original report

Enrichment of FGFR3-TACC3 Fusions in Patients With Bladder Cancer Who Are Young, Asian, or Have Never Smoked

abstract

Purpose ***FGFR3-TACC3*** **(fibroblast growth factor receptor 3–transforming acidic coiled coil-containing protein 3) fusions have recently been identified as driver mutations that lead to the activation of *FGFR3* in bladder cancer and other tumor types and are associated with sensitivity to tyrosine kinase inhibitors. We examined the clinical and molecular characteristics of patients with *FGFR3-TACC3* fusions and hypothesized that they are enriched in a subset of patients with bladder cancer.**

Materials and Methods **We correlated somatic *FGFR3-TACC3* fusions with clinical and molecular features in two cohorts of patients with bladder cancer. The first cohort consisted of the muscle-invasive bladder cancer (MIBC) data set (n = 412) from The Cancer Genome Atlas. The second cohort consisted of patients with MIBC or high-grade non-MIBC at the Dana-Farber Cancer Institute that had targeted capture sequencing of a selected panel of cancer genes (n = 356). All statistical tests were two sided. The clinical response of one patient with *FGFR3-TACC3* bladder cancer to an FGFR3 inhibitor was investigated.**

Results **Overall, 751 patients with high-grade bladder cancer without *FGFR3-TACC3* fusions and 17 with *FGFR3-TACC3* fusions were identified in the pooled analysis of the data sets from The Cancer Genome Atlas and the Dana-Farber Cancer Institute. *FGFR3-TACC3* fusions were enriched in patients age ≤ 50 years versus age 51 to 65 years versus those older than 65 years (pooled, *P* = .002), and were observed in four (12%) of 33 patients age ≤ 50 years in the pooled analysis. Similarly, *FGFR3-TACC3* fusions were significantly more common in Asians (13%) compared with African Americans (4%) and whites (2%; pooled, *P* < .001), as well as in never smokers (5.6%) compared with ever smokers (1.1%; pooled, *P* < .001). One patient with the fusion who was treated with an FGFR3 inhibitor achieved complete remission for 10 months.**

Conclusion **Clinical testing to identify *FGFR3* fusions should be prioritized for patients with bladder cancer who are younger, never smokers, and/or Asian.**

Amin H. Nassar
Kevin Lundgren
Mark Pomerantz
Eliezer Van Allen
Lauren Harshman
Atish D. Choudhury
Mark A. Preston
Graeme S. Steele
Kent W. Mouw
Xiao X. Wei
Bradley A. McGregor
Toni K. Choueiri
Joaquim Bellmunt
David J. Kwiatkowski
Guru P. Sonpavde

Author affiliations and support information (if applicable) appear at the end of this article.

Corresponding author: Guru P. Sonpavde, MD, Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, 450 Brookline Ave, DANA 1230, Boston, MA 02215; e-mail: GuruP_Sonpavde@dfci.harvard.edu.

INTRODUCTION

Bladder cancer remains a major contributor to cancer-related morbidity and mortality. In 2017, 79,030 new cases of bladder cancer are expected to be diagnosed, and approximately 16,870 deaths are predicted to occur from the disease in the United States.[1,2] Compared with other cancer subtypes, advances in the management of bladder cancer have been limited in the past three decades, and there is an unmet need to develop novel therapeutic agents that target potentially actionable alterations.[3,4]

Genomic alterations in fibroblast growth factor receptors (*FGFR*s) are among the most frequent events during bladder cancer development. FGFRs are receptor tyrosine kinases that orchestrate various cellular processes, including cell proliferation, differentiation, and survival.[5] *FGFR* mutations lead to developmental syndromes when present in the germline, and contribute to cancer growth when acquired somatically.[6] *FGFR* fusions with an intact kinase domain have been identified in several cancer

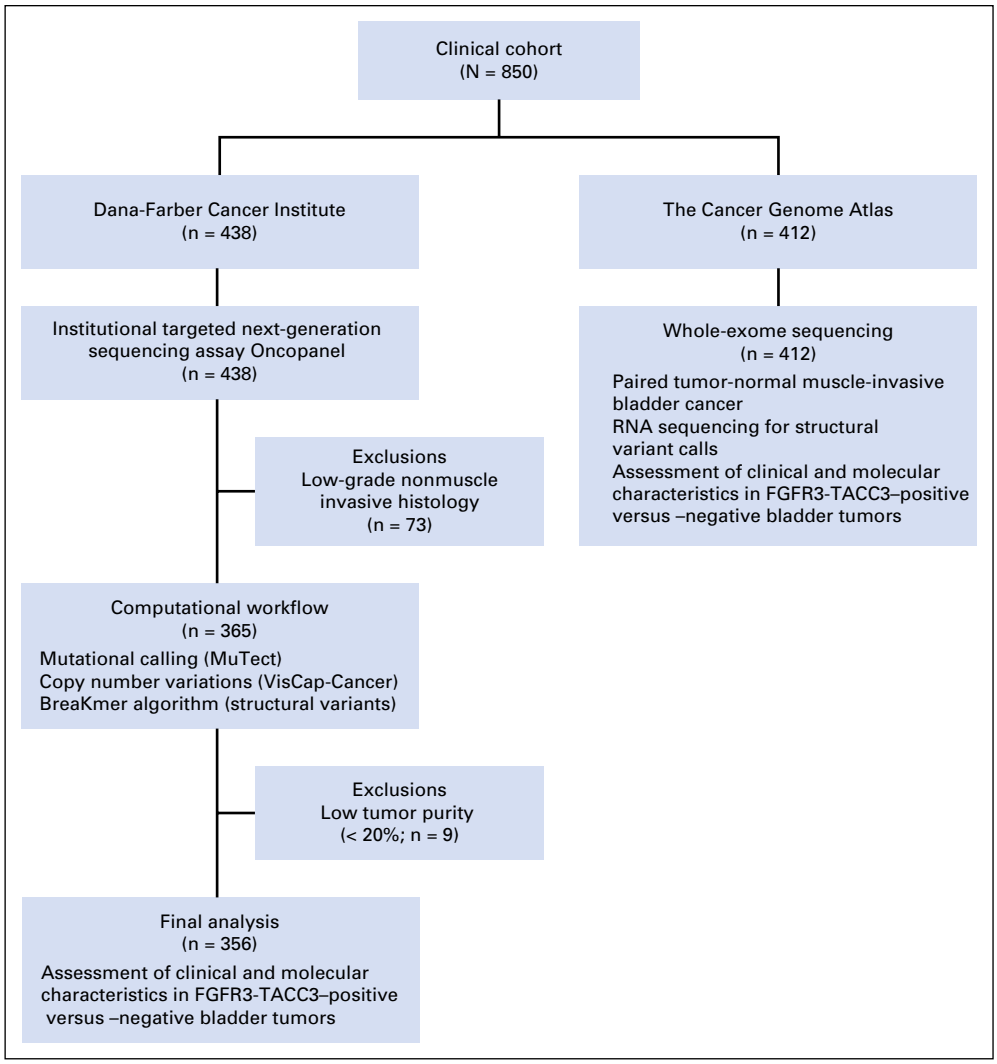

Fig 1. CONSORT diagram for 850 patients with bladder cancer.

types, including cervical cancer, bladder carcinoma, glioblastoma multiforme, squamous lung carcinoma, and head and neck cancer.[7-15] *FGFR3*, a member of this family, has been reported to be involved in fusions with several genes in bladder carcinoma, including *TACC3* (transforming acidic coiled coil-containing protein 3). The *TACC3* gene is located just 48 kb away from FGFR3 on 4p16.3, which likely predisposes *FGFR3* and *TACC3* to fusion events. TACC3 normally is thought to mediate the stabilization and organization of the mitotic spindle during mitosis.[14]

In The Cancer Genome Atlas (TCGA) muscle-invasive bladder cancer (MIBC) cohort, in-frame activating *FGFR3-TACC3* fusions—observed in 10 (2.4%) of 412 patients—were the most common gene fusions identified.[7] FGFR3-TACC3 fusion proteins consist of the immunoglobulin, transmembrane, and tyrosine kinase domains of FGFR3, fused to the coiled-coil domain of TACC3. Through the promotion of dimerization, these fusions lead to a constitutively active FGFR3 kinase protein that has been demonstrated to promote cell proliferation in vivo and in vitro.[7-9,13] Phase I and II trials of FGFR inhibitors have reported promising antitumor activity in patients with FGFR genetic alterations, especially bladder cancer.[16]

Certain genetic alterations, particularly gene fusion events, are enriched in clinical subsets of patients with cancer. For example, never-smoking patients with lung adenocarcinoma have more frequent *EGFR* mutations and *ALK* and *ROS1* fusions[17,18]; therefore, we hypothesized that a similar association may exist between somatic FGFR3-TACC3 fusions and patient characteristics in bladder cancer.[17-19]

METHODS

TCGA and Dana-Farber Cancer Institute Data

We tested our hypotheses in two cohorts, one from TCGA and one from the Dana-Farber Cancer Institute (DFCI). For the TCGA MIBC cohort (n = 412), we examined the clinical and molecular characteristics of the 10 *FGFR3-TACC3* fusion patients who were identified on the basis of analysis of RNA sequencing data compared with the remaining 402 patients.[7] For the DFCI cohort (n = 356), we identified 240 patients who were diagnosed with MIBC and 116 with high-grade[20] non-MIBC (n = 116). Patients with MIBC and high-grade non-MIBC were pooled together in the DFCI cohort as there is substantial evidence that the two subtypes are biologically and genomically similar.[21-23] Overall, seven patients with *FGFR3-TACC3* fusions were identified in the DFCI cohort using an institutional targeted next-generation sequencing assay[24] (Oncopanel). Figure 1 shows the sample inclusion and exclusion criteria and workflow.

Tissue Collection and DNA Extraction

Tumor specimens and clinicopathologic information were collected with institutional review board approval at DFCI. Board-certified genitourinary pathologists at DFCI reviewed and verified the diagnosis, tumor grade, stage, and histology. Tumor areas that contained at least 20% of tumor cells (mean tumor purity, 58%; range, 20% to 100%) were isolated from normal tissue and chosen for DNA extraction. DNA was then isolated using the QIAamp DNA formalin-fixed, paraffin-embedded tissue kit (Qiagen, Wetzlar, Germany) according to manufacturer instructions. DNA was quantified by Nanodrop and pico-Green assays.

Targeted Sequencing

Two hundred nanograms of genomic DNA from each sample was subjected to targeted exon capture and sequencing using Oncopanel_v1 to v3 cancer gene panels at Brigham and Women's Hospital (Boston, MA). The Oncopanel gene panel includes capture probes for 275 to 560 cancer-associated genes, as well as intronic portions of 60 genes for rearrangement detection, including FGFR3.[24] Sample DNA was captured using Oncopanel_v1 to v3 bait sets using a solution-phase Agilent SureSelect hybrid capture kit (Agilent Technologies, Santa Clara, CA). Sequencing libraries were prepared from captured DNA as described in detail elsewhere. Paired-end sequencing was performed on an Illumina HiSEquation 2500 sequencer (Illumina, San Diego, CA). Reads were demultiplexed using Picard tools (http://picard.sourceforge.net) and aligned to human reference genome b37 using the Burrows-Wheeler Aligner[25] (http://bio-bwa.sourceforge.net/bwa.shtml). Low-quality reads and duplicates were filtered out and eliminated using Picard. Single-nucleotide variants and small indels were analyzed using MuTect version 1 0.27200 (https://confluence.broadinstitute.org/display/CGATools/MuTect; accessed May 2013) and annotated by Oncotator (http://www.broadinstitute.org/oncotator; accessed May 2013). Copy number alterations were analyzed using a custom R-based tool[26,27] (VisCap-Cancer).

Mean depth of read coverage for the targeted genes was ×283. Mean, median, and range of percentage of target bases with read depth > ×30 was 98%, 99%, and 78% to 99%, respectively.

Identification of Rearrangements and Analysis of Genomic Breakpoints

FGFR3 fusion sequences were identified using the BreaKmer algorithm[28] and were manually reviewed using Integrated Genomic Viewer[29] to exclude sequencing or alignment artifacts. All analyses of sequencing data and mutation and fusion calls were performed blinded to clinical data.

Clinical Response to Anti-FGFR3 Therapy

One patient with *FGFR3-TACC3* MIBC received anti-FGFR3 therapy along with docetaxel and the clinical response was monitored.

Statistical Analysis

We used Fisher's exact test for categorical data and the Wilcoxon rank-sum test for quantitative data. All statistical tests were two sided and a P value ≤ .05 was considered statistically significant. Statistical correction for multiple comparisons was not performed, as we considered these analyses exploratory.

Table 1. Baseline Demographic and Clinical Characteristics of Patients in the TCGA and DFCI Cohorts

Characteristic	TCGA	DFCI		
		HG NMIBC + MIBC DFCI	MIBC DFCI	HG NMIBC DFCI
Total	412 (100)	356 (100)	240 (100)	116 (100)
Age, years				
≤ 50	25 (6)	8 (2)	5 (2)	3 (3)
51-65	137 (33)	95 (27)	57 (24)	38 (33)
> 65	250 (61)	253 (71)	178 (74)	75 (65)
Gender				
Male	304 (74)	273 (77)	185 (77)	88 (76)
Female	108 (26)	83 (23)	55 (23)	28 (24)
Race				
Asian	44 (11)	3 (1)	2 (1)	1 (1)
African American	23 (6)	5 (1)	4 (2)	1 (1)
White	327 (79)	339 (95)	228 (95)	111 (96)
ND	18 (4)	9 (3)	6 (3)	3 (3)
Ethnicity				
Hispanic	9 (2)	1 (0)	1 (0)	0 (0)
Non-Hispanic	371 (90)	304 (85)	210 (88)	94 (81)
ND	32 (8)	51 (14)	29 (12)	22 (19)
Smoking status				
Never smoker	111 (27)	84 (24)	53 (22)	31 (27)
Ever smoker	288 (70)	261 (73)	178 (74)	83 (72)
ND	13 (3)	11 (3)	9 (4)	2
Histology				
Papillary	133 (32)	213 (60)	104 (43)	109 (94)
Nonpapillary	274 (67)	141 (40)	135 (56)	6 (5)
ND	5 (1)	2 (1)	1 (0)	1 (2)
T stage				
Ta	0 (0)	65 (18)	0 (0)	65 (56)
Tis	0 (0)	1 (0)	0 (0)	1 (1)
T1	1 (0)	45 (13)	0 (0)	45 (39)
T2	123 (30)	71 (20)	71 (30)	0 (0)
T3	196 (48)	78 (22)	78 (33)	0 (0)
T4	59 (14)	73 (21)	73 (30)	0 (0)
Tx	1 (0)	10 (3)	10 (4)	0 (0)
ND	32 (8)	13 (4)	8 (3)	5 (4)
Nodal status				
X	36 (9)	43 (12)	37 (15)	6 (5)
0	238 (58)	250 (70)	140 (58)	110 (95)
1	47 (11)	29 (8)	29 (12)	0 (0)
2	76 (18)	28 (8)	28 (12)	0 (0)
3	9 (2)	6 (2)	6 (3)	0 (0)
ND	6 (1)	0 (0)	0 (0)	0 (0)

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Table 1. Baseline Demographic and Clinical Characteristics of Patients in the TCGA and DFCI Cohorts (Continued)

Characteristic	TCGA	DFCI		
		HG NMIBC + MIBC DFCI	MIBC DFCI	HG NMIBC DFCI
Metastasis				
X	203 (49)	171 (48)	156 (65)	15 (13)
M0	196 (48)	150 (42)	49 (20)	101 (87)
M1	11 (3)	35 (10)	35 (15)	0 (0)
ND	2 (0)	0 (0)	0 (0)	0 (0)
FGFR3-TACC3 fusion				
No	402 (98)	349 (98)	235 (98)	114 (98)
Yes	10 (2)	7 (2)	5 (2)	2 (2)

NOTE. Data are presented as No. (%), unless otherwise noted.

Abbreviations: DFCI, Dana-Farber Cancer Institute; *FGFR3*, fibroblast growth factor receptor 3; HG, high grade; ND, not determined; NMIBC, nonmuscle invasive bladder cancer; *TACC3*, transforming acidic coiled-coil-containing protein 3; TCGA, The Cancer Genome Atlas.

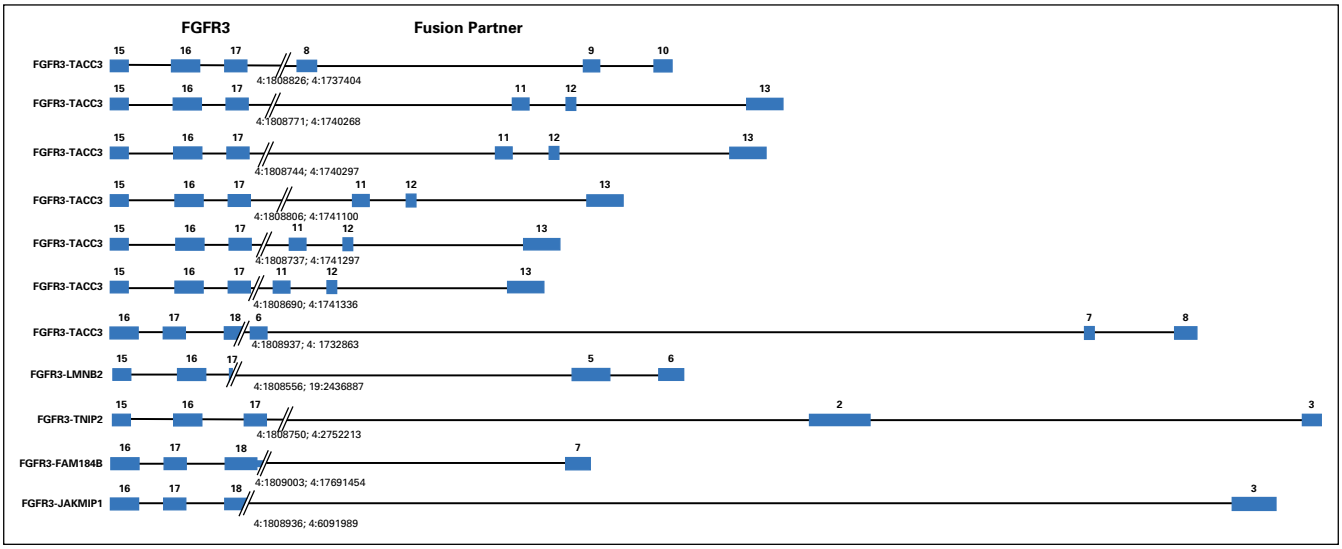

Fig 2. Schematic representation of the genomic rearrangements observed in 11 tumor samples that harbor fibroblast growth factor receptor 3 (*FGFR3*) fusion variants identified using the Oncopanel assay in the Dana-Farber Cancer Institute cohort. All exons and introns are drawn to scale. *FAM184B*, family with sequence similarity 184 member B; *LMNB2*, lamin B2; *JAKMIP1*, Janus kinase and microtubule interacting protein 1; *TACC3*, transforming acidic coiled-coil-containing protein 3; *TNIP2*, TNFAIP3 interacting protein 2.

RESULTS

Formalin-fixed, paraffin-embedded tumor specimens were obtained from 438 patients at DFCI. We excluded 82 tumors from the analysis because they were of low-grade nonmuscle invasive histology (n = 73) or had low (< 20%) tumor purity (n = 9). Three hundred fifty-six patients from the DFCI data set were analyzed, including three Asian patients, five African American patients, 339 white patients, and nine of unknown race.

Ten (2.4%) of 412 patients in the TCGA cohort (mean age at diagnosis, 68 years [range 34 to 90 years]; median age at diagnosis, 69 years), and seven (2.0%; MIBC, n = 5; non-MIBC, n = 2) of 356 patients in the DFCI cohort (mean age at diagnosis, 71 years [range, 12 to 96 years]; median age at diagnosis, 72 years) harbored *FGFR3-TACC3* fusions. Table 1 lists the baseline clinicopathologic characteristics of the 768 patients. We mapped the genomic breakpoints of *FGFR3* and its corresponding fusion partners that were identified in the DFCI cohort, which included four non-*TACC3* fusions (Fig 2). All *FGFR3-TACC3* fusions occurred in the exon 17 to 18 intron (n = 6) or in exon 18 (n = 1) of *FGFR3*, which led to a small C-terminal truncation of FGFR3 with preservation of the kinase domain. *FGFR3* was fused to various exons of *TACC3*, most commonly exon 11, all of which maintain the TACC3 coiled-coil domain in the fusion protein.

FGFR3-TACC3 fusions were enriched in the TCGA cohort in patients age ≤ 50 years compared with those age 51 to 65 years and those older than 65 years, with three (12%) of 25

Table 2. Associations Between FGFR3-TACC3 Fusions and Clinical Features in Bladder Cancer

Clinical Characteristic	TCGA (n = 412)			DFCI (n = 356)			Pooled (N = 768)		
	FGFR3-TACC3 Fusion			*FGFR3-TACC3* Fusion			*FGFR3-TACC3* Fusion		
	Yes	No	Total	Yes	No	Total	Yes	No	Total
Age, years									
≤ 50	3 (12)	22 (88)	25	1 (13)	7 (87)	8	4 (12)	29 (88)	33
51-65	2 (1)	135 (99)	137	5 (5)	90 (95)	95	7 (3)	225 (97)	232
> 65	5 (2)	245 (98)	250	1 (0)	252 (100)	253	6 (1)	497 (99)	503
Total	10	402	412	7	349	356	17	751	768
P		.03			.001			.002	
Race									
Asian	6 (14)	38 (86)	44	0 (0)	3 (100)	3	6 (13)	41 (87)	47
African American	1 (4)	22 (96)	23	0 (0)	5 (100)	5	1 (4)	27 (96)	28
White	3 (1)	324 (99)	327	7 (2)	332 (98)	339	10 (2)	656 (98)	666
Total	10	384	394	7	340	347	17	724	741
P		< .001			> .99			< .001	
Smoking status									
Never smoker	8 (7)	103 (93)	111	3 (4)	81 (96)	84	11 (6)	184 (94)	195
Ever smoker	2 (1)	286 (99)	288	4 (2)	257 (98)	261	6 (1)	543 (99)	549
Total	10	389	399	7	338	345	17	727	744
P		< .001			.37			< .001	

NOTE. Data are presented as No. (%), unless otherwise noted.

Abbreviations: DFCI, Dana-Farber Cancer Institute; *FGFR3*, fibroblast growth factor receptor 3; *TACC3*, transforming acidic coiled-coil-containing protein 3; TCGA, The Cancer Genome Atlas.

patients age ≤ 50 harboring a fusion (P = .03; Table 2). *FGFR3-TACC3* fusions in TCGA were also more frequent in Asians (six [14%] of 44 patients) compared with other races (P < .001), as well as in never smokers (eight [7.2%] of 111 patients) compared with ever smokers (P < .001; Table 2). Similarly, *FGFR3-TACC3* fusions were more common in DFCI patients age ≤ 50 years (one [12%] of eight patients) compared with other age groups (P = .001; Table 2). Race and smoking status were not associated with fusions in the DFCI cohort as a result of small numbers of patients in these categories and lack of statistical power.

Analysis of the pooled TCGA and DFCI cohorts (N = 768) confirmed significant associations between *FGFR3-TACC3* fusions and age ≤ 50 years (12%; P = .002), Asian race (13%; P < .001), and never-smoking status (5.6%; P < .001; Table 2). Eleven (65%) of 17 patients with *FGFR3-TACC3* fusions were associated with least one of these three clinical characteristics, and three (18%) of the 17 patients were Asian never smokers age ≤ 50 years.

We next examined whether tumors with *FGFR3-TACC3* fusions had molecular features that distinguished them from other tumors. We examined 33 genes that were defined as being significantly mutated in the TCGA analysis and were also tested in the Oncopanel assay. As the Oncopanel analysis was performed on tumor samples only, we excluded variants that were observed at any frequency in the Exome Aggregation Consortium database,[30] as they were considered likely germline variants. The 17 patients whose tumors harbored *FGFR3-TACC3* fusions were enriched for *CDKN1A* mutations (5 [29%] of 17 *v* 76 [10%] of 751; P = .03; Table 3). Conversely, *FGFR3-TACC3* fusion-positive tumors had significantly fewer *TP53* mutations (P = .02), and none had *RB1* mutations (P = .054; Table 3). Somatic copy number alterations were also analyzed in both cohorts using criteria for loss, deletion, gain, and amplification that were developed and applied independently in the two cohorts (Table 3). Analysis of the pooled cohorts demonstrated significant associations between *FGFR3-TACC3* fusions and *FGFR3* gain (P = .003),

Table 3. Associations Between FGFR3-TACC3 Fusions and Molecular Features in Bladder Cancer

Molecular Characteristic	TCGA (n = 412)			DFCI (n = 356)			Pooled (N = 768)		
	FGFR3-TACC3 Fusion			*FGFR3-TACC3* Fusion			*FGFR3-TACC3* Fusion		
	Yes	No	Total	Yes	No	Total	Yes	No	Total
CDKN1A mutation									
Yes	3 (30)	34 (8)	37	2 (28.6)	42 (12)	44	5 (29)	76 (10)	81
No	7 (70)	368 (92)	375	5 (71.4)	307 (88)	312	12 (71)	675 (90)	687
Total	10	402	412	7	349	356	17	751	768
P		.052			.21			.03	
TP53 mutation									
Yes	1 (10)	197 (49)	198	2 (29)	151 (43)	153	3 (18)	348 (46)	351
No	9 (90)	205 (51)	214	5 (71)	198 (57)	203	14 (82)	403 (54)	417
Total	10	402	412	7	349	356	17	751	768
P		.02			.70			.02	
RB1 mutation									
Yes	0 (0)	72 (18)	72	0 (0)	67 (19)	67	0 (0)	139 (19)	139
No	10 (100)	330 (82)	340	7 (100)	282 (81)	289	17 (100)	612 (81)	629
Total	10	402	412	7	349	356	17	751	768
P		.22			.36			.054	
FGFR3 amplification									
Amplification	0 (0)	5 (1)	5	0 (0)	2 (1)	2	0 (0)	7 (1)	7
Gain	2 (20)	3 (1)	5	3 (43)	33 (9)	36	5 (29)	36 (5)	41
No	8 (80)	390 (98)	398	4 (57)	314 (90)	318	12 (71)	704 (94)	716
Total	10	398	408	7	349	356	17	747	764
P		.01			.06			.003	
MDM2 amplification									
Amplification	0 (0)	25 (6)	25	0 (0)	23 (7)	23	0 (0)	48 (6)	48
Gain	3 (30)	7 (2)	10	1 (14)	37 (11)	38	4 (24)	44 (6)	48
No	7 (70)	366 (92)	373	6 (86)	289 (83)	295	13 (76)	655 (88)	668
Total	10	398	408	7	349	356	17	747	764
P		.002			.74			.04	
ERBB2 amplification									
Amplification	0 (0)	6 (2)	6	1 (14)	7 (2)	8	1 (6)	13 (2)	14
Gain	0 (0)	15 (4)	15	2 (29)	44 (13)	46	2 (12)	59 (8)	61
No	10 (100)	377 (94)	387	4 (57)	298 (85)	302	14 (82)	675 (90)	689
Total	10	398	408	7	349	356	17	747	764
P		1.0			.04			.25	
PTEN deletion									
Deletion	2 (20)	6 (2)	8	0 (0)	1 (0)	1	2 (12)	7 (1)	9
Loss	1 (10)	46 (12)	47	0 (0)	57 (16)	57	1 (6)	103 (14)	104
No	7 (70)	346 (87)	353	7 (100)	291 (83)	298	14 (82)	637 (85)	651
Total	10	398	408	7	349	356	17	747	764
P		.02			.61			.02	

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Table 3. Associations Between FGFR3-TACC3 Fusions and Molecular Features in Bladder Cancer (Continued)

Molecular Characteristic	TCGA (n = 412)			DFCI (n = 356)			Pooled (N = 768)		
	FGFR3-TACC3 Fusion			*FGFR3-TACC3* Fusion			*FGFR3-TACC3* Fusion		
	Yes	No	Total	Yes	No	Total	Yes	No	Total
CDKN2A deletion									
Deletion	4 (40)	88 (22)	92	4 (57)	62 (18)	66	8 (47)	150 (20)	158
Loss	3 (30)	80 (20)	83	2 (29)	72 (21)	74	5 (29)	152 (20)	157
No	3 (30)	230 (58)	233	1 (14)	215 (62)	216	4 (24)	445 (60)	449
Total	10	398	408	7	349	356	17	747	764
P		.16			.01			.0033	
SNV count									
Median	128.5	224	224	6	6	6	—	—	—
P		.04			.37			—	
CNV count									
Median	4	2	2	3	3	3	—	—	—
P		.09			.76			—	

NOTE. TCGA cohort: amplification: log2(copy ratio) > 1, gain: 0.59 ≤ log2(copy ratio) < 1; deletion: log2(copy ratio) < –1, loss: –1 ≤ log2(copy ratio) < –0.42. DFCI cohort: amplification: log2(copy ratio) > 1.8, gain: 1.1 ≤ log2(copy ratio) < 1.8; deletion: log2(copy ratio) < –2, loss: –2 ≤ log2(copy ratio) < –1.
Abbreviations: CNV, copy number variation; DFCI, Dana-Farber Cancer Institute; *FGFR3*, fibroblast growth factor receptor 3; SNV, single nucleotide variant; *TACC3*, transforming acidic coiled-coil-containing protein 3; *MDM2*, murine double minute 2; TCGA, The Cancer Genome Atlas.

MDM2 (murine double minute 2) gain (*P* = .04), deletion of *PTEN* (*P* = .02), and deletion of *CDK2NA* (*P* = .0033; Table 3).

As a result of differences in the extent of genome sequencing in the TCGA and DFCI cohorts, we analyzed the overall mutational burden in each cohort separately. In the TCGA cohort, the nonsynonymous somatic mutation rate across 18,862 genes was significantly higher in patients without *FGFR3-TACC3* fusions compared with those with fusions (median 224 *v* 128; *P* = 0.04; Table 3). In the DFCI cohort, which analyzed a smaller number of genes, no significant difference in mutational burden was observed (Table 3). In addition, there were no significant differences in the frequency of somatic copy number alterations in either the TCGA or DFCI cohorts (Table 3).

One patient who harbored the *FGFR3-TACC3* fusion in MIBC in the DFCI cohort was treated with an FGFR3 inhibitor and docetaxel and experienced complete remission for approximately 10 months.

DISCUSSION

Our results demonstrate that patients with bladder cancer with *FGFR3-TACC3* fusions have distinct clinical and molecular features compared with the general population of patients with bladder cancer. We observed significant enrichment for these fusions in patients age ≤ 50 years (12% of patients), of Asian race (13%), and who were never smokers (5.6%). In addition, *FGFR3-TACC3* fusions were associated with a low frequency of *TP53* and *RB1* mutations and a higher frequency of *CDKN1A* mutations, *FGFR3* and *MDM2* amplifications, and *PTEN* deletions. Because *FGFR3-TACC3* fusion-positive tumors can be sensitive to FGFR inhibitors,[9,31,32] these observations suggest that molecular testing to detect *FGFR3-TACC3* fusions in bladder cancer should be prioritized for patients who are young (age ≤ 50 years), of Asian race, and/or who have never smoked. Most strikingly, we observed that all patients with bladder cancer who were Asian never smokers younger than age 50 years (n = 3) had *FGFR3-TACC3* fusions.

We emphasize that our study has significant limitations as a result of the small number of patients with *FGFR3-TACC3* fusions included (n = 17), which reflects that this is a relatively rare molecular subset of bladder cancer. We began this study with a specific hypothesis about associations between clinical features and FGFR3-TACC3 fusion mutations, and that hypothesis was validated; however, we recognize that there may be other associations of clinical and pathologic features with FGFR3-TACC3 fusion mutations that

we did not explore here. Most importantly, we strongly advocate additional studies of this association to extend and confirm these findings.

In conclusion, FGFR3-TACC3 fusion-positive bladder cancer is highly enriched in Asians, never smokers, and those age ≤ 50 years. This association suggests that patients in these demographic categories should be prioritized for molecular testing, and, if the FGFR3-TACC3 fusion is found, enrolled in appropriate clinical trials that are using emerging targeted therapies against FGFR3.

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AUTHOR CONTRIBUTIONS

Conception and design: Amin H. Nassar, Kevin Lundgren, Mark Pomerantz, Toni K. Choueiri, Joaquim Bellmunt, David J. Kwiatkowski, Guru P. Sonpavde

Financial support: Toni K. Choueiri

Administrative support: Toni K. Choueiri, David J. Kwiatkowski

Provision of study material or patients: Amin H. Nassar, Lauren Harshman, Graeme S. Steele, Toni K. Choueiri, Joaquim Bellmunt, Guru P. Sonpavde

Collection and assembly of data: Amin H. Nassar, Kevin Lundgren, Graeme S. Steele, Toni K. Choueiri, Joaquim Bellmunt, David J. Kwiatkowski, Guru P. Sonpavde

Data analysis and interpretation: Amin H. Nassar, Eliezer Van Allen, Lauren Harshman, Atish D. Choudhury, Mark A. Preston, Kent W. Mouw, Xiao X. Wei, Bradley A. McGregor, Toni K. Choueiri, Joaquim Bellmunt, David J. Kwiatkowski, Guru P. Sonpavde

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Amin H. Nassar
No relationship to disclose

Kevin Lundgren
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Mark Pomerantz
Honoraria: Bayer

Eliezer Van Allen
Stock and Other Ownership Interests: Synapse, Tango Therapeutics, Genome Medical
Consulting or Advisory Role: Synapse, Roche, Third Rock Ventures, Takeda, Novartis, Genome Medical, InVitae
Speakers' Bureau: Illumina
Research Funding: Bristol-Myers Squibb, Novartis

Lauren Harshman
Consulting or Advisory Role: Medivation, Astellas Pharma, Pfizer, Genentech, Theragene, KEW, Corvus Pharmaceuticals, Merck, Exelixis, Bayer
Research Funding: Medivation, Astellas Pharma (Inst), Bayer (Inst), Sotio (Inst), Genentech (Inst), Dendreon (Inst), Bristol-Myers Squibb (Inst), Takeda (Inst), Merck (Inst), Janssen Oncology (Inst), Pfizer (Inst)
Travel, Accommodations, Expenses: Bayer

Atish D. Choudhury
Employment: LeMaitre Vascular (I)
Honoraria: Bayer, Astellas Pharma
Research Funding: Janssen Pharmaceuticals (Inst)

Mark A. Preston
No relationship to disclose

Graeme S. Steele
No relationship to disclose

Kent W. Mouw
Consulting or Advisory Role: Pfizer, EMD Serono

Xiao X. Wei
No relationship to disclose

Bradley A. McGregor
Consulting or Advisory Role: Bayer, Seattle Genetics, Astellas Pharma, Exelixis, AstraZeneca, Genentech, Nextar

Toni K. Choueiri
Honoraria: National Comprehensive Cancer Network, UpToDate
Consulting or Advisory Role: Pfizer, Bayer, Novartis, GlaxoSmithKline, Merck, Bristol-Myers Squibb, Genentech, Eisai, Foundation Medicine, Cerulean Pharma, AstraZeneca, Peloton Therapeutics, Exelixis, Prometheus Laboratories, Alligent, Ipsen, Corvus Pharmaceuticals
Research Funding: Pfizer (Inst), Novartis (Inst), Merck (Inst), Exelixis (Inst), TRACON Pharma (Inst), GlaxoSmithKline (Inst), Bristol-Myers Squibb (Inst), AstraZeneca (Inst), Peloton Therapeutics (Inst), Genentech (Inst), Celldex (Inst), Agensys (Inst), Eisai (Inst)

Joaquim Bellmunt
Honoraria: UpToDate
Consulting or Advisory Role: Pierre Fabre, Astellas Pharma, Pfizer, Merck, Genentech, Novartis, AstraZeneca, MedImmune, Bristol-Myers Squibb

Research Funding: Millennium Pharmaceuticals (Inst), Sanofi (Inst)

Travel, Accommodations, Expenses: Pfizer, MSD Oncology

David J. Kwiatkowski
Consulting or Advisory Role: AstraZeneca, Genentech
Research Funding: AADi

Guru P. Sonpavde
Honoraria: UpToDate
Consulting or Advisory Role: Bayer, Genentech, Sanofi, Merck, Novartis, Pfizer, Argos Therapeutics, Agensys, Eisai, AstraZeneca, Janssen Pharmaceuticals, Amgen, Bristol-Myers Squibb, Exelixis
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Other Relationship: Boehringer Ingelheim, AstraZeneca

Affiliations

Amin H. Nassar, **Mark A. Preston**, **Graeme S. Steele**, and **David J. Kwiatkowski**, Brigham and Women's Hospital, Harvard Medical School; and **Kevin Lundgren**, **Mark Pomerantz**, **Eliezer Van Allen**, **Lauren Harshman**, **Atish D. Choudhury**, **Kent W. Mouw**, **Xiao X. Wei**, **Bradley A. McGregor**, **Toni K. Choueiri**, **Joaquim Bellmunt**, and **Guru P. Sonpavde**, Dana-Farber Cancer Institute, Boston, MA.

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