Pattern of placental alkaline phosphatase (PLAP) expression in human tumors: a tissue microarray study on 12,381 tumors

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Abstract

Placental alkaline phosphatase (PLAP) is commonly expressed at high levels in testicular germ cell tumors. PLAP immunohistochemistry (IHC) is thus often used to confirm this diagnosis, especially in cases of putative metastasis. However, other tumors can also express PLAP. To comprehensively determine PLAP expression in normal and tumor tissue, a tissue microarray containing 16,166 samples from 131 different tumor types and subtypes as well as 608 samples from 76 different normal tissue types was analyzed by IHC. Moderate to strong PLAP positivity was found in 27 (21%) of 131 different tumor types including seminoma (96%), embryonal carcinoma (85%), and yolk sac tumors of the testis (56%); endometrioid carcinoma of the endometrium (28%) and the ovary (20%); gastric adenocarcinoma (22%); serous carcinoma (not otherwise specified) of the ovary (17%) and the uterus (11%); adenocarcinoma of the ampulla of Vater (15%); carcinosarcoma of the ovary (11%) and the uterus (8%); esophageal adenocarcinoma (10%); invasive urethelial carcinoma (4%); cholangiocarcinoma (2%); and adenocarcinoma of the lung (1%). Low-level PLAP immunostaining, often involving only a small fraction of tumor cells, was seen in 21 additional tumor entities. The clinical significance of PLAP expression may vary between tumor types as high PLAP expression was linked to advanced pathological tumor stage \( p = 0.0086 \), nodal metastasis \( p = 0.0085 \), and lymphatic \( p = 0.0007 \) and blood vessel invasion \( p = 0.0222 \) in colorectal cancer, but to low pathological tumor stage in endometrial cancer \( p = 0.0043 \). In conclusion, our data identify several tumor entities that can show PLAP expression at comparable levels to testicular germ cell tumors. These tumor entities need to be considered in cases of PLAP-positive metastasis. Low-level PLAP expression can be found in various other tumor entities and should generally not be viewed as a strong argument for germ cell neoplasia.

Keywords: immunohistochemistry; PLAP; tissue microarray

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Conflict of interest statement: The PLAP antibody clone MSVA-350R was provided by MS Validated Antibodies GmbH (owned by a family member of GS).

Introduction

Placental alkaline phosphatase (PLAP), also known as alkaline phosphatase, placental type (ALPP), is encoded by the ALPP gene at chromosome 2q37.1 [1]. PLAP is a dimer of 65 kDa consisting of 535 amino acids and is thought to play a role in guiding migratory cells and transport specific molecules over the plasma membrane [1,2]. PLAP is expressed in the placenta from the ninth week of gestation and its concentration increases continually throughout pregnancy [2]. PLAP can be separated into three distinct isoenzymes corresponding to early, mid, and term placenta [3]. In normal human tissues, the expression of PLAP is largely restricted to the placenta but low-level RNA expression has also been reported for uterine cervix, fallopian tube, and – to a lower level – the lung [4,5].

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PLAP expression also occurs in tumors [4,6–60]. This is particularly known for testicular germ cell tumors [6–8,10–36]. PLAP expression, often at high levels, has been described to occur in up to 100% of testicular germ cell neoplasia in situ [20,28,30], up to 100% of seminoma [4,6,7,9,20–23,30,35,36,38,41,42], up to 100% of embryonal carcinoma [35,42], up to 87% of yolk sac tumors [30], and up to 100% of choriocarcinomas [12,16,19]. Antibodies targeting PLAP are thus regularly used for the detection and classification of testicular tumors [61]. Various studies have demonstrated, however, that PLAP expression can also occur in non-germinal cell tumors, but immunohistochemical data are controversial. For example, positive PLAP immunostaining has been described to occur in 0–100% of adenocarcinomas of the ampulla of Vater [4,35], 0–38% of gastric adenocarcinoma [4,35], 0–100% of rhabdomyosarcomas [44,56], 20–80% of high-grade serous carcinomas of the ovary [43,59], and 0–18% of clear cell renal cell carcinomas [4,35]. These conflicting data are probably caused by the use of different antibodies, immunostaining protocols, and criteria to determine PLAP positivity in these studies.

To better understand the prevalence of PLAP immunostaining in different tumor types, a comprehensive study analyzing a large number of neoplastic and non-neoplastic tissues under highly standardized conditions is needed. We thus analyzed PLAP expression in more than 16,000 tumor tissue samples from 131 different tumor types and subtypes as well as 76 different normal tissue types by immunohistochemistry (IHC) in a tissue microarray (TMA) format.

Materials and methods

Tissue microarrays

TMAs composed of normal and tumor tissues were employed for this study. The normal TMA contained eight samples from eight different donors from each of 76 different normal tissue types. The cancer TMAs contained a total of 16,166 primary tumors from 131 tumor types and subtypes. Histopathological data including grade, pathological tumor (pT) stage, and pathological lymph node (pN) status were available from 583 ovarian cancers, 259 endometrial cancers, and 1,784 colorectal cancers. The dataset on colorectal cancer also included molecular information on mismatch repair protein deficiency. The composition of both normal and cancer TMAs is described in the Results section. All samples were from the archives of the Institutes of Pathology, University Hospital of Hamburg, Germany; the Institute of Pathology, Clinical Center Osnabrueck, Germany; and Department of Pathology, Academic Hospital Fuert, Germany. Tissues were fixed in 4% buffered formalin and then embedded in paraffin. TMA tissue spot diameter was 0.6 mm. 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 was carried out in compliance with the Helsinki Declaration.

Immunohistochemistry

Freshly cut TMA sections were immunostained under the same experimental conditions. Two different primary antibodies were used for PLAP detection: MSVA-350R (rabbit recombinant; MS Validated Antibodies, Hamburg, Germany) and IR779 (mouse monoclonal 8A9, Agilent DAKO, Santa Clara, CA, USA). The normal tissue array was analyzed with both MSVA-350R and IR779, while the multitumor array was analyzed with MSVA-350R only. For MSVA-350R, slides were deparaffinized with xylol, rehydrated through a graded alcohol series, and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121°C in pH 9.0 Target Retrieval Solution (Agilent). Endogenous peroxidase activity was blocked with Peroxidase Blocking Solution (Agilent) for 10 min. The primary antibody was diluted 1:150 and applied for 60 min at 37°C. For IR779, the slides were deparaffinized and rehydrated as described previously, and exposed to heat-induced antigen retrieval for 15 min in Agilent’s PT Link pretreatment module at 95°C in pH 9.0 retrieval buffer. Slides were transferred to an Autostainer Link 48 device (Agilent) for peroxidase blocking (5 min) and incubation of the primary antibody (ready to use prediluted for 20 min at room temperature). Both antibodies were visualized using the respective EnVision reagents (Agilent) for manual and automated staining according to the manufacturer’s directions. One pathologist (NG) analyzed all immunostainings. For tumor tissues, the percentage of positive neoplastic cells was estimated, and the staining intensity was semiquantitatively recorded (0, 1+, 2+, and 3+). For statistical analyses, the staining results were categorized into four groups. Tumors without any staining were considered negative. Tumors with 1+ staining intensity in ≤70% of cells and 2+ intensity in ≤30% of cells were considered weakly positive. Tumors with 1+ staining intensity in >70% of cells, 2+ intensity in 31–70%, or 3+ intensity in ≤30% were considered moderately positive. Tumors with 2+ intensity in

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>70% or 3+ intensity in >30% of cells were considered strongly positive.

Statistics
Statistical calculations were performed with JMP 14 software (SAS Institute Inc., Cary, NC, USA). Contingency tables and the chi-square test were performed to search for associations between PLAP and tumor phenotype. Survival curves were calculated according to Kaplan–Meier. The log-rank test was applied to detect significant differences between groups. A P value of ≥0.05 was considered as statistically significant.

Results
Technical issues
A total of 12,381 (76.6%) of 16,166 tumor samples were interpretable in our TMA analysis. Non-interpretable samples demonstrated lack of unequivocal tumor cells or loss of the tissue spot during technical procedures. A sufficient number of samples of each normal tissue type was evaluable.

PLAP in normal tissues
With two different antibodies (MSVA-350R and IR779), particularly strong PLAP immunostaining was found in the placenta. Here, strong PLAP positivity was seen in chorion cells as well as in cyto- and syncytiotrophoblast of mature placenta (Figure 1A,D). Staining was only moderate and limited to the surface cell membrane in the trophoblast of early placenta, and only weak in amnion cells. Also, for both antibodies, weak PLAP staining was seen at the apical membrane of epithelial cells in the endocervix (Figure 1B,E), endometrium, and the fallopian tube, although this did not occur in all samples analyzed. PLAP immunostaining was lacking for both antibodies in most other tissues including all epithelial...
Table 1. PLAP immunostaining in human tumors.

| Tumor entity                                      | On TMA (n) | Analyzable (n) | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | Positive (%) |
|--------------------------------------------------|------------|----------------|--------------|----------|--------------|------------|--------------|
| **Tumors of the skin**                           |            |                |              |          |              |            |              |
| Pilomatrixoma                                     | 35         | 32             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Basal cell carcinoma                              | 88         | 56             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Benign nevus                                      | 29         | 26             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Squamous cell carcinoma of the skin               | 90         | 82             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Malignant melanoma                                | 48         | 44             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Merkel cell carcinoma                             | 46         | 44             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| **Tumors of the head and neck**                   |            |                |              |          |              |            |              |
| Squamous cell carcinoma of the larynx             |            |                |              |          |              |            |              |
| Squamous cell carcinoma of the pharynx            |            |                |              |          |              |            |              |
| Oral squamous cell carcinoma (floor of the mouth) |            |                |              |          |              |            |              |
| Pleomorphic adenoma of the parotid gland          |            |                |              |          |              |            |              |
| Warthin tumor of the parotid gland                |            |                |              |          |              |            |              |
| Adenocarcinoma, NOS (papillary cystadenocarcinoma) |            |                |              |          |              |            |              |
| Salivary duct carcinoma                           |            |                |              |          |              |            |              |
| Acinic cell carcinoma of the salivary gland       |            |                |              |          |              |            |              |
| Adenocarcinoma NOS of the salivary gland          |            |                |              |          |              |            |              |
| Adenoid cystic carcinoma of the salivary gland    |            |                |              |          |              |            |              |
| Basal cell adenocarcinoma of the salivary gland   |            |                |              |          |              |            |              |
| Basal cell adenoma of the salivary gland          |            |                |              |          |              |            |              |
| Epithelial–myoepithelial carcinoma of the salivary gland |    |                |              |          |              |            |              |
| Mucoepidermoid carcinoma of the salivary gland    |            |                |              |          |              |            |              |
| Myoepithelial carcinoma of the salivary gland     |            |                |              |          |              |            |              |
| Myoepithelioma of the salivary gland              |            |                |              |          |              |            |              |
| Oncocytic carcinoma of the salivary gland         |            |                |              |          |              |            |              |
| Polymorphous adenocarcinoma, low grade, of the salivary gland | | | | | | | |
| Polymorphous adenoma of the salivary gland        |            |                |              |          |              |            |              |
| **Tumors of the lung, pleura, and thymus**        |            |                |              |          |              |            |              |
| Adenocarcinoma of the lung                        | 246        | 160            | 79.4         | 19.4     | 0.6          | 0.6        | 20.6         |
| Squamous cell carcinoma of the lung               | 130        | 65             | 98.5         | 1.5      | 0.0          | 0.0        | 1.5          |
| Small cell carcinoma of the lung                  | 20         | 16             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Mesothelioma, epithelioid                         | 39         | 32             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Mesothelioma, other types                         | 76         | 63             | 98.4         | 1.6      | 0.0          | 0.0        | 1.6          |
| Thymoma                                           | 29         | 29             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| **Tumors of the female genital tract**             |            |                |              |          |              |            |              |
| Squamous cell carcinoma of the vagina             | 78         | 63             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Squamous cell carcinoma of the vulva              | 130        | 116            | 94.8         | 5.2      | 0.0          | 0.0        | 5.2          |
| Squamous cell carcinoma of the cervix             | 130        | 124            | 91.9         | 6.5      | 0.0          | 1.6        | 8.1          |
| Endometrioid endometrial carcinoma                | 236        | 223            | 40.4         | 31.8     | 9.0          | 18.8       | 59.6         |
| Endometrial serous carcinoma                      | 82         | 72             | 56.9         | 31.9     | 4.2          | 6.9        | 43.1         |
| Carcinosarcoma of the uterus                      | 48         | 38             | 68.4         | 23.7     | 2.6          | 5.3        | 31.6         |
| Endometrioid carcinoma, high grade, G3            | 13         | 13             | 84.6         | 15.4     | 0.0          | 0.0        | 15.4         |
| Endometrioid clear cell carcinoma                 | 8          | 7              | 85.7         | 14.3     | 0.0          | 0.0        | 14.3         |
| Endometrioid carcinoma of the ovary               | 110        | 91             | 41.8         | 38.5     | 6.6          | 13.2       | 58.2         |
| Serous carcinoma of the ovary (NOS)               | 559        | 462            | 50.2         | 32.9     | 8.4          | 8.4        | 49.8         |
| Mucinous carcinoma of the ovary                   | 96         | 71             | 85.9         | 7.0      | 2.8          | 4.2        | 14.1         |
| Clear cell carcinoma of the ovary                 | 50         | 40             | 90.0         | 10.0     | 0.0          | 0.0        | 10.0         |
| Carcinosarcoma of the ovary                       | 47         | 38             | 60.5         | 28.9     | 5.3          | 5.3        | 39.5         |
| Brenner tumor                                     | 9          | 9              | 77.8         | 22.2     | 0.0          | 0.0        | 22.2         |
| **Tumors of the breast**                          |            |                |              |          |              |            |              |
| Invasive breast carcinoma of no special type      | 1,391      | 1185           | 99.2         | 0.8      | 0.0          | 0.0        | 0.8          |
| Lobular carcinoma of the breast                   | 294        | 236            | 99.6         | 0.0      | 0.4          | 0.0        | 0.4          |
| Medullary carcinoma of the breast                 | 26         | 26             | 96.2         | 3.8      | 0.0          | 0.0        | 3.8          |
| Tubular carcinoma of the breast                   | 27         | 26             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Mucinous carcinoma of the breast                  | 58         | 44             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Phylodes tumor of the breast                      | 50         | 47             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
| Adenomatous polyv, dysplasia                      | 1,000      | 98             | 100.0        | 0.0      | 0.0          | 0.0        | 0.0          |
(Continues)
| Tumor entity                                                | PLAP immunostaining  |
|------------------------------------------------------------|----------------------|
| Tumors of the digestive system                             |                      |
| Adenocarcinoma of the colon                                | 956 (721) 89.7 9.3 0.7 0.3 10.3 |
| Gastric adenocarcinoma, diffuse type                       | 226 (130) 87.7 8.5 0.8 3.1 12.3 |
| Gastric adenocarcinoma, intestinal type                    | 224 (134) 70.1 22.4 5.2 2.2 29.9 |
| Gastric adenocarcinoma, mixed type                         | 62 (48) 77.1 18.8 2.1 2.1 22.9 |
| Adenocarcinoma of the esophagus                            | 133 (60) 76.7 13.3 5.0 5.0 23.3 |
| Squamous cell carcinoma of the esophagus                   | 124 (42) 100.0 0.0 0.0 0.0 0.0 |
| Squamous cell carcinoma of the anal canal                  | 91 (78) 98.7 1.3 0.0 0.0 1.3 |
| Cholangiocarcinoma                                         | 114 (108) 93.5 4.6 0.0 1.9 6.5 |
| Hepatocellular carcinoma                                  | 50 (50) 100.0 0.0 0.0 0.0 0.0 |
| Ductal adenocarcinoma of the pancreas                      | 663 (459) 78.4 19.2 1.5 0.9 21.8 |
| Pancreatic/ampullary adenocarcinoma                        | 119 (76) 72.4 13.2 10.5 3.9 27.6 |
| Actin cell carcinoma of the pancreas                       | 13 (12) 100.0 0.0 0.0 0.0 0.0 |
| Gastrointestinal stromal tumor                             | 50 (49) 100.0 0.0 0.0 0.0 0.0 |
| Tumors of the urinary system                               |                      |
| Urothelial carcinoma, pT2–4 G3                             | 1,207 (613) 78.0 18.4 1.6 2.0 22.0 |
| Small cell NEC of the bladder                              | 18 (18) 94.4 5.6 0.0 0.0 5.6 |
| Sarcomatoid urothelial carcinoma                           | 25 (24) 95.8 4.2 0.0 0.0 4.2 |
| Clear cell renal cell carcinoma                            | 1,226 (759) 99.9 0.1 0.0 0.1 |
| Papillary renal cell carcinoma                             | 320 (208) 100.0 0.0 0.0 0.0 |
| Clear cell (tubulo) papillary renal cell carcinoma         | 28 (19) 100.0 0.0 0.0 0.0 |
| Chromophobe renal cell carcinoma                           | 151 (118) 100.0 0.0 0.0 0.0 |
| Oncocytoma                                                 | 199 (147) 100.0 0.0 0.0 0.0 |
| Tumors of the male genital organs                          |                      |
| Adenocarcinoma of the prostate (primary)                   | 248 (232) 100.0 0.0 0.0 0.0 0.0 |
| Adenocarcinoma of the prostate (recurrence)                | 261 (231) 100.0 0.0 0.0 0.0 0.0 |
| Small cell NEC of the prostate                             | 17 (16) 93.8 6.3 0.0 0.0 6.3 |
| Seminoma                                                   | 621 (444) 0.7 3.2 12.2 84.0 99.3 |
| Embryonal carcinoma of the testis                          | 50 (39) 9.6 12.8 20.5 64.1 97.4 |
| Yolk sac tumor                                             | 50 (32) 25.0 18.8 3.1 53.1 75.0 |
| Teratoma                                                   | 50 (44) 95.5 2.3 2.3 0.0 4.5 |
| Squamous cell carcinoma of the penis                       | 80 (66) 98.5 1.5 0.0 0.0 1.5 |
| Tumors of the endocrine organs                             |                      |
| Adenoma of the thyroid gland                               | 114 (108) 100.0 0.0 0.0 0.0 0.0 |
| Papillary thyroid carcinoma                                | 392 (361) 99.4 0.6 0.0 0.0 0.6 |
| Follicular thyroid carcinoma                               | 158 (147) 100.0 0.0 0.0 0.0 0.0 |
| Medullary thyroid carcinoma                                | 107 (100) 100.0 0.0 0.0 0.0 0.0 |
| Anaplastic thyroid carcinoma                               | 45 (43) 100.0 0.0 0.0 0.0 0.0 |
| Adrenal cortical adenoma                                   | 50 (44) 100.0 0.0 0.0 0.0 0.0 |
| Adrenal cortical carcinoma                                 | 26 (26) 100.0 0.0 0.0 0.0 0.0 |
| Phaeochromocytoma                                          | 50 (50) 100.0 0.0 0.0 0.0 0.0 |
| Appendix, NET                                              | 22 (12) 91.7 8.3 0.0 0.0 8.3 |
| Colorectum, NET                                            | 11 (10) 100.0 0.0 0.0 0.0 0.0 |
| Ileum, NET                                                 | 49 (46) 100.0 0.0 0.0 0.0 0.0 |
| Lung, NET                                                  | 19 (17) 100.0 0.0 0.0 0.0 0.0 |
| Pancreas, NET                                              | 99 (95) 98.9 1.1 0.0 0.0 1.1 |
| Colorectum, NEC                                            | 12 (10) 100.0 0.0 0.0 0.0 0.0 |
| Gallbladder, NEC                                           | 4 (4) 100.0 0.0 0.0 0.0 0.0 |
| Pancreas, NEC                                              | 15 (15) 100.0 0.0 0.0 0.0 0.0 |
| Tumors of hematopoietian and lymphoid tissues               |                      |
| Hodgkin lymphoma                                           | 103 (76) 100.0 0.0 0.0 0.0 0.0 |
| Non-Hodgkin lymphoma                                       | 62 (54) 100.0 0.0 0.0 0.0 0.0 |
| Small lymphocytic lymphoma, B-cell type (B-SLL/B-CLL)      | 50 (30) 100.0 0.0 0.0 0.0 0.0 |
| DLBCL                                                      | 114 (94) 100.0 0.0 0.0 0.0 0.0 |
| Follicular lymphoma                                        | 88 (63) 100.0 0.0 0.0 0.0 0.0 |
| T-cell non-Hodgkin lymphoma                                | 24 (16) 100.0 0.0 0.0 0.0 0.0 |

(Continues)
cells of the gastrointestinal and the genitourinary tract, gallbladder, liver, pancreas, salivary and bronchial glands, breast glands, Brunner glands, thyroid, pituitary gland, adrenal gland, parathyroid gland, testis, epididymis, seminal vesicle, prostate, non-keratinizing and keratinizing squamous epithelium of various different sites, skin appendages, hematopoietic and immune cells, and the brain. Staining of muscular tissues revealed complete absence of staining by MSVA-350R (Figure 1C) while Agilent Dako IR779 showed moderate to strong staining of smooth muscle (Figure 1F) and weak to moderate staining of skeletal muscle. These latter findings were considered to be due to cross-reactivity.

PLAP in cancer

By using MSVA-350R, positive PLAP immunostaining was detectable in 1,503 (12.1%) of the 12,381 analyzable tumors, including 761 (6.1%) with weak, 184 (1.5%) with moderate, and 558 (4.5%) with strong immunostaining. Overall, 48 (36.6%) of 131 tumor categories showed detectable PLAP expression with 22 (16.8%) tumor categories showing strong positivity in at least one case (Table 1). Representative images of PLAP-positive tumors are shown in Figure 2. The highest rate of positive staining was found in testicular tumors, followed by tumors of the female genital tract, gastroesophageal, and pancreaticobiliary cancers. It is of note that only weak PLAP immunostaining was occasionally found in 21 different tumor entities. In most of these tumors, PLAP immunostaining was limited to a small fraction of tumor cells (Figure 2D,E). None of the 48 leiomyomas, 84 leiomyosarcomas, 7 rhabdomyosarcomas, or 91 angiomyolipomas showed any PLAP staining. A graphical representation of the rank order of PLAP positive and strongly positive cancers is shown in Figure 3.

PLAP expression and histopathological parameters

The relationship between PLAP expression and histopathological data in ovarian, endometrial, and colorectal cancers is summarized in Table 2. The data show that high PLAP expression is linked to advanced pT stage (p = 0.0086), nodal metastasis (p = 0.0085),

| Tumor entity | On TMA (n) | Analyzable (n) | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | Positive (%) |
|--------------|------------|----------------|--------------|----------|--------------|------------|--------------|
| Mantle cell lymphoma | 18 | 13 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Marginal zone lymphoma | 16 | 10 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| DLBCL in the testis | 16 | 13 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Burkitt lymphoma | 5 | 1 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table 1. Continued

| Tumor entity | On TMA (n) | Analyzable (n) | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | Positive (%) |
|--------------|------------|----------------|--------------|----------|--------------|------------|--------------|
| Tumors of soft tissue and bone | | | | | | | |
| Tenosynovial giant cell tumor | 45 | 44 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Granular cell tumor | 53 | 44 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Leiomyoma | 50 | 48 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Leiomyosarcoma | 87 | 84 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Liposarcoma | 132 | 129 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Malignant peripheral nerve sheath tumor | 13 | 11 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Myofibrosarcoma | 26 | 26 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Angiosarcoma | 73 | 66 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Angiomylolipoma | 91 | 91 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Dermatofibrosarcoma protuberans | 21 | 18 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ganglioneuroma | 14 | 13 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Kaposi sarcoma | 8 | 6 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Neurofibroma | 117 | 96 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sarcoma, NOS | 75 | 59 | 98.3 | 1.7 | 0.0 | 0.0 | 1.7 |
| Paraganglioma | 41 | 37 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ewing sarcoma | 23 | 18 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Rhabdomyosarcoma | 7 | 7 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Schwannoma | 121 | 106 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Synovial sarcoma | 12 | 11 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Osteosarcoma | 43 | 35 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Chondrosarcoma | 38 | 17 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |

B-SLL/B-CLL, B-cell small lymphocytic/chronic lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; NOS, not otherwise specified.
and lymphatic \((p = 0.007)\) and blood vessel invasion \((p = 0.0222)\) in colorectal cancer, while low PLAP expression was found to be associated with advanced pT stage in endometroid carcinoma of the endometrium \((p = 0.0043)\). Associations between PLAP expression and tumor phenotype were not found in serous (not otherwise specified) and endometrioid ovarian cancer.

**Discussion**

In an immunohistochemical analysis of more than 10,000 tumors analyzed by IHC, it is important to use suitable reagents and protocols. The International Working Group for Antibody Validation (IWGAV) has proposed that antibody validation for IHC on formalin-fixed tissues should include either a comparison of the findings obtained by two different independent antibodies or a comparison with expression data obtained by another independent method [62]. Here, 76 different normal tissue categories were included in the antibody comparison experiment to ensure that any antibody cross-reactivity would be detected in our validation experiment. The fact that the antibodies MSVA-350R and Agilent IR779 both showed strong PLAP staining in chorion and trophoblastic cells of the placenta and weak staining of amnion cells and apical membranes of endocervical, endometrial, and fallopian tube epithelium confirms that these findings are PLAP specific. Most of these results are also consistent with data from three independent RNA screening studies, including the Human Protein Atlas (HPA) RNA-seq tissue dataset [63], the FANTOM5 project [64,65], and the Genotype-Tissue Expression (GTEx) project [5], which also suggest that the uterine cervix is the organ with the second highest PLAP expression following placenta. PLAP RNA expression was not described for endometrium and fallopian tube, but this may be due to the small fraction of the total cells of these organs expressing PLAP. RNAs derived

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**Figure 2.** PLAP immunostaining in cancer. The panels show strong PLAP positivity in (A) seminoma, (B) embryonal carcinoma, (C) high-grade serous carcinoma of the ovary, (D) adenocarcinoma of the pancreas, and (E) gastric adenocarcinoma, and weak focal PLAP positivity in (F) an adenocarcinoma of the lung with the antibody MSVA-350R.
Figure 3. Ranking order of PLAP immunostaining in human tumors. Both the frequency of positive cases (blue dots) and the frequency of strongly positive cases (orange dots) are shown. Eighty-three additional tumor entities without any PLAP-positive cases are not shown due to space restrictions.
from small structures or rare cell types are largely underrepresented and thus potentially missed in RNA analyses. Lung was also described to produce very limited amounts of PLAP RNA but this is not supported by the findings in our IHC analysis. It is of note that the strong immunostaining of smooth muscle derived from various organs seen with clone 8A9 was not seen with MSVA-350R and is thus considered to reflect cross-reactivity. In line with this interpretation, PLAP RNA expression has previously not been described in smooth muscle cells [5,64,65]. Based on these data, the antibody MSVA-350R was solely used for our tumor tissue analyses. Studies using clone 8A9 have previously described PLAP expression in leiomyoma [49], leiomyosarcoma [49], and angiomyolipoma of the kidney [49,57]. As we did not find any PLAP staining in a total of 223 tumors of these categories, it appears certain that earlier results were caused by antibody cross-reactivity and not by true PLAP expression.

The successful analysis of PLAP expression in 12,381 cancers of 131 different tumor types and subtypes confirmed a high frequency of PLAP expression in testicular tumors but also showed that frequent and high-level PLAP immunostaining occurs in various other tumor types, most commonly derived from the female genital tract, the gastroesophageal, and the pancreaticobiliary system. Our findings observed for seminomas (99%), embryonal carcinoma (97%), and yolk sac tumors (75%) of the testis are largely consistent with the literature [4,6–9,12,16,19–23,26,30,33,35,36,38,41,42]. That the highest PLAP positivity rates in extra-testicular cancers were found in tumors of the female genital tract

| Table 2. PLAP immunostaining and cancer phenotype. | PLAP immunostaining |
|---|---|
| | Analyzable (n) | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | P value |
| **Colorectal adenocarcinoma** | | | | | | |
| All cancers | 652 | 89.7 | 9.2 | 0.8 | 0.3 | 0.0086 |
| pT1 | 29 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0086 |
| pT2 | 120 | 92.5 | 7.5 | 0.0 | 0.0 | 0.0086 |
| pT3 | 350 | 91.4 | 8.3 | 0.3 | 0.0 | 0.0086 |
| pT4 | 147 | 82.3 | 14.3 | 2.7 | 0.7 | 0.0086 |
| pN0 | 320 | 93.8 | 5.9 | 0.3 | 0.0 | 0.0086 |
| pN+ | 324 | 86.1 | 12.3 | 1.2 | 0.3 | 0.0086 |
| V0 | 472 | 91.9 | 7.6 | 0.4 | 0.0 | 0.0086 |
| V+ | 169 | 84.6 | 13.0 | 1.8 | 0.6 | 0.0086 |
| L0 | 326 | 94.5 | 4.9 | 0.6 | 0.0 | 0.0086 |
| L1 | 291 | 84.9 | 13.7 | 1.0 | 0.3 | 0.0086 |
| Left | 571 | 89.7 | 9.3 | 0.7 | 0.4 | 0.0086 |
| Right | 76 | 89.5 | 9.2 | 1.3 | 0.0 | 0.0086 |
| MMR deficient | 21 | 85.7 | 9.5 | 4.8 | 0.0 | 0.0086 |
| MMR proficient | 535 | 90.1 | 9.0 | 0.6 | 0.4 | 0.0086 |
| **Endometrioid endometrial carcinoma** | | | | | | |
| All cancers | 173 | 37.6 | 31.2 | 11.0 | 20.2 | 0.0043 |
| pT1 | 114 | 28.9 | 30.7 | 14.9 | 25.4 | 0.0043 |
| pT2 | 24 | 45.8 | 37.5 | 0.0 | 16.7 | 0.0043 |
| pT3–4 | 32 | 56.3 | 31.3 | 6.3 | 6.3 | 0.0043 |
| pN0 | 49 | 32.7 | 42.9 | 4.1 | 20.4 | 0.0043 |
| pN+ | 29 | 55.2 | 27.6 | 6.9 | 10.3 | 0.0043 |
| **Endometrioid ovarian carcinoma** | | | | | | |
| All cancers | 34 | 35.3 | 35.3 | 5.9 | 23.5 | 0.1211 |
| pT1 | 24 | 29.2 | 37.5 | 8.3 | 25.0 | 0.6409 |
| pT2 | 6 | 66.7 | 16.7 | 0.0 | 16.7 | 0.6409 |
| pT3 | 4 | 25.0 | 50.0 | 0.0 | 25.0 | 0.6409 |
| pN0 | 22 | 40.9 | 31.8 | 4.5 | 22.7 | 0.7939 |
| pN1 | 7 | 28.6 | 42.9 | 4.5 | 22.7 | 0.7939 |
| **Serous ovarian carcinoma (NOS)** | | | | | | |
| All cancers | 348 | 49.7 | 32.8 | 7.9 | 9.8 | 0.6000 |
| pT1 | 29 | 41.4 | 27.6 | 13.8 | 17.2 | 0.6000 |
| pT2 | 40 | 50.0 | 37.5 | 5.0 | 7.5 | 0.6000 |
| pT3 | 237 | 51.9 | 32.9 | 7.2 | 8.0 | 0.6000 |
| pN0 | 74 | 48.6 | 36.5 | 4.1 | 10.8 | 0.5481 |
| pN1 | 153 | 56.2 | 30.7 | 5.9 | 7.2 | 0.5481 |

pT, pathological tumor stage; pN, pathological lymph node status; L, lymphatic invasion status; V, blood vessel invasion status; MMR, mismatch repair.
Figure 4. Graphical comparison of PLAP data from this study (x) in comparison with the previous literature (dots). Red: $n = 1–9$, orange: $n = 10–50$, green: $n > 51$. For comparison purposes, studies that did not differentiate between different tumor subtypes were marked with blue dots and the overall positivity rate was applied to the different tumor subtypes present in our tumor microarrays. All studies are referred to in the reference list.
fits well with the distribution of PLAP expression in normal tissues and also with earlier studies. Several authors have previously described variable levels of PLAP expression in high-grade serous carcinomas [35,43,45,52,54,59], endometrioid carcinomas [4,35,45,52], and other variants [4,35,52,58] of ovarian cancer as well as in endometrial cancer [4,35]. Adenocarcinomas of the stomach and of the esophagus were also among the commonly PLAP-positive tumors. Previous studies have reported 67% PLAP positivity in a study on 6 adenocarcinomas of the esophagus [4] and in 38% of 8 [4], 23% of 107 [48], and in 0 of 2 gastric adenocarcinomas [35]. Moreover, the TCGA database described elevated PLAP expression in 60% of 354 gastric adenocarcinomas [1].

From a diagnostic point of view, it is important to keep in mind that very high PLAP expression levels, which are often considered characteristic for germ cell tumors, predominated in germ cell tumors but also occurred in multiple additional tumor entities. These included – in addition to those mentioned above – further clinically important and frequent cancer types such as adenocarcinoma of the lung, urothelial cancer, colorectal adenocarcinoma as well as mucoepidermoid carcinoma of salivary glands. It is also noteworthy that weak PLAP expression limited to a small subset of tumor cells can occur in a wide variety of tumor entities and must not be viewed as a strong argument for the germ cell origin of a cancer. It was not within the scope of our study to analyze molecular mechanisms and functional consequences of PLAP expression in these cancers. However, the availability of clinico-pathological data for some of the tumor entities that expressed PLAP in a significant fraction of cases enabled an analysis of the potential clinical significance of PLAP expression. Finding a link between PLAP upregulation and colon cancer aggressiveness supports the concept of targeting PLAP in colon cancers [66]. That the respective findings were inverse between endometrial and colorectal cancer might suggest that the tumor biologic role of PLAP expression can vary between tumor entities.

The data from this study provide a comprehensive ranking list of tumors according to their PLAP expression across a large variety of tumor entities. It is a strongpoint of our study that all tissues were stained in 1 day under exactly the same experimental conditions and that one expert pathologist interpreted all immunostains, resulting in as much standardization as possible. It is almost certain that the use of different protocols, antibodies, interpretation criteria, and thresholds used to define ‘positivity’ have jointly caused the high diversity of literature data on PLAP expression in cancer (summarized in Figure 4). The frequencies described in this study are thus specific to the reagents and protocols used in our laboratory. It is expected that different experimental conditions would have changed the PLAP positivity rates – especially in the cancers with low expression levels – but would have little impact on the tumor ranking based on the PLAP positivity rates.

In summary, our data show that PLAP can be highly expressed in a variety of tumor types. Besides germ cell tumors, which show the highest PLAP expression prevalence, high-level PLAP expression can be found in cancers from the female genital tract, the gastrointestinal, and the pancreaticobiliary system as well as in a few other tumor types. Low-level PLAP expression can be found in various other tumor entities and should generally not be viewed as a strong argument for germ cell neoplasia.

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Author contributions statement

VR, TK, RS and GS designed the study. VR, NG, AML, EB, AM, CW, SW, CF, KM, PL, RU, WW, FJ, SM, CB, AM, SS and TK performed the immunohistochemical analyses and/or contributed to the pathological validation of the tumors, the TMA construction, and data collection. MK, CH-M and RS carried out the data analyses. GS, RS, TK and VR wrote the first draft of the manuscript. All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to the published, and agree to be accountable for all aspects of the work.

References

1. Grossman RL, Heath AP, Ferretti V, et al. Toward a shared vision for cancer genomic data. N Engl J Med 2016; 375: 1109–1112.
2. Dabare AA, Nouri AM, Cannell H, et al. Profile of placental alkaline phosphatase expression in human malignancies: effect of tumour cell activation on alkaline phosphatase expression. Urol Int 1999; 63: 168–174.
3. Millán JL, Fishman WH. Biology of human alkaline phosphatases with special reference to cancer. Crit Rev Clin Lab Sci 1995; 32: 1–39.
4. Hamilton-Dutoit SJ, Lou H, Pallesen G. The expression of placental alkaline phosphatase (PLAP) and PLAP-like enzymes in normal
and neoplastic human tissues. An immunohistochemical study using monoclonal antibodies. *APMIS* 1990; 98: 797–811.

5. The GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; 45: 580–585.

6. Biermann K, Klingmüller D, Koch A, et al. Diagnostic value of markers M2A, OCT3/4, AP-2gamma, PLAP and c-KIT in the detection of extragonadal seminomas. *Histopathology* 2006; 49: 290–297.

7. Brehmer-Andersson E, Ljungdahl-Ståhle E, Koshida K, et al. Isoenzymes of alkaline phosphatases in seminomas. An immunohistochemical and biochemical study. *APMIS* 1990; 98: 977–982.

8. Burke AP, Mostofi FK. Placental alkaline phosphatase immunohistochemistry of intratubular malignant germ cells and associated testicular germ cell tumors. *Hum Pathol* 1988; 19: 663–670.

9. Cheville JC, Rao S, Iczkowski KA, et al. Cytokeratin expression in seminoma of the human testis. *Am J Clin Pathol* 2000; 113: 583–588.

10. Dekker I, Rozeboom T, Delemarre J, et al. Immunohistochemical and immunohistochemical features of primary central nervous system germ cell tumors: a 24-years experience. *Int J Clin Exp Pathol* 2014; 7: 6965–6972.

11. Epifanov NS. Joseph Lister (on the 150th anniversary of his birth). *Khirurgiya (Mosk)* 1977: 144–145.

12. Gao Y, Jiang J, Liu Q. Clinicopathological and immunohistochemical features of primary central nervous system germ cell tumors: a 24-years experience. *Int J Clin Exp Pathol* 2014; 7: 6965–6972.

13. Giwercman A, Cantell L, Marks A. Placental-like alkaline phosphatase as a marker of carcinoma-in-situ of the testis. Comparison with monoclonal antibodies M2A and 43-9F. *APMIS* 1991; 99: 586–594.

14. Hattab EM, Tu PH, Wilson JD, et al. OCT4 immunohistochemistry is superior to placental alkaline phosphatase (PLAP) in the diagnosis of central nervous system germinoma. *Am J Surg Pathol* 2005; 29: 368–371.

15. Heidenreich A, Sesterhenn IA, Mostofi FK, et al. Immunohistochemical expression of monoclonal antibody 43-9F in testicular germ cell tumours. *Int J Androl* 1998; 21: 283–288.

16. Hustin J, Collette J, Franchimont P. Immunohistochemical demonstration of placental alkaline phosphatase in various states of testicular development and in germ cell tumours. *Int J Androl* 1987; 10: 29–35.

17. Iczkowski KA, Butler SL, Shanks JH, et al. Trials of new germ cell immunohistochemical stains in 93 extragonadal and metastatic germ cell tumors. *Hum Pathol* 2008; 39: 275–281.

18. Inoue HK, Naganuma H, Ono N. Pathobiology of intracranial germ-cell tumors: immunohistochemical, immunohistochemical, and electron microscopic investigations. *J Neurooncol* 1987; 5: 105–115.

19. Jacobsen GK, Nørgaard-Pedersen B. Placental alkaline phosphatase in testicular germ cell tumours and in carcinoma-in-situ of the testis. An immunohistochemical study. *Acta Pathol Microbiol Immunol Scand A* 1984; 92: 323–329.

20. Kersemakers AM, Honecker F, Stoop H, et al. Identification of germ cells at risk for neoplastic transformation in gonadoblastoma: an immunohistochemical study for OCT3/4 and TSPY. *Hum Pathol* 2005; 36: 512–512.

21. Kraggerud SM, Berner A, Bryne M, et al. Spermatocytic seminoma as compared to classical seminoma: an immunohistochemical and DNA flow cytometric study. *APMIS* 1999; 107: 297–302.

22. Liu A, Cheng L, Du J, et al. Diagnostic utility of novel stem cell markers SALL4, OCT4, NANOG, SOX2, UTF1, and TCL1 in primary mediastinal germ cell tumors. *Am J Surg Pathol* 2010; 34: 697–706.

23. Loftus BM, Gilmartin LG, O’Brien MJ, et al. Intratubular germ cell neoplasia of the testis: identification by placental alkaline phosphatase immunostaining and argyrophilic nucleolar organizer region quantification. *Hum Pathol* 1990; 21: 941–948.

24. Mosbech CH, Svingen T, Nielsen JE, et al. Expression pattern of clinically relevant markers in paediatric germ cell- and sex-cord stromal tumours is similar to adult testicular tumours. *Virchows Arch* 2014; 465: 567–577.

25. Nakamura H, Takeshima H, Makino K, et al. C-kit expression in germinoma: an immunohistochemistry-based study. *J Neurooncol* 2005; 75: 163–167.

26. Niehans GA, Manivel JC, Copland GT, et al. Immunohistochemistry of germ cell and trophoblastic neoplasms. *Cancer* 1988; 62: 1113–1123.

27. Pantazis G, Harter PN, Capper D, et al. The embryonic stem cell factor UTF1 serves as a reliable diagnostic marker for germinomas. *Pathology* 2014; 46: 225–229.

28. Rajpert-De Meyts E, Kvist M, Skakkebaek NE. Heterogeneity of immunohistochemical tumour markers in testicular carcinoma in situ: pathogenetic relevance. *Virchows Arch* 1996; 428: 133–139.

29. Shinoda I, Miwa Y, Sakai N, et al. Immunohistochemical study of placental alkaline phosphatase in primary intracranial germ-cell tumours. *J Neurosurg* 1985; 63: 733–739.

30. Stoop H, Honecker F, van de Geijn GJ, et al. Stem cell factor as a novel diagnostic marker for early malignant germ cells. *J Pathol* 2008; 216: 43–54.

31. Suster S, Moran CA, Dominguez-Malagon H, et al. Germ cell tumors of the mediastinum and testis: a comparative immunohistochemical study of 120 cases. *Hum Pathol* 1998; 29: 737–742.

32. Suzuki T, Sasano H, Aoki H, et al. Immunohistochemical comparison between anaplastic seminoma and typical seminoma. *Acta Pathol Jpn* 1993; 43: 751–757.

33. Wang F, Liu A, Peng Y, et al. Diagnostic utility of SALL4 in extragonadal yolk sac tumors: an immunohistochemical study of 59 cases with comparison to placental-like alkaline phosphatase, alpha-fetoprotein, and glypican-3. *Am J Clin Pathol* 2009; 33: 1529–1539.

34. Weissferdt A, Kalhor N, Rodriguez Canales J, et al. Primary mediastinal yolk sac tumors: an immunohistochemical analysis of 14 cases. *Appl Immunohistochem Mol Morphol* 2019; 27: 125–133.

35. Wick MR, Swanson PE, Manivel JC. Placental-like alkaline phosphatase reactivity in human tumors: an immunohistochemical study of 520 cases. *Hum Pathol* 1987; 18: 946–954.

36. Yamamoto H, Uchibayashi T, Koshida K, et al. Immunopathology of alkaline phosphatase isozymes in seminoma. *Urol Int* 1993; 50: 33–35.

37. Fadare O, Shaker N, Alghamdi A, et al. Endometrioid tumors with yolk sac tumor-like morphologic patterns or immunophenotypes: an expanded appraisal. *Mod Pathol* 2019; 32: 1847–1860.

38. Som A, Zhu R, Guo CC, et al. Recurrent seminomas: clinical features and biologic implications. *Urol Oncol* 2012; 30: 494–501.
39. Washiyama K, Sekiguchi K, Tanaka R, et al. Immunohistochemical study on AFP, HCG and PLAP in primary intracranial germ cell tumors. Prog Exp Tumor Res 1987; 30: 296–306.

40. Uzuka T, Aoki H, Natsumeda M, et al. Indication of intraoperative immunohistochemistry for accurate pathological diagnosis of brain tumors. Brain Tumor Pathol 2011; 28: 239–246.

41. Braendstrup O. HLA class I antigens are expressed by Sertoli cells of intratubular germ cell neoplasia. APMIS 1996; 104: 579–582.

42. Bailey D, Marks A, Stratis M, et al. Immunohistochemical staining of germ cell tumors and intratubular malignant germ cells of the testis using antibody to placental alkaline phosphatase and a monoclonal anti-spermatocytic antibody. Mod Pathol 1991; 4: 167–171.

43. Orsaria M, Londero AP, Marzinotto S, et al. Placental type alkaline phosphatase tissue expression in ovarian serous carcinoma. Cancer Biomark 2016; 17: 479–486.

44. Goldsmith JD, Pawel B, Goldblum JR, et al. Detection and diagnostic utilization of placental alkaline phosphatase in muscular tissue and tumors with myogenic differentiation. Am J Surg Pathol 2002; 26: 1627–1633.

45. Nouwen EJ, Hendrix PL, Dauwe S, et al. Tumor markers in the human ovary and its neoplasms. A comparative immunohistochemical study. Am J Pathol 1987; 126: 230–242.

46. Tian BL, Gao AF, Xu C, et al. Clinicopathologic analysis of spermatocytic seminoma. Zhonghua Bing Li Xue Za Zhi 2012; 41: 752–755.

47. Chen YP, Zhu WF, Chen LF, et al. Clinicopathologic features and expression of OCT4 protein in testicular diffuse large B cell lymphoma. Zhonghua Bing Li Xue Za Zhi 2017; 46: 383–387.

48. Watanabe H, Tokuyama H, Ohta H, et al. Expression of placental alkaline phosphatase in gastric and colorectal cancers. An immunohistochemical study using the prepared monoclonal antibody. Cancer 1990; 66: 2575–2582.

49. Wong NA, Wingate J, Colling R. A study of alpha5 chain of collagen IV, caldesmon, placental alkaline phosphatase and smoothelin as immunohistochemical markers of gastrointestinal smooth muscle neoplasms. J Clin Pathol 2014; 67: 105–111.

50. Rais G, Andaloussi MM, Raissouni SS, et al. Spermatocytic seminoma at the National Institute of Oncology in Morocco. BMC Res Notes 2011; 4: 218.

51. Haroon S, Tariq MU, Fatima S, et al. Spermatocytic seminoma: a 21 years’ retrospective study in a tertiary care hospital in Pakistan. Int J Clin Exp Pathol 2013; 6: 2350–2356.

52. Nakopoulos L, Stefanaki K, Janinis J, et al. Immunohistochemical expression of placental alkaline phosphatase and vimentin in epithelial ovarian neoplasms. Acta Oncol 1995; 34: 511–515.

53. Saad RS, Landreneau RJ, Liu Y, et al. Utility of immunohistochemistry in separating thymic neoplasms from germ cell tumors and metastatic lung cancer involving the anterior mediastinum. Appl Immunohistochem Mol Morphol 2003; 11: 107–112.

54. Bollinger DJ, Wick MR, Dehner LP, et al. Peritoneal malignant mesothelioma versus serous papillary adenocarcinoma. A histochemical and immunohistochemical comparison. Am J Surg Pathol 1989; 13: 659–670.

55. Decaussin M, Borda A, Bouvier R, et al. Spermatocytic seminoma. A clinicopathological and immunohistochemical study of 7 cases. Ann Pathol 2004; 24: 161–166.

56. Zhang HT, Guo L, Su Q. Clinicopathologic analysis of spindle cell rhabdomyosarcoma: report of 8 cases. Zhonghua Zhong Liu Za Zhi 2008; 30: 141–143.

57. Cho NH, Shim HS, Choi YD, et al. Estrogen receptor is significantly associated with the epithelioid variants of renal angiomyolipoma: a clinicopathological and immunohistochemical study of 67 cases. Pathol Int 2004; 54: 510–515.

58. McDicken IW, McLaughlin PJ, Tromans PM, et al. Detection of placental-type alkaline phosphatase in ovarian cancer. Br J Cancer 1985; 52: 59–64.

59. Khoury N, Raju U, Crissman JD, et al. A comparative immunohistochemical study of peritoneal and ovarian serous tumors, and mesotheliomas. Hum Pathol 1990; 21: 811–819.

60. Moch H, Oberholzer M, Dalquen P, et al. Diagnostic tools for differentiating between pleural mesothelioma and lung adenocarcinoma in paraffin embedded tissue. Part I: immunohistochemical findings. Virchows Arch A Pathol Anat Histopathol 1993; 423: 19–27.

61. Boccelliino M, Vanacore D, Zappavigna S, et al. Testicular cancer from diagnosis to epigenetic factors. Oncotarget 2017; 8: 104654–104663.

62. Uhlen M, Bandrowski A, Carr S, et al. A proposal for validation of antibodies. Nat Methods 2016; 13: 823–827.

63. Human Protein Atlas. ALPP, RNA Database, Version 20.1. Release date 24 February 2021. Available from: https://www.proteinatlas.org

64. Li X, Berahovich R, Zhou H, et al. PLAP-CAR T cells mediate high specific cytotoxicity against colon cancer cells. Front Biosci (Landmark Ed) 2020; 25: 1765–1786.