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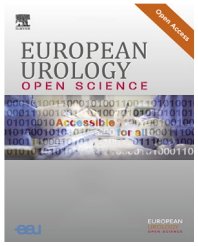

European Association of Urology

Urothelial Cancer

Clinical and Genomic Landscape of FGFR3 Alterations Across Different Stages of Urothelial Cancer

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Abstract

Background and objective: Our aim was to provide a comprehensive analysis of the prevalence of potentially targetable activating *FGFR3* alterations and their impact on oncological outcomes across different urothelial carcinoma (UC) stages.
Methods: We retrospectively analyzed clinical data and *FGFR3* results for 1509 formalin-fixed, paraffin-embedded tissue specimens. Actionable activating *FGFR3* mutations were assessed using a well-established multiplex SNaPshot polymerase chain reaction approach. *FGFR3* fusion testing was performed with a Qiagen Therascreen kit.
Key findings and limitations: In the study population of 1509 patients, 202 (13%) had stage pTa, 380 (25%) had stage pT1, 258 (17%) had localized muscle-invasive bladder cancer (MIBC), 556 (37%) had locally advanced MIBC, 91 (6.0%) had metastatic UC of the bladder (mUCB), and 22 (1.5%) had metastatic upper tract UC (mUTUC). Of the *FGFR3* alterations detected in 373 patients (25%), 104 (52%) were in stage pTa, 158 (42%) were in pT1, 42 (16%) were in localized MIBC, 53 (9.5%) were in locally advanced MIBC, nine (9.9%) were in mUCB, and seven (32%) were in mUTUC. *FGFR3* alterations were associated with better progression-free survival and overall survival in the overall population ($p < 0.001$), but not in subgroup analyses for different disease stages. Study limitations include the retrospective design and heterogeneous patient cohort.

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Conclusions and clinical implications: *FGFR3* alterations occur at a stage-dependent frequency and are more prevalent in lower tumor stages. We were unable to demonstrate an independent prognostic effect of *FGFR3* alterations on oncological outcomes after adjusting for tumor stage.

Patient summary: We analyzed a protein called fibroblast growth factor receptor 3 (FGFR3) in patients with cancer of the urinary tract. We found that more aggressive tumors had fewer genetic changes in FGFR3 in comparison to less aggressive tumors. However, genetic changes in FGFR3 were not related to survival for these patients.

1. Introduction

Urothelial carcinoma (UC) of the bladder is the tenth most common malignancy worldwide, accounting for 3% of global cancer diagnoses [1]. UC is a highly heterogeneous disease covering a wide range of tumors with different levels of aggressiveness, which is also reflected by considerable differences on a pathological and molecular level. [2] One of the central biological pathways driving UC tumor growth involves *FGFR3*. The *FGFR3* gene encodes a cell-surface receptor that leads to ligand-independent dimerization of the receptor and activation of downstream signaling pathways promoting cell growth, proliferation, differentiation, and angiogenesis [3,4]. Selective targeting of activating *FGFR3* alterations represents an attractive therapeutic strategy, and *FGFR* inhibitors are already used in clinical practice [5]. Pathogenic activating *FGFR3* alterations exhibit a stage-dependent frequency. Papillary noninvasive UC and a relevant proportion of stroma-invasive UC tumors mainly arise via a hyperplasia sequence driven by activating *FGFR3* alterations. By contrast, the majority of muscle-invasive UCs and a relevant subset of aggressive stroma-invasive metastatic UCs arise via a flat carcinoma in situ (CIS) sequence that is mainly driven by chromosomal aberrations and inactivating *TP53* mutations [2]. Hence, the frequency of *FGFR3* alterations across different tumor stages varies from 10–15% in muscle-invasive or metastatic UC to up to 80% in noninvasive UC [6]. However, studies investigating the frequency and distribution of *FGFR3* alterations across different tumor stages are scarce, and frequencies so far have only been reported in small cohort studies focused on specific tumor stages.

To provide a comprehensive overview of the prevalence of potentially targetable activating *FGFR3* alterations in different UC stages, we profiled 1509 UCs using well-established molecular pathology workflows. This represents the largest longitudinal study to date on activating *FGFR3* alterations in UC. Our aim was to explore whether *FGFR3* alterations are independently associated with UC oncological outcomes after adjusting for tumor stage. Given the retrospective nature of our analysis, we did not seek to establish a definitive causal relationship but rather to assess whether any association observed persists after accounting for known confounders.

2. Patients and methods

2.1. Study population

We analyzed 1509 UCs of different stages for the prevalence of actionable activating *FGFR3* alterations. The study population consisted of: (1) 202 papillary noninvasive pTa UCs (non–muscle-invasive bladder cancer, NMIBC) from the Comprehensive Cancer Center Erlangen-EMN (CCC-EMN) bladder cancer registry; (2) 289 stroma-invasive pT1 UCs from the University of Regensburg cohort; (3) 91 stroma-invasive pT1 UCs from the CCC-EMN bladder cancer registry; (4) 407 muscle-invasive bladder cancers (MIBCs) from the CCC-EMN bladder cancer registry; (5) 407 MIBCs from The Cancer Genome Atlas (TCGA) bladder cancer cohort [7]; and (6) 113 metastatic UCs (mUCs) from a multicenter cohort of patients treated with immune checkpoint inhibitors (FOSMIC cohort; [8]). *FGFR3* mutational and gene fusion data were downloaded and adapted from the most recent data set release of the TCGA bladder cancer cohort (BLCA, PanCancer Atlas version) from the cBioPortal for cancer genomics [9,10]. All tissue samples were re-reviewed centrally by two experienced uropathologists (A.H. and M.E.) according to the 2022 World Health Organization (WHO) classification of tumors and the latest Union for International Cancer Control staging manual (8th edition) [11]. An overview of the pathological characteristics of the different cohorts is provided in Table 1.

2.2. Assessment of actionable FGFR3 alterations in UC samples

The term "*FGFR3* alteration" covers both *FGFR3* mutations and *FGFR3* fusions. DNA and RNA for *FGFR3* assessment was isolated from formaldehyde-fixed, paraffin-embedded tissue (FFPE) using an automated procedure (Promega Maxwell; Promega, Madison, WI, USA). Five 10-µm FFPE sections with at least 50% tumor content were used per patient tumor and microdissected to maximize tumor purity. Sections were deparaffinized and suspended in 300 µl of incubation buffer and digested with proteinase K at 56°C overnight with shaking at 550 rpm. DNA or RNA was then isolated from lysates using a Promega DNA or RNA FFPE purification kit according to the manufacturer's instructions. The DNA and RNA samples were then purified and quantified, with quality control

Table 1 – Clinical and histopathological characteristics and outcomes for study cohort stratified by *FGFR3* status

Parameter	*FGFR3* alteration (*n* = 373)	*FGFR3* wildtype (*n* = 1136)	*p* value
Male sex, *n* (%)	296 (79)	832 (73)	**0.018**
Median age, yr (IQR)	71 (63–78)	70 (62–78)	0.3
Tumor stage, *n* (%)			**<0.001**
pTa	104 (28)	98 (8.6)	
pT1	158 (42)	222 (20)	
Localized MIBC (pT2)	42 (11)	216 (19)	
Locally-advanced MIBC (≥pT3)	53 (14)	503 (44)	
Metastatic UCB	9 (2.4)	82 (7.2)	
Metastatic UTUC	7 (1.9)	15 (1.3)	
Pathological T stage, *n* (%)			**<0.001**
pTa	104 (28)	98 (8.6)	
pT1	159 (43)	226 (20)	
pT2	44 (12)	234 (21)	
pT3	48 (13)	436 (38)	
pT4	18 (4.8)	142 (13)	
Concomitant carcinoma in situ, *n* (%)			**<0.001**
Yes	67 (18)	354 (31)	
No	228 (61)	340 (30)	
Data not available	78 (21)	442 (39)	
WHO 1973 grade, *n* (%)			**<0.001**
G1	22 (5.9)	21 (1.8)	
G2	144 (39)	96 (8.5)	
G3	207 (56)	1019 (90)	
WHO 2004/2016 low-grade tumor, *n* (%)	86 (23)	52 (4.6)	**<0.001**
Histological subtype, *n* (%)			**<0.001**
Not otherwise specified	333 (89)	763 (67)	
Histological variant	29 (7.8)	147 (13)	
Squamous	11 (2.9)	198 (17)	
Glandular	0 (0)	2 (0.18)	
Trophoblastoid	0 (0)	3 (0.26)	
Neuroendocrine	0 (0)	23 (2.0)	
Pathological N stage, *n* (%)			**<0.001**
pN0	69 (19)	456 (40)	
pN1	17 (4.6)	129 (11)	
pN2	19 (5.1)	169 (15)	
NX	268 (72)	382 (34)	
Metastases, *n* (%)	16 (4.3)	97 (8.5)	**0.008**
mFU for surviving patients, mo (IQR)	36 (14–71)	35 (16–65)	0.5
Recurrence, *n* (%)	114 (31)	445 (39)	**<0.001**
Median recurrence-free survival, mo (IQR)	22 (8–53)	18 (7–47)	**0.015**
Progression, *n* (%)	65 (17)	392 (35)	**<0.001**
Median progression-free survival, mo (IQR)	31 (12–63)	19 (7.3–51)	**<0.001**
Death, *n* (%)	103 (28)	530 (47)	**<0.001**
Death from disease, *n* (%)	65 (17)	395 (35)	**<0.001**
Median overall survival, mo (IQR)	32 (13–63)	21 (11–49)	**<0.001**

MIBC = muscle-invasive bladder cancer; UCB = urothelial carcinoma of the bladder; UTUC = upper tract urothelial carcinoma; mFU = median follow-up; IQR = interquartile range; WHO = World Health Organization.

performed using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

For assessment of actionable activating *FGFR3* mutations, we used a well-established multiplex SNaPshot polymerase chain reaction (PCR) approach as previously reported [12–14]. In brief, three regions of the *FGFR3* gene on exons 7, 10, and 15 are amplified in a multiplex PCR, followed by extension of mutation-specific primers with labeled dideoxynucleotides. After removal of excess primers and dNTPs, eight SNaPshot primers detecting nine *FGFR3* mutations were annealed to the PCR products and extended with a labeled dideoxynucleotide and analyzed on an automatic sequencer. This protocol allows sensitive detection of 11 well-known activating *FGFR3* mutations that account for more than 99% of known activating *FGFR3* mutations in UC (p.R248C, p.S249C, p.G372C, p.G382R, p.S373C, p.Y375C, p.A393E, p.K652E, p.K652Q, p.K652M, and p.K652T).

FGFR3 fusion testing was performed using a Therascreen *FGFR* kit (Qiagen, Hilden, German). Reverse transcription of RNA was carried out according to the manufacturer's instructions, followed by fusion transcript–typing for *FGFR3::TACC3* v1/v3 and *FGFR3::BAIAP2L1* via quantitative PCR. All reactions were controlled and analyzed using the RGQ (PCR cycler) and RGAM software provided by Qiagen.

Mutational and structural variant data from the TCGA bladder cancer cohort were adapted from previous reports on this cohort [7].

2.3. Statistical analysis

Statistical analyses were conducted with SPSS v29.0 (IBM, Armonk, NY, USA) and GraphPad Prism v10 (GraphPad, San Diego, CA, USA). Recurrence-free survival (RFS) was defined as time from diagnosis to the first histologically proven cancer recurrence with at most the same tumor stage. The censoring date for RFS was defined as the date of the last clinical and sonographic follow-up with unremarkable findings, including normal cystoscopy. Progression-free survival (PFS) was defined as time from diagnosis (transurethral resection of bladder tumor) to the first histologically tumor recurrence with a higher tumor

stage than at initial diagnosis or, in cases of metastatic disease, during further tumor monitoring. The censoring date for PFS was the date of the last clinical, cystoscopic, and, when available, radiographic assessment confirming no evidence of disease progression. In patients who had already undergone radical cystectomy (RC), detection of local tumor recurrence or metastases was considered progression. Each tumor progression event was also considered as recurrence, unless recurrence had occurred earlier. Overall survival (OS) was defined as time from diagnosis to death, irrespective of the cause of death. The censoring date for OS was defined as the date of last follow-up for patients who were still alive. Cancer-specific survival (CSS) was defined as the time from diagnosis to death due to the underlying UC. The censoring date for CSS was defined as the date of last follow-up for patients who were still alive or had died from causes unrelated to UC. RFS, PFS, CSS, and OS rates were depicted by Kaplan–Meier analyses and tested for significance with the log rank test. Multivariable Cox regression analyses were used to assess the impact of *FGFR3* alterations and other clinically significant variables on RFS, PFS, CSS, and OS. Group differences between the *FGFR3* alteration and wildtype groups were evaluated using χ^2 and Fisher's exact tests for dichotomous variables. The χ^2 test was applied for data counts $\geq$5, while Fisher's exact test was used for expected data counts <5. The Mann-Whitney U test was used to assess differences between *FGFR3* alteration and wildtype groups for ordinal or continuous variables. A p value <0.05 indicated statistical significance. All analyses were considered two-tailed.

2.4. Ethics approval

All findings and data acquisition and processing comply with the ethical standards described in the latest Declaration of Helsinki. The study was approved by the local ethics committees at the University of Regensburg (ethics reference 19-1396-101), Erlangen (approval numbers 22-343-B, 97_18 Bc, and 329_16 B), and Heidelberg (reference 2018-545N-MA).

3. Results

3.1. Patient cohort

The median age was 71 yr (interquartile range [IQR] 62–78) and 75% of the patients were male. Tumor recurrence occurred in 559 patients (37%) and progression to higher tumor stages in 457 patients (30%), and 633 patients (42%) died during the study (31% cancer-specific). Median follow-up for surviving patients was 35 mo (IQR 15–67). Patients with an *FGFR3* alteration were more often male (79% vs 73%; p = 0.018), which was even more pronounced in the *FGFR3* fusion subgroup (91% male). The group with an *FGFR3* alteration had lower tumor stages and lower rates of histological variants. Table 1 provides a detailed comparison of patients with and without an *FGFR3* alteration in the study cohort.

3.2. Overall cohort

3.2.1. Histopathological parameters and FGFR3 alteration status

In the overall cohort, 202 patients (13%) had stage pTa, 380 (25%) had stage pT1, 258 (17%) had localized MIBC, 556 (37%) had locally advanced MIBC, 91 (6.0%) had mUCB, and 22 (1.5%) had metastatic upper tract UC (mUTUC). *FGFR3* alterations were present in 373 patients (25%), of whom 35 (2.3%) had an *FGFR3* fusion. In the group of 373 patients with an *FGFR3* alteration, 104 (52%) had stage pTa, 158 (42%) had pT1, 42 (16%) had localized MIBC, 53 (9.5%) had locally advanced MIBC, nine (9.9%) had mUCB, and seven (32%) had mUTUC (Fig. 1). An *FGFR3* alteration was present in 22 grade 1 (G1) tumors (51%), 144 G2 tumors (60%), and 207 G3 tumors (17%). Table 2 provides detailed information regarding specific *FGFR3* alterations across different tumor stages.

3.2.2. Factors associated with survival

Multivariable Cox regression analyses revealed that age, disease stage, and WHO 1973 grade (all p < 0.001) were

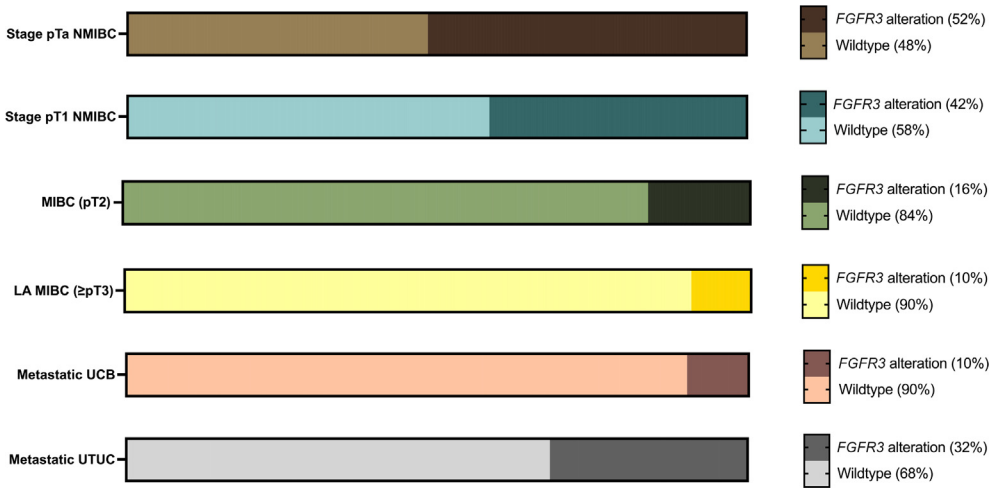

Fig. 1 – *Proportion of patients with an FGFR3 alteration across different disease stages.* NMIBC = non–muscle-invasive bladder cancer; MIBC = muscle-invasive bladder cancer; LA = locally advanced; UCB = urothelial carcinoma of the bladder; UTUC = upper urinary tract urothelial carcinoma.

Table 2 – *FGFR3* alterations across different tumor stages

FGFR3 status	Patients, *n* (%)						
	pTa	pT1	Localized MIBC (pT2)	Locally advanced MIBC (≥pT3)	mUCB	mUTUC	Total
***FGFR3* mutation**							
Wildtype	98 (49)	240 (63)	221 (86)	506 (91)	83 (91)	16 (73)	1164 (77)
A393E	0 (0)	0 (0)	1 (0.39)	0 (0)	0 (0)	0 (0)	1 (0.07)
G235D	0 (0)	0 (0)	1 (0.39)	0 (0)	0 (0)	0 (0)	1 (0.07)
G372C	4 (2)	13 (3.4)	3 (1.2)	4 (0.72)	0 (0)	0 (0)	24 (1.6)
G382R	0 (0)	0 (0)	1 (0.4)	2 (0.36)	0 (0)	0 (0)	3 (0.19)
K652E	0 (0)	0 (0)	0 (0)	2 (0.36)	0 (0)	0 (0)	2 (0.13)
R248C	6 (3)	21 (5.5)	3 (1.2)	2 (0.36)	0 (0)	0 (0)	32 (2.1)
S249C	66 (33)	73 (19)	21 (8.1)	30 (5.4)	4 (4.4)	6 (27)	200 (13)
S249C/G370C	1 (0.49)	1 (0.26)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.13)
S249C/R248C	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.1)	0 (0)	1 (0.07)
S249C/Y375C	1 (0.49)	2 (0.53)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.2)
V306I	0 (0)	0 (0)	0 (0)	1 (0.18)	0 (0)	0 (0)	1 (0.07)
Y375C	26 (12)	30 (7.9)	7 (2.7)	9 (1.3)	3 (3.3)	0 (0)	75 (5)
Total	202 (100)	380 (100)	258 (100)	556 (100)	91 (100)	22 (100)	1509 (100)
***FGFR3* fusion**							
No *FGFR3* fusion	201 (100)	357 (94)	253 (98)	553 (100)	89 (98)	21 (96)	1474 (98)
FGFR3::TACC3	1 (0.49)	23 (6.1)	5 (1.9)	3 (0.54)	2 (2.2)	1 (4.5)	35 (2.3)

MIBC = muscle-invasive bladder cancer; mUCB = metastatic urothelial carcinoma of the bladder; UTUC = upper tract urothelial carcinoma.

independent risk factors for worse OS. Disease stage and WHO 1973 grade (both $p < 0.001$) were independent risk factors for worse CSS. *FGFR3* alterations were not significantly associated with either OS ($p = 0.4$) or CSS ($p = 0.4$). Table 3 provides further details. Fig. 2 and Fig. 3 show Kaplan-Meier survival curves for different disease stages.

3.3. Stage pTa NMIBC

For patients with stage pTa NMIBC, the *FGFR3* alteration group had a higher rate of low-grade tumors according to the WHO 2004 scheme (74% vs 52%; $p = 0.001$) and lower rate of G3 tumors according to the WHO 1973 scheme (12% vs 34%; $p < 0.001$). There were no significant differences in age, sex distribution, concomitant CIS, tumor focality, RFS, PFS, or OS between the *FGFR3* alteration and wildtype groups. Supplementary Table 1 provides further details.

3.4. Stage pT1 NMIBC

For patients with stage pT1 NMIBC, the *FGFR3* alteration had a significantly lower rate of concomitant CIS (28% vs 42%; $p = 0.005$), a higher rate of WHO 2004 low-grade tumors (5.7% vs 0.45%; $p = 0.002$), and a lower rate of WHO 1973 G3 tumors (54% vs 80%; $p < 0.001$). There were no significant differences in tumor focality, age, sex distribution, RFS, PFS, or OS between the *FGFR3* alteration and wildtype groups (Supplementary Table 2).

3.5. Localized MIBC

For patients with localized MIBC, median OS was longer in the *FGFR3* alteration group. There were no differences in RFS, PFS, grade, N stage, sex distribution, or age between the FGFR3 alteration and wildtype groups (Supplementary Table 3).

3.6. Locally advanced MIBC

For patients with locally advanced MIBC, the *FGFR3* alteration group did no significantly differ in sex distribution, age, N stage, grade, RFS, PFS, or OS from the wildtype group (Supplementary Table 4).

Table 3 – Multivariable Cox regression results for overall survival and cancer-specific survival

Parameter	OR (95% CI)	*p* value
Overall survival		
Age (continuous)	1.02 (1.01–1.02)	**<0.001**
Female sex	1.06 (0.89–1.26)	0.6
Disease stage	1.35 (1.28–1.42)	**<0.001**
WHO 1973 grade	1.95 (1.39–2.75)	**<0.001**
Carcinoma in situ	1.07 (0.96–1.19)	0.2
FGFR3 alteration	0.90 (0.71–1.13)	0.4
FGFR3 fusion	1.05 (0.56–1.98)	0.9
Cancer-specific survival		
Age (continuous)	1.01 (1.00–1.01)	0.2
Female sex	1.06 (0.86–1.30)	0.6
Disease stage	1.4 (1.3–1.5)	**<0.001**
WHO 1973 grade	4.98 (2.54–9.79)	**<0.001**
Carcinoma in situ	1.06 (0.94–1.20)	0.4
FGFR3 alteration	0.89 (0.67–1.19)	0.4
FGFR3 fusion	1.246 (0.592–2.624)	0.6

OR= odds-ratio; CI = confidence interval; WHO, World Health Organization.

3.7. Metastastic UCB and metastatic UTUC

For patients with metastatic UCB or metastatic UTUC, there were no differences in age, sex distribution, pathological T stage, N stage, grade, or survival outcomes between the *FGFR3* alteration and wildtype groups. Supplementary Tables 5 and 6 provide further details.

4. Discussion

In our evaluation of the *FGFR3* alteration status in 1509 patients with UC across all disease stages, we observed a decrease in the frequency of *FGFR3* alterations with increasing disease stage and grade. A large meta-analysis of 916 tumors also showed a decrease in the frequency of *FGFR3* mutations with increasing stage and grade. The authors

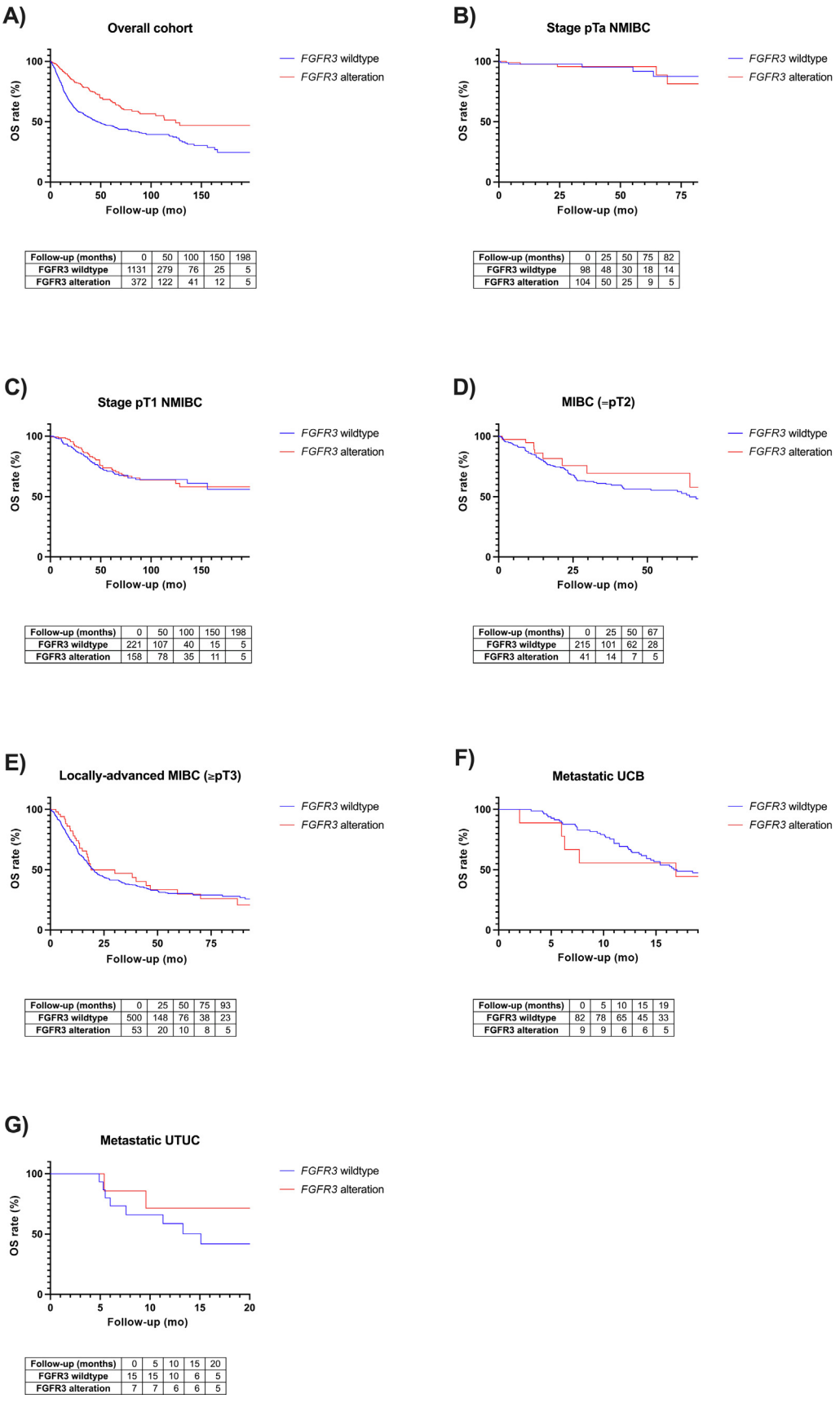

Fig. 2 – Kaplan-Meier curves for overall survival (OS) by *FGFR3* status across different disease stages. Log-rank tests were used to assess statistical significance. (A) Overall cohort ($p < 0.001$). (B) Stage pTa non–muscle-invasive bladder cancer (NMIBC; $p = 0.3$). (C) Stage pT1 NMIBC ($p = 0.7$). (D) Localized muscle-invasive bladder cancer (MIBC; $p = 0.3$). (E) Locally advanced MIBC ($p = 0.9$). (F) Metastatic urothelial carcinoma of the bladder (UCB; $p = 0.3$). (G.) Metastatic upper urinary tract urothelial carcinoma (UTUC; $p = 0.09$).

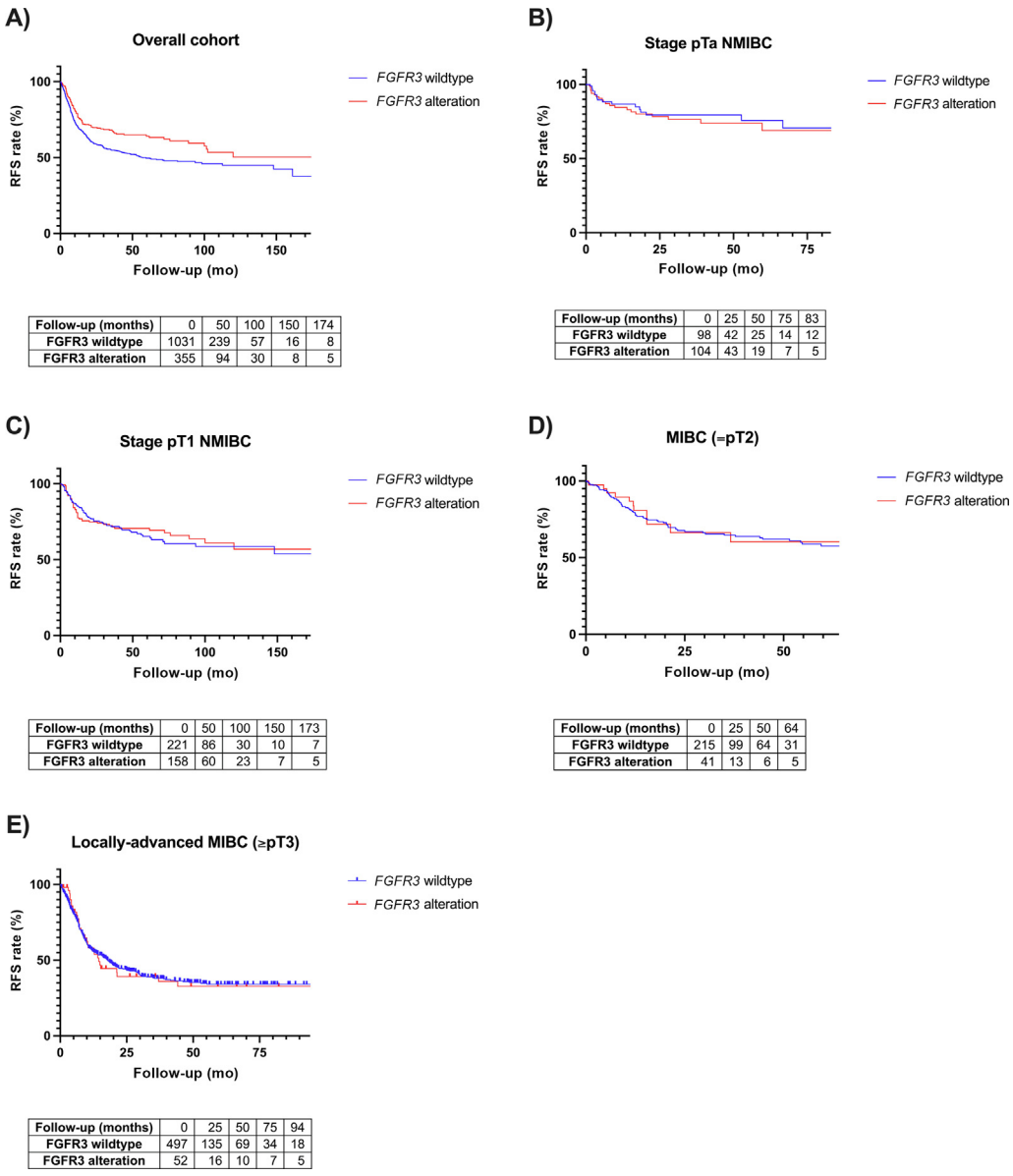

Fig. 3 – Kaplan-Meier curves for recurrence-free survival (RFS) by *FGFR3* status across different disease stages. Log-rank tests were used to assess statistical significance. (A) Overall cohort ($p < 0.001$). (B) Stage pTa non–muscle-invasive bladder cancer (NMIBC; $p = 0.7$). (C) Stage pT1 NMIBC ($p = 0.8$). (D) Localized muscle-invasive bladder cancer (MIBC; $p = 0.8$). (E) Locally advanced MIBC ($p = 0.7$).

reported FGFR3 mutations in 65% of pTa, 30% of pT1, and 12% of pT2–4 tumors, and in 70% of G1, 68% of G2, and 19% of G3 tumors [15], which is consistent with our findings. Another study reported *FGFR3* mutations in 74% of pTa, 21% of pT1, and 16% of pT2–4 tumors, and in 84% of G1, 55% of G2, and 7% of G3 tumors. However, it should be noted that this study included only 132 patients, which limits the significance of the results [6].

In the overall cohort, the proportion of male patients was higher in the *FGFR3* alteration group than in the wildtype group, which was especially true for the *FGFR3* fusion subgroup (91% male). Another interesting finding is that the wildtype group had higher T and N stages and more metastases. This could explain the difference in sex distribution, as women usually present with more advanced tumor stage and a higher number of lymph-node and distant metastases in comparison to age-matched men [16,17]. This is consistent with our subgroup analyses by disease stage, in which a balanced sex distribution was observed for each stage.

While the stage-dependent frequency of *FGFR3* alterations is well recognized, their impact on oncological outcomes is still a subject of debate, and the available data are contradictory.

In a prospective study involving 221 patients with NMIBC, Burger et al [18] found better PFS in the group with *FGFR3* mutations. Further studies confirmed better PFS and OS in stage pT1 NMIBC with an *FGFR3* mutation [19–21]. By contrast, Hernández et al [22] found an association between *FGFR3* mutations and higher recurrence rates in stage Ta G1 NMIBC, whereas other studies found no association between *FGFR3* alterations and RFS, PFS, CSS, or OS at all [23,24]. Van Rhijn et al [25] investigated *FGFR3* mutations

across different UC stages and found favorable CSS prognosis for patients with an *FGFR3* mutation. It should be noted that the frequency of *FGFR3* alterations in their study, similar to the other studies mentioned above, decreased significantly with increasing tumor stage, which may have influenced the correlation observed. Another study involving 1000 RC specimens found that *FGFR3* mutations were associated with NMIBC, lower pT stage, grade 1–2 disease, the absence of CIS, pN0 stage, and longer CSS [26]. However, no subgroup analyses for different tumor stages were performed in these studies [25,26]. A major strength of our study is that we examined the impact of *FGFR3* alterations on oncological outcomes both in the overall cohort and in various subgroups by disease stage. Our results confirm better RFS and OS for patients with *FGFR3* alterations in the overall cohort (Table 1 and Figs. 2 and 3). However, this effect did not extend to subgroup analyses of the different disease stages, for which Kaplan-Meier analyses revealed no difference in RFS or OS. Hence, it appears that the effect on oncological outcomes is primarily driven by the effect in lower tumor stages, in which *FGFR3* alterations are more likely to occur, rather than the alterations themselves. This hypothesis is reinforced by the multivariable Cox regression results, in which more aggressive disease—as reflected by disease stage, CIS presence, and high-grade tumors—rather than *FGFR3* alterations seemed to be the driver for OS and CSS.

Irrespective of their prognostic ability, activating *FGFR3* alterations represent an attractive target for new drug therapies. The pan-FGFR inhibitor erdafitinib was evaluated for the treatment of locally advanced or metastatic UC in patients with *FGFR2* or *FGFR3* alterations and disease progression after platinum-based chemotherapy. The phase 3 trial revealed significantly longer median OS (12 vs 7.8 mo; $p = 0.005$) and PFS (5.6 vs 2.7 mo; $p < 0.001$) with erdafitinib versus chemotherapy [5]. The drug was subsequently approved by the US Food and Drug Administration and is now being used in these patients.

Ongoing research is also investigating the significance of *FGFR* as a therapeutic target in NMIBC and MIBC. The THOR-2 trial is evaluating erdafitinib in patients with *FGFR3* or *FGFR2* alterations and disease recurrence after bacillus Calmette Guérin treatment who were ineligible for or refused RC [27]. While encouraging efficacy has been observed for erdafitinib, with significantly higher RFS rates (96% at 6 mo and 77% at 12 mo) than with intravesical chemotherapy (73% at 6 mo and 41% at 12 mo; $p = 0.008$), it also led to severe adverse events in 31% of patients and was discontinued by 29% [27]. Erdafitinib application via an intravesical delivery system (TAR-210) represents a promising approach with a potentially more favorable side-effect profile and comparable efficacy. In a phase 1 study involving patients with *FGFR2/3*-altered NMIBC, the device was generally well tolerated and demonstrated a PFS rate of 82% in high-risk NMIBC and a complete response rate of 87% in intermediate-risk NMIBC [28]. Results from the ongoing open-label, multicenter, randomized phase 3 MoonRISe-1 trial (NCT06319820), which is comparing the efficacy and safety of intravesical erdafitinib delivery with the TAR-210 system to intravesical chemotherapy with mitomycin or gemcitabine in intermediate-risk NMIBC, are eagerly awaited.

Our study population comprises retrospective cohorts from a time before *FGFR3* targeted therapy was available. Thus, none of the patients from the CCC-EMN, FOSMIC, and Regensburg cohorts received *FGFR*-targeted therapy, so a potential influence on our results can be excluded.

4.1. Limitations

A major limitation of our study is the retrospective design. However, owing to the structured follow-up, which typically included clinical sonographic and cystoscopy evaluations, as well as radiographic imaging in more advanced disease stages, and the relatively long follow-up period, we were able to conduct a robust assessment of oncological status for patients. Consequently, the likelihood that recall or selection bias significantly influenced our results or main conclusions is considered to be low. Another limitation is that the sample size varied for the different disease stages, which complicates comparability and needs to be considered when interpreting the data.

5. Conclusions

FGFR3 alterations occur at a stage-dependent frequency and are more prevalent in lower tumor stages. We were unable to demonstrate an independent prognostic effect of *FGFR3* alterations on oncological outcomes after adjusting for tumor stage.

Author contributions: Maximilian Haas had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Haas, Breyer, Eckstein.
Acquisition of data: Mayr, Sikic, Klümper, Erben, Wirtz, Bolenz, Roghmann, Stöhr, Bahlinger, Breyer.
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Appendix A. Supplementary material

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