



# PD-L1 expression and FGFR-mutations among Danish patients diagnosed with metastatic urothelial carcinoma: A retrospective and descriptive study

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Checkpoint inhibitors have changed the treatment landscape of advanced urothelial carcinoma (mUC), and recently, a fibroblast-growth-factor-receptor (FGFR) inhibitor has been introduced. This study aimed at estimating programmed death-ligand 1 (PD-L1) expression in primary tumors (PTs) and the PD-L1 expression concordance between PTs and paired metastases in 100 patients with UC managed in the real-world setting. Further, the aim was to investigate FGFR1–3 aberrations and the correlation between FGFR1–3 aberrations and PD-L1 expression. PD-L1 immunohistochemistry was performed on 100 formalin-fixed paraffin-embedded archival primary UC samples and 55 matched metastases using the 22C3 PD-L1 assay. PD-L1 expression was determined by the combined positive score, considered positive at  $\geq 10$ . Targeted next-generation sequencing on the S5+/Prime System with the OncoPrint Comprehensive Assay version 3 was used to detect FGFR1-3 aberrations in PTs. We found that 29 of 100 PTs had positive PD-L1 expression. The PD-L1 concordance rate was 71%. FGFR1-3 aberrations were observed in 18% of PTs, most frequently FGFR3 amplifications or mutations. We found no association between FGFR1-3 aberrations and PT PD-L1 expression ( $p = 0.379$ ). Our data emphasize the need for further studies in predictive biomarkers.

**Key words:** Descriptive study; urothelial carcinomas; PD-L1 expression; FGFR aberrations.

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For decades, cisplatin-based combination chemotherapy has been the recommended treatment for locally advanced, unresectable, or metastatic urothelial carcinoma (mUC). However, approximately 50% of mUC patients are cisplatin-ineligible, and in most patients treated, cisplatin-resistance develops within 9 months [1,2]. Hence, development of new effective treatment modalities is needed.

Although urothelial carcinoma (UC) is associated with a high frequency of somatic mutations, only few drugs targeting mechanisms involved in carcinogenesis have been available [3]. However, since 2016, several immune-checkpoint-inhibitors (ICIs) have been approved by the US Food and Drug

Administration (FDA) and European Medicines Agency (EMA) for the management of mUC in the first-line and second-line treatment settings [4].

In the first-line setting, the KEYNOTE-052 study reported a 24% objective response rate to pembrolizumab [5]. The trial demonstrated a subgroup of patients responding exceptionally well to pembrolizumab with long-term responses, particularly in patients with high combined positive score (CPS) PD-L1 expression in tumor tissue. However, responses were not reserved for patients with PD-L1 positive tumors but were observed across varying degrees of PD-L1 expression [5]. Biomarkers to predict effect of ICIs have been extensively studied, although no clear correlation has been found. Nevertheless, PD-L1 expression is incorporated in clinical practice after recommendation from EMA

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restricting the use of first-line pembrolizumab and atezolizumab to patients with PD-L1 positive tumors with different companion diagnostic tests and cut-offs used for each ICI [6]. Notably, the recommendations do not address whether PD-L1 testing ought to be performed on biopsies from primary tumor or metastases, despite emerging evidence that PD-L1 expression differ significantly between primary tumors and metastases [7].

Inhibition of fibroblast growth factor receptor (FGFR) constitutes another new target for mUC treatment. Genomic alterations in FGFR3 are among the most frequent reported somatic mutations in UC of the upper urinary tract (UUT) and bladder [8]. In addition, gene amplifications of FGFR1 and FGFR3 are common in UC with reported rates of 6% and 13%, respectively [9]. Although primarily associated to UC of low malignant potential, FGFR alterations are present in mUC as well, albeit the prevalence is poorly investigated [10,11].

With this exploratory, retrospective study, we aimed to establish the frequency of PD-L1 positive (CPS  $\geq$  10) tumors and the concordance rate of PD-L1 expression between primary tumors and paired metastases in a real-world Danish cohort of 100 treatment-naïve UC patients. We further wanted to investigate the prevalence of FGFR aberrations in the cohort.

## METHODS

### Patient cohort and diagnostic material

This single-institution study was approved by the regional scientific ethical committee of the capital region of Denmark (H-19041420) and included a total of 100 patients with mUC treated at the Department of Oncology, Rigshospitalet, Denmark, between January 2010 and March 2016. The patients form part of a larger previously described cohort, and were retrospectively and consecutively selected as the latest 100 patients treated at the department, fulfilling inclusion criteria for the present study [12]. Only patients with high-grade UC including all variants of infiltrating UC were included. Inclusion was irrespective of primary tumor location in the urinary tract and pT stage. Patients with pure squamous cell carcinoma, patients previously receiving neoadjuvant chemotherapy, radiation therapy, or ICI were excluded from analysis.

For all patients, PD-L1 expression and FGFR analysis were evaluated on archival formalin-fixed and paraffin-embedded (FFPE) tissue blocks from the primary tumor sampled by biopsy, transurethral resection of bladder tumor (TURBT), or originating from primary surgical tumor resection. Cytologic specimens were disregarded. Time interval between primary biopsy and subsequent systemic treatment was not registered. Further, if available, PD-L1 expression was evaluated on tissue blocks representing distant metastases either synchronous metastases, that is, metastases diagnosed before or within a three-

month interval of primary tumor diagnosis or metachronous metastases, defined as metastases diagnosed more than 3 months after primary diagnosis. Prior to analysis, original slides from all patients were reviewed by a pathologist to confirm diagnosis, stage, and relevant pathological features. Tumor was graded using WHO-classification for high-grade or low-grade.

### PD-L1 staining and evaluation

From each patient, one representative block of vital tumor tissue from the most infiltrative area of the primary tumor, and if available, one block of representative matched metastatic tissue was selected. For PD-L1 IHC staining, the 22C3 pharmDx kit was chosen and used on the Dako ASL48 platform according to manufacturer's recommendations. New two  $\mu$ m whole tissue sections were cut and stained following the protocol and for each slide and positive and negative controls were included and evaluated per manufacturer's instruction. This evaluation was done by the primary investigator trained in a Merck Sharp & Dohme sponsored training program for interpretation of PD-L1 expression in UC. PD-L1 expression was determined according to CPS, defined as the number of PD-L1 positive tumor cells (partial or complete linear membranous staining) added by the number of PD-L1 positive lymphocytes and macrophages (membranous and/or cytoplasmic staining) divided by the total number of viable tumor cells, multiplied by 100. Only cases with a minimum of 100 viable invasive cells available for PD-L1 evaluation were included in the analysis. Identification of tumor cells and immune cells was based on morphologic features alone assisted by Hematoxylin and Eosin (H&E) slides. Necrotic and burned areas were disregarded. Each H&E and corresponding PD-L1-22C3 slide was scanned using Hamamatsu nanozoomer and evaluated on the Hamamatsu NDP viewer v1.23.beta. PD-L1 staining was considered positive with a CPS score  $\geq$  10, the recommended cut-off as included in the PD-L1 IHC 22C3 pharmDx Interpretation Manual [13].

### FGFR1-3 alteration analysis

From all patient samples, new four times 10  $\mu$ m FFPE sections were cut from the same tissue block from primary tumor used for PD-L1 analysis. The H&E slides were used to identify the most invasive and vital tumor areas and used as template for macro-dissection to increase the number of tumor cells. Targeted NGS (next-generation sequencing) was carried out on the S5+/Prime System with the OncoPrint Comprehensive Assay version 3 according to manufacturer's instructions (Thermo Fisher Scientific). We decided to abstain from RNA-analysis (FGFR fusions) as most of the material from the macro dissected FFPE blocks were too sparse and RNA concentrations therefore not high enough for analysis. Thus, the analysis only included FGFR mutations and amplifications. Further details regarding the FGFR-analysis can be found in the [Appendix S1](#).

### Statistics

Statistical analysis was performed using STATA IC.14 (Statistical Software, College Station, TX, USA).

Differences in PD-L1 expression and FGFR1-3 aberration status according to the baseline characteristics were calculated using Pearson-Chi-Square test and Fisher's exact test. To calculate differences in PD-L1 expression in primary tumors and paired metastases, McNemar's Chi-Square test was used. Statistical significance was considered at  $p < 0.05$ .

## RESULTS

### Patient characteristics

Baseline characteristics of the 100 study patients are presented in Table 1. More men (78%) than women (22%) were included. Median age at primary diagnosis was 66 years (range 39–86 years). In most patients, the primary tumor was in the bladder (88%), and most tumors were stage pT2 (65%) or pT1 (16%) as defined by histology at initial diagnosis of UC.

### PD-L1 expression in primary tumors and their paired metastases

About 29 of 100 primary tumors had a CPS  $\geq 10$ . We observed no significant differences in CPS score ( $\geq 10$  vs.  $< 10$ ) according to gender, primary tumor location, tumor stage, primary tumor histology, or the presence or absence of FGFR aberrations (Table 2).

For 55 patients, metastatic tissue was available for PD-L1 expression analysis (Table 1); 32 cases representing a synchronous metastasis and 23 cases representing a metachronous metastasis. In Table 3, PD-L1 expression (e.g., CPS  $\geq 10$  or  $< 10$ ) in primary tumors compared with their paired distant metastases are shown. 71% ( $n = 39/55$ ) of primary tumors had concordant PD-L1 expression with their paired metastasis, while 13% ( $n = 7/55$ ) converted from positive to negative and 16% ( $n = 9/55$ ) converted from negative to positive PD-L1 expression ( $p = 0.617$ ). Considering only metachronous metastases, 78% ( $n = 18/23$ ) of primary tumors had concordant PD-L1 expression with their paired metastasis, while nine % ( $n = 2/23$ ) and 13% ( $n = 3/23$ ) converted from positive to negative and from negative to positive PD-L1 expression, respectively ( $p = 0.655$ ) (Table 4).

### FGFR aberrations and PD-L1 expression

Ninety-five of 100 patients had enough primary tumor tissue left for FGFR1-3 analysis of which 17 (18%) harbored a FGFR1-3 aberration. The majority of FGFR aberrations (15%,  $n = 14/95$ ) were FGFR3 alterations, eight patients harboring FGFR3 amplifications ( $\geq 5$  copies), and seven

**Table 1.** Baseline clinicopathological characteristics

	Total no. (%)
All	100
Sex	
Male	78
Female	22
Age at primary diagnosis (median, years)	
All	66
Male	67
Female	66
Location of primary tumor (includes material used for PD-L1 analysis)	
Lower urinary tract, bladder	88
Upper urinary tract, renal pelvis/ureter	12
T-stage	
pT1 <sup>1</sup>	16
pT2	65
pT3	6
pT4	8
Unknown	5
Histological subtype	
Urothelial carcinoma (UC) NOS <sup>2</sup>	66
UC with squamous differentiation <sup>3</sup>	14
UC with divergent histological differentiation <sup>4</sup>	20
Location of available metastatic tissue used for PD-L1 analysis	
Lymph node pelvis/perivesical	38 (69%)
Visceral/bone metastasis <sup>5</sup>	17 (31%)
Time from primary tumor to metastasis	
Synchronous metastasis (–5 month to 3 month)	32
4–6 months	7
7–12 months	8
>1 year	8

<sup>1</sup>Includes 3 case with pT1a tumors, 9 cases with pT1b and 3 cases with pT1 tumors not further subclassified.

<sup>2</sup>Defined as pure infiltrating UC.

<sup>3</sup>Defined as the presence of infiltrating UC and squamous differentiation in the same tumor.

<sup>4</sup>Includes UC with divergent histological features including one or more of the following: Plasmacytoid-, neuroendocrine-, sarcomatoid-, glandular-, clear cell- and lymphoepithelioma-like differentiation along with poorly differentiated UC.

<sup>5</sup>Includes a total of two cases diagnosed with bone metastasis.

patients harboring FGFR3 mutations (Table 5). The most common FGFR3 mutation identified in this study was p.Y373C (three observations), followed by p.R248C, p.S249C, and p.G370C. Table 6 lists the frequencies of FGFR1-3 aberrations correlated to patient characteristics. We found no differences in frequency according to gender or stage. We observed significantly more FGFR1-3 aberrations among patients with UUT UC (45%,  $n = 5/11$ ) than among patients with bladder UC (14%,  $n = 12/84$ ) ( $p = 0.024$ ). However, if only including cases with FGFR3 mutations in the analysis, we

**Table 2.** PD-L1<sup>1</sup> by baseline clinicopathological characteristics and FGFR 1–3<sup>2</sup> alteration status

Characteristics	PD-L1 positive CPS ≥ 10 (%)	PD-L1 negative CPS < 10 (%)	Total, n (%)	p-value
Primary tumor (PT)	29 (29%)	71 (71%)	100 (100%)	
PT with available metastatic tissue	19 (35%)	36 (65%)	55 (100%)	
Metastatic tissue	21 (38%)	34 (62%)	55 (100%)	
Sex				
Male	23 (29%)	55 (71%)	78 (100%)	0.840 <sup>§</sup>
Female	6 (27%)	16 (73%)	22 (100%)	
Location				
Lower urinary tract, bladder	27 (31%)	61 (69%)	88 (100%)	0.500*
Upper urinary tract, renal pelvis/ureter	2 (17%)	10 (83%)	12 (100%)	
Stage primary tumor				
pT1-pT2	25 (31%)	56 (69%)	81 (100%)	0.697 <sup>§</sup>
pT3-pT4	3 (21%)	11 (79%)	14 (100%)	
Unknown	1 (20%)	4 (80%)	5 (100%)	
Histology primary tumor				
UC NOS	15 (23%)	51 (77%)	66 (100%)	0.231 <sup>§</sup>
UC with squamous diff.	6 (43%)	8 (57%)	14 (100%)	
UC with divergent histological diff.	7 (35%)	13 (65%)	20 (100%)	
FGFR <sup>2</sup> mutation/amplification				
FGFR 1–3 wild-type	24 (30%)	54 (69%)	78 (100%)	0.379*
FGFR 1–3 alteration	3 (18%)	14 (82%)	17 (100%)	

<sup>1</sup>Programmed death ligand 1.<sup>2</sup>Fibroblast growth factor receptor 1–3.<sup>§</sup>Chi<sup>2</sup> test.

\*Fisher's exact test.

**Table 3.** PD-L1 expression in primary tumors compared to their paired distant metastasis

Primary tumor	Distant metastases		p-value
	PD-L1 negative (CPS < 10)	PD-L1 positive (CPS ≥ 10)	
PD-L1 negative (CPS <10)	27 (49%) <sup>1</sup>	9 (16%)	0.617*
PD-L1 positive (CPS ≥10)	7 (13%)	12 (22%)	

<sup>1</sup>Percentages may not sum to 100 due to rounding.\*McNemar's chi<sup>2</sup> test.

found no differences between groups (9% UUT vs. 8% lower urinary tract,  $p = 0.509$ ). Numerically, FGFR aberrated samples were less likely to be PD-L1 positive (18%,  $n = 3/17$ ) than FGFR wild type samples (31%,  $n = 24/78$ ) (Table 2), though this did not reach statistical significance, ( $p = 0.379$ ).

## DISCUSSION

In this retrospective study, we examined PD-L1 expression in primary tumors and paired metastatic lesions as well as FGFR aberrations in primary tumors of 100 patients with UC, who all eventually developed mUC and were treated in the real-world clinical setting at the Department of Oncology, Rigshospitalet. The present cohort is representative of mUC patients treated at the department during the inclusion period [12]. To the best of our knowledge, this is one of few studies evaluating PD-L1

expression and FGFR aberrations in the same subset of patients.

We found that 29% of primary tumors had a PD-L1 CPS score of  $\geq 10$  (*i.e.*, positive PD-L1 expression). This finding is in line with other studies based on the 22C3 antibody, in which 20–40% of UC tumors have a reported PD-L1 CPS of  $\geq 10$  [14,15]. We found no difference in primary tumor PD-L1 expression according to gender, and in line with one recent study nor according to stage or histological subtype [16].

The concordance rate of PD-L1 expression between primary tumors and paired metastases was 71% overall, and 78% for patients with metachronous metastases. Thus, in 29% and 22% of cases, respectively, a previously PD-L1 negative or positive primary tumor changed expression status in the metastasis.

Similar to our study, de Jong *et al.* [16] compared PD-L1 expression in matched urothelial bladder cancer specimens using the SP142 assay and

**Table 4.** PD-L1 expression in primary tumors compared to their paired distant metastasis diagnosed more than 3 months after primary diagnosis

Primary tumor	Distant metastases		p-value
	PD-L1 negative (CPS < 10)	PD-L1 positive (CPS ≥ 10)	
PD-L1 Negative (CPS <10)	11 (48%) <sup>1</sup>	3 (13%)	0.655*
PD-L1 Positive (CPS ≥10)	2 (9%)	7 (30%)	

<sup>1</sup>Percentages may not sum to 100 due to rounding.\*McNemar's  $\chi^2$  test.

found a PD-L1 expression concordance rate of 81%. Likewise, Burgess *et al.* found poor agreement in high expression rates between primary tumors and metastatic lesions, also based on the SP142 antibody [17]. Interestingly, Tretiakova *et al.* found a concordance rate of 90% between primary UC and paired metastatic lesions based on four antibodies (22C3, 28.8, SP142, and E1L3N) and PD-L1 expression status evaluated on tissue microarrays (TMA) [18]. However, the high concordance rate reported in their study might be explained by sample size and tumor heterogeneity, as they evaluated PD-L1 expression on small TMA tumor samples and not whole sections.

Our findings suggest that PD-L1 expression might be spatially dynamic with some subclones being

negative while others are strongly positive. Furthermore, our findings of PD-L1 expression discordance can be due to dynamic changes during the disease trajectory. Both spatial and temporal heterogeneity have been proposed as reasons for discordance in PD-L1 expression between primary tumors and paired metastases and highlight the relevance of analyzing metastatic tissue prior to treatment decision-making, as PD-L1 expression in primary tumors may not accurately predict PD-L1 status in metastatic lesions [17]. This might be particularly relevant following treatment, as changes in PD-L1 expression after neoadjuvant chemotherapy have been described [16]. In other cancer types, changes in PD-L1 expression after anticancer treatment have been observed too [19,20]. However, as we explored a previously untreated patient cohort, this issue is not relevant in the present study.

In recent years, it has become evident that the predictive value of PD-L1 expression by IHC alone is limited and its value as a predictive biomarker for treatment selection in mUC is under debate [21–23]. In a newly published review summarizing data on the five FDA-approved ICIs used in mUC, the authors concluded that across all five drugs a significant number of patients with defined PD-L1 negative tumors still responded to ICI [22]. Like in mUC, similar observations have been made across a number of different cancers including cervical cancer, malignant melanoma, and small cell lung cancer [24]. The

**Table 5.** Patient characteristics according to PD-L1 expression and FGFR 1–3 aberrations in primary tumor tissue

Patient nr.	Age	Gender	Primary tumor	Stage	Histology	PD-L1 CPS score	FGFR <sup>1</sup> 1–3 aberrations
1.	69	M	Bladder	pT2	UC NOS	0.5	<b>FGFR1 aberrations</b> Mutation p.D585H
2.	64	M	Bladder	pT2	UC NOS	10.0	<b>FGFR2 aberrations</b> FGFR2 amplification (copies 16)
3.	75	M	Bladder	pT2	UC DHD <sup>2</sup>	5.5	Mutation H254Y <b>FGFR3 aberrations</b>
4.	63	F	Bladder	pT1	UC NOS	1.9	Mutation p.G370C
5.	59	M	Bladder	pT2	UC NOS	1.4	FGFR3 amplification (copies 10) and Mutation p.Y373C
6.	71	M	Bladder	pT2	UC NOS	11.2	FGFR3 amplification (copies 9)
7.	56	F	Bladder	pT2	UC SD <sup>3</sup>	13.1	FGFR3 amplification (copies 5)
8.	53	F	Renal pelvis	pT4	UC NOS	0.1	FGFR3 amplification (copies 33)
9.	73	M	Bladder	pT2	UC NOS	0.5	FGFR3 amplification (copies 23)
10.	66	M	Renal pelvis	pT4	UC NOS	4.6	FGFR3 amplification (copies 9) FGFR1 amplification (copies 6)
11.	69	M	Renal pelvis	pT3	UC NOS	0	FGFR3 amplification (copies 14)
12.	69	M	Ureter	pT3	UC NOS	4.4	FGFR3 amplification (copies 5)
13.	61	M	Bladder	pT1b	UC NOS	5.4	Mutation p.R248C
14.	62	M	Bladder	pT2b	UC DHD <sup>2</sup>	4.5	Mutation p.S249C
15.	52	F	Renal pelvis	pT4	UC DHD <sup>2</sup>	0.4	Mutation p.R248C
16.	81	F	Bladder	pT2	UC NOS	1.9	Mutation p.Y373C
17.	71	M	Bladder	pT1	UC NOS	0.2	Mutation p.Y373C

<sup>1</sup>Fibroblast growth factor receptor.<sup>2</sup>UC DHD, Urothelial carcinoma with divergent histological differentiation.<sup>3</sup>UC SD, Urothelial carcinoma with squamous differentiation.

**Table 6.** Frequency of FGFR1-3 aberrations according to patient characteristics

Characteristics	No FGFR1-3 aberrations	FGFR 1–3 aberrations	Total number	p-value*
Gender				
Male	61 (84%)	12 (16%)	73 (100%)	0.532
Female	17 (77%)	5 (23%)	22 (100%)	
Primary site				
Lower UT <sup>1</sup>	72 (86%)	12 (14%)	84 (100%)	<b>0.024</b>
Upper UT <sup>2</sup>	6 (55%)	5 (45%)	11 (100%)	
Stage				
pT1-pT2	65 (84%)	12 (16%)	77 (100%)	0.104
pT3-pT4	8 (62%)	5 (38%)	13 (100%)	
(unknown)	5 (100%)	0 (–)	5 (100%)	

<sup>1</sup>Lower UT (lower urinary tract, bladder).

<sup>2</sup>Upper UT (upper urinary tract, renal pelvis/ureter).

\*Fisher's exact test.

Bold indicates statistical significant value (p<0.05)

apparent limited predictive value of IHC based PD-L1 expression is likely multifactorial; besides spatial and temporal heterogeneity in PD-L1 expression this may be related to individual drugs being linked to specific antibodies and associated PD-L1 assays, which in turn are based on different drug-specific algorithms for evaluation of PD-L1 expression. Finally, there is no uniform definition of PD-L1 positivity [6]. Consequently, data on the predictive value of PD-L1 expression are often heterogeneous, conflicting, and difficult to compare [21].

In this study, 18% of the primary tumors were found to harbor an alteration in FGFR1-3. All the identified FGFR3 mutations are known to be activating mutations and are among the most commonly reported mutations in UC [9,25]. One case harbored an FGFR1 mutation, which to the best of our knowledge, has not been linked to UC. The FGFR3 mutation rate found in this study is slightly lower than previously reported. The study sample size along with the older age of the FFPE tissue used in our analysis might contribute to this discrepancy, as the FFPE sample storage period has been demonstrated to influence DNA integrity [26].

We observed that FGFR1-3 alterations were more frequent in UUT UC than in UC of the lower urinary tract (45% vs. 12%, p = 0.009); however, we found no difference in FGFR3 mutation frequency between the two groups. Contrary to this, Sfakianos *et al.* found that FGFR3 mutations as well as FGFR-TACC3 fusions were more common in UUT UC compared with UC of the bladder (36% vs. 22% and 8.5% vs. 2%, respectively) [27]. Similarly, Necchi *et al.* found FGFR3 mutations to be more common in UUT UC than in lower urinary tract UC (21% vs. 14%, p = 0.002), while amplifications and rearrangements occurred at similar rates [28].

It has previously been proposed, that FGFR3 mutations are associated with low T-cell infiltrates suggesting that FGFR3 mutated tumors may

respond suboptimally to ICIs [29,30]. However, using data from the IMVIGOR210 and Checkmate-275 clinical trials, Wang *et al.* showed that patients with FGFR3 mutations responded similarly to ICI as did patients without such mutations [29]. Based on these results, the authors concluded that patients with mUC harboring FGFR3 mutations should not be denied ICI treatment. This is supported by the results in the present study where no association between FGFR1-3 mutation status and PD-L1 expression status was found (p = 0.379).

Multiple factors may influence response to ICI in addition to PD-L1 expression, such as tumor mutational burden (TMB) and presence of tumor infiltrating lymphocytes (TILs) [31]. A dysregulation of the growth receptor signaling pathway due to FGFR gene aberrations, including amplifications, fusions, and mutations, enhances tumor proliferation, invasion, angiogenesis, and immune evasion through reduced cytokine signaling [32]. These aberrations may influence the tumor microenvironment with subsequently reduced TILs. This can be part of the explanation for the observed poorer response rates to ICI for tumors with FGFR gene mutations in some studies [29,30].

The relatively high frequency of FGFR3 alterations observed in UC has increased the interest in FGFR as a therapeutic target in mUC. Erdafitinib, a pan-FGFR tyrosine kinase inhibitor, recently received FDA approval as second-line treatment for platinum-resistant mUC but has not yet been approved by EMA [33]. Clinical phase I/II trials are investigating combination treatment with FGFR-inhibition and ICI to disrupt immune evasion in the tumor microenvironment, and initial data are promising (ClinicalTrials.gov number, NCT03473756) [34].

The results presented in the present study add to the growing evidence that a more personalized treatment strategy in the management of mUC patients is warranted. Further studies in predictive

biomarkers including the changes of these following treatment as well as the optimal order of treatment regimens are needed.

This study has some limitations, primarily related to sample size and the retrospective design. The varying storage time of the material might, as previously mentioned, influence the DNA integrity and the sample storage time, which also forced us to abstain from RNA analysis in our FGFR analysis. We were therefore unable to examine potential fusion proteins including FGFR3-TACC3 and FGFR3-BAIAP2L1, which have also been linked to UC [35]. In order to reflect a real-world setting, this consecutive study also included two cases of bone metastasis. In one of these cases, PD-L1 expression changed from negative in the primary tumor to positive in the paired metastasis and in the other case, the opposite switch was observed. Thus, although it has been shown, that due to decalcification, samples from bone metastases may lower the proportion and intensity of IHC PD-L1 stained cells, it seems unlikely, that the two included bone metastases have biased our results [36].

It can be speculated if the storage time of the FFPE tissue block affected the immunohistochemical reaction of the PD-L1 antibody. However, the FFPE tissue blocks used in this study have all been stored according to standards and furthermore, all our analyses were carried out on newly cut FFPE sections. Reassuringly, the proportion of PD-L1 positive tumors found in this retrospective study is in accordance with the KEYNOTE-045 clinical trial, in which both archival tumor samples and newly obtained biopsies were used for PD-L1 assessment [14].

## CONCLUSION

Approximately 30% of UC patients treated in the real-world clinical setting has a PD-L1 positive tumor, that is, have a CPS  $\geq$  10. In about 30% of cases, discordance exists in PD-L1 expression between primary tumors and paired metastatic lesions. Little less than 20% of patients harbor an FGFR1-3 aberration. Our data emphasize the need of further extensive studies in predictive biomarkers including intra-patient heterogenic biomarker expression with the goal of implementation of a personalized treatment approach in mUC.

## AUTHOR CONTRIBUTIONS

T. Grantzau had full access to all the data in the study. T. Grantzau and L. Høj Omland had full access to the clinical data and acquisition of data. T. Grantzau and LC. Melchior had full access to the molecular data and take responsibility of the

integrity of the data and the accuracy of the data analysis. Pappot, Grantzau, and Grønkær Toft were involved in the study conception and design. Grantzau, Melchior, and Elversang were involved in the analysis and interpretation of data. Grantzau, Melchior, Stormoen, and Omland involved in drafting the manuscript. Grønkær Toft, Stormoen, Omland, and Pappot involved in revision of the manuscript.

## CONFLICT OF INTEREST

This work was supported by a research grant from Roche. The sponsor had no role in the study design, data collection, analysis, interpretation, writing, or decision to submit the manuscript for publication. The authors have no conflicts of interest to declare.

## ETHICAL APPROVAL

This single-institution study was approved by the regional scientific ethical committee of the capital region of Denmark (H-19041420). Informed consent is not required with approval from regional scientific ethical committee.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1.** Supplementary material.