Prognostic markers in invasive bladder cancer: *FGFR3* mutation status versus P53 and KI-67 expression: a multi-center, multi-laboratory analysis in 1058 radical cystectomy patients

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Abstract

Objectives: To determine the association between the *FGFR3* mutation status and immuno-histochemistry (IHC) markers (p53 and Ki-67) in invasive bladder cancer (BC), and to analyze their prognostic value in a multicenter, multi-laboratory radical cystectomy (RC) cohort.

Patients and methods: We included 1058 cN0M0, chemotherapy-naive BC patients who underwent RC with pelvic lymph-node dissection at 8 hospitals. The specimens were reviewed by uro-pathologists. Mutations in the *FGFR3* gene were examined using PCR-SNaPshot; p53 and Ki-67 expression were determined by standard IHC. *FGFR3* mutation status as well as p53 (cut-off>10%) and Ki-67 (cut-off>20%) expression were correlated to clinicopathological parameters and disease specific survival (DSS).

Results: pT-stage was <pT2 in 80, pT2 in 266, pT3 in 513 and pT4 in 199 patients, respectively. Cancer-positive nodes were found in 410 (39%) patients. An *FGFR3* mutation was detected in 107 (10%) and aberrant p53 and Ki-67 expression in 718 (68%) and 581(55%) tumors, respectively. The *FGFR3* mutation was associated with lower pT-stage (P<0.001), lower grade (P<0.001), pN0 (P=0.001) and prolonged DSS (P<0.001). Aberrant Ki-67 and p53 expression were associated with higher pT-stage and G3-tumors, but not with pN-stage or worse DSS, even if these IHC-biomarkers were combined (P=0.81). Significant predictors for DSS in multivariable analysis were pT-stage (HR1.5, 95%CI:1.3-1.6; P<0.001), lympho-vascular invasion (LVI) (HR1.4, 95%CI:1.2-1.7; P=0.001), pN-stage (HR1.9, 95%CI:1.6-2.4; P<0.001) and *FGFR3* mutation status (HR1.6, 95%CI:1.1-2.2; P=0.011).

Conclusion: The *FGFR3* mutation selectively identified patients with favorable BC at RC while p53 and Ki-67 were only associated with adverse tumor characteristics. Our results suggest that, besides tumor-stage, nodal-status and LVI, the oncogenic *FGFR3* mutation may represent a valuable tool to guide adjuvant treatment and follow-up strategies after RC.

Keywords: Bladder; Cancer; Urothelial carcinoma; Cystectomy; FGFR3; Mutation; Immunohistochemistry; p53; Ki-67

1. Introduction

Bladder cancer (BC) is one of the most common urological malignancies worldwide. It is histologically divided into 2 major entities: non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). Radical cystectomy (RC) is the standard treatment for BCG-refractory, very high-risk NMIBC and non-metastatic MIBC [1]. While the 5-year survival of patients with NMIBC is generally good, patients with MIBC have an unfavorable prognosis with a 5-year survival of approximately 50% despite major pelvic surgery [2−4]. This highlights the need for reliable prognostic markers for risk-stratification and guidance of management decisions, such as follow-up after RC and the use of (neo)adjuvant therapy.

Traditional prognostic variables at RC for BC include tumor-stage (pT), nodal-stage (pN) and lympho-vascular invasion (LVI) [1,5,6]. Along with histological staging, several molecular markers predicted clinical outcome after RC and could identify patients who would benefit from additional treatment [7]. Most studies used IHC as their method to assess prognosis [7−12]. Among the countless IHC markers, p53 and Ki-67 are the most widely used [8,12]. Aberrant IHC expression of p53 has shown negative prognostic impact for both NMIBC and MIBC in most studies [7−14]. Overexpression of Ki-67 has also been correlated with poor survival [12,13,15]. Although their prognostic value was tested in single and multi-center settings, IHC-analyses from multiple centers were done in a single laboratory [8,12].

Mutations in the gene Fibroblast Growth Factor Receptor 3 (*FGFR3*) have gained interest as a prognostic BC biomarker [13−16]. In contrast to p53 and Ki-67 expression, *FGFR3* mutations were associated with favorable prognosis BC [16] and are predominantly found in genetically stable BC of lower stage/grade [13,17]. While *FGFR3* mutations are less common (10%-20%) in invasive BC [13−16], the first reports suggested that the *FGFR3* mutation is associated with favorable features and prognosis in MIBC as well [15−20].

Despite the assumed value of both the *FGFR3* mutations status as well as p53 and Ki-67 IHC, only little is known about their combined prognostic value. Furthermore, there is a great unmet need to validate these markers among multiple laboratories. In the present study, we aimed to determine the association between *FGFR3* mutations and p53 & Ki-67 expression and analyzed their prognostic value in a multi-center, multi-laboratory setting.

2. Patients and methods

2.1. Study population

We analyzed 1058 cN0M0, chemotherapy-naive patients who underwent RC with pelvic lymph-node dissection (PLND) for cT1-4aN0M0 urothelial BC (based on transurethral resection and preoperative imaging) with residual tumor at RC, at 8 centers between 1986-2016. The cystectomy specimens were locally reviewed. The slides of the Toronto, Dallas and Turku cohorts were reviewed by TvdK. For this study, appropriate ethical approval was obtained at each site according to national regulations and the principles of the Declaration of Helsinki. Local ethics

committees and/or translational research boards (see supplementary file) approved the study, which was conducted in accordance with Good Clinical Practice guidelines. The tissue was secondarily used according to the Code of Conduct for responsible use.

2.2. DNA extraction and FGFR3 mutation analysis

The methods for DNA extraction and *FGFR3* mutation analysis have been previously described [16,17]. In brief, Hematoxylin and Eosin (HE) slides of RC-specimens served as templates for manual macro-dissection on the formalin fixed, paraffin embedded (FFPE) tissue-block or blank slides. The dissected samples contained a minimum of 70% tumor cells, as assessed by histological examination. DNA was extracted from the tissues according to the manufacturer's protocol using the DNeasy Tissue Kit. In the Paris cohort, the Maxwell 16 FFPE Plus LEV DNA Purification Kit and an automated Maxwell platform (Promega) were used for DNA isolation.

At 6 laboratories, the *FGFR3* mutation status was examined using PCR-SNaPshot assay. The cases from Turku and Dallas were analyzed in Toronto. Details of this method were previously reported [16,17]. The three frequently mutated regions on the exons 7, 10 and 15, which represent approximately 99% of oncogenic *FGFR3* mutations in BC, were simultaneously amplified by PCR. After removing excess primers and deoxynucleotides, specific SNaPshot primers were annealed to the PCR-products, subsequently separated by capillary electrophoresis and analyzed in an automatic sequencer (Prism 3100 genetic analyzer). A total of 11 known oncogenic *FGFR3* mutations can be detected with this PCR-SNaPshot method. The codon numbering refers to the cDNA open reading frame of the FGFR3b isoform expressed in epithelia [19]. The reading of the assays was done without knowledge of clinical or IHC-expression data.

2.3. Immunohistochemistry (IHC)

Standard immunohistochemistry was used to assess protein expression of p53 and Ki-67. The freshly cut slides were routinely processed with a monoclonal antibodies against p53 (DO-7, Dako, CA) and Ki-67 (MIB-1, Dako, CA). Overexpression of p53 and Ki-67 was defined if >10% (p53) and >20% (Ki-67) of the cells stained positive [10−13,16,20]. Standard tissue microarray (TMA) technology [20] was used in 7 participating laboratories. Whole slide staining was applied in the Regensburg cohort. The cases from Turku (TMA) were stained and analyzed in Toronto. The slides were assessed by 2 observers at each of the 7 laboratories and agreement on different scores was reached in a second combined session.

2.4. Statistical analysis

Of descriptive variables, medians were reported along with their interquartile range (IQR). Differences in distributions between *FGFR3* mutation status, p53 and Ki-67 expression levels were correlated to clinicopathological parameters: age, gender, stage, grade, CIS, LVI, nodal status, using the Chi-square or Fisher's exact test, as appropriate. Binary logistic regression analyses were conducted to determine the relationship between the variables. Disease-specific survival (DSS) was assessed from the date of RC to the date of death from BC. The data of patients who did not experience disease-specific death were censored at the date of last follow-up. DSS was calculated using the log-rank test (Kaplan-Meier). Cox-proportional hazard models were used for the multivariable DSS analysis with adjustments for variables that were significant ($P<0.05$) prognostic factors according to the univariable analyses. Then, the cooperative prognostic effect of combinations of markers was assessed by stepwise stratification of the cohort based on the number of altered biomarkers [21,22] and related to DSS. All tests were 2-sided and statistical significance was set at $P<0.05$. Statistical analyses were performed using R Version 3.6.3 (R-Foundation for Statistical-Computing, Vienna, Austria, 2020).

3. Results

3.1. Clinicopathological characteristics

Clinicopathological characteristics of the 1058 RC-patients are displayed in Table 1. The median age was 67 years (IQR; 58-74 years). *FGFR3* mutations were found in 107 (10%), aberrant p53 in 718 (68%) and aberrant Ki-67 in 581 (55%) RC-specimens, respectively.

3.2. Clinicopathologic characteristics according to FGFR3 mutation, p53- and Ki-67 expression

The *FGFR3* mutation was significantly associated with lower pT-stage ($P<0.001$), lower grade ($P<0.001$), absence of CIS ($P=0.02$), pN0 ($P=0.001$) and normal p53 and Ki-67 expression ($P<0.001$ and $P=0.001$, respectively) (Table2a, supplementary files). Aberrant p53 was associated with higher pT-stage ($P=0.006$), higher grade ($P<0.001$), absence of CIS ($P=0.004$) and not with nodal stage (Table2b, suppl files). Aberrant Ki-67 was associated with higher pT-stage ($P=0.05$), higher grade ($P=0.003$), absence of LVI ($P=0.03$) and not with nodal stage (Table2c, supplementary files). Furthermore, aberrant Ki-67 was associated with aberrant p53 expression ($P<0.001$). There was no association between any of the biomarkers and gender or age.

On multivariable analysis, the *FGFR3* mutation was associated with lower stage ($P=0.003$), lower grade ($P<0.001$) and pN0 ($P=0.04$) (Table 3). p53 expression

Table 1
Patient and tumor characteristics of the 1058 patients (cT1-4aN0M0) who underwent radical cystectomy. Median follow-up for the 538 survivors was 4.4 years (inter-quartile range; 2.2-7.4 years) while 520 patients died of bladder cancer. Median, interquartile range and percentages are shown for the variables.

Variable		Frequency	Percentage
Age (Median; IQR)		67 years	58 − 74 years
Sex	Female	222	21%
pT-stage at cystectomy	pTa/is/1	24/7/49	7.6%
	pT2	266	25%
	pT3	513	49%
	pT4	199	19%
Grade (WHO1973)	G1	1	0.%
	G2	91	9%
	G3	966	91%
Carcinoma in situ		403	38%
Lympho-vascular invasion		498	47%
pN-stage	pN0	648	61%
	pN+	410	39%
	pN1	151	14%
	pN2-3	259	25%
Lymph nodes removed (Median; IQR)		13	8.0 − 18
Adjuvant radiotherapy	No	989	94%
	Yes	69	6%
Adjuvant chemotherapy	No	765	72%
	Yes	293	28%
FGFR3 mutation	All	107	10%
	S249C	67	6% (63% of mutations)
	Other mutations	40	4% (37% of mutations)
p53 expression	Normal	340	32%
	Aberrant	718	68%
Ki-67 expression	Normal	477	45%
	Aberrant	581	55%
City / Hospital / Laboratory	Regensburg	156	15%
	Toronto	104	10%
	Dallas**	132	13%
	Turku*	54	5%
	Rotterdam	147	14%
	Amsterdam	195	18%
	Erlangen	98	9%
	Paris	172	16%
Total		1058	100%

* Molecular analyses and pathology review of the cases from Turku were done in Toronto.
** *FGFR3* mutation analyses and pathology review of the cases from Dallas were done in Toronto.
Abbreviations: pT-stage = pathological tumor-stage & pN-stage = pathological nodal-stage (according to the 2002 TNM classification of urothelial carcinoma of the urinary bladder). IQR = interquartile range.

remained associated with higher grade ($P<0.001$) and absence of CIS ($P=0.001$) while Ki-67 expression remained associated with higher grade ($P=0.005$) and absence of LVI ($P=0.01$).

3.3. Clinical outcome according to FGFR3 mutation status, p53 and Ki-67 expression

Median follow up time of the entire cohort was 5.3 years (reverse Kaplan Meier method). Overall, the *FGFR3* mutation was significantly associated with favorable DSS (Fig. 1A): Median survival for patients with mutant versus wild type tumors not reached versus 3.7 years ($P<0.001$; IQR 2.5-4.9 years). In contrast, neither aberrant p53 expression nor aberrant Ki-67 expression corresponded with worse DSS (Fig. 1B and Fig. 1C).

Zooming in on patients with locally advanced tumors (≥pT3 and/or pN+; n=793), the difference in DSS between *FGFR3* mutant vs *FGFR3* wild type tumors was not statistically significant (median DSS 3.6 years vs 2.5 years; $P=0.13$). Again, there was no statistically significant difference in DSS between p53 normal expression vs overexpression (median DSS 2.4 vs 2.7, respectively; $P=0.66$) nor between Ki-67 normal expression vs overexpression

Table 3
Multivariable binary logistic regression models displaying the relation between *FGFR3* mutation status and the immunohistochemical biomarkers p53 and Ki-67, and the clinico-pathological variables (listed in the rows of the table) of the 1058 radical cystectomy patients.

	FGFR3 mutation		p53 expression		Ki-67 expression	
Variable	OR (95% CI)	*P*	OR (95% CI)	P	OR (95% CI)	*P*
pT						
pTa/1/is	ref.	0.003	ref.	0.22	ref.	0.12
pT2	0.28 (0.14-0.56)		1.6 (0.91-2.7)		1.6 (0.93-2.7)	
pT3	0.33 (0.17-0.66)		1.5 (0.87-2.5)		1.3 (0.77-2.2)	
pT4	0.40 (0.18-0.89)		1.9 (1.0-3.4)		1.1 (0.60-1.9)	
pN						
pN0	ref.	0.05	ref.	-	ref.	-
pN1	0.50 (0.23-0.99)		1.1 (0.84−1.5)	0.41	1.1 (0.84-1.5)	0.46
pN2/3	0.61 (0.33-0.98)					
Grade						
G1 - G2	ref.	-	ref.	-	ref.	-
G3	0.18 (0.10-0.30)	< 0.001	2.9 (1.8-4.6)	<0.001	1.92 (1.2-3.0)	0.005
CIS						
Negative	ref.	-	ref.	-	ref.	-
Positive	0.66 (0.41-1.0)	0.08	0.64 (0.49-0.84)	0.001	0.82 (0.64-1.1)	0.12
LVI						
Negative	ref.	-	ref.	-	ref.	-
Positive	1.5 (0.89-2.4)	0.13	0.94 (0.70-1.3)	0.6	0.69 (0.52-0.91)	0.01

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; *FGFR3* = fibroblast growth factor receptor 3; LVI = lympho-vascular invasion; OR = odds ratio; ref. = reference

(median DSS 2.5 years vs 2.6 years; *P*=0.95). Zooming in on pT2N0 patients (*n*=192), median DSS was not reached in this subgroup. The 2-year DSS probability of *FGFR3* mutant tumors was 1.0 (95%CI, 0.81-1.0) vs 0.85 (95%CI, 0.79-0.90) for *FGFR3* wild type tumors; *P*=0.08. The 2-year DSS for normal p53 expression was 0.90 (95%CI, 0.80-0.95) vs 0.85 (95%CI, 0.77-0.95) for aberrant p53 expression; *P*=0.61 and 2-year DSS for normal Ki-67 expression was 0.89 (95%CI, 0.79-0.95) vs aberrant Ki-67 0.85 (95%CI, 0.77-0.95); *P*=0.27.

In the multivariable Cox regression model (Table 4), the *FGFR3* mutation remained significantly associated with DSS, while p53 and Ki-67 were not prognostic. pT-stage, LVI, pN-stage and age also remained significantly associated with DSS in this multivariable analysis.

3.4. Clinical outcome according to combinations of biomarkers

To assess the cooperative prognostic effect of the IHC-markers, we combined altered p53 and Ki-67. We found that 435 patients (41%) had both aberrant p53 and Ki-67; 429 (41%) had one altered IHC-biomarker and 194 (18%) had normal expression of both IHC-biomarkers. The frequencies of altered IHC-biomarkers, as well as the distribution of the *FGFR3* mutation status among these groups, is displayed in Figure2a, supplementary files).

Stratifying for the number of altered IHC-biomarkers, we found no statistically significant difference in DSS among patients with no altered IHC-biomarkers versus patients with 1 or 2 altered IHC-biomarkers (median DSS; 0-altered: 4.3 years; 1-altered: 4.3 years; 2-altered: 3.9 years; *P*=0.81) (Figure2b, supplementary files). Moreover, if we tested the number of altered IHC-biomarkers in the *FGFR3* mutant and wild type sub-groups, the number of altered IHC markers was not associated with a statistically significant difference in DSS; neither in the wild type group (median DSS; 0-altered: 3.3 years; 1-altered: 3.8 years; 2-altered: 3.9 years; *P*=0.59), nor in the *FGFR3* mutant group (median DSS not reached - 75 percentile DSS; 0-altered: 1.7 years; 1-altered: 2.9 years; 2-altered: 1.1 years; *P*=0.15) (Figure2c, supplementary files).

4. Discussion

The aim of the present study was to determine the association between *FGFR3* mutations and p53 and Ki-67 expression in a large cohort of patients with invasive BC treated by RC, and to analyze their prognostic value in a large and unique multicenter, multi-laboratory setting. We found that altered expression of Ki-67 and p53 was associated with higher pT-stage and grade disease, while *FGFR3* mutations were associated with lower stage, lower grade, pN0-disease and also an independent favorable prognosticator in multivariable analysis. So, *FGFR3* mutations selectively identified patients with favorable disease at RC and proved a more robust biomarker for clinical outcome than p53 and/or Ki-67 IHC.

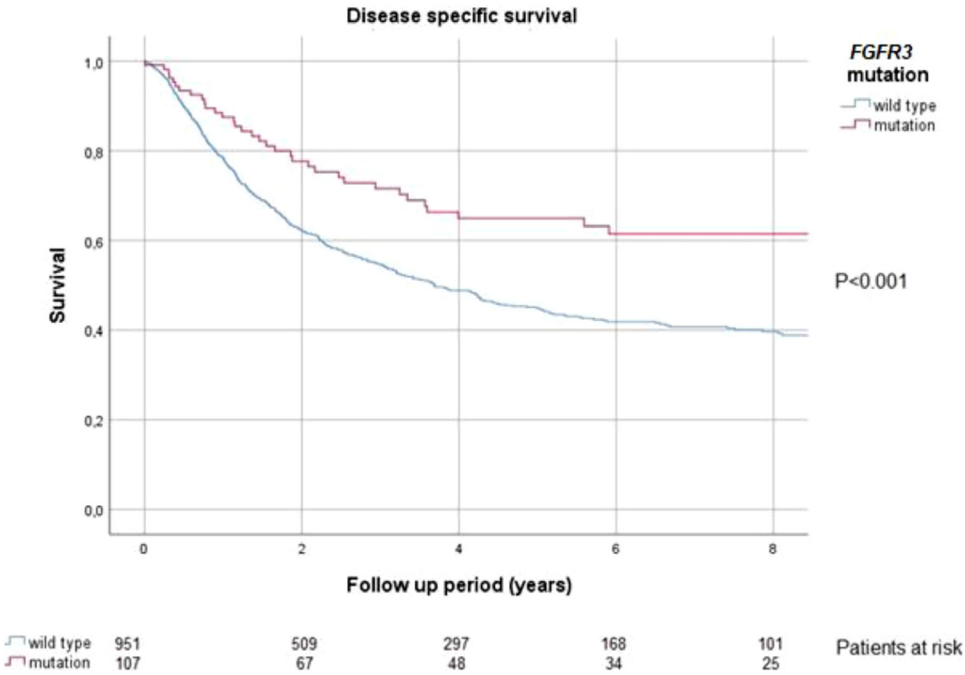

Fig. 1. Kaplan Meier plots (a-c) showing disease specific survival (DSS) for *FGFR3* mutation status (1a), p53 expression (1b) and Ki-67 expression (1c). Follow-up was truncated at 8 years.

Fig. 1A. This plot shows DSS stratified by the presence of the *FGFR3* mutation. The 2-year DSS probability for *FGFR3* mutant tumors was 0.79 (95%CI, 0.69-0.86) versus 0.66 (95%CI, 0.60-0.66) for wild type tumors.

Fig. 1B. This plot shows DSS stratified by the presence of the aberrant versus normal p53 expression. The median DSS of p53 aberrant cases was 3.9 years (Inter-quartile range (IQR); 2.8-5.0 years) versus 4.5 years (IQR; 3.1-5.9 years) for the cases with normal p53 expression.

Fig. 1C. This plot shows DSS stratified by the presence of aberrant versus normal Ki-67 expression. The median DSS of Ki-67 aberrant cases was 4.2 years (IQR; 3.1-5.3 years) versus 3.8 years (IQR; 2.5-5.1 years) for the cases with normal Ki-67 expression.

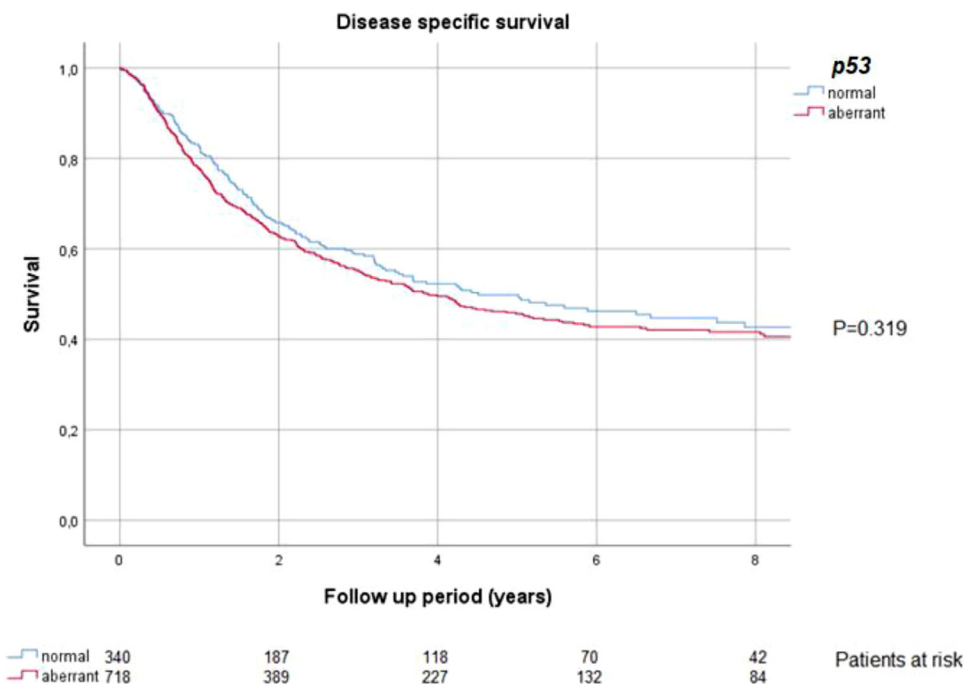

Fig. 1. Continued

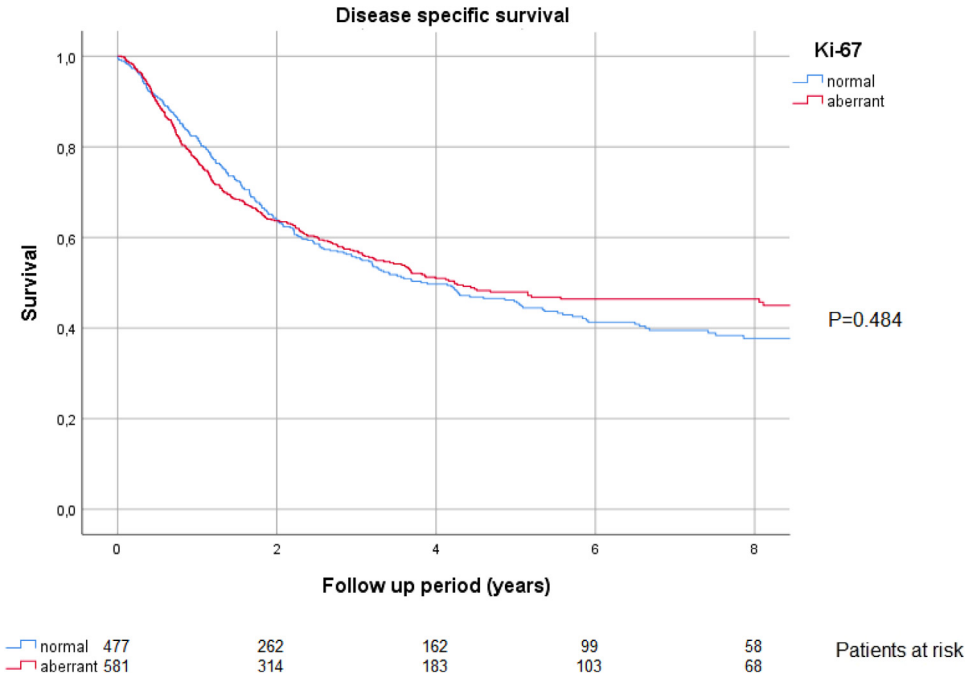

Fig. 1. Continued

Our findings regarding the *FGFR3* mutation are in line with previous studies demonstrating its association with favorable prognosis in NMIBC and some studies on MIBC [13−20]. Simultaneously, only little is known about the prognostic value of the *FGFR3* mutations in combination with IHC-markers. This is of particular interest, given the observation that *FGFR3*-over-expressing tumors can show high proliferation [23] − a paradox of coexisting favorable and adverse features - which can be detected by IHC with the proliferation marker Ki-67. Furthermore, earlier work in NMIBC showed the cooperative effect of *FGFR3* with p53 and Ki-67 where *FGFR3* mutations were related to a favorable prognosis and aberrant p53 and Ki-67 expression to unfavorable disease [13]. In the present study, however, *FGFR3* mutations remained the only prognostic marker, in addition to histo-pathological characteristics and in contrast to p53 and Ki-67 expression.

Another remarkable finding of our study is the observation that p53 and Ki-67 were not only associated with risk factors for worse disease (e.g., higher grade) but also with a favorable factor: either absence of CIS (p53) or absence of LVI (Ki-67). This illustrates the ambiguity of both IHC-markers within our RC-cohort. This is also reflected by our observation that their combined status did not improve prognostic impact, which is in contrast to the reports by Shariat et al. [21] and Lotan et al. [22]. Whereas overexpression of p53 and Ki-67 are reported markers of worse BC prognosis in literature [7−14], they did not hold their value as prognostic markers in the present study; neither as a single marker nor as a combination.

Distinctive features of the present study are the focus on the value of the combination of the *FGFR3* mutations and p53 & Ki-67 IHC and the multi-center, multi-laboratory setting. Our results on the "favorable" *FGFR3* mutation were in line with the literature. The differences between our findings on p53 and Ki-67 and those reported in literature may be explained by the multi-laboratory setting of the present study. So far, single and multi-institution studies reported high accuracy of IHC in determining p53 and Ki-67 status in BC-tissue samples. However, these studies were performed in a single-laboratory setting [8,12]. IHC has been criticized because of lack of inter-laboratory standardization and reproducibility in interpretation of the results, leading to discrepancies in expression assessment and even showing excessive variation if measuring centrally stained slides from the same cases [24]. Particularly in a multi-laboratory setting like ours (with both TMA and whole slide staining), these issues could have led to rather unexpected yet realistic results. On the other hand, the PCR-based *FGFR3* mutation analysis has shown a 100% concordance if DNA-samples were analyzed in multiple laboratories [17].

Another possible explanation regarding the unexpected results for p53 and Ki-67 is the variation of threshold values differentiating IHC-positive from IHC-negative tumors. In this study, we took 20% nuclear positivity as the cut-off value for high expression of Ki-67 and 10% for p53, as these values have previously been reported as prognostic in terms of recurrence, progression and disease-free survival in many previous studies [7-13,16,21-26]. However, recent work suggested that reassessment of this threshold (taking into account null-type mutations) in invasive high-grade BC shows a better correlation with TP53 mutations and oncological outcome [27,28]. Whether the use of a different

Table 4
Multivariable Cox regression analysis of *FGFR3* mutation status and the immunohistochemical biomarkers (p53 & Ki-67) including the other factors potentially influencing disease specific survival of the 1058 patients who were treated with radical cystectomy.

Variable	HR (95% CI)	*P*-value
Gender		
Male	ref.	
Female (vs. male)	1.0 (0.82 - 1.3)	0.87
Age (years)		
<67 years	ref.	
>67 years	1.2 (1.0 - 1.5)	0.02
pT		
pTa/1/is	ref.	0.01
pT2	1.1 (0.65-1.8)	
pT3	1.8 (1.1-2.9)	
pT4	2.5 (1.5-4.2)	
CIS		
Negative	ref.	
Positive	1.2 (1.0-1.4)	0.07
LVI		
Negative	ref.	
Positive	1.9 (1.4-2.0)	<0.001
pN		
pN0	ref.	
pN1	1.9 (1.6-2.4)	<0.001
pN2/3	2.1 (1.4-2.4)	
FGFR3 mutation status		
Wild type	ref.	
Mutation	0.65 (0.46-0.94)	0.02
p53 expression		
Normal	ref.	
Aberrant	1.0 (0.83-1.2)	0.99
Ki-67 expression		
Normal	ref.	
Aberrant	0.93 (0.78-1.11)	0.44

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; *FGFR3* = fibroblast growth factor receptor 3; LVI = lympho-vascular invasion; HR = hazard ratio; ref = reference.

threshold would lead to different results in our multi-laboratory setting needs to be evaluated in further analyses and represents a limitation to the present study.

Other limitations of our study are the selection of patients with residual disease at RC, which may reflect more advanced disease with subsequent higher tumor-load. Also, patients were included over a relatively long time period. Furthermore, we only studied *FGFR3* mutations and not *FGFR3* gene fusions. Given the large number of RCs in the present study and the low reported frequency of *FGFR3* fusions compared with *FGFR3* mutations in MIBC [15], we anticipate that this has not substantially influenced our conclusions on the favorable prognostics of the *FGFR3* mutation in RC-patients. Despite its limitations, our study may have important implications for clinical practice. Establishing clinical relevance of a marker for guiding therapy decisions requires that it classifies patients into distinct subgroups with different management recommendations. Our study confirmed that the *FGFR3* mutation would be a reliable biomarker for patient selection regarding adjuvant therapy. This prompts further exploration of anti-*FGFR3*-targeted therapies in the peri-operative setting [15,17,18,29,30]. Currently, prospective trials in the adjuvant setting are recruiting and use FGFR-inhibitors for selected patients with *FGFR3*-alterations after RC. The next step may be to focus on the ability to select patients for FGFR-inhibitors. For this purpose, future studies should focus on the reliability of the mutation in the TUR specimen [17].

In conclusion, the present large multi-center, multi-laboratory RC-study demonstrated that *FGFR3* mutations selectively identify patients with favorable disease at RC, while p53 and Ki-67 expression were only associated with some adverse tumor characteristics. Our results suggest that, besides tumor-stage, nodal-status and LVI, the oncogenic *FGFR3* mutation may represent a valuable tool to guide adjuvant treatment and follow-up strategies after RC.

5. Site specific approval and protocol numbers

- Amsterdam: The Institutional Review Board of the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital (CFMPB-160 & IRBd18126).
- Rotterdam: The medical-ethical board of Erasmus MC, Rotterdam (MEC 168.922/1998/55).
- Regensburg: Medical ethical board of the University of Regensburg ([16]-101-0218).
- Paris: The regional ethics board of Ile-de-France IX – Comité de protection des personnes – Ile-de-France IX - Créteil ([11]-052).
- Toronto: The regional ethics board of the University Health Network, Toronto (02-0515-C, 08-0263-T, 09-0826-CE and 09-0556-TE).
- Turku: Ethical committee of the hospital district of South-West Finland, No: ETMK 6/2006.
- Erlangen: The ethical and translational boards, Erlangen (217_18 Bc and 329_16-B).
- Dallas: The Institutional Review Board for the Protection of Human Subjects at University of Texas Southwestern Medical School: STU 102014-008.

Conflict of interest

FC and YN received a European Urological Scholarship for the European Association of Urology. The authors have declared no conflicts of interest related to the current manuscript.

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Supplementary materials

Supplementary material associated with this article can be found online.

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