TRIM27 to affect cisplatin resistance and cancer metastasis in esophageal squamous cell carcinoma by regulating L1CAM expression. *Drug Resist Updat*. 2024;76:101096. <https://doi.org/10.1016/j.drup.2024.101096>
- [10] Giordano M, Decio A, Battistini C, Baronio M, Bianchi F, Villa A, et al. L1CAM promotes ovarian cancer stemness and tumor initiation via FGFR1/SRC/STAT3 signaling. *J Exp Clin Cancer Res*. 2021;40(1):319. <https://doi.org/10.1186/s13046-021-02117-z>
- [11] Fogel M, Gutwein P, Mechttersheimer S, Riedle S, Stoeck A, Smirnov A, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. *Lancet*. 2003;362(9387):869–75. [https://doi.org/10.1016/S0140-6736\(03\)14342-5](https://doi.org/10.1016/S0140-6736(03)14342-5)
- [12] Altevogt P, Ben-Ze'ev A, Gavert N, Schumacher U, Schafer H, Sebens S. Recent insights into the role of L1CAM in cancer initiation and progression. *Int J Cancer*. 2020;147(12):3292–6. <https://doi.org/10.1002/ijc.33177>
- [13] Joe S, Lee M, Kang J, Kim J, Hong SH, Lee SJ, et al. Enhanced risk stratification in early-stage endometrial cancer: integrating POLE through droplet digital PCR and L1CAM. *Cancers (Basel)*. 2023;15(19):4899. <https://doi.org/10.3390/cancers15194899>
- [14] Kim JH, Lee KW, Ahn DG, Oh KY, Yoon HJ. Clinical significance of L1CAM expression and its biological role in the progression of oral squamous cell carcinoma. *Oncol Rep*. 2023;49(4):67. <https://doi.org/10.3892/or.2023.8504>
- [15] Versluis M, Plat A, de Bruyn M, Matias-Guiu X, Trovic J, Krakstad C, et al. L1CAM expression in uterine carcinosarcoma is limited to the epithelial component and may be involved in epithelial-mesenchymal transition. *Virchows Arch*. 2018;473(5):591–8. <https://doi.org/10.1007/s00428-018-2444-8>
- [16] Schrevel M, Corver WE, Vegter ME, Ter Haar NT, Dreef EJ, Beltman JJ, et al. L1 cell adhesion molecule (L1CAM) is a strong predictor for locoregional recurrences in cervical cancer. *Oncotarget*. 2017;8(50):87568–81. <https://doi.org/10.18632/oncotarget.20976>
- [17] Dos Santos MV, Holth A, Lindemann K, Staff AC, Davidson B. Clinical significance of L1CAM expression in metastatic tubo-ovarian high-grade serous carcinoma. *Gynecol Oncol*. 2023;176:76–81. <https://doi.org/10.1016/j.ygyno.2023.07.004>
- [18] Yu H, Zhou P, Li D, Li W. L1CAM-positive expression is associated with poorer survival outcomes in resected non-small cell lung cancer patients. *Int J Clin Exp Pathol*. 2019;12(7):2665–71.
- [19] Tsutsumi S, Morohashi S, Kudo Y, Akasaka H, Ogasawara H, Ono M, et al. L1 Cell adhesion molecule (L1CAM) expression at the cancer invasive front is a novel prognostic marker of pancreatic ductal adenocarcinoma. *J Surg Oncol*. 2011;103(7):669–73. <https://doi.org/10.1002/jso.21880>
- [20] Zhao X, Liu S, Chen X, Zhao J, Li F, Zhao Q, et al. L1CAM overexpression promotes tumor progression through recruitment of regulatory T cells in esophageal carcinoma. *Cancer Biol Med*. 2021;18(2):547–61. <https://doi.org/10.20892/j.issn.2095-3941.2020.0182>
- [21] Kaifi JT, Reichelt U, Quaas A, Schurr PG, Wachowiak R, Yekebas EF, et al. L1 is associated with micrometastatic spread and poor outcome in colorectal cancer. *Mod Pathol*. 2007;20(11):1183–90. <https://doi.org/10.1038/modpathol.3800955>
- [22] Wachowiak R, Fiegel HC, Kaifi JT, Quaas A, Krickhahn A, Schurr PG, et al. L1 is associated with favorable outcome in neuroblastomas in contrast to adult tumors. *Ann Surg Oncol*. 2007;14(12):3575–80. <https://doi.org/10.1245/s10434-007-9608-0>
- [23] Doberstein K, Harter PN, Haberkorn U, Bretz NP, Arnold B, Carretero R, et al. Antibody therapy to human L1CAM in a transgenic mouse model blocks local tumor growth but induces EMT. *Int J Cancer*. 2015;136(5):E326–39. <https://doi.org/10.1002/ijc.29222>
- [24] Yuan Y, Li J, Chen J, Han L, Wang L, Yue Y, et al. Characterization of a novel T cell-engaging bispecific antibody for elimination of L1CAM-positive tumors. *Biomed Pharmacother*. 2024;174:116565. <https://doi.org/10.1016/j.biopha.2024.116565>
- [25] Lindenblatt D, Fischer E, Cohrs S, Schibli R, Grunberg J. Paclitaxel improved anti-L1CAM lutetium-177 radioimmunotherapy in an ovarian cancer xenograft model. *EJNMMI Res*. 2014;4(1):54. <https://doi.org/10.1186/s13550-014-0054-2>
- [26] Kunkele A, Taraseviciute A, Finn LS, Johnson AJ, Berger C, Finney O, et al. Preclinical assessment of CD171-directed CAR T-cell adoptive therapy for childhood neuroblastoma: CE7 epitope target safety and

- product manufacturing feasibility. *Clin Cancer Res.* 2017;23(2):466–77. <https://doi.org/10.1158/1078-0432.CCR-16-0354>
- [27] Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med.* 1998;4(7):844–7. <https://doi.org/10.1038/nm0798-844>
- [28] Dancau AM, Simon R, Mirlacher M, Sauter G. Tissue microarrays. *Methods Mol Biol.* 2016;1381:53–65. [https://doi.org/10.1007/978-1-4939-3204-7\\_3](https://doi.org/10.1007/978-1-4939-3204-7_3)
- [29] Vermij L, Jobsen JJ, Leon-Castillo A, Brinkhuis M, Roothaan S, Powell ME, et al. Prognostic refinement of NSMP high-risk endometrial cancers using oestrogen receptor immunohistochemistry. *Br J Cancer.* 2023;128(7):1360–8. <https://doi.org/10.1038/s41416-023-02141-0>
- [30] Romanova M, Zidlik V, Javurkova V, Konde A, Simetka O, Klat J. L1CAM is not a predictive factor in early-stage squamous-cell cervical cancer. *In Vivo.* 2023;37(5):2334–9. <https://doi.org/10.21873/invivo.13337>
- [31] Adnan Y, Ali SMA, Farooqui HA, Kayani HA, Idrees R, Awan MS. High CD44 immunoeexpression correlates with poor overall survival: assessing the role of cancer stem cell markers in oral squamous cell carcinoma patients from the high-risk population of Pakistan. *Int J Surg Oncol.* 2022;2022:9990489. <https://doi.org/10.1155/2022/9990489>
- [32] Ali SMA, Adnan Y, Ali SM, Ahmad Z, Chawla T, Farooqui HA. Immunohistochemical analysis of a panel of cancer stem cell markers and potential therapeutic markers in pancreatic ductal adenocarcinoma. *J Cancer Res Clin Oncol.* 2023;149(6):2279–92. <https://doi.org/10.1007/s00432-022-04315-4>
- [33] Soovares P, Pasanen A, Butzow R, Lassus H. L1CAM expression associates with poor outcome in endometrioid, but not in clear cell ovarian carcinoma. *Gynecol Oncol.* 2017;146(3):615–22. <https://doi.org/10.1016/j.ygyno.2017.06.010>
- [34] Rawnaq T, Kleinhans H, Uto M, Schurr PG, Reichelt U, Cataldegirmen G, et al. Subset of esophageal adenocarcinoma expresses adhesion molecule I1 in contrast to squamous cell carcinoma. *Anticancer Res.* 2009;29(4):1195–9.
- [35] Inaguma S, Wang Z, Lasota JP, Miettinen MM. Expression of neural cell adhesion molecule L1 (CD171) in neuroectodermal and other tumors: an immunohistochemical study of 5155 tumors and critical evaluation of CD171 prognostic value in gastrointestinal stromal tumors. *Oncotarget.* 2016;7(34):55276–89. <https://doi.org/10.18632/oncotarget.10527>
- [36] Stelloo E, Nout RA, Osse EM, Jurgensliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts. *Clin Cancer Res.* 2016;22(16):4215–24. <https://doi.org/10.1158/1078-0432.CCR-15-2878>
- [37] Giannini A, D'Oria O, Corrado G, Bruno V, Sperduti I, Bogani G, et al. The role of L1CAM as predictor of poor prognosis in stage I endometrial cancer: a systematic review and meta-analysis. *Arch Gynecol Obstet.* 2024;309(3):789–99. <https://doi.org/10.1007/s00404-023-07149-8>
- [38] Vizza E, Bruno V, Cutillo G, Mancini E, Sperduti I, Patrizi L, et al. Prognostic role of the removed vaginal cuff and its correlation with L1CAM in low-risk endometrial adenocarcinoma. *Cancers (Basel).* 2021;14(1):34. <https://doi.org/10.3390/cancers14010034>
- [39] Geels YP, Pijnenborg JM, Gordon BB, Fogel M, Altevogt P, Masadah R, et al. L1CAM expression is related to non-endometrioid histology, and prognostic for poor outcome in endometrioid endometrial carcinoma. *Pathol Oncol Res.* 2016;22(4):863–8. <https://doi.org/10.1007/s12253-016-0047-8>
- [40] Van Gool IC, Stelloo E, Nout RA, Nijman HW, Edmondson RJ, Church DN, et al. Prognostic significance of L1CAM expression and its association with mutant p53 expression in high-risk endometrial cancer. *Mod Pathol.* 2016;29(2):174–81. <https://doi.org/10.1038/modpathol.2015.147>
- [41] Park S, Cho Y, Kim HS. Mesonephric-like adenocarcinoma of the uterine corpus: clinicopathological and prognostic significance of L1 cell adhesion molecule (L1CAM) over-expression. *Anticancer Res.* 2023;43(10):4559–71. <https://doi.org/10.21873/anticancer.16650>
- [42] Zhang J, Yang F, Ding Y, Zhen L, Han X, Jiao F, et al. Overexpression of L1 cell adhesion molecule correlates with aggressive tumor progression of patients with breast cancer and promotes motility of breast cancer cells. *Int J Clin Exp Pathol.* 2015;8(8):9240–7.
- [43] Soovares P, Pasanen A, Simila-Maarala J, Butzow R, Lassus H. Clinical factors and biomarker profiles associated with patient outcome in endometrioid ovarian carcinoma – emphasis on tumor grade. *Gynecol Oncol.* 2022;164(1):187–94. <https://doi.org/10.1016/j.ygyno.2021.10.078>
- [44] Boo YJ, Park JM, Kim J, Chae YS, Min BW, Um JW, et al. L1 expression as a marker for poor prognosis, tumor progression, and short survival in patients with colorectal cancer. *Ann Surg Oncol.* 2007;14(5):1703–11. <https://doi.org/10.1245/s10434-006-9281-8>
- [45] Min JK, Kim JM, Li S, Lee JW, Yoon H, Ryu CJ, et al. L1 cell adhesion molecule is a novel therapeutic target in intrahepatic cholangiocarcinoma. *Clin Cancer Res.* 2010;16(14):3571–80. <https://doi.org/10.1158/1078-0432.CCR-09-3075>
- [46] Guo X, Xiong L, Zou L, Sun T, Zhang J, Li H, et al. L1 cell adhesion molecule overexpression in hepatocellular carcinoma associates with advanced tumor progression and poor patient survival. *Diagn Pathol.* 2012;7:96. <https://doi.org/10.1186/1746-1596-7-96>
- [47] Cave DD, Hernando-Momblona X, Sevillano M, Minchiotti G, Lonardo E. Nodal-induced L1CAM/CXCR4 subpopulation sustains tumor growth and metastasis in colorectal cancer derived organoids. *Theranostics.* 2021;11(12):5686–99. <https://doi.org/10.7150/thno.54027>
- [48] Caccavale J, Fiumara D, Stapf M, Sweitzer L, Anderson HJ, Gorky J, et al. A simple and accurate rule-based modeling framework for simulation of autocrine/paracrine stimulation of glioblastoma cell motility and proliferation by L1CAM in 2-D culture. *BMC Syst Biol.* 2017;11(1):124. <https://doi.org/10.1186/s12918-017-0516-z>
- [49] Issa Y, Nummer D, Seibel T, Muerkoster SS, Koch M, Schmitz-Winnenthal FH, et al. Enhanced L1CAM expression on pancreatic tumor endothelium mediates selective tumor cell transmigration. *J Mol Med (Berl).* 2009;87(1):99–112. <https://doi.org/10.1007/s00109-008-0410-7>
- [50] Pompili SVB, Fanzini S, Schachner M, Chen S. In vitro and in vivo studies of melanoma cell migration by antagonistic mimetics of adhesion molecule L1CAM. *Int J Mol Sci.* 2024;25(9):4811. <https://doi.org/10.3390/ijms25094811>
- [51] Ben Q, An W, Fei J, Xu M, Li G, Li Z, et al. Downregulation of L1CAM inhibits proliferation, invasion and arrests cell cycle progression in pancreatic cancer cells in vitro. *Exp Ther Med.* 2014;7(4):785–90. <https://doi.org/10.3892/etm.2014.1519>
- [52] Uhlen M, Bandrowski A, Carr S, Edwards A, Ellenberg J, Lundberg E, et al. A proposal for validation of antibodies. *Nat Methods.* 2016;13(10):823–7. <https://doi.org/10.1038/nmeth.3995>
- [53] GTEx Consortium. The genotype-tissue expression (GTEx) project. *Nat Genet.* 2013;45(6):580–5. <https://doi.org/10.1038/ng.2653>
- [54] Lizio M, Harshbarger J, Shimoji H, Severin J, Kasukawa T, Sahin S, et al. Gateways to the FANTOM5 promoter level mammalian expression atlas. *Genome Biol.* 2015;16(1):22. <https://doi.org/10.1186/s13059-014-0560-6>
- [55] Lizio M, Abugessaisa I, Noguchi S, Kondo A, Hasegawa A, Hon CC, et al. Update of the FANTOM web resource: expansion to provide additional transcriptome atlases. *Nucleic Acids Res.* 2019;47(D1):D752–8. <https://doi.org/10.1093/nar/gky1099>
- [56] Thul PJ, Akesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal H, et al. A subcellular map of the human proteome. *Science.* 2017;356(6340):eaal3321. <https://doi.org/10.1126/science.aal3321>
- [57] Maten MV, Reijnen C, Pijnenborg JMA, Zegers MM. L1 cell adhesion molecule in cancer, a systematic review on domain-specific functions. *Int J Mol Sci.* 2019;20(17):4180. <https://doi.org/10.3390/ijms20174180>