Immunohistochemical expression of TROP-2 (TACSTD2) on the urothelial carcinoma of the urinary bladder and other types of cancer

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Abstract. In metastatic or locally advanced urothelial carcinoma (UC), therapeutic options have been limited to chemotherapy and immune checkpoint inhibitors. Novel targets and drugs such as antibody drug conjugates have been developed, and enfortumab vedotin targeting nectin-4 and sacituzumab govitecan (SG) targeting trophoblast cell surface antigen 2 (TROP-2), the protein product of the TACSTD2 gene, have been approved. The expression of TROP-2 was investigated within UC and other types of carcinomas, and within the tissue of different healthy organs to understand treatment responses and toxicities. The expression of TROP-2 in the tissues of 42 patients with UC, 13 patients with other types of cancer and in the normal tissues of 11 patients was retrospectively analyzed. Immunohistochemical staining of the TROP-2 protein was performed on a BenchMark ULTRA IHC/ISH System (Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) according to accredited staining protocols in a routine immunohistochemistry accredited and certified facility of the laboratory of immunohistochemistry at the Institute of Pathology (Gerhard-Domagk Institute)- University Hospital Muenster (UKM)-Muenster-Germany]. Different expression levels of TROP-2 were observed, and the highest expression rate of TROP-2 was observed in UC, independent of the tumor stage. However, normal urothelial cells had similar expression levels. Except for ductal carcinoma *in situ*, the expression of TROP-2 was reduced in other types of cancer and in the healthy tissues from other organs, including pancreas, gall bladder, colon and prostate. Given the treatment response based on the expression level of TROP-2, SG would be effective in almost all cases of UC. However, it would also have an effect on the normal urothelium.

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Introduction

Bladder cancer is the 10th most common cancer worldwide (1). This type of cancer is particularly challenging to treat as it is most frequent (>50%) within the elderly population, and these patients often have underlying morbidities and reduced functional status (2). The standard treatment for metastatic disease is chemotherapy, including treatment with gemcitabine and cisplatin, or treatment with methotrexate, vinblastine, doxorubicin and cisplatin (3,4), followed by maintenance immunotherapy with avelumab (5). For all patients, and specifically for the elderly, treatment is challenging due to side effects or contraindications. Besides avelumab, other immune checkpoint inhibitors (ICIs) and monoclonal antibodies have been approved since 2016. The ICIs used for the treatment of bladder cancer, including atezolizumab, durvalumab, nivolumab and pembrolizumab, target either programmed cell death protein-1 (PD-1) or programmed death ligand-1 (PD-L1), and they can be used as first- or later-line treatment of metastatic and refractory bladder cancer (6-12). The response rates to ICI treatment are relatively low, and these drugs are expensive with a high economic burden (13). There are numerous adverse effects that influence the pathway of therapy with ICIs, such as neurological complications, gastrointestinal tract irritation, cardiovascular adverse effects and possibly myocarditis, musculoskeletal toxicity in different body parts and hemotoxicity (14). Therefore, novel targeted approaches such as fibroblast growth factor receptor 3 (FGFR3) inhibition and antibody drug conjugates (ADCs) have been investigated, resulting in approval of erdafitinib (15). After chemotherapy and immunotherapy, enfortumab vedotin, an ADC-targeting nectin-4 has been approved for the treatment of metastatic urothelial carcinoma (UC) (16). In April 2021, sacituzumab govitecan (SG), an ADC targeting trophoblast cell surface antigen 2 (TROP-2), became a treatment option for patients

with locally advanced or metastatic UC, who also previously received platinum-containing chemotherapy and either a PD-1 or PD-L1 inhibitor (17).

TROP-2, a protein encoded by the TACSTD2 gene, is a transmembrane glycoprotein that is upregulated in all types of cancer, which is independent of the baseline levels of TROP-2 expression (18). TROP-2 is expressed in numerous solid tumors with a reduced expression observed in normal tissues and has a role in stem cell biology (18). It regulates cancer growth, invasion and spread by several signaling pathways, for example in thyroid cancer cell invasion, TROP-2 signal transduction has been seen as a downstream effect of the ERK and JNK pathways (19). Stoyanova *et al* (20) demonstrated that TROP-2 signaling enhances stem cell-like properties of cancer cells, as TROP-2 regulates proliferation and self-renewal through β-catenin signaling (2). It has been speculated that phosphatidylinositol 4,5-bisphosphate (PIP2) may regulate TROP-2 phosphorylation and calcium signal transduction, as the cytoplasmic domain of TROP-2 contains a PIP2-binding sequence overlapping with a protein kinase C phosphorylation site (21).

High TROP-2 expression levels are associated with poor prognosis in pancreatic, hilar cholangiocarcinoma, cervical, gastric and other types of cancer (22,23).

In the present study, the aim was to investigate the expression of TROP-2 through immunohistochemical analysis within the tumor tissue and tissue of different healthy organs for an improved understanding of treatment responses and toxicities.

Materials and methods

Patient cohort. The tumor tissues of patients with UC at different stages of invasion as well as different sporadically normal tissues as a control were retrospectively investigated. Patients were treated at the University Hospital in Muenster, Germany. The majority of biopsies originated from invasive UC (n=17), fewer from non-invasive UC, papillary carcinomas of the urinary bladder (n=11), urothelial carcinoma *in situ* (CIS) of the urinary bladder (n=10), invasive UC of the renal pelvis (n=3) and poorly differentiated neuroendocrine carcinoma of the urinary bladder (n=2). The age range of patients was 47-84 years. Sex distribution was 67% men and 33% women. The tissues were collected in 4-5 months, at first the tumor tissue and as a control of the staining, the normal tissue biopsies. Tissues were processed and stained with hematoxylin and eosin: Fixation in 4% buffered formalin then processed automatically in Leica-Biosystem-Apparat (Histocore Peloris 3; Leica Microsystems GmbH); formalin for 44 min at 45˚C then ascending in different six concentrated ethanol runs in 270 min with temperature 45˚C, then three runs though xylene in 180 min. with temperature 45˚C. This was followed by 3 runs of paraffin at 65˚C. The blocks were sectioned at 2 μm. The slides were placed in oven for 20 min at 63˚C for deparaffination and placed in xylene with at 37˚C. The treatment was repeated to remove the wax. Staining was performed in the Leica-Biosystem-Apparat starting with hydration through decreasing concentration of alcohol baths (100, 90, 80 and 70%) and water. Staining was in hematoxylin for 3-5 min. at room temperature.

The sections were washed in running tap water until blue for ≤5 min. Differentiation was with 1% acid alcohol (1% HCl in 70% alcohol) for a few sec. After rinsing in running tap water, the section was dipped in ammonia water until the sections become blue, followed by tap water wash. The sections were counterstained in 1% Eosin Y for 10 min. at room temperature.

After washing in tap water for 1-5 min the slides were dehydrated in increasing concentration of alcohols. The slides were placed in two xylene baths for clearing and then mounted in DPX before observation under a light microscope. Tissues from other types of cancer were also investigated: Colonic adenocarcinoma (n=2), primary prostate cancer (n=3), specimens from lymph node metastases of prostate carcinoma (PC; n=2), endometrial carcinoma (n=2) and ductal carcinoma *in situ* (DCIS) of the breast (n=2). Regarding normal tissues obtained from patients without cancer, colonic tissue (n=2), gall bladder tissue (n=2), pancreatic tissue (n=2) and prostate tissue (n=3) were investigated. The clinicopathological data of the patient cohort are summarized in Table I. Tumor samples were systematically reviewed by two uropathologists according to the 8th Tumor-Node-Metastasis cancer staging system [Union for International Cancer Control, UICC (24)] and the 2022 WHO classification for genitourinary tumors (25).

Immunohistochemistry. Immunohistochemical staining of the TROP-2 protein was carried out on a BenchMark ULTRA IHC/ISH System (Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) according to accredited staining protocols in a routine laboratory of immunohistochemistry of the Institute of Pathology (Gerhard-Domagk Institute)-University Hospital Muenster (UKM)-Muenster-Germany, accredited and certified according to the D-15-13021-01-00 (DAKKS (Deutsche Akkreditierungsstelle). Tissues were cut into 4-μm thick paraffin sections and incubated with the polyclonal anti-TROP-2 primary antibody (cat. no. HPA055067; MilliporeSigma; clone TACSTD2, Rabbit; 1:300) for 32 min at 37˚C, and antigen retrieval was carried out using the CC1 buffer from Roche Tissue Diagnostics; Roche Diagnostics, Ltd. with incubation at 95˚C for 32 min. The staining protocol was established using the specific membranous TROP-2 protein expression on normal bladder urothelium. The final titration of 1:300 was identified from a dilution row of 1:50, 1:100, 1:150, 1:200, 1:300 and 1:400 with the 1:300 primary antibody dilution revealing strong (intensity 3+) specific membranous TROP-2 protein expression in normal urothelial cells without any detectable unspecific background staining, using optiView DAB IHC detection kit and examined under light microscopy. The surrounding smooth muscle tissue of bladder samples served as an internal control. Tonsil tissue served as an additional external negative control. Membranous TROP-2 expression was evaluated by two board-certified uropathologists. Specific TROP-2 immunoreactivity localized to the cell membrane of ≥10% of tumor cells was considered positive. TROP-2 expression was then classified as either negative (0), weak (1+), moderate (2+) or strong (3+) (13).

Ethics approval. The present study was approved by the local ethical commission (approval no. 2016-483-f-S for urothelial carcinoma and 2007-467-F-S for prostate carcinoma) of the University Hospital Muenster and from the Medical Association

Table I. Clinicopathological data of the cohort.

Type of cancer	Strong (3+) or moderate (2+) TROP-2 protein expression, n (% of normal cells)	Strong (3+) TROP-2 protein expression, n (% of tumor cells)	Moderate (2+) TROP-2 protein expression, n (% of tumor cells)	Weak (1+) TROP-2 protein expression, n (% of tumor cells)	Absence (0) of TROP-2 protein expression, n (% of tumor cells)
Invasive UC (n=17)	3+/12 (100)	12 (100)	0 (0)	0 (0)	0 (0%)
	2+/4 (100)	4 (20)	4 (70)	0 (0)	0 (10)
	3+/1 (100)	1 (20)	0 (0)	0 (0)	0 (80%)
	0 (0)	0 (0)	1 (10)	0 (0)	0 (90%)
Poorly differentiated neuroendocrine carcinoma (n=2)	3+/2 (100)	2 (20)	0 (0)	0 (0)	0 (80%)
Papillary non-invasive UC (n=11)	3+/10 (100)	10 (100)	1 (70)	0 (0)	0 (30)
UC *in situ* (n=10)	3+/9 (100)	9 (100)	1 (100)	0 (0)	0 (0)
Invasive UC of renal pelvis (n=3)	3+/3 (100)	0 (0)	2 (90)	1 (10)	0 (0)
Primary PC (n=3)	3+/3 (100)	2 (85)	0 (0)	0 (0)	0 (15)
Metastatic PC (n=2)	0 (0)	1 (90)	0 (0)	1 (80)	0 (10-20)
Normal colon mucosa (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (100%)
Colon adenocarcinoma (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (100%)
Normal pancreatic tissue	0 (0)	0 (0)	0 (0)	1 (70)	0 (30)
(n=2)	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)
Endometrial carcinoma (n=2)	0 (0)	0 (0)	0 (0)	2 (10)	0 (90)
Normal gall bladder epithelium (n=2)	0 (0)	0 (0)	0 (0)	2 (10)	0 (90)
Ductal *in situ* carcinoma of the breast (n=2)	2+/2 (100)	0 (0)	2 (90)	0 (0)	0 (10)

UC, urothelial carcinoma; PC, prostate carcinoma; TROP-2, trophoblast cell surface antigen 2.

of Westfalen-Lippe-Germany. All investigations were carried out according to guidelines and regulations. Written informed consent was obtained from the patients before the investigation.

Results

Expression of TROP-2 in tissues of invasive UC of the bladder. In 12/19 cases with histological diagnosis of invasive UC at stages tumor (T)1-T4, there was a strong complete cell membrane expression of TROP-2 (3+) in 100% of tumor cells. Normal urothelial cells (Fig. 1A-C) also demonstrated strong expression of TROP-2 (3+). In 4 cases of invasive UC, only 20% of tumor cells demonstrated strong expression of TROP-2 (3+), and 70% of tumor cells demonstrated moderate expression of TROP-2 (2+). Normal urothelium within these tumors showed expression of TROP-2 with strength (2-3+) (Fig. 1D). In a single case of invasive UC, 20% of tumor cells demonstrated strong expression of TROP-2 (3+), while normal urothelium was detected in 100% (3+) of tumor cells. In 1 case of carcinoma *in situ*, 100% of tumor cells demonstrated expression of 3+. In 2 cases of poorly differentiated neuroendocrine carcinoma, only 20% of tumor cells demonstrated strong expression of TROP-2 (3+) in 1 of the 2 cases, while 10% of tumor cells demonstrated moderate expression of TROP-2 (2+) in the other case (Fig. 1E).

Expression of TROP-2 in tissues of invasive UC of the renal pelvis. In 2/3 cases of invasive UC of the renal pelvis, a moderate expression of TROP-2 (2+) was observed in 90% of the tumor cells, while the normal epithelium of the tubules demonstrated strong expression of TROP-2 (3+). In the third case of invasive UC of the renal pelvis, only 10% of tumor cells demonstrated a weak TROP-2 expression (1+), and 100% of the associated normal urothelial cells had a strong TROP-2 expression (3+; Fig. 1F).

Expression of TROP-2 in tissues of papillary non-invasive UC of the bladder. In 10/11 cases of papillary non-invasive UC, there was strong membrane expression of TROP-2 (3+) in 100% of tumor cells as well as in the associated normal urothelial cells. In a single case, 70% of tumor cells demonstrated moderate expression of TROP-2 (2+; Fig. 1G and H).

Expression of TROP-2 in tissues of CIS of the bladder. In 9/10 cases with CIS of the bladder, there was strong expression

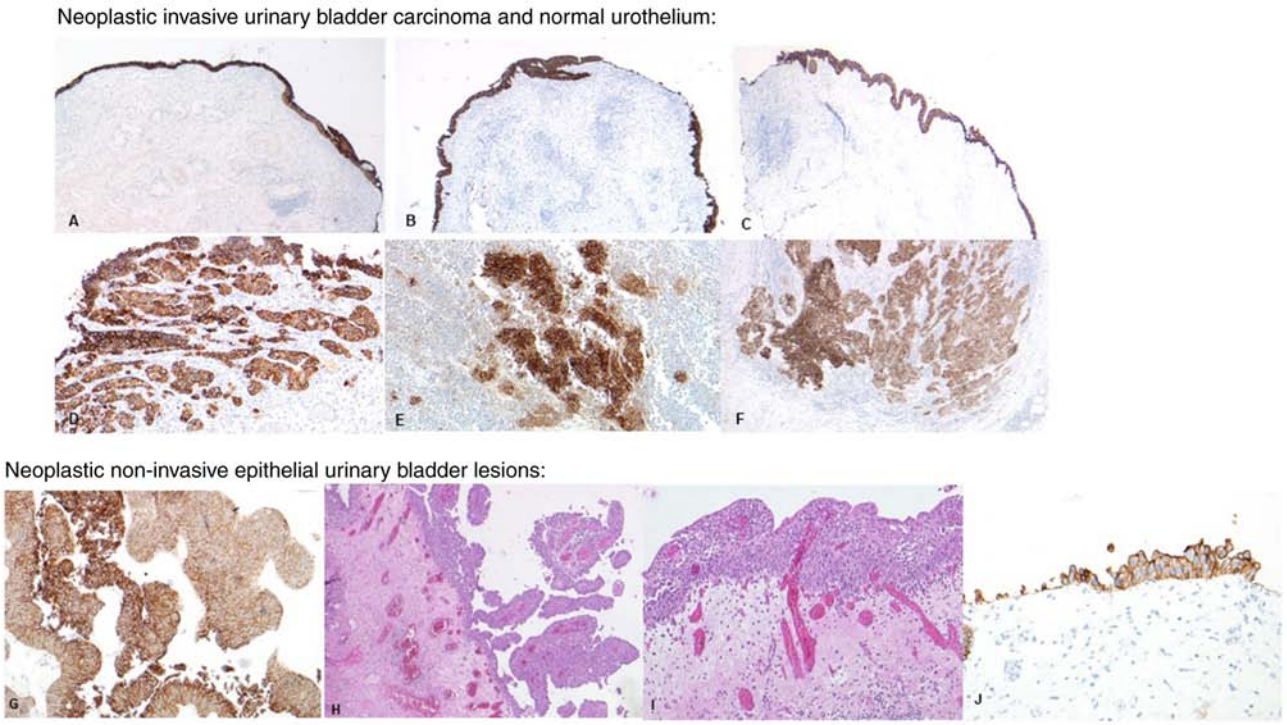

Figure 1. Immunohistochemical expression of TROP-2 on different subtypes of uothelial carcinoma of the urinary bladder and renal pelvis, normal urothelium and non-invasive urothelial tumors. (A-C) Normal urothelial cells of the bladder with strong expression of TROP-2 (3+) on the surface of 100% of the cells (magnification, x10). (D) Invasive UC of the bladder with strong expression of TROP-2 (3+) on the membrane of 100% of the tumor cells (magnification, x10). (E) Invasive high-grade neuroendocrine carcinoma of the bladder with strong expression of TROP-2 on the membrane and in cytoplasm in the central group of tumor cells (magnification, x10). (F) Invasive UC of the renal pelvis with strong expression of TROP-2 (3+) on the membrane of 100% of the tumor cells (magnification, x10). (G) Papillary non-invasive UC of the bladder with strong expression of TROP-2 (3+) on the surface of 100% of the tumor cells (magnification, x10). (H) Papillary non-invasive UC of the bladder with papillary projections of the neoplastic urothelial cells (magnification, x10; stained with H&E). (I) Urothelial CIS of the bladder with flat lesions of the dysplastic urothelial cells (magnification, x20; stained with H&E). (J) Urothelial CIS of the bladder with strong expression of TROP-2 (3+) on the surface of 100% of the tumor cells (magnification, x20). TROP-2, trophoblast cell surface antigen 2; UC, urothelial carcinoma; H&E, hematoxylin and eosin; CIS, carcinoma *in situ*.

of TROP-2 (3+), and only 1 case demonstrated moderate expression of TROP-2 (2+). A strong expression of TROP-2 (3+) was observed within the associated normal urothelial cells of all cases (Fig. 1I and J).

Expression of TROP-2 in tissues of primary and metastatic PC. In 3 cases with primary PC, TROP-2 expression was weak (1+) in 80% of the tumor cells in 1 case, and 85% of the tumor cells in the other 2 cases had a moderate to strong TROP-2 expression (2-3+) (Fig. 2A and B). Normal prostatic glands (Fig. 2C) within these tumors were associated with a strong TROP-2 expression (3+). The 2 cases with metastatic PC within the lymph nodes demonstrated different levels of TROP-2 expression (Fig. 2D); in 1 case, only 10% of the tumor cells had a weak TROP-2 expression (1+), and in the other case, 90% of the tumor cells demonstrated a strong TROP-2 expression (3+).

Expression of TROP-2 in tissues other than urogenital organs. There was no expression of TROP-2 in normal colon mucosa and in adenocarcinoma of the colon (Fig. 2E). Weak TROP-2 expression (1+) was observed in 10-70% of the glandular epithelial cells of the normal pancreatic tissue (Fig. 2F). In endometrial carcinoma, 10% of the tumor cells demonstrated a weak TROP-2 expression (1+) (Fig. 2G). In the gall bladder (Fig. 2H), only 10% of the epithelial cells demonstrated weak to moderate TROP-2 expression (1-2+). DCIS of the breast was associated with a moderate TROP-2 expression (2+) in 90% of the tumor cells (Fig. 2I).

Discussion

The treatment landscape for advanced UC has changed over the past number of years. Until 2017, treatment options were limited to chemotherapy. During that time, PD-1/PD-L1-based ICIs such as atezolizumab, durvalumab, nivolumab and pembrolizumab were approved, resulting in improved responses to treatment (6-12). However, only 25-30% of patients demonstrate an objective response, and even though immunotherapy is generally well-tolerated, toxicities with durable consequences, such as cutaneous toxicity, gastrointestinal toxicity, pulmonary and hepatic adverse effects, have been reported in a study by George *et al* (14).

Therefore, novel targeted approaches such as FGFR3 inhibition and ADCs have been investigated, resulting in the approval of erdafitinib (15), enfortumab vedotin and SG (16,17). The ADC SG is approved by the US Food and Drug Administration for the treatment of advanced or metastatic UC after chemotherapy and immunotherapy (17).

To the best of the authors' knowledge, there are only a small number of large studies analyzing the expression of TROP-2. One study carried out by Wucherpfennig *et al* (26) aimed to

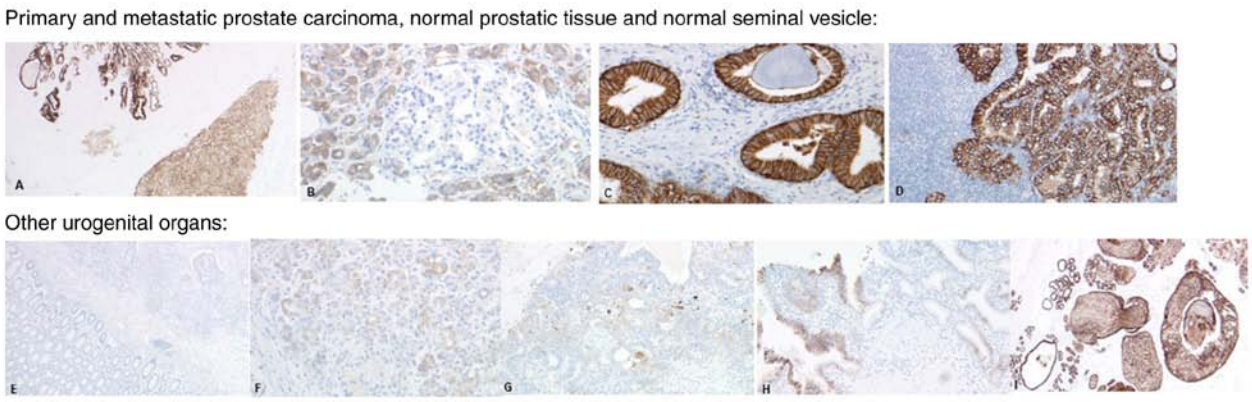

Figure 2. Expression of TROP-2 on the primary and metastatic prostate carcinoma, normal prostatic tissue and other non-urogenital organs. (A) Primary PC with moderate expression of TROP-2 (2+) on the cell membrane of 100% of the tumor cells. Normal cells of the seminal vesicle have strong expression of TROP-2 (3+) on the membrane of 100% of the cells (magnification, x10). (B) Primary PC with weak expression of TROP-2 (1+) on the cell membrane of 100% of the tumor cells of Gleason grade 3, but no TROP-2 expression on the tumor cells of Gleason grade 4 in the middle of the field (magnification, x10). (C) Normal prostate tissue with strong expression of TROP-2 (3+) on the surface of 100% of the glandular epithelial cells (magnification, x20). (D) Metastatic PC in a lymph node with strong expression of TROP-2 (3+) on the cell membrane of 100% of the tumor cells (magnification, x10). (E) On the right, there is no expression of TROP-2 in invasive colon adenocarcinoma. On the left, there is also no expression of TROP-2 in non-neoplastic colon mucosa (magnification, x10). (F) Normal pancreatic tissue with weak expression of TROP-2 (1+) on the cell membrane of acinar cells (magnification, x10). (G) Weak expression of TROP-2 (1+) on the membrane of cells of invasive endometrial glands (magnification, x10). (H) Normal epithelium of the gall bladder with moderate expression of TROP-2 (2+) on the surface of ~10% of the normal glandular epithelium (magnification, x10). (I) Intraluminal cells of ductal carcinoma *in situ* of the breast demonstrated moderate expression of TROP-2 (2+) on the membrane of 100% of the cells. Cells of normal glands and ducts presented in the same section on the lefthand side had strong expression of TROP-2 (3+) on the cell membrane (magnification, x4). PC, prostate carcinoma; TROP-2, trophoblast cell surface antigen 2.

investigate the histopathological and immunohistochemical expression of TROP-2 within different subtypes of urinary bladder carcinoma, including small cell neuroendocrine carcinoma, urachus carcinoma, adenocarcinoma and squamous cell carcinoma. These subtypes of urinary bladder carcinoma are rare in western countries, as the most common type of bladder cancer is urothelial carcinoma, which accounts for > 90% of all bladder cancers. Other rare subtypes like squamous cell carcinoma, adenocarcinoma and neuroendocrine carcinoma account for <10% (25).

In the present cohort, only 2 cases of neuroendocrine carcinoma were included and had only partly higher expression of TROP-2 comparison with the expression within the invasive urothelial carcinoma. Another large study by Dum *et al* (27) with ≥16,000 samples that could be interpreted and evaluated demonstrated 109 tumor categories were TROP-2 positive, with urothelial cancer being one of the types of cancer with high positivity rates and expression levels. High positivity rates were associated with a less advanced T-stage, whereas expression levels were similar across stages. Since the aim of the present study was to investigate common subtypes of urothelial carcinoma, UC was analyzed, and cases were subdivided into invasive and non-invasive carcinomas. Non-invasive UC was further divided into CIS and papillary non-invasive UC. UC of the renal pelvis was also included, such as in the study by Tomiyama *et al* (28), which demonstrated that non-invasive UC had an increased TROP-2 expression compared with muscle-invasive cancer subtypes. The present study is in agreement with the study by Tomiyama *et al* (28) as it was revealed that non-invasive carcinomas had a stronger expression of TROP-2 compared with invasive carcinomas. By contrast, the findings of the present study demonstrated a wide but weak expression of TROP-2 in UC of the renal pelvis.

In the papillary, non-invasive tumors with CIS, there was no relevant difference in the strength, rate and pattern of expression of TROP-2. The majority of the papillary non-invasive tumors and the CIS demonstrated a high expression of TROP-2. Compared with the study by Tomiyama *et al* (28), the expression of TROP-2 in normal tissue from an area nearby the tumor was also investigated, and it was demonstrated that normal urothelial cells had a high expression of TROP-2 in all investigated lesions. Since results or interpretation of expression within normal tissue were not found, normal tissues of the urinary bladder, prostate and renal pelvis were analyzed, and it was revealed that these tissues had a strong TROP-2 expression. The analysis of normal tissues from the gall bladder and pancreas revealed a weak expression of TROP-2. It was also observed that the expression of TROP-2 expression in PC at Gleason grade 3 was higher compared with that in PC at Gleason grade 4. Next, different tumor types including colon adenocarcinoma, endometrial carcinoma and DCIS of the breast were analyzed. In summary, except for prostate cancer and DCIS of the breast, other types of cancer demonstrated low or no expression of TROP-2. For treatment purposes, the aforementioned types of cancer should be immunohistochemically analyzed to investigate the expression level of TROP-2. High expression of TROP-2 in urothelial cells should be taken into consideration before treatment initiation. Stepan *et al* (29) also detected high expression of TROP-2 in several mouse tissue samples.

In contrast to the majority of patients with UC, patients with other types of cancer or UC of the upper tract still express the urothelial cells relevant amounts of TROP-2, what might lead to toxicities of the urinary tract. According to the study by Dum *et al* (27), there is an absence of TROP-2 staining in cells of the gastrointestinal tract; in the present study, it was observed that there was no TROP-2 expression within the

normal colonic mucosa, but there was still weak expression within gall bladder and pancreatic tissues. This may explain the gastrointestinal toxicities that have been reported after treatment with SG (17). In the current study, diarrhea (65%) and nausea (60%) accounted for the majority of common toxicities across all grades. Another explanation would be that the toxicity is more closely associated with the chemotherapeutic agents and less with the expression of TROP-2.

Liu *et al* (30), Zhang *et al* (31) and Avellini *et al* (32) reported that strong expression of TROP-2 is associated with poor prognosis and aggressiveness of UC. Tomiyama *et al* (28) reported contradictory results demonstrating that high expression of TROP-2 in carcinomas of the upper urinary tract is associated with good prognosis, and concluded that high TROP-2 expression should be used as a biomarker serving to decide what the best therapeutic option for a patient is.

In summary, high expression of TROP-2 was observed in UC cells and normal urothelial cells, which points to the association between the expression level and response rate. SG would be effective in the majority of cases of invasive and non-invasive UC, but with a possible hazardous effect to the normal and healthy urothelium. Other types of cancer, except for DCIS of the breast and prostate cancer, demonstrated low or no expression of TROP-2. The main limitation of the present study is the small number of cases; future investigations will have an increased cohort in which more UC cases from the upper tract and from other organs such as breast, lung and stomach will be analyzed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MA, KS, CB, MB, EW and AS conceived the idea of the study and developed the study design, interpreted the results and drafted the manuscript. MA, KS, CB and BH collected data, interpreted the results and provided final approval. MT participated in the last revision of the work and processed other samples from normal tissues. MA and BH were involved in sample selection and analysis of results. MA and KS confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the local ethical commission (approval no. 2016-483-f-S) of the University Hospital Muenster and the medical association of Westfalen-Lippe-Germany. All investigations were carried out according to guidelines and regulations. Written informed consent was obtained from the patients before the investigation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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