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Research Article

Evaluation of Nectin-4 and Trop-2: Implications for Patient Outcomes and Therapy in Penile Cancer

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ABSTRACT

Metastatic penile cancer (PC) continues to have a poor prognosis because of inadequate treatment options. Enfortumab vedotin and sacituzumab govitecan are antibody-drug conjugates (ADCs) that have significantly improved the prognosis of several other tumor types and are, therefore, promising candidates for the successful treatment of metastatic PC. We examined the expression of ADC targets Nectin-4 and Trop-2 in an international multicenter cohort of 203 PC patients both in the primary tumor and in metastases. In addition, we evaluated their prognostic values. Either intermediate or high Nectin-4 membrane expression was found in 28.0% of primary tumors and 18.8% of metastases. The expression in primary tumor decreased significantly with increasing T stage ($P \leq .001$). It did not correlate with human papillomavirus status ($P = .307$) and was not associated with metastasis-free survival, cancer-specific survival, or overall survival. Nectin-4 expression levels at the tumor front and in metastases were significantly associated with each other ($P = .005$). Trop-2 was detected on the membrane of almost all samples with intermediate or high expression (primary tumor, 98.1%; metastasis, 100%). It was not associated with metastasis-free survival, cancer-specific survival, or overall survival. The expression at tumor front was significantly increased in human papillomavirus −positive tumors ($P = .005$). Neither Nectin-4 nor Trop-2 was found to be of prognostic value in PC. Enfortumab vedotin therapy seems promising for selected patients with high Nectin-4 expression, which should be confirmed in PC metastases before treatment is considered. Trop-2 is highly expressed in almost all PCs, and therefore, it is a very interesting potential target for sacituzumab govitecan therapy. Both ADCs warrant investigation in clinical trials focusing on PC.

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Introduction

Penile cancer (PC) is a rare disease within developed countries and fatal in the metastatic stage because of the lack of effective treatment options. Therefore, potential new therapeutics, including immune checkpoint inhibitors, have been tested; however, unfortunately, they have largely shown negative results in PC.[1,2] Recently, antibody-drug conjugates (ADCs) have led to significant improvements in prognosis in several tumor entities.[3,4] Enfortumab vedotin (EV), an ADC against Nectin-4, has been shown to drastically increase the overall survival (OS) of patients with urothelial carcinoma and recently received approval as first-line therapy in combination with pembrolizumab.[3,5] Sacituzumab govitecan (SG), which targets Trop-2, has also been approved by the Federal Drug Administration for the treatment of locally advanced or metastasized urothelial carcinoma and triple-negative breast carcinoma.[4,6] Both Nectin-4 and Trop-2 are membrane proteins that are involved in cell-cell adhesion and associated with tumor progression.[7-9] The expression of ADC targets, including Nectin-4 and Trop-2, is a prerequisite of therapy response.[10] Only limited data are available concerning Nectin-4 and Trop-2 expression in primary tumors of PC, and data on PC metastases are very rare.[11,12]

In this study, we investigated the expression of Nectin-4 and Trop-2 in both primary tumors and paired metastases within a large international cohort of PC patients to evaluate the rationale of using the 2 ADCs in PC. Furthermore, we analyzed their possible prognostic values and associations with clinical and histopathologic parameters.

Materials and Methods

Patient Cohort and Tissue Microarray Construction

Tumor tissues and clinical data from 203 patients who underwent treatment for PC at multiple centers in Germany, Russia, and Portugal between 1992 and 2018 were included. The TNM classification was updated to the eighth edition for malignant tumors.[13] The human papillomavirus (HPV) status was determined as previously described via HPV PCR and $p16^{INK4a}$ immunostaining (clone 1D7D2A1; Abcam).[14] Samples were considered HPV positive if both the HPV PCR and p16 staining were positive.

Tissue microarrays (TMAs) were constructed using tumor samples with a spot size of 1.5 mm from the tumor center (CENTER), tumor front (FRONT), lymph node metastases (MET), and normal penile skin tissue (SKIN), with the location of punches being determined by experienced uropathologists (A.P., C.G., A.H.). Two punches were used for the TMAs per patient and localization. Immunohistochemistry for Nectin-4 was performed on a Ventana BenchMark ULTRA autostainer (Ventana) using EPR15613-68 rb (Abcam) at a 1:100 dilution. The procedure included the following steps: first, heat retrieval was conducted at 100 °C for 4 minutes with CC1; then, incubation was performed with CC1 for another 64 minutes, followed by primary antibody incubation for 32 minutes, and finally, washing, detection, and counterstaining were conducted with hematoxylin for 4 minutes and then with bluing reagent for another 4 minutes. Trop-2 immunostaining was carried out using the antibody SP295 (Abcam) at a 1:500 dilution and the same pinpoints as for Nectin-4, except for the duration of time taken for the hematoxylin and bluing reagents (8 minutes each instead of 4 minutes). This staining protocol has been validated in a previous study.[15]

The H score was used for staining quantification. The samples were classified as negative (H score, 0-14), weak (H score, 15-99), moderate (H score, 100-199), or strong (H score, 200-300), as previously described (Supplementary Figs. 1 and 2).[15,16] This study was approved by the Saarland ethical committee (permit number 220/19) for analyses of patient data and tumor samples and conducted in accordance with the Declaration of Helsinki.

Statistical Analyses

Statistical analyses were performed using SPSS statistics 28 (SPSS; IBM) and GraphPad Prism (version 10.1.2; GraphPad). A Kaplan-Meier estimator was used for survival analyses, and statistical significance was assessed with the log-rank test. Nonparametric Mann-Whitney and Kruskal-Wallis tests were used for statistical comparisons between 2 or more groups. Relationship tests between paired samples were performed using Spearman rank correlation coefficient. All P values were calculated as 2-sided; $P < .05$ was considered statistically significant.

Results

Patient Characteristics

The patient characteristics are summarized in Table 1. High-risk HPV (hrHPV) and $p16^{INK4a}$ overexpression were simultaneously detected in tumors from 63 patients (41.4%), which were therefore defined as being HPV related. In 6 patients, hrHPV was detected without the overexpression of $p16^{INK4a}$, whereas 10 patients had $p16^{INK4a}$ overexpression without HPV detection. The usual type (55.5%) was the most common histologic subtype. During a median follow-up period of 31.50 months (IQR, 15.00-72.75 months), tumor recurrence or metachronous metastasis was found in 59 patients. In all, 76 patients died during the follow-up period, 46 of them died because of the tumor disease. For both markers, we investigated membranous and cytoplasmic staining separately, but we focused mainly on the former as the prerequisite of an ADC treatment effect.

Nectin-4

Nectin-4 membrane staining was negative in 40.4% of FRONT, 31.7% of CENTER, and 56.3% of MET samples (Fig. 1, Table 2). The median H score of membranous Nectin-4 expression was 30 (IQR, 0-80) in FRONT, 50 (IQR, 10-100) in CENTER, and 0 (IQR, 0-87.5) in MET. Moderate or strong Nectin-4 staining was found in 28% of CENTER and 23.4% of FRONT samples, but the proportion was reduced to 18.8% in MET; none of the latter samples displayed high expression.

Nectin-4 expression levels in the membrane and cytoplasm were significantly associated with each other in FRONT (Spearman, $P \leq .001$), CENTER ($P \leq .001$), and MET ($P \leq .001$). Although significant correlations of membranous Nectin-4 expression between FRONT and CENTER ($P \leq .001$) and between FRONT and MET ($P = .005$) were shown, there was no significant association between CENTER and MET ($P = .892$).

In comparisons of membranous expression in FRONT and MET, 47% of patients had a consistent level of expression of Nectin-4, although 23.5% of cases showed an increase and 29.4% showed a decrease in their H score in metastases. In contrast, the H scores were consistent between CENTER and MET in only 25% of cases,

Table 1
Clinical and histopathologic characteristics of patient cohort (N = 203)

	N	%
Median age (y)	61.5 (51.25-72.75)	
Primary surgical procedure		
Partial penectomy	81	45.3
Total penectomy	65	36.3
Circumcision	21	11.7
Glansectomy	12	6.7
N/A	24	
Primary tumor		
pT1a	33	19.5
pT1b	26	15.4
pT2	64	37.9
pT3	41	24.3
pT4	5	3.0
N/A	34	
Regional lymph nodes		
c/pN0	116	69.5
pN1	13	7.8
pN2	13	7.8
pN3	25	15.0
N/A	36	
Grading		
G1	26	13.3
G2	100	51.0
G3/G4	70	35.7
N/A	7	
Lymphovascular invasion		
LVI0	138	71.5
LVI1	55	28.5
N/A	10	
Perineural invasion		
Pn0	112	80.6
Pn1	27	19.4
N/A	64	
HPV ($+p16^{INK4a}$)		
Negative	89	58.6
Positive	63	41.4
N/A	51	
Non–HPV-related squamous cell carcinoma		
Usual type	110	55.5
Pseudohyperplastic	10	5.1
Pure verrucous	11	5.6
Carcinoma cuniculatum	2	1.0
Sarcomatoid	3	1.5
Mixed tumors	1	0.5
HPV-related squamous cell carcinoma		
Basaloid	19	9.6
Warty basaloid	27	13.6
Papillary basaloid	2	1.0
Warty	12	6.1
Clear cell	1	0.5

HPV, human papillomavirus; N/A, not available.

although an increase or a decrease in the H score in metastases was present in 37.5% for each (Supplementary Fig. S3).

Membranous Nectin-4 expression decreased significantly in CENTER ($P \leq .001$; Fig. 2) and FRONT ($P = .023$; Supplementary Fig. S4) with an increase in the T stage. However, no differences in expression were found regarding the nodal status at the time of diagnosis in FRONT ($P = .265$) or CENTER ($P = .739$; Supplementary Fig. S5).

Membranous Nectin-4 expression was not significantly associated with HPV status (CENTER, $P = .307$; FRONT, $P = .051$), but a weak trend toward higher expression in HPV-positive tumors

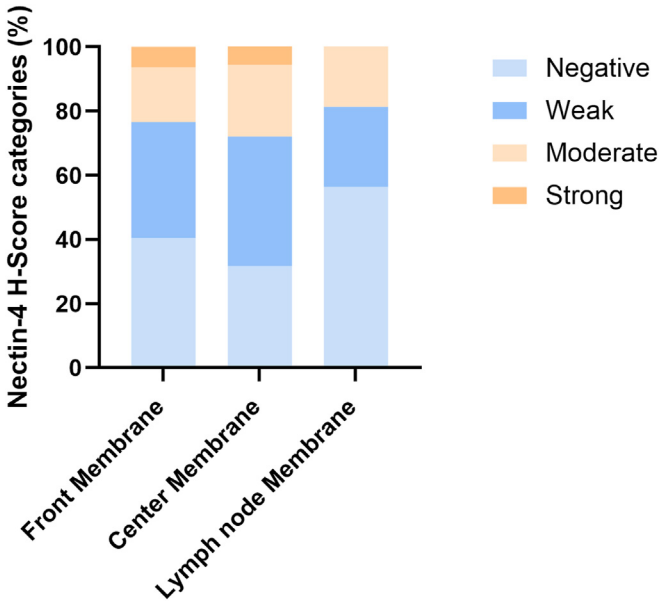

Figure 1.
Categorized membranous expression of Nectin-4 in tumor front, tumor center, and lymph nodes.

occurred in FRONT. Regarding histologic subtypes, significantly higher expression in the basaloid subtype compared with the usual type ($P = .037$) was found in FRONT (Supplementary Fig. S6), although no significant differences were observed between the other subtypes or in CENTER ($P = .412$; Supplementary Fig. S7). Neither membranous nor cytoplasmic Nectin-4 expression was associated with metastasis-free survival, cancer-specific survival, or OS (Supplementary Table S1; membrane: Supplementary Fig. S8; cytoplasm: Supplementary Fig. S9).

Trop-2

Trop-2 was expressed on the membrane of almost all samples (Fig. 3, Table 3). Moderate or high Trop-2 expression was found in 88.1% of FRONT, 98.1% of CENTER, and 100% of MET with median H scores of 240 in FRONT, 270 in CENTER, and 300 in MET. The cytoplasmic expression was lower, with median H scores of 80 in CENTER, 70 in FRONT, and 145 in MET and significantly associated with the membranous expression in FRONT (Spearman, $P = .010$) and MET ($P = .002$) but not in CENTER ($P = .674$). The membranous expression in CENTER was significantly associated with that in MET ($P = .035$), but this was not confirmed for FRONT ($P = .107$).

Membranous Trop-2 expression was not associated with the T stage or nodal status, neither in FRONT (T stage, $P = .261$; nodal status, $P = .203$) nor in CENTER (T stage, $P = .470$; nodal status, $P = .752$). However, FRONT showed significantly higher Trop-2 expression on the membrane of HPV-positive tumors ($P = .005$; Supplementary Fig. S10). There were no significant differences between the histologic subtypes ($P = .162$). In CENTER, no significant differences were found in terms of either HPV status ($P = .861$) or histologic subtype ($P = .066$).

Likewise, no significant association of Trop-2 expression on the membrane or in the cytoplasm with the metastasis-free survival, cancer-specific survival, or OS could be detected in either FRONT or CENTER (Supplementary Table S2; membrane: Supplementary Fig. S11; cytoplasm: Supplementary Fig. S12).

Table 2
Nectin-4 expression in tumor front, tumor center, lymph nodes, and normal skin tissue (N = 203)

	n	%	Median H score (IQR)
Nectin-4 FRONT membrane			
Negative	57	40.4	30 (0-80)
Low	51	36.2	
Moderate	24	17.0	
Strong	9	6.4	
Nectin-4 FRONT cytoplasm			
Negative	41	29.1	40 (10-80)
Low	78	55.3	
Moderate	20	14.2	
Strong	2	1.4	
Nectin-4 CENTER membrane			
Negative	51	31.7	50 (10-100)
Low	65	40.4	
Moderate	36	22.4	
Strong	9	5.6	
Nectin-4 CENTER cytoplasm			
Negative	26	16.1	60 (30-100)
Low	91	56.5	
Moderate	39	24.2	
Strong	5	3.1	
Nectin-4 average membranous H score primary tumor			
Negative	25	19.4	45 (18.75-95)
Low	76	58.8	
Moderate	26	20.2	
Strong	2	1.6	
Nectin-4 lymph nodes membrane			
Negative	18	56.3	0 (0-87.5)
Low	8	25.0	
Moderate	6	18.8	
Strong	0	0	
Nectin-4 lymph nodes cytoplasm			
Negative	11	34.4	30 (10-60)
Low	16	50.0	
Moderate	4	12.5	
Strong	1	3.1	
Nectin-4 normal tissue membrane			
Negative	23	23.7	90 (20-170)
Low	29	29.9	
Moderate	27	27.8	
Strong	18	18.6	
Nectin-4 normal tissue cytoplasm			
Negative	21	21.6	50 (20-85)
Low	60	61.9	
Moderate	13	13.4	
Strong	3	3.1	

Validation of Tissue Microarray Results Using Whole Slide Sections

To evaluate the data obtained from the TMA analysis, 15 representative whole slide sections were stained against Nectin-4 and 10 against Trop-2 (Supplementary Figs. S13 and S14). A strong correlation in Nectin-4 staining between the TMA samples and the corresponding whole slides occurred, both for the membrane (Spearman correlation coefficient, 0.920; $P \leq .001$) and for the cytoplasm (Spearman correlation coefficient, 0.777; $P \leq .001$). Although the H score category was identical in 93.3% of cases in the TMA and whole sections, a higher H score was defined for the whole slide (weak) than for the TMA section average (negative) in 1 case.

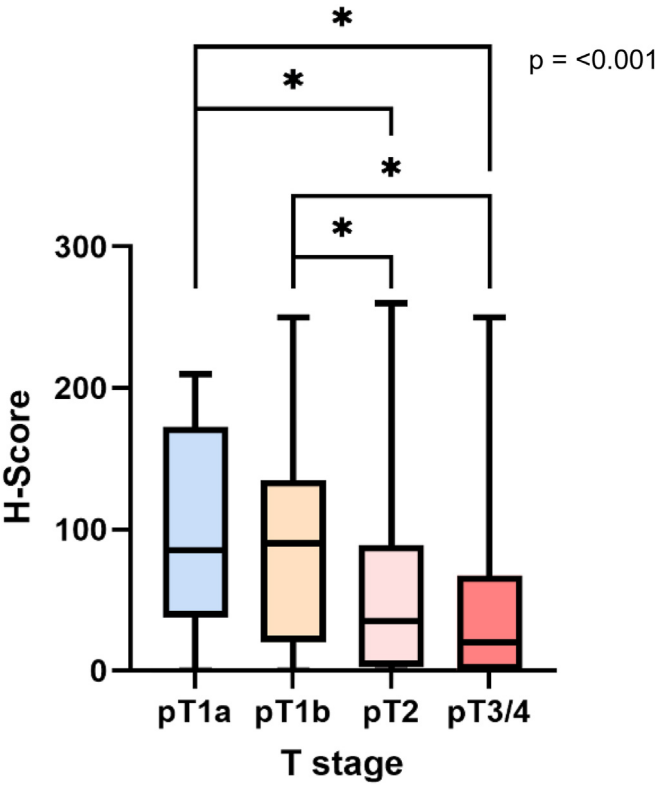

Figure 2.
Membranous Nectin-4 expression in tumor center (CENTER) depending on the T stage.

Trop-2 staining also showed a significant correlation in expression between the TMA and whole slide sections for both the membrane (Spearman correlation coefficient, 0.833; $P = .003$) and

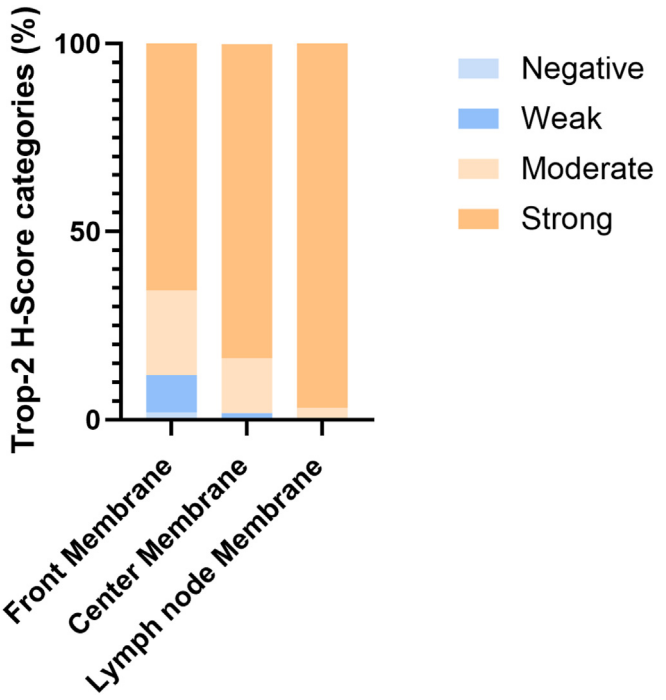

Figure 3.
Categorized membranous expression of Trop-2 in tumor front, tumor center, and lymph nodes.

Table 3
Trop-2 expression in tumor front, tumor center, lymph nodes, and normal skin tissue (N = 203)

	n	%	Median H score (IQR)
Trop-2 FRONT membrane			
Negative	3	2.0	240 (180-280)
Low	15	9.9	
Moderate	34	22.5	
Strong	99	65.6	
Trop-2 FRONT cytoplasm			
Negative	21	14.0	70 (30-120)
Low	72	48.0	
Moderate	40	26.7	
Strong	17	11.3	
Trop-2 CENTER membrane			
Negative	0	0	270 (220-290)
Low	3	1.8	
Moderate	24	14.5	
Strong	138	83.6	
Trop-2 CENTER cytoplasm			
Negative	32	19.4	80 (20-160)
Low	62	37.6	
Moderate	45	27.3	
Strong	26	15.8	
Trop-2 average membranous H score primary tumor			
Negative	0	0	250 (200-275)
Low	3	2.2	
Moderate	30	22.1	
Strong	103	75.7	
Trop-2 lymph nodes membrane			
Negative	0	0	300 (270-300)
Low	0	0	
Moderate	1	3.2	
Strong	30	96.8	
Trop-2 lymph nodes cytoplasm			
Negative	0	0.0	145 (110-292,5)
Low	1	4.5	
Moderate	11	50.0	
Strong	10	45.5	
Trop-2 normal tissue membrane			
Negative	0	0	300 (290-300)
Low	0	0	
Moderate	0	0	
Strong	99	100	
Trop-2 normal tissue cytoplasm			
Negative	2	2.0	230 (170-290)
Low	1	1.0	
Moderate	29	29.6	
Strong	66	67.3	

the cytoplasm (Spearman correlation coefficient, 0.794; $P = .006$). The H score category for the whole slide section (intermediate) differed in 2 cases from that of the TMA (weak).

Discussion

New therapeutic options are urgently needed for PC because it remains a rare disease with a poor prognosis in the metastatic stage.[17] In this context, ADC therapies, such as EV or SG, could be helpful to overcome the present therapeutic limitations in PC. However, the presence of targets must be evaluated as a prerequisite of therapy response. Therefore, we investigated Nectin-4 and Trop-2 expression in both primary tumors and paired metastases and correlated it with clinical parameters in a well-characterized PC cohort. This is currently the largest published cohort that has been analyzed for Nectin-4 or Trop-2 expression.

We observed intermediate or high expression of Nectin-4 in only approximately 25% of cases, which was also reflected in a low median H score of 50, although low expression was very common. This is consistent with data from a study by Tekin et al,[12] in which high expression of Nectin-4 was present in approximately 10% of patients. In another study, more frequent Nectin-4 expression was detected, but separate results for cytoplasmic and membranous expression were not reported.[11] The differences in expression results between individual studies could be because of heterogeneous Nectin-4 expression in tumor tissue, as has been shown in several tumor entities.[12,18,19] In contrast to other studies, we used TMAs with representative cores from both the tumor front and center. We did not observe significant differences in expression between the distinct areas. When the average H scores were calculated for the FRONT and CENTER, the H score category switched in several cases from negative to low, reflecting minimal heterogeneity between the areas.

We further demonstrated a significant decrease in Nectin-4 expression with increasing tumor stage, which could limit the effectiveness of the therapy in advanced tumor stages. A further decrease in expression was seen in lymph node metastases compared with primary tumors, which has also been reported for other tumor entities.[15] To our knowledge, this is the first study to analyze Nectin-4 expression in PC metastases. ADC therapy appears to be particularly useful in the metastatic stage, and therefore, knowledge of expression in metastases is of decisive importance for therapy planning. To identify patients who could benefit from EV therapy, a previous metastasis biopsy seems necessary because the expression in the primary tumor is not predictive of that in metastases in many cases, as shown in our study and in accordance with data on bladder cancer.[15] A recently published case report demonstrated that treatment with EV might be appropriate in metastatic PC in the case of high Nectin-4 expression.[20]

In our study, Nectin-4 expression was independent of HPV status in contrast to the findings of previous studies in relation to PC and other squamous tumor entities.[11,12,21] The significantly higher expression in the basaloid subtype, which is mostly HPV positive, could at least in part explain a possible association with HPV, as found by Tekin et al[12]; in their study, the proportion of basaloid tumors was 14.9% and, thus, significantly higher than the 9.6% in our cohort. Further evaluations should be carried out to evaluate whether there is an association with HPV status or histologic subtype, especially because a recently published study demonstrated potentially higher expression of Nectin-4 and Trop-2 in HPV-positive tumors without evidence of a *TP53* mutation.[22]

We did not observe any prognostic value of Nectin-4 expression. This is consistent with results obtained by Grass et al,[11] who also found no significant differences with regard to survival. In contrast, another study described significantly longer progression-free survival in patients with high Nectin-4 expression, although this must be critically assessed because of the small number of patients with high expression.[12] Here, a higher proportion of low-stage tumors, which are characterized by higher Nectin-4 expression, as shown in our study, could be a reason for the contradictory results.[14,23] To summarize, it appears that Nectin-4 is less useful as a prognostic marker in PC than nodal status or other clinical parameters.

In contrast to Nectin-4, Trop-2 is almost always highly expressed both in the primary tumor and in metastases, which makes it a highly desirable target for potential ADC therapy using

SG. High Trop-2 expression in the primary tumor has been reported in PC and in other squamous cell tumor entities, in concordance with our results.[12,24-27] There is consistently high expression of Trop-2 in both the primary tumor and metastases, and therefore, SG therapy in metastasized PC appears to be appropriate even without previous testing or biopsy.

The optimal H score threshold of target expression to predict the efficacy of EV or SG remains unclear. Although in vitro studies have demonstrated a correlation between treatment response and biomarker expression, a definitive cutoff value has yet to be established.[28-30] In a study by Klümper et al,[15] the response to EV therapy in urothelial carcinoma was significantly lower in cases with low or absent Nectin-4 expression than in those with moderate or high expression. Similarly, Loriot et al[31] evaluated the treatment response to SG of urothelial carcinoma by comparing patients with Trop-2 H scores above and below 215. Although a higher response rate and longer progression-free survival were observed in patients with a higher H score, the difference did not reach statistical significance, leaving uncertain the precise threshold for treatment response.

In our study, Trop-2 expression was not associated with prognosis because almost all tumors had a high or moderate expression. This is in contrast to some other studies concerning squamous cell carcinomas, which might be because of different H score cutoff values.[12,27,32] Furthermore, we were able to show that Trop-2 expression was significantly increased in hrHPV-positive tumors, especially in the tumor front, without significant differences in histologic subtypes. Higher Trop-2 expression in HPV-positive tumors has already been reported, and previous studies have discussed a possible influence of Trop-2 on the *E6/E7* activity of HPV or changes in the *PI3K* signaling pathway.[12,26] A more detailed analysis of Trop-2-associated molecular mechanisms is required, and in parallel, preclinical studies are necessary to evaluate the rationale and biological effects of these promising new therapeutic options.

A limitation of our study may be the use of TMAs instead of whole slide sections when describing Nectin-4 expression in tumor tissues. However, several representative cores from the tumor front and center were analyzed. In addition, the evaluation using whole slide sections confirmed the eligibility of TMAs as representative sources to analyze large patient cohorts. The use of different antibodies, along with the possible differences among patient cohorts concerning tumor stages and histologic subtypes, may also explain differences in the expression frequency, particularly that of Nectin-4, compared with other limited published data. Another limitation may be the age of the samples because older samples could be suboptimal for molecular testing. Therefore, the number of real HPV-positive cases could be slightly higher. However, the frequency of HPV-positive patients corresponds to the incidence found in larger meta-analyses.[33] We also included p16 overexpression as a surrogate marker for HPV status because this correlates specifically with HPV as shown in squamous cell carcinomas of the oropharynx.[34] Furthermore, it must be discussed as to whether grading should be carried out in HPV-positive PC. In squamous cell carcinomas of the oropharynx, HPV-associated tumors are usually nonkeratinizing and strongly basaloid, that is, conventionally G3, although they are clinically much less aggressive than non–HPV-associated carcinomas with G2 differentiation.[34] However, because of the rarity of PC and the lack of evidence in large cohorts, no valid conclusions can be drawn as to whether tumor grading is appropriate. Given the uncertain prognostic value of histologic grading in HPV-positive PC, assessments of *TP53* status may offer a more reliable approach to evaluating tumor aggressiveness. In their study, Elst et al[22] demonstrated that *TP53* loss of function is a strong driver of poor prognosis, independent of HPV status. Nevertheless, the strengths of our study lie in the well-characterized and substantially large international multicenter patient cohort, which enabled a detailed analysis of Nectin-4 and Trop-2 in primary tumors and, for the first time, in a representative number of metastases of PC.

In conclusion, neither Nectin-4 nor Trop-2 was of prognostic value in PC in our study. Trop-2 was highly expressed in almost all PC cases, making it an exciting target for potential ADC therapy. Nectin-4 was expressed less frequently, especially in the advanced stage, and hence, EV therapy appears to be useful for selected patients with confirmed Nectin-4 expression; therefore, biopsies of metastases are recommended. Further preclinical and clinical studies on both drugs should be carried out to improve the limited therapeutic options for metastatic PC.

Author Contributions

J.N.M. and K.J. performed study concept and design; J.N.M., M.E., A.H., and C.G. developed methodology; J.N.M., K.J., and M.E. wrote the original draft; J.N.M., M.E., J.H., A.H., M.S., R.H., and V.M. involved in writing, review, and editing; J.N.M., M.E., A.P., S.L., K.B., and R.M.B. performed investigation; O.K., J.L., R.H., H.L., J.S., C.J., H.W., R.M.B., M.S., and V.M. provided resources; K.J., A.H., M.S., and V.M. performed supervision; and J.N.M., M.E., and K.J. provided formal analysis.

Data Availability

Data generated and analyzed during the study are available on reasonable request to the corresponding author.

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Declaration of Competing Interest

The authors report no relevant conflicts of interest.

Ethics Approval and Consent to Participate

This study was approved by the Saarland ethical committee (permit number 220/19) for analyses of patient data and tumor samples and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients involved in this study. If this was impossible because of the retrospective character of this study over 30 years, data have been handled in an anonymous way according to the European Union General Data Protection Regulation.

Supplementary Material

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