Clinical Cancer Research

Molecular Markers Increase Precision of the European Association of Urology Non–Muscle-Invasive Bladder Cancer Progression Risk Groups

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Abstract

Purpose: The European Association of Urology (EAU) guidelines for non–muscle-invasive bladder cancer (NMIBC) recommend risk stratification based on clinicopathologic parameters. Our aim was to investigate the added value of biomarkers to improve risk stratification of NMIBC.

Experimental Design: We prospectively included 1,239 patients in follow-up for NMIBC in six European countries. Fresh-frozen tumor samples were analyzed for *GATA2*, *TBX2*, *TBX3*, and *ZIC4* methylation and *FGFR3*, *TERT*, *PIK3CA*, and *RAS* mutation status. Cox regression analyses identified markers that were significantly associated with progression to muscle-invasive disease. The progression incidence rate (PIR = rate of progression per 100 patient-years) was calculated for subgroups.

Results: In our cohort, 276 patients had a low, 273 an intermediate, and 555 a high risk of tumor progression based on the EAU NMIBC guideline. Fifty-seven patients (4.6%) progressed to muscle-invasive disease. The limited number of progressors in this large cohort compared with older studies is likely due to improved treatment in the past two decades. Overall, wild-type *FGFR3* and methylation of *GATA2* and *TBX3* were significantly associated with progression (HR = 0.34, 2.53, and 2.64, respectively). The PIR for EAU high-risk patients was 4.25. On the basis of *FGFR3* mutation status and methylation of *GATA2*, this cohort could be reclassified into a good class (PIR = 0.86, 26.2% of patients), a moderate class (PIR = 4.32, 49.7%), and a poor class (PIR = 7.66, 24.0%).

Conclusions: We conclude that the addition of selected biomarkers to the EAU risk stratification increases its accuracy and identifies a subset of NMIBC patients with a very high risk of progression. *Clin Cancer Res; 24(7); 1586–93. ©2018 AACR.*

Introduction

Patients with non–muscle-invasive bladder cancer (NMIBC) have a recurrence rate of approximately 70%, and in up to 15% of cases, the tumor progresses to muscle-invasive bladder cancer (MIBC; refs. 1–3). Hence, patients with NMIBC need to be monitored frequently for many years. This contributes to the fact that the management of patients accounts for 3% of all cancer costs in the EU (€143 billion in 2012; ref. 4).

The European Association of Urology (EAU) has developed widely adopted guidelines for the treatment and follow-up of NMIBC patients. In these guidelines, patients are stratified into low-, intermediate-, and high-risk groups based on clinicopathologic characteristics (5). These guidelines are based on European

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

The EAU guideline for non–muscle-invasive bladder cancer (NMIBC) recommends risk stratification based on clinicopathologic parameters only. This large prospective international study shows that the addition of *FGFR3* mutation status and *GATA2* methylation status to this risk stratification reclassifies EAU high-risk patients in good, moderate, or poor progression risk subsets. On the basis of this biomarker subclassification of EAU high-risk patients, these patients could be allocated to different treatment strategies. Patients at very high risk of progression could receive more intensive surveillance and additional treatments or may even be considered for early cystectomy. We conclude that the addition of selected biomarkers to the EAU risk stratification increases its accuracy and that the use of these progression markers has potential for implementation in the EAU NMIBC guidelines. Further validation is however recommended.

Organisation for Research and Treatment of Cancer (EORTC) nomograms (1). Patients included in this large study were recruited between 1979 and 1989. Treatment and follow-up differed from current practice. For instance, BCG was not FDA approved until 1990 and the mandatory retransurethral resection (TUR) for high-risk tumors was not standard practice in the 1980s. Over the past decades, these items have changed NMIBC management, and insight on the molecular architecture of bladder tumors has dramatically increased. The EAU risk stratification does not include molecular markers, and it is important and challenging to determine whether the molecular knowledge can improve the management of NMIBC patients, particularly those at high risk of progression.

Previous work showed that activating point mutations in the *FGFR3* gene were associated with a lower chance of progression of pTa and pT1 tumors (6–8). Methylation markers for progression of NMIBC (*GATA2*, *TBX2*, *TBX3*, and *ZIC4)* were identified and validated in two small patient series (9). Further validation of the four genes was done by Beukers and colleagues on 192 formalin-fixed paraffin-embedded (FFPE) bladder cancer samples enriched for progressing cases (10). In this large international prospective study of NMIBC (FP7: UROMOL), we aimed to evaluate the added value of these markers to the well-established risk stratification of the EAU NMIBC guidelines (5).

Materials and Methods

Patient selection and data collection

A total of 1,239 patients in follow-up for NMIBC (urothelial carcinoma) were prospectively included in hospitals in Denmark ($n = 581$), Germany ($n = 386$), Serbia ($n = 77$), Spain ($n = 75$), Sweden ($n = 84$), and the Netherlands ($n = 36$; Table 1). Inclusion criteria were patients diagnosed with NMIBC and not previously diagnosed with MIBC. Of these primary and recurrent patients, 884 were in follow-up for stage pTa disease, 310 for pT1 disease, and 45 for pTis. Only one tumor per patient was included in this study. Because treatment and follow-up regimens were based on the original pathology reports, we used these for staging and grading. Fresh-frozen tumor tissue was collected. Sections with at least 50% tumor cells were selected for DNA isolation.

Table 1. Patient and tumor characteristics of all included patients ($N = 1,239$)

Patient and tumor characteristics		
Patient characteristics		
Age	Mean (range)	70 (21–96)
		n (%)
Gender	Male	961 (77.6)
	Female	278 (22.4)
Smoking	Never	154 (12.4)
	Former	390 (30.8)
	Current	312 (25.2)
	Unknown	*383 (30.9)*
Country of inclusion	Denmark	581 (46.9)
	Germany	386 (31.2)
	Netherlands	36 (2.9)
	Serbia	77 (6.2)
	Spain	75 (6.1)
	Sweden	84 (6.8)
Ever diagnosed with *CIS*	Yes	191 (15.4)
	No	1,048 (84.6)
Intravesical instillation	Yes	464 (37.4)
(BCG/MMC/Chemo ever)	No	774 (62.5)
	Unknown	*1 (0.1)*
Tumor characteristics		
Tumor type	Primary	583 (47.1)
	Recurrent	656 (52.9)
Stage	pTa	884 (71.3)
	pT1	310 (25.0)
	pT*is*	45 (3.6)
Grade[a]	Low grade	849 (68.5)
	High grade	353 (28.5)
	PUNLMP	12 (1.0)
	Unknown	*25 (2.0)*
Multiplicity	Solitary	879 (70.9)
	Multiple	344 (27.8)
	Unknown	*16 (1.3)*
Tumor size	<3 cm	663 (53.5)
	≥3 cm	162 (13.1)
	Unknown	*414 (33.4)*
EAU risk category	Low	276 (22.3)
	Intermediate	273 (22.0)
	High	555 (44.8)
	Unknown	*135 (10.9)*
Prior recurrence rate	<1/year	157 (12.4)
	>1/year	86 (6.8)
	Unknown	*1,028 (82.9)*
Progression to T2	Yes	57 (4.6)
	No	1,182 (95.4)
Months of follow-up	Median (range)	27.0 (0-81)
		Positive test
Mutation analyses	*FGFR3*	424/762 (55.6)
	TERT	571/770 (74.2)
	PIK3CA	171/778 (22.0)
	RAS	58/774 (7.5)
Methylation dichotomized	*GATA2*	241/792 (30.4)
	TBX2	447/792 (56.4)
	TBX3	273/792 (34.5)
	ZIC4	242/792 (30.6)

Abbreviations: BCG, Bacillus Calmette-Guerin; MMC, Mitomycin C.
[a]Low grade included "low grade," "grade 1," and "grade 2"; high grade included "high grade," "grade 3," and "grade 4. "

Clinicopathologic parameters were entered in an online database. There was no attempt to modify current clinical practice at the participating centers; follow-up took place according to the national guidelines. The study was approved by the Central Denmark Region Committees on Biomedical Research Ethics (#1994/2920), the ethics committee of the University Hospital

Erlangen (Erlangen, Germany; #3755), the ethics committee of the technical University of Munich (Munich, Germany; #2792/10), Medical Ethics Committee of Erasmus MC (Rotterdam, the Netherlands; MEC#168.922/1998/55; Rotterdam), the Uppsala Region Committee on Biomedical Research Ethics (#2008/252), the Ethical Committee of Faculty of Medicine, University of Belgrade (Belgrade, Serbia; #440/VI-7), the Ethics Committee (CEIC) of Institut Municipal d'Assistència Sanitària/Hospital del Mar (Barcelona, Spain; 2008/3296/I), and the ethics committee of the University Hospital Jena (Jena, Germany; #4774-4/16). Patients either gave their written informed consent, or samples were used according to "The Code for Proper Secondary Use of Human Tissues in the Netherlands" (http://www.federa.org/). The study was conducted according to the principles of the Declaration of Helsinki. In all centers, standardized procedures were applied for sampling, freezing, and shipment of the samples (11).

Molecular analyses

DNA was isolated using Puregene DNA Isolation kit (Thermo Fisher Scientific). Methylation of the *GATA2*, *TBX2*, *TBX3*, and *ZIC4* genes was determined as described by Kandimalla and colleagues (9). In short, DNA was converted with bisulfite (EZ-DNA Methylation Gold 30TM, Zymo Research Corp.). The converted DNA samples were amplified in a bisulfite-specific PCR. Primer and probe details can be found in Supplementary Table S1. After completion, the samples were treated with an Exonuclease I (EXOI)/Shrimp Alkaline Phosphatase (SAP) mixture to remove excess primers and dNTPs. Next, a single-nucleotide probe extension SNaPshot analysis was performed. Then, the sample was placed in an automatic sequencer (ABI PRISM 3130 XL Genetic Analyzer, Applied Biosystems). SNaPshot data were analyzed by use of GeneMarker version 2.4 (SoftGenetics). Point mutations in the *FGFR3*, *PIK3CA*, *TERT*, and *RAS* oncogenes were likewise determined using a probe extension SNaPshot analysis following PCR of selected regions (12, 13).

Statistical analysis

Analyses were performed retrospectively on the data that were collected in real time. Each methylation marker was dichotomized as hypomethylated versus hypermethylated. The ROC curve was used to set a cutoff for each methylation marker by determining the optimum between sensitivity and specificity of the methylation ratio for predicting progression to MIBC. Progression-free survival (PFS) curves were estimated using the Kaplan–Meier method, followed by log-rank analysis to determine the difference between both groups. Progressive disease is defined as progression to stage T2 or higher stage disease, development of nodal or distant metastases, or death of disease. Patients that died of other cause prior to progression were censored at the time of death. Univariate and multivariable Cox regression analyses were performed to test the prognostic relevance of the different variables. Harrell c-statistic was defined to measure the predictive capacity. The progression incidence rate (PIR) was used to determine the impact of a new risk stratification. The PIR is the number of progressors divided by the amount of person-years in that risk group, times 100, and can be interpreted as the rate of progression per 100 person-years of follow-up and is cumulative (14). Statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp.). Two-sided P values lesser than 0.05 were considered statistically significant.

Results

Patient and tumor characteristics

Over 47% of patients were included with a primary tumor, and 37.4% received any type of intravesical instillation (Table 1). Age and gender distribution was in accordance with the literature (2). Numbers of tumors with methylation or mutations in the analyzed genes are depicted in Table 1. The determined cutoffs, sensitivities, and specificities for all methylation markers are listed in Supplementary Table S2. Distribution of clinical characteristics per country is depicted in Supplementary Table S3.

Relation of potential predictor variables to progression of NMIBC

According to the EAU risk stratification, 276 NMIBC patients had a low-risk, 273 an intermediate-risk, and 555 patients had a high-risk tumor (Table 1; ref. 5). Progression to muscle-invasive disease was seen in one (0.4%) of low-risk tumors, eight (2.9%) of intermediate-risk tumors, and 45 (8.1%) of high-risk patients; the remaining nine progressions occurred in patients of an unknown EAU risk category. In all included patients, univariate Cox regression analysis identified a significantly higher HR of progression for increasing age (HR = 1.04, $P = 0.004$) and EAU risk category (HR = 5.92, $P < 0.001$; Table 2A). Many of the clinical parameters included in the EAU risk category were significantly correlated to progression [carcinoma *in situ* (*CIS*), stage, grade, and tumor size]. Of the biomarkers, *FGFR3* mutations were associated with a lower HR for progression (HR = 0.34, $P = 0.002$) and methylation of *GATA2* and *TBX3* with a significantly higher HR (HR = 2.53, $P = 0.003$ and HR = 2.64, $P = 0.002$, respectively). All other potential biomarkers, mutation status of *TERT*, *PIK3CA*, *RAS*, and methylation status of *TBX2* and *ZIC4*, were not significantly associated with progression. Overall, c-statistics were highest for EAU risk category (0.70), grade (0.70), stage (0.69), *FGFR3* mutation status (0.66), and methylation of *GATA2* (0.62; Table 2A). PFS was significantly poorer in patients with higher EAU risk categorization, *FGFR3* wild-type, and *GATA2* and *TBX3* methylated tumors (Supplementary Fig. S5).

The combination of EAU risk category, *FGFR3* mutation status, *GATA2*, and *TBX3* methylation status resulted in an overall predictive capacity of 76% as calculated by the Harrell c-statistic (Table 2B), and this was 0.72 for the biomarker combination without EAU risk category.

Potential predictor variables for progression of high-risk NMIBC

Because progression to MIBC was most prominent in the EAU high-risk group and a more personalized risk stratification for this group is of most benefit for patients, we next focused on this group. In this subgroup, age was again significantly associated with progression, even though the HR was low (HR = 1.04, $P = 0.021$; Table 3A). Furthermore, grade and hypermethylation of *GATA2* resulted in a significantly higher HR for progression (HR = 2.28, $P = 0.018$ and HR = 2.04, $P = 0.046$, respectively). In contrast, intravesical instillations and *FGFR3* mutations were associated with a lower HR of progression to MIBC (HR =

Table 2A. Univariate Cox regression analysis of potential predictor variables and time to progression in all patients ($N = 1,239$)

Variables	*n*[a]	HR (95% CI)	*P*	c-statistic
Clinical and tumor variables				
Age	1,034	1.04 (1.01–1.07)	**0.004**	0.58
Gender	1,038	0.79 (0.40–1.56)	0.489	0.51
Smoking (ever)	794	1.13 (0.51–2.54)	0.760	0.52
Intravesical instill.	1,037	0.62 (0.36–1.07)	0.084	0.59
Primary/recurrent	1,038	1.12 (0.67–1.89)	0.664	0.51
Ever *CIS*	1,038	2.23 (1.25–3.97)	**0.007**	0.57
Stage	1,038	1.73 (1.31–2.29)	<**0.001**	0.69
Grade	1,020	4.80 (2.81–8.18)	<**0.001**	0.70
Tumor size	742	2.12 (1.14–3.94)	**0.018**	0.56
Multiplicity	1,025	1.59 (0.94–2.70)	0.086	0.53
EAU risk category (low + interm. = reference)	946	5.92 (2.89–12.11)	<**0.001**	0.70
Mutation markers				
FGFR3	659	0.34 (0.17–0.68)	**0.002**	0.66
TERT	667	2.23 (0.87–5.73)	0.095	0.55
PIK3CA	676	1.21 (0.59–2.49)	0.605	0.49
RAS	671	0.44 (0.06–3.20)	0.416	0.51
Methylation markers				
GATA2	688	2.53 (1.36–4.71)	**0.003**	0.62
TBX2	688	1.90 (0.96–3.73)	0.064	0.56
TBX3	688	2.64 (1.41–4.92)	**0.002**	0.59
ZIC4	688	1.50 (0.79–2.81)	0.213	0.54

NOTE: *P* values in bold are less than 0.05.
[a]*n*, number of patients included in that specific univariate analysis.

Table 2B. Multivariable Cox regression analysis of potential biomarker predictor variables and time to progression in all patients (patients with missing values were excluded)

Variables in model	HR (95% CI)	*P*	Complete model *P*	c-statistic
Biomarkers + EAU category ($n = 614$)				
EAU category (low + interm. = reference)	3.08 (1.31–7.23)	0.010	<0.001	0.76
FGFR3 mutation	0.57 (0.28–1.20)	0.139		
GATA2 methylation	1.90 (0.98–3.66)	0.057		
TBX3 methylation	1.68 (0.84–3.34)	0.141		
Biomarkers ($n = 659$)				
FGFR3 mutation	0.43 (0.21–0.87)	0.019	<0.001	0.72
GATA2 methylation	2.23 (1.16–4.31)	0.016		
TBX3 methylation	1.85 (0.94–3.65)	0.076		

0.35, $P = 0.002$ and HR = 0.31, $P = 0.010$). The c-statistic was moderate for both biomarkers (*FGFR3* 0.63 and *GATA2* 0.58; Table 3A). PFS was significantly poorer in patients that did not receive intravesical instillation. In addition, higher grade, *GATA2* hypermethylation, or *FGFR3* wild type were associated with a higher risk of progression (see Supplementary Fig. S6).

Multivariable analysis showed that combining both *FGFR3* mutation status and *GATA2* methylation status resulted in an overall significant model ($P = 0.005$), with a predictive capacity of 67% (Table 3B). The PFS curves diverged significantly, $P < 0.01$ (Fig. 1: good class "hypomethylated *GATA2* and mutated *FGFR3*," moderate class "either hypermethylated *GATA2* and mutated *FGFR3*," or "hypomethylated *GATA2* and wild-type *FGFR3*," and poor class "hypermethylated *GATA2* and wild-type *FGFR3*"). Supplementary Figures S7 and S8 show further comparisons of EAU low-risk versus EAU high-risk good class patients and a comparison with overall high-risk EAU patients. Table 4 illustrates the distribution of characteristics of the subclassified patients.

Addition of biomarkers improves EAU high-risk stratification precision

To compare the EAU high-risk group to the subclassified risk groups, the PIRs per risk group were calculated. In Fig. 2 and Supplementary Table S4, the PIRs for all risk groups are shown. The original EAU high-risk group had a PIR of 4.25. Using a combination of *GATA2* methylation status and *FGFR3* mutation status, 26.2% of the EAU high-risk patients could be subclassified in a good class (PIR = 0.86). This is a 4.8 times lower progression risk than the original EAU high-risk stratification. In contrast, 24.0% of the original EAU high-risk patients would be subclassified as having a very high risk for progression, with a PIR of 7.66 (Fig. 2; Supplementary Table S4). Overall, the proportion of high-grade tumors and the proportion of patients ever diagnosed with *CIS* increased over the increasing risk groups (Table 4). Progression to muscle-invasive disease was seen in 2.1% of the good class patients, increasing to 14.9% in the poor class patients (Table 4). On the basis of this biomarker subclassification of EAU high-risk patients, these patients could be allocated to different management strategies (Fig. 2).

Discussion

Currently, the EAU risk groups are used to stratify NMIBC patients for treatment and follow-up. Scoring according to this system is based on the clinicopathologic parameters: tumor size, multiplicity, primary tumor, stage, grade, and *CIS* (5). In particular, grading has been shown to be subject to interobserver variation, and not all variables are always taken into account

Table 3A. Univariate Cox regression analysis of potential predictor variables and time to progression in patients with high-risk tumors according to the EAU guidelines ($n = 555$)

Variables	n^a	HR (95% CI)	*P*	c-statistic
Clinical and tumor variables				
Age	465	1.04 (1.01–1.07)	**0.021**	0.57
Gender	467	1.00 (0.50–2.02)	0.999	0.51
Smoking (ever)	366	0.72 (0.30–1.73)	0.460	0.50
Intravesical instill.	467	0.35 (0.18–0.68)	**0.002**	0.64
Primary/recurrent	467	1.22 (0.67–2.19)	0.518	0.53
Ever *CIS*	467	1.00 (0.54–1.84)	0.997	0.50
Stage	467	1.10 (0.76–1.59)	0.629	0.57
Grade	453	2.28 (1.15–4.52)	**0.018**	0.61
Tumor size	283	1.28 (0.64–2.56)	0.483	0.52
Multiplicity	455	1.28 (0.70–2.36)	0.423	0.52
Mutation markers				
FGFR3	321	0.31 (0.13–0.75)	**0.010**	0.63
TERT	322	2.16 (0.75–6.20)	0.151	0.55
PIK3CA	328	1.04 (0.43–2.53)	0.933	0.50
RAS	323	0.49 (0.07–3.63)	0.494	0.52
Methylation markers				
GATA2	333	2.04 (1.01–4.10)	**0.046**	0.58
TBX2	333	1.36 (0.65–2.82)	0.413	0.52
TBX3	333	1.71 (0.86–3.43)	0.129	0.55
ZIC4	333	1.43 (0.70–2.89)	0.325	0.54

[a]*n*, number of patients included in that specific univariate analysis.

(15). Furthermore, the recommendation of the recent WHO classification (2016) to grade bladder cancer in two categories only (low and high grade) eliminates the importance of distinguishing grade 2 from grade 3 tumors for estimating progression in NMIBC (16). Robust biomarkers that can improve risk assessment are therefore needed.

The purpose of this international prospective study was to investigate the previously discovered and validated methylation of *GATA2*, *TBX2*, *TBX3*, and *ZIC4* as well as the hotspot mutations in the *FGFR3*, *PIK3CA*, *RAS*, and *TERT* genes for the risk of progression of NMIBC (9, 10). We investigated whether these biomarkers are of added value to the EAU risk groups for the stratification of NMIBC patients for the treatment and follow-up protocol. Here, we confirm that methylation of *GATA2* and mutation of *FGFR3* was associated with progression. Furthermore, a combination of *GATA2* and *FGFR3* status stratified EAU high-risk patients into three classes with different progression risks. The rate of progression per 100 patient-years (PIR) was calculated and compared among EAU high-risk plus biomarker subgroups. These data demonstrated that adding the *FGFR3* and *GATA2* biomarkers resulted in an improved prediction model. For instance, 24.0% of EAU high-risk patients were found to have a PIR of 7.66, which is 1.8 times higher than the entire high-risk group and almost 9 times higher than patients with the lowest risk of progression (26.2% of high-risk patients, who were subclassified in a good class). This finding suggests that these patients should receive more intensive surveillance and additional treatments or should even be considered for early cystectomy. Thus, we show that by adding the biomarkers, stratification of high-risk patients can be improved.

Table 3B. Two multivariable Cox regression analyses of potential predictor variables and time to progression in patients with high-risk tumors according to the EAU guidelines (patients with missing values were excluded)

Variables in model	HR (95% CI)	*P*	Complete model *P*	c-statistic
Markers only ($n = 321$)				
FGFR3 mutation	0.33 (0.13–0.80)	0.014	0.005	0.67
GATA2 methylation	1.88 (0.91–3.89)	0.087		
Biomarker predictor variables + grade ($n = 312$)				
Grade	1.60 (0.64–4.01)	0.318	0.010	0.69
FGFR3 mutation	0.40 (0.15–1.06)	0.064		
GATA2 methylation	1.83 (0.86–3.86)	0.116		

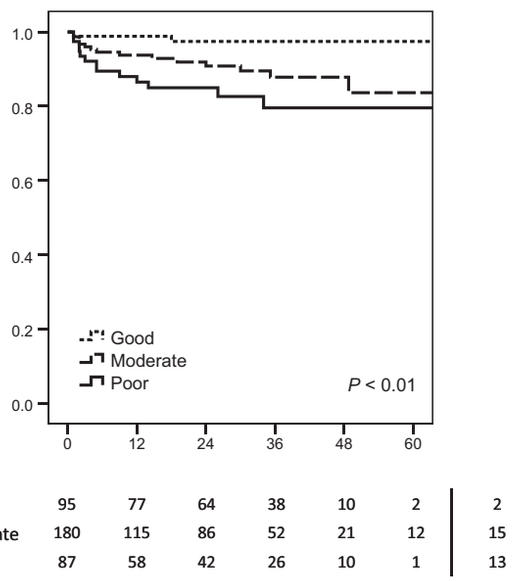

Figure 1.

PFS curve in patients with EAU high-risk tumors of a combination of *FGFR3* and *GATA2* status. Dotted line, good status (hypomethylated *GATA2* and mutated *FGFR3*); dashed line, moderate status (either hypermethylated *GATA2* and mutated *FGFR3* or hypomethylated *GATA2* and wild-type *FGFR3*); or solid line, poor status (hypermethylated *GATA2* and wild type *FGFR3*). Progressive disease is defined as progression to stage T2 or higher stage disease. *P* value is based on log-rank test.

Table 4. Patient and tumor characteristics of all reclassified patients based on a combination of *FGFR3* mutation status and *GATA2* methylation status (only high-risk patients with known marker results and known time of follow-up were included; $n = 362$)

EAU risk group		High risk ($n = 362$)		
Newly suggested risk groups		**Good ($n = 95$)**	**Moderate ($n = 180$)**	**Poor ($n = 87$)**
Patient characteristics				
Age	Mean (range)	69 (47–90)	68 (37–96)	72 (51–93)
		n (%)	*n* (%)	*n* (%)
Gender	Male	69 (72.6)	139 (77.2)	72 (82.8)
	Female	26 (27.4)	41 (22.8)	15 (17.2)
Intravesical instill.	Yes	36 (37.9)	82 (45.6)	45 (51.7)
	No	59 (62.1)	98 (54.4)	42 (48.3)
Tumor characteristics				
Tumor type	Primary	59 (62.1)	87 (48.3)	51 (58.6)
	Recurrent	36 (37.9)	93 (51.7)	36 (41.4)
Stage	pTa	29 (30.5)	65 (36.1)	34 (39.1)
	pT1	62 (65.3)	104 (57.8)	49 (56.3)
	pTis	4 (4.2)	11 (6.1)	4 (4.6)
Grade	Low grade	59 (62.1)	60 (33.3)	14 (17.5)
	High grade	34 (35.8)	111 (61.7)	73 (83.9)
	Unknown	*2 (2.1)*	*9 (5.0)*	—
Ever *CIS*	Yes	13 (13.7)	64 (35.6)	32 (36.8)
	No	82 (86.3)	116 (64.4)	55 (63.2)
Follow-up				
Progression to T2	Yes	2 (2.1)	15 (8.3)	13 (14.9)
	No	93 (97.9)	165 (91.7)	74 (85.1)

The value of this cohort is its large sample size, its prospective nature, and its multi-institutional and multinational character. From a statistical point of view, the low number of progressing patients (4.6% overall) is a limitation. However, the patients in this cohort were recruited between 2008 and 2013, and their progression risk is that of NMIBC patients in a 21st century urology clinic. In the large retrospective study by Sylvester and colleagues, which formed the basis for the EAU risk group

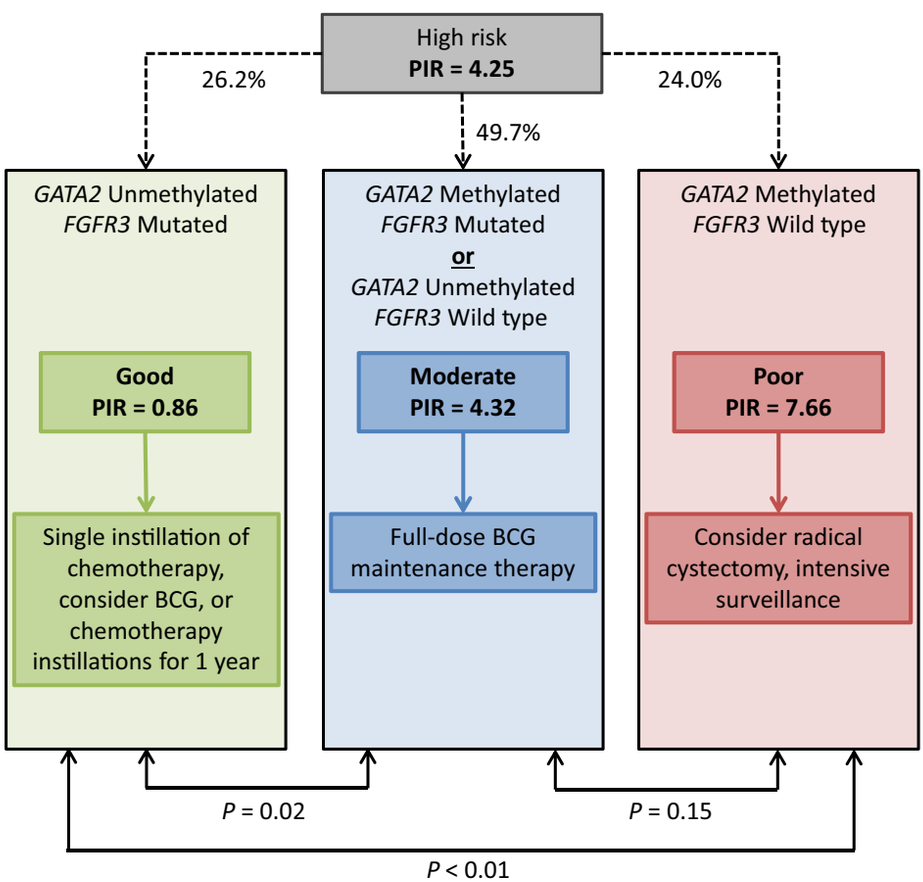

Figure 2.
Reclassification of EAU high-risk group patients identifying a group with very low and very high risk, suggesting that treatment for these patients should be changed. The PIRs are depicted in the box of each risk group. The PIR is the number of progressors divided by the amount of person-years in that risk group, times 100, and can be interpreted as the rate of progression per 100 person-years. The percentages at each arrow indicate the proportion of reclassified patients that were originally classified in the EAU high-risk group. *P* values are based on log-rank test.

stratification, 11% of patients progressed to MIBC (1). The latter patients were recruited between the years 1979 and 1989. Obviously, treatment and follow-up in those years differed considerably from current practice. In fact, the low numbers of patients with progression in our study are most probably the result of improved treatment and surveillance protocols, for example, MMC and BCG instillations improved quality of endoscopic instruments and re-TUR in pT1 cases.

There is robust literature on the favorable disease course of NMIBC patients having *FGFR3*-mutant tumors (6, 7, 17, 18). In addition, van Rhijn and colleagues described the importance of *FGFR3* mutation status in pT1 tumors; *FGFR3* mutations occurred in 28% of pT1 tumors, and multivariable analysis showed an HR of 2.2 for nonprogression in the multivariable analysis ($P = 0.05$) (19). Although we found a higher mutation frequency in pT1 tumors (45%), we confirmed the favorable outcome of *FGFR3*-mutant tumors.

Recently, a progression score based on expression of 12 genes was shown to add prognostic information beyond the EORTC risk score described by Sylvester and colleagues (1, 20). This 12-gene signature was developed on a subset of the patient cohort in the current study. Univariate Cox regression analysis of the 12 gene score resulted in an HR of 2.39 ($P < 0.001$) for the continuous and HR 5.08 ($P < 0.001$) for the dichotomized risk score. Moreover, the 12-gene progression score was also able to predict PFS within the EORTC high-risk patients ($P = 0.035$ or $P = 0.041$, depending on the cutoff used; ref. 20). In this study, using *FGFR3* and *GATA2*, we were able to identify three groups with different progression risks within the EAU high-risk patients (Fig. 1, $P < 0.01$). On the basis of these data, we conclude that both gene expression profiling and *FGFR3*+*GATA2* analysis can be very useful to further differentiate risk groups in the EAU highest risk category. Both types of assays can be performed in a molecular pathology laboratory.

In this study, DNA was isolated from fresh-frozen tissue; therefore, we had to redetermine cutoffs for the methylation markers. Still, for clinical implementation, an FFPE-based analysis is more suitable. That the assays perform well also on FFPE tissue was already shown in the study by Beukers and colleagues (10). The biomarker assays that we used are not very expensive (€20 material cost/sample); however, a cost-effectiveness analysis of the proposed stratification should be conducted. Finally, given the number of progressing patients in the EAU high-risk group, it would be wise to further validate the combination of *FGFR3* mutations and *GATA2* methylation in a case-enriched series.

Conclusion

Addition of *FGFR3* mutation status and *GATA2* methylation status to the EAU risk stratification increases its accuracy and, moreover, identifies a subset of NMIBC patients with a very high yearly risk of progression. In these very high risk patients, intensive surveillance is warranted and early cystectomy should be considered. We conclude that the use of these progression markers has potential for implementation in the EAU NMIBC guidelines. Further validation in high-risk NMIBC patients is recommended.

Disclosure of Potential Conflicts of Interest

J.L. Boormans is a consultant/advisory board member for Janssen Pharma BV, MSD, and Roche Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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