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Original Article

Comprehensive Landscape of Cyclin Pathway Gene Alterations and Co-occurrence with *FGF/FGFR* Aberrations Across Urinary Tract Tumors

Denis L.F. Jardim[1,2,*,‡], Sherri Z. Millis[3,‡], Jeffrey S. Ross[3,4], Scott Lippman[5], Siraj M. Ali[3,*], Razelle Kurzrock[6,7,8]

[1]Department of Clinical Oncology, Hospital Sirio Libanes, São Paulo, Brazil
[2]Latin American Cooperative Oncology Group (LACOG), Porto Alegre, RS, Brazil
[3]Foundation Medicine, Cambridge, MA, USA
[4]Departments of Pathology and Urology, Upstate Medical University, Syracuse, NY, USA
[5]Center for Personalized Cancer Therapy and Division of Hematology and Oncology, University of California, San Diego, CA, USA
[6]WIN Consortium for Personalized Cancer Therapy, Paris, France
[7]Medical College of Wisconsin, Milwaukee, WI, USA
[8]University of Nebraska (adjunct)

*Corresponding author: Denis L.F. Jardim, MD, PhD, Department of Clinical Oncology, Hospital Sirio Libanes, Adma Jafet Street, 91. Bela Vista, 01308-050, São Paulo, Brazil. Tel: +55 (11) 2344 3080; Email: denis.ljardim@hsl.org.br
‡Contributed equally.

Abstract

Background: Cyclin pathway gene alterations are frequent in urothelial tumors and may co-exist with other important aberrations, leading to therapeutic opportunities. We characterized the landscape of cyclin gene alterations in urothelial and non-urothelial urinary tract (UT) malignancies.

Patients and Methods: Overall, 6842 urothelial and 897 non-urothelial UT cancers were analyzed (hybrid-capture-based comprehensive genomic profile (Foundation Medicine)). Alteration frequency in cyclin-sensitizing and -resistance genes, and co-occurrence with fibroblast growth factor receptor (FGFR) gene abnormalities were evaluated.

Results: Cyclin-activating gene alterations were detected in 47.3% of urothelial and 37.9% of non-urothelial UT cancers. Frequency varied by histology and tumor site. *CDKN2A* and *CDKN2B* loss were the most frequent alterations in urothelial tumors (present in 38.5% and 30.4% of patients, respectively). Both genes were less frequently altered in adenocarcinomas (15.2% and 8.9%), but commonly altered in squamous cell carcinomas (74.4% and 39%). Tumors of neuroendocrine origin were relatively silent in activating cyclin alterations, but frequently displayed *Rb1* alterations (86% and 83.7% of neuroendocrines and small cell carcinomas). Urachal tumors ($n = 79$) presented a distinct landscape of cyclin alterations relative to other UT cancers, with less frequent alterations overall. *FGF/FGFR* genes were altered in 34.9% of urothelial (22.1% in *FGFR3),* and 19.4% of non-urothelial urinary tract tumors (6.8% *FGFR3*). Cyclin-activating alterations frequently co-occurred with *FGF/FGFR* alterations but were in general mutually exclusively with cyclin resistance alterations (*RB1/CCNE1*).

Conclusions: Cyclin pathway activating alterations are common in urinary tract tumors, but frequency varies with histology and tumors sites. Co-occurrence of cyclin and FGFR pathway alterations may inform therapeutic opportunities.

Key words: cell cycle; CDK4; CDK6; molecular genetics; precision oncology targeted therapy.

Implications for Practice

Cyclin gene alterations are common in urinary tract tumors and often co-occur with *FGF/FGFR* aberrations. However, the alteration/co-alteration pattern varies by site/histology and may be distinct in rare/ultra-rare urinary tract tumor subtypes. Defining the cyclin/FGFR landscape may inform therapeutic actionability.

Introduction

A variety of tumors arise in the urinary tract, from the renal pelvis to the urethra. Bladder urothelial carcinoma is the most frequent malignant tumor in this setting and is currently the ninth most common cancer in incidence worldwide.[1] Recent effort, including The Cancer Genome Atlas Program (TCGA), provided important insights into the molecular landscape of muscle-invasive bladder cancer, which is the lethal presentation of bladder cancer.[2] According to TCGA, the cell cycle genes constitute the most

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frequent signaling pathway components to harbor molecular alterations in bladder cancer, wherein aberrations are found in up to 89% of cases.[2] The frequency of alterations may vary according to the molecular subtypes of bladder cancer (luminal, basal, or neuronal). Cyclins play a major role in regulating the cell cycle and its interaction with regulatory proteins and other counterparts (including *Rb1*) are of great importance for the proliferation and de-regulation of bladder cancer cells.[3] Cyclin activation derived from genomic abnormalities is frequently detected in other solid tumors as well, including a variety of additional urothelial cancers.[4-7] Relevant genomic targets may vary in frequency according to the urinary tract site (upper vs. lower system), as well as when less frequent histologies are diagnosed.

A substantial proportion of bladder cancers also present actionable genomic alterations affecting tyrosine kinase receptor signals.[2] Erdafitinib is one example of successful biomarker-based approval for advanced urothelial cancers harboring fibroblast growth factor receptor (*FGFR*) mutations or fusions in *FGFR2* and *FGFR3* genes.[8] Similar to the cyclin pathway, the *FGFR* pathway also regulates proliferation, migration, and invasiveness of cells[9] and may suffer molecular alterations leading to constitutional activation.[10] In addition, crosstalk between both pathways can amplify the progression of bladder cancer cells, leading to suboptimal results of isolated blockage of each pathway[11] Of note, palbociclib (CDK4/6 inhibitor) as monotherapy was unsuccessful for bladder cancer, perhaps in part because of the presence of driver genomic co-alterations.[12-15] We hypothesized that a deep analysis of cyclin alterations in different urinary tract tumors along with co-occurrence analysis with the FGFR pathway may help to understand potential challenges in targeting the cyclin pathway for therapeutic purposes.

In this study, we analyzed the landscape of molecular alterations affecting the cyclin signals and potential co-drivers in key pathways, such as FGFR, in 7739 samples. We included rare subtypes of urinary tract cancers, whose molecular profiles have not previously been well interrogated, with the aim of identifying unique alteration patterns and potential therapeutic opportunities.

Patients and Methods

Study Population

Consecutive samples submitted from 2012 through April 2020 by physicians worldwide were analyzed from the Foundation Medicine (Foundation Medicine, Cambridge, MA, USA) database. We included patients with urothelial carcinoma from any origin, and tumors arising from the entire urinary tract of any histology (renal pelvis, ureter, bladder, urachus, and urethra) (primary or metastatic). The histology of all cases was centrally reviewed by a group of experienced board-certified pathologists at Foundation Medicine. Histology classification was determined and submitted by local pathologists and confirmed during central revision, following recommendations from the World Health Organization. As recommended, any amount of small cell histology, even when present with predominantly urothelial elements, warrants the classification of primary small cell bladder cancer.[16] DNA was extracted from formalin-fixed, paraffin-embedded tissue, as previously described[17] and contained a minimum of 20% tumor nuclei. Patient identification was redacted for the study. Approval for this study, including a waiver of informed consent and HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (protocol 20152817).

Next-Generation Sequencing (NGS)

Using a CLIA-certified laboratory (Foundation Medicine), DNA was extracted from formalin-fixed, paraffin-embedded sections, and comprehensive genomic profiling was performed on hybridization-captured, adaptor ligation-based libraries to a median depth of coverage of >500×.[17] The platform simultaneously sequenced the coding regions of 315-324 cancer-related genes plus select introns from 31 genes frequently rearranged in cancer. Results were analyzed for base substitutions, short insertions/deletions (indels), rearrangements, and copy number alterations (amplification and homozygous deletion). Benign germline events were removed by custom filtering.

Genomic alterations of interest were classified either as activators of the cyclin pathway (8 genes, including *CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* (loss), *CDKN2A* (loss), and *SMARCB1*) or related to potential resistance pathways to CDK4/6 inhibition (*RB1* and *CCNE1*). In addition, genomic alterations in the fibroblast growth factor pathway (7 genes, *FGFR*1 to 4, *FGF3*, *FGF4,* and *FGF19*) were included in the analysis because of their importance in urothelial cancers. Analysis of frequencies was performed by tumor site and histology subtype.

Statistical Analysis

The study objectives were to describe the frequency of cyclin genomic alterations in urothelial bladder cancers, and rare urinary sites/histologies, as well as the co-occurrence of alterations in cyclin pathway genes and resistance genes or *FGFR* pathway genes. For co-occurrence and comparison analysis, the odds ratio (OR) and its respective 95% CIs were estimated. Statistical analysis was performed using GraphPad Prism. Python 2.7 (GraphPad Prism, RRID:SCR_002798; https://scicrunch.org/resources/Any/record/nlx_144509-1/SCR_002798/resolver?q=*&l=) and Anaconda version 4-4.3.21 (IPython, RRID:SCR_001658; https://scicrunch.org/resolver/SCR_001658).

Results

Tumor Samples Characteristics

We analyzed 7739 tumor samples from urinary tract tumors. Of these, 6842 samples were from urothelial tumors (71.5% bladder; 16.7% upper tract; 0.9% urethral; and the remainder from unknown sites). We also included 897 samples of non-urothelial cancers (most representative histologies included 25% adenocarcinomas; 20.5%, small cell or neuroendocrine carcinomas; and 19.2%, squamous cell carcinoma) and 79 cases of urachal carcinomas.

Overall, any cyclin gene alterations were detected in 47.3% of urothelial cancers, 37.9% of non-urothelial cancers and 30% of urachal carcinomas. Most genomic cyclin pathway gene aberrations were copy number alterations (Supplementary Tables S1–S3).

Cyclin Pathway Alterations in Urothelial Urinary Tract Tumors

The most frequent alterations in cyclin pathway genes included *CDKN2A* and *CDKN2B* loss in 38.5% and 30.4% of patients, respectively. *CCND1* amplification was present

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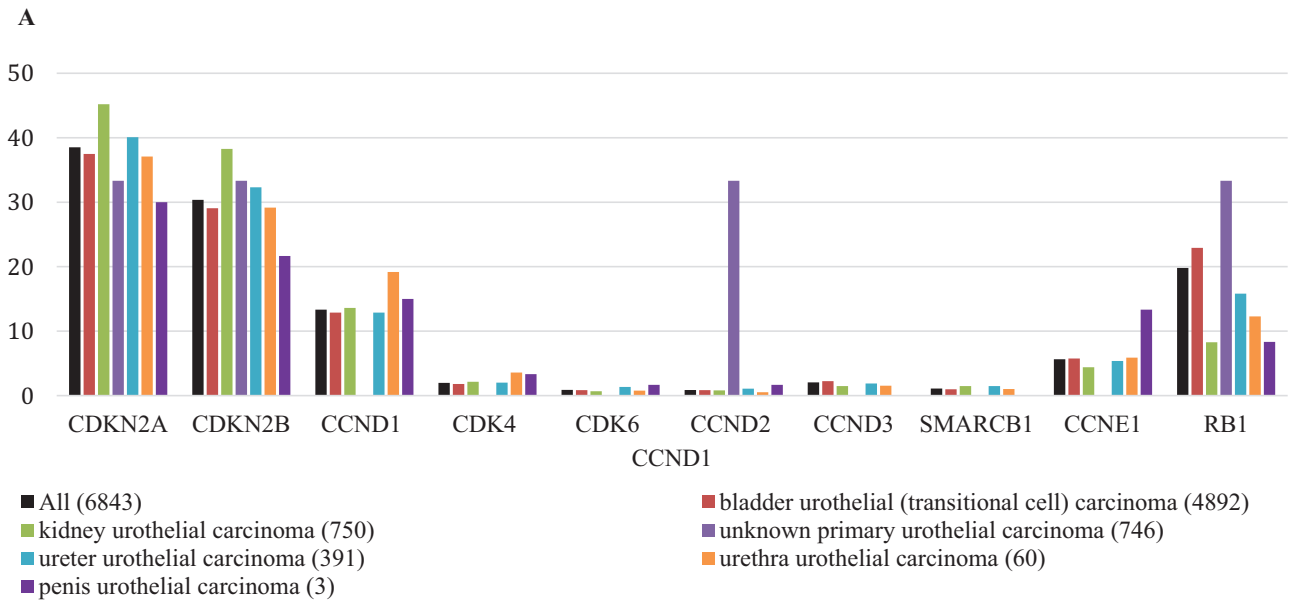

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Tumor type	% Cases with gene alterations*	*CDKN2A*	*CDKN2B*	*CCND1*	*CDK4*	*CDK6*	*CCND2*	*CCND3*	*SMARCB1*	*CCNE1*	*RB1*
All (6842)	**47.3**	**38.5**	**30.4**	**13.3**	**2.0**	**0.9**	**0.9**	**2.0**	**1.1**	**5.6**	**19.8**
Bladder urothelial carcinoma (4892)	45.6	37.5	29.1	12.9	1.8	0.8	0.8	2.2	1.0	5.7	22.9
Kidney urothelial carcinoma (750)	55.5	45.2	38.3	13.6	2.1	0.7	0.8	1.5	1.5	4.4	8.3
Unknown primary urothelial carcinoma (746)	33.3	33.3	33.3	0.0	0.0	0.0	33.3	0.0	0.0	0.0	33.3
Ureter urothelial carcinoma (391)	48.9	40.1	32.3	12.9	2.0	1.3	1.1	1.9	1.5	5.4	15.8
Urethra urothelial carcinoma (60)	50.4	37.1	29.2	19.2	3.6	0.8	0.5	1.5	1.0	5.9	12.3
Penis urothelial carcinoma (3)	40.0	30.0	21.7	15.0	3.3	1.7	1.7	0.0	0.0	13.3	8.3

Figure 1. Landscape of genomic alterations in cyclin sensitizing genes (*CDK4, CDK6, CCND1, CCND2, CCND3, CDKN2B, CDKN2A,* and *SMARCB1*) and resistant genes (*RB1* and *CCNE1)* in urothelial tumors (see also Supplementary Table S1 for types of alterations [copy number changes versus mutations]). (**A**) Analysis of specific gene alteration by urothelial tumor site. Percent of patients with alterations is shown on *y*-axis. (**B**) Chart of alterations (%) in cyclin pathway genes. *Percent in the first column includes only cyclin sensitizing genes. Numbers in brackets represent numbers of patients analyzed. The percentage of patients with an alteration is shown. Pink denotes percentage of patients in that disease subtype with alterations above the percentage for all patients; yellow denotes percentage for that subgroup being below the percentage for all patients.

in 13.3% of samples, while abnormalities in the remaining 5 genes (*CDK4, CDK6, CCND2, CCND3, and SMARCB1*) were detected in less than 2% of cases each (Fig. 1).

According to tumor location, kidney urothelial carcinomas presented a higher frequency of cyclin alterations (defined as≥ 1 cyclin gene altered) compared to bladder urothelial tumors, 55.5% vs. 45.6%, respectively (OR = 1.49, 95%CI, 1.27-1.74; $P < .0001$). Interestingly, urothelial cancers of unknown origin presented a substantial higher incidence of *CCND2* alterations compared to overall urothelial cancers (33.3% vs.0.9%; OR = 54.46, 95%CI, 40.63-72.98; $P < .0001$).

Cyclin Pathway Alterations in Non-Urothelial Urinary Tract Tumors, Including Rare Tumor Subtypes

The patterns of cyclin gene alterations varied according to the histologic type of urinary tract tumors. Adenocarcinomas presented a lower frequency of *CDKN2A* (15.2%) and *CDKN2B* (8.9%) alterations compared to urothelial cancers (38.5% [OR = 0.29, 95%CI, 0.20-0.41; $P < .0001$] and 30.4% [OR = 0.22, 95%CI, 0.14-0.36; $P < .0001$], respectively), but a higher frequency of *CDK6* (4.9% vs. 0.9% [OR = 5.65, 95%CI, 2.93-10.88; $P < .0001$) and *CCND2* (4% vs. 0.9% [OR = 4.58, 95%CI, 2.25-9.33; $P < .0001$) alterations (Fig. 2). Conversely, squamous cell carcinomas were enriched in *CDKN2A* (74.4%) and *CDKN2B* (39%) alterations, but also *CCND1* amplification (18%).

Tumors of neuroendocrine origin were relatively silent in cyclin pathway genetic abnormalities, with alterations in any cyclin gene detected in 9.3% of bladder neuroendocrine carcinomas and 6.1% of bladder small cell carcinomas. The distribution of alterations in cyclin genes in urachal tumors was more balanced, with the highest frequency of alterations of 7.6% in *CDKN2A*, *CCND1*, and *CDK6* (all presenting the same frequency).

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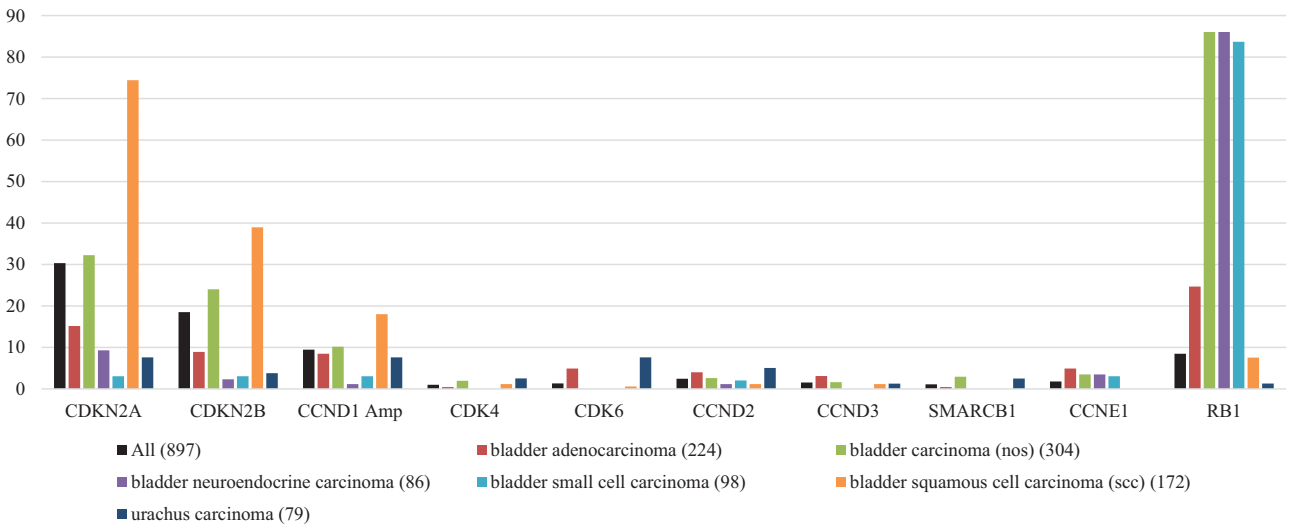


B

Histology	% Cases with gene alterations*	*CDKN2A*	*CDKN2B*	*CCND1*	*CDK4*	*CDK6*	*CCND2*	*CCND3*	*SMARCB1*	*CCNE1*	*RB1*
All (897)	**37.9**	**30.3**	**18.5**	**9.5**	**1.0**	**1.3**	**2.5**	**1.6**	**1.1**	**2.9**	**29.7**
Bladder adenocarcinoma (224)	31.3	15.2	8.9	8.5	0.4	4.9	4.0	3.1	0.4	1.8	8.5
Bladder carcinoma (nos) (304)	43.1	32.2	24.0	10.2	2.0	0.0	2.6	1.6	3.0	4.9	24.7
Bladder gist (1)	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bladder neuroendocrine carcinoma (86)	9.3	9.3	2.3	1.2	0.0	0.0	1.2	0.0	0.0	3.5	86.0
Bladder sarcoma (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bladder small cell carcinoma (98)	6.1	3.1	3.1	3.1	0.0	0.0	2.0	0.0	0.0	3.1	83.7
Bladder squamous cell carcinoma (172)	72.1	74.4	39.0	18.0	1.2	0.6	1.2	1.2	0.0	0.0	7.6
Bladder leiomyosarcoma (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	33.3

Figure 2. Landscape of genomic alterations in cyclin sensitizing genes (*CDK4, CDK6, CCND1, CCND2, CCND3, CDKN2B, CDKN2A,* and *SMARCB1*) and resistant genes (*RB1* and *CCNE1)* in non-urothelial tumors of the urinary tract (see also Supplementary Tables S2 and S3 for types of alterations (copy number changes vs. mutations)). (**A**) Analysis of specific gene alteration by tumor site. Percent of patients with alterations is shown on *y*-axis. (**B**) Chart of alterations (%) in cyclin pathway genes. *Percent in first column includes only cyclin sensitizing genes. Numbers in brackets represent numbers of patients analyzed. The percent of patients with an alteration are shown. Pink denotes percentage of patients in that disease subtype with alterations above the percentage for all patients; yellow denotes percentage for that subgroup being below the percentage for all patients. *Abbreviations*: GIST, gastrointestinal stromal tumors; NOS, not otherwise specified.

Cyclin Pathway Resistant Genes (*RB1* and *CCNE1*) Alterations and Co-occurrence with Cyclin Sensitizing Pathway Alterations

Overall, *RB1* and *CCNE1* were altered in 19.8% and 5.6% of urothelial tumors, respectively (Fig. 1). Single nucleotide variations (SNVs, 85%) were more frequent for *RB1*, while copy number alterations (99.2%) for *CCNE1*. *RB1* alterations were more frequent in the bladder and unknown primary urothelial cancers (22.9% and 33.3%), while less common in primary kidney sites (8.3%).

As for non-urothelial histologies, including rare cancers, the overall frequency of *RB1* and *CCNE1* alterations were 29.7% and 2.9%, respectively (Fig. 2). *RB1* alterations were a hallmark of bladder neuroendocrine and small cell carcinomas (86% and 83.7% of altered samples, respectively). Urachal tumors presented a low frequency of *RB1* (2.5%) and *CCNE1* (1.3%) alterations.

In general, cyclin pathway sensitizing and resistant mutations were less likely to occur together than either type of alteration separately (Table 1 and Supplementary Figs. S1 and S2).

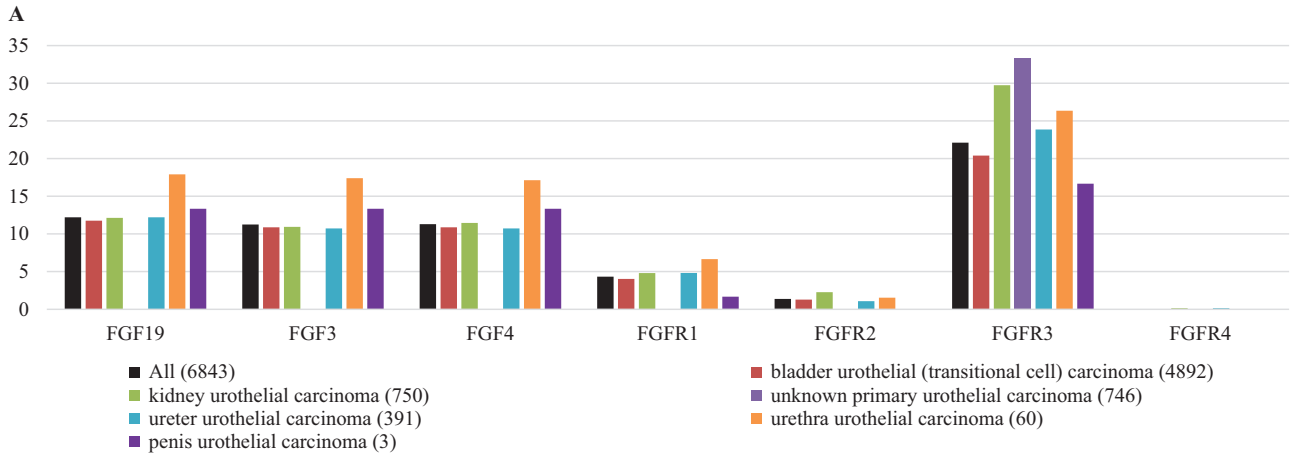
FGF/FGFR Alterations and Co-occurrence with Cyclin Pathway Alterations

FGF/FGFR pathway genes were altered in 34.9% of urothelial tumors: 22.1% of patients for *FGFR3;* 1.4%, *FGFR2* (Fig. 3). Alterations in genes coding the FGFR ligands (*FGF3, FGF4, FGF19*) were exclusively copy number alterations, while in *FGFR3* SNVs prevailed in 77% of cases, in addition to 17% gene rearrangements and 6% copy number alterations

Table 1 Co-occurrence of alterations in cyclin activating/sensitizing (*CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* (loss), *CDKN2A* (loss), and *SMARCB1* and resistance genes (*RB1* and *CCNE1*) in the cyclin pathway.

Tumor type	Cyclin sensitizing alterations only	*RB1/CCNE1* alterations only	Both cyclin sensitizing and *RB1/CCNE1*	Neither cyclin sensitizing or *RB1/CCNE1*	OR co-occurrence* (95% CI)	*P*-value**
Bladder urothelial carcinoma	2074	1108	157	1239	0.08 (0.07-0.1)	.0001
Kidney urothelial carcinoma	401	72	15	232	0.12 (0.07-0.22)	<.0001
Unknown primary urothelial carcinoma	343	123	22	214	0.11 (0.07-0.18)	<.0001
Ureter urothelial carcinoma	188	59	9	117	0.09 (0.05-0.20)	<.0001
Urethra urothelial carcinoma	21	10	3	20	0.29 (0.07-1.19)	.111
Bladder adenocarcinoma	66	17	4	129	0.46 (0.15-1.42)	.2223
Bladder carcinoma (NOS)	119	75	12	75	0.10 (0.05-0.20)	<.0001
Bladder neuroendocrine carcinoma	3	65	5	3	0.08 (0.01-0.48)	.0136
Bladder small cell carcinoma	3	72	3	14	0.19 (0.04-1.06)	.0743
Bladder squamous cell carcinoma	122	10	2	34	0.06 (0.01-0.27)	<.0001
Urachus	11	2	1	65	2.95 (0.25-35.43)	.3942

*OR < 1 indicates mutual exclusivity.
**Derived from two-tailed Fisher's exact test.
Abbreviations: NOS, not other specified; OR, odds ratio.



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Histology	% Cases with gene alterations	*FGF19*	*FGF3*	*FGF4*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*
All (6842)	**34.9**	**12.2**	**11.3**	**11.3**	**4.3**	**1.4**	**22.1**	**0.0**
Bladder urothelial carcinoma (4892)	32.3	11.8	10.9	10.9	4.0	1.3	20.4	0.0
Kidney urothelial carcinoma (750)	42.4	12.1	10.9	11.5	4.8	2.3	29.7	0.1
Unknown primary urothelial carcinoma (746)	33.3	0.0	0.0	0.0	0.0	0.0	33.3	0.0
Ureter urothelial carcinoma (391)	36.6	12.2	10.7	10.7	4.8	1.1	23.9	0.1
Urethra urothelial carcinoma (60)	43.2	17.9	17.4	17.1	6.6	1.5	26.3	0.0
Penis urothelial carcinoma (3)	28.3	13.3	13.3	13.3	1.7	0.0	16.7	0.0

Figure 3. Landscape of genomic alterations in FGF/FGFR genes in urothelial tumors (see also Supplementary Table S1 for types of alterations (copy number changes versus mutations)). (**A**) Analysis of specific gene alteration by urothelial tumor site. Percent of patients with alterations is shown on *y*-axis. (**B**) Chart of alterations (%) in cyclin pathway genes. Numbers in brackets represent numbers of patients analyzed. The percent of patients with an alteration are shown. Pink denotes percentage of patients in that disease subtype with alterations above the percentage for all patients; yellow denotes percentage for that subgroup being below the percentage for all patients.

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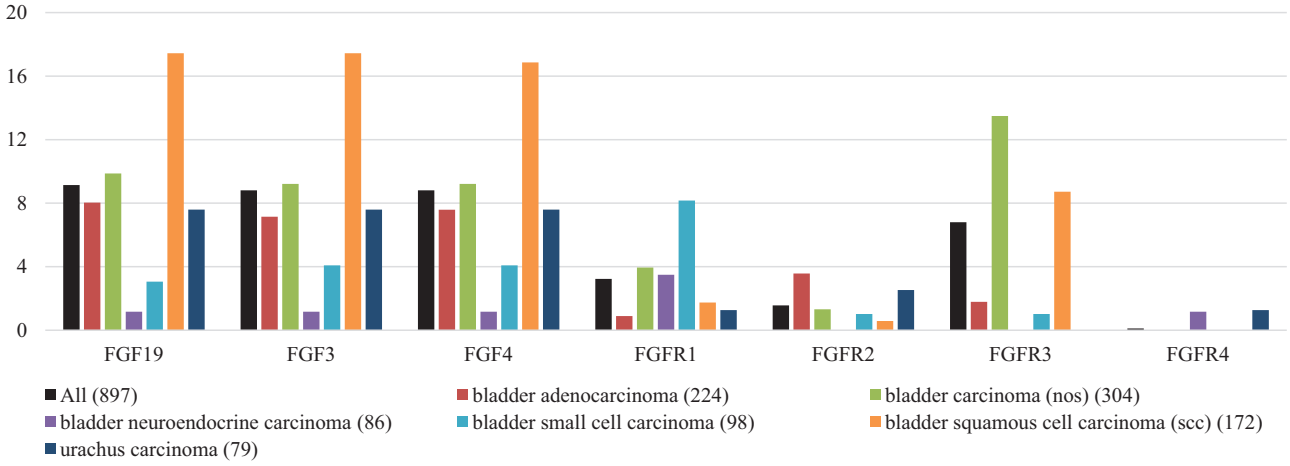

B

Histology	% Cases with gene alterations	*FGF19*	*FGF3*	*FGF4*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*
All (897)	**19.4**	**9.1**	**8.8**	**8.8**	**3.2**	**1.6**	**6.8**	**0.1**
Bladder adenocarcinoma (224)	13.8	8.0	7.1	7.6	0.9	3.6	1.8	0.0
Bladder carcinoma (nos) (304)	26.0	9.9	9.2	9.2	3.9	1.3	13.5	0.0
Bladder gist (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bladder neuroendocrine carcinoma (86)	5.8	1.2	1.2	1.2	3.5	0.0	0.0	1.2
Bladder sarcoma (3)	33.3	0.0	0.0	0.0	33.3	0.0	0.0	0.0
Bladder small cell carcinoma (98)	11.2	3.1	4.1	4.1	8.2	1.0	1.0	0.0
Bladder squamous cell carcinoma (172)	27.3	17.4	17.4	16.9	1.7	0.6	8.7	0.0
Bladder leiomyosarcoma (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Figure 4. Landscape of genomic alterations in FGF/FGFR genes in non-urothelial tumors of the urinary tract (see also Supplementary Tables S2 and S3 for types of alterations (copy number changes versus mutations)). (**A**) Analysis of specific gene alteration by tumor site. Percent of patients with alterations is shown on *y*-axis. (**B**) Chart of alterations (%) in cyclin pathway genes. Numbers in brackets represent numbers of patients analyzed. The percent of patients with an alteration are shown. Pink denotes percentage of patients in that disease subtype with alterations above the percentage for all patients; yellow denotes percentage for that subgroup being below the percentage for all patients. *Abbreviations*: GIST, gastrointestinal stromal tumors; NOS, not otherwise specified.

(Supplementary Table S1). Kidney urothelial cancers had a higher frequency of *FGFR3* alterations than primary bladder urothelial cancer (29.7% vs. 20.4%; OR = 1.65, 95%CI, 1.39-1.96; $P < .0001$).

Non-urothelial tumors, including rare cancer types, presented in 19.4% of patients with FGFR pathway alterations (Fig. 4), mostly in the *FGF3, FGF4,* and *FGF19* genes. *FGFR3* alterations were a rare event in adenocarcinomas (1.8%), urachal tumors (0%), neuroendocrine (0%), and small cell carcinomas (1%), but present in 8.7% of squamous cell carcinomas.

We described a positive co-occurrence between cyclin sensitizing and FGF/FGFR pathway genomic alterations (Table 2) in all histologies and tumor sites analyzed (Fig. 5 and Supplementary Fig. S3). For bladder urothelial cancer, the co-occurrence analysis demonstrated an OR of 5.32 (95% CI, 4.66-6.07; $P = .0001$) for concomitant vs. isolated alterations.

Discussion

The cell cycle pathway is the subject of frequent molecular alterations in urinary tract tumors. To our knowledge, this is the largest dataset of patients ($n = 7739$) with urothelial and other tumor types arising from the urinary tract describing cyclin pathway alterations using comprehensive genomic profiling. We demonstrated that any cyclin activating gene alteration was detected in 47.3% of urothelial and 37.9% of non-urothelial cancers. Due to the large sample size, we also were able to describe significant differences in the landscape of cyclin alterations in rare tumor types and histologies.

We demonstrated that urothelial tumors are enriched in *CDKN2A/2B* loss and *Rb1* alterations, as described in prior smaller series.[2,18] The type of genomic alteration detected in cyclin genes is variable, with a predominance of copy number variation (Supplementary Tables S1-S3). Hence, a comprehensive genomic profiling approach, instead of hot-spot panel, is needed to identify the full spectrum of alterations that might occur in the cyclin pathway. Especially for genes such as *CDKN2A*, inactivation is mediated by homozygous deletions and/or other concomitant genetic abnormalities leading to haploinsufficiency.[3] *CDKN2A* alterations were recently described as a potential predictive marker of poor responses to checkpoint inhibitors in urothelial cancer,[19] increasing the importance of its detection. *CCND1* alterations were also detected in 13.3% of patients and were previously linked to higher metastatic potential and worse prognosis.[20] Interestingly, *CCND1* alterations were not detected in urothelial tumors of the unknown primary sites; conversely, these

Table 2 Co-occurrence of alterations in cyclin activating/sensitizing (*CDK4* amplification, *CDK6* amplification, *CCND1*, *CCND2*, *CCND3*, *CDKN2B* (loss), *CDKN2A* (loss), and (*SMARCB1*) and genes from FGF/FGFR pathway.

Tumor type	Cyclin sensitizing alterations only	*FGF/FGFR* alterations only	Both cyclin sensitizing and *FGF/FGFR*	Neither cyclin or *FGF/FGFR*	OR co-occurrence* (95% CI)	*P*-value**
Bladder urothelial carcinoma	1089	438	1142	2223	5.32 (4.66-6.07)	.0001
Kidney urothelial carcinoma	179	81	237	253	4.14 (3.01-5.68)	<.0001
Unknown primary urothelial carcinoma	172	80	193	301	4.22 (3.06-5.82)	<.0001
Ureter urothelial carcinoma	68	40	129	154	7.30 (4.63-11.51)	<.0001
Urethra urothelial carcinoma	13	6	11	30	4.23 (1.29-13.89)	.02
Bladder adenocarcinoma	45	6	25	148	13.70(5.29-35.49)	<.0001
Bladder carcinoma (NOS)	73	21	58	153	5.79 (3.27-10.25)	<.0001
Bladder neuroendocrine carcinoma	7	4	1	74	2.64 (0.26-27.01)	.39
Bladder small cell carcinoma	3	8	3	84	10.5 (1.81-60.85)	.018
Bladder squamous cell carcinoma	83	6	41	42	3.46 (1.36-8.80)	.007
Urachus	17	2	7	53	10.91 (2.07-57.60)	.0027

*OR < 1 indicate mutual exclusivity.
**Derived from 2-tailed Fisher's exact test.
Abbreviations: FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; NOS, not other specified; OR, odds ratio.

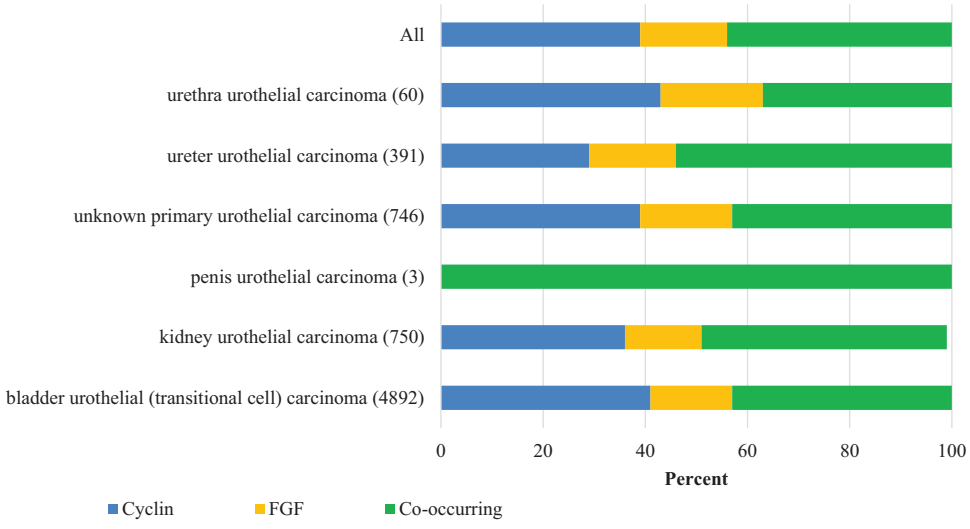

Figure 5. Co-alteration analysis of FGF/FGFR pathway and cyclin pathway in urothelial tumors. The ratio of alterations in the cyclin pathway sensitizing only, the FGF/FGFR pathway only, or alterations in both pathways is shown for urothelial tumors.

tumors were enriched in *CCND2* alterations as compared to other urothelial tumors.

Non-urothelial urinary tract tumors comprise a group of rare and heterogeneous diseases. The major types in this category include squamous cell carcinoma, adenocarcinomas, and neuroendocrine tumors. These tumors lack standardized protocols of therapy, highlighting the importance of further genomic characterization of these entities. As expected, the landscape of cyclin alterations is also heterogeneous in these tumors. Squamous cell cancer demonstrated a pattern of alterations that resemble urothelial cancers, while adenocarcinoma seems to present a more distinct pattern.

Urachal carcinomas are predominantly glandular morphology tumors with substantially fewer alterations in cyclin genes. In fact, this entity is rarely detected as a non–glandular tumor type and molecularly resembles gastro–intestinal tumors.[21] Interestingly, the distribution and frequency of cyclin alterations in urachal tumors described herein (any alteration in 30%; CDKN2A in 7.6%; CCND1 in 7.6%, CDK6 in 7.6%), are similar to our prior description of cyclin

alterations in non–colorectal gastrointestinal tumors, such as gastric cancers (any alteration in 22.9%; CDKN2A in 11.8%; CCND1 in 4.8%, CDK6 in 5.0%).[22]

Small cell and neuroendocrine carcinomas of the urinary tract are aggressive diseases, frequently characterized by *Rb1* inactivation,[23] and our largest database confirmed this finding.

As for therapeutic opportunities, important observations can be suggested from our findings. The presence of *Rb1* alterations can drive resistance to cyclin inhibitors but were also associated with responses to platinum-based chemotherapy in urothelial tumors.[24] According to our data, this would be important for some urothelial tumors and especially for tumors of neuroendocrine origin. In addition, the frequent mutual exclusivity between cyclin-activating alterations and resistance alterations (*Rb1* and *CCNE1*) reported herein, suggests a potential targeted strategy for these tumors using cyclin inhibition. Even so, a prior clinical trial with palbociclib (CDK 4/6 inhibitor) failed to demonstrate meaningful clinical activity in urothelial tumors.[12] For this trial, patients were selected by immunohistochemistry (IHC) demonstrating positivity for Rb and negativity for p16 (*CDKN2A*). Molecular selection of patients for cyclin-activating alterations also failed in a basket trial of solid tumors testing the CDK4/6 inhibitor ribociclib.[25] This trial included 7 patients with urothelial tumors, and there was one pronounced response to ribociclib (37% tumor reduction; duration, 254 days) (urothelial bladder cancer refractory to multiple therapies harboring *CCND1* amplification). This alteration is present in 13.3% of urothelial tumors in our dataset. As exemplified in breast cancer, for which cyclin inhibitors received regulatory approval, molecular markers for patient selection continue to be a matter of debate.[26] In addition, a combination strategy (in breast cancer with antihormonal agents) was the successful approach for cyclin inhibition.[26] Further emphasizing the role of combination approaches that address co-alterations, co-targeting cyclin and MEK signaling showed activity in 56% of patients (5 of 9 participants) with tumors harboring genomic co-alterations that activate both of these pathways.[13,14]

In this context, we explored the relationship between molecular markers of cyclin activation and FGFR alterations. The FGF/FGFR pathway is frequently altered in urothelial tumors and alterations including *FGFR3* mutations and fusions lead to clinical responses to FGFR inhibitors.[8] In our large dataset, *FGFR3* alterations were common in the bladder and upper tract urothelial tumors, and a rare event in non-urothelial tumors (except for 8.7% of FGFR3 alterations in squamous cell carcinomas). Other alterations in the pathway are possible, but their role in predicting responses to FGFR inhibitors is under further evaluation.[27] According to our data, FGF/FGFR alterations beyond *FGFR3* are frequent and could potentially expand the role of FGFR inhibitors. We reported a significant co-occurrence of FGF/FGFR and cyclin alterations. This finding validates prior observations of the association between *CDKN2A* and *FGFR3* alterations.[2,11] There are potential clinical implications of this finding. Cyclin alterations can modulate the evolution of FGFR-altered urothelial tumors, leading to aggressiveness and poor prognosis of these tumors[28,29]; FGFR activation could lead to resistance to cyclin inhibition, as reported in breast cancer,[30] decreasing the therapeutic index of cyclin inhibitors as monotherapy; and, finally, a potential combination strategy with FGFR and cyclin inhibitors is justifiable.[31] Indeed, this combination is already in clinical development for breast cancer.[32,33]

There are several limitations to our study. As the patient selection was based on physician orders worldwide, a possible selection bias toward more refractory or aggressive tumors could occur. Hence, although this is the largest database reporting on urinary tract tumors so far, frequencies and genomic associations might not apply to all tumor stages. In addition, lack of full clinical annotation prevents associations with clinical staging, therapeutic responses, and prognosis. Nonetheless, the data generated here for therapeutic opportunities, including in rare urinary tract cancers, merits further investigation.

Conclusion

Our large dataset provides valuable information about the landscape of cyclin alterations in urothelial and non-urothelial urinary tract tumors, including rare and ultra-rare subtypes. These alterations are frequent in a variety of urinary tract tumor types, but the specific altered genes and patterns of alterations vary by tumor histology, especially for non-urothelial urinary tract tumors. Intrinsic genomic mechanisms of resistance to cyclin inhibitors (*Rb1* and *CCNE1* alterations) are not common in the presence of cyclin activating alterations; but co-occurrence of activating cyclin alterations with other potential driver alterations, such as *FGF/FGFR* aberrations, suggests that combination strategies co-targeting cyclins and FGF/FGFR are warranted.

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Conflict of Interest

Denis L. Jardim: Janssen, Bristol-Myers Squibb, Libbs (C/A), Roche, Janssen, Astellas, MSD, Bristol-Myers Squibb, Pfizer, AztraZeneca, Libbs (speaker fees); **Sherri Z. Millis:** Foundation Medicine (E [former]); **Jeffrey S. Ross:** Tango Therapeutics, Celsius Therapeutics (C/A OI), Foundation Medicine (E, OI); **Siraj M. Ali:** Foundation Medicine (E [former]); **Razelle Kurzrock:** Boehringer Ingelheim, Debiopharm, Foundation Medicine, Genentech, Grifols, Guardant, Incyte, Konica Minolta, Medimmune, Merck Serono, Omniseq, Pfizer, Sequenom, Takeda, TopAlliance (RF), Actuate Therapeutics, Bicara Therapeutics, Inc., Biological Dynamics, Ilyon, Neomed, Pfizer, Roche, TD2/Volastra, Turning Point Therapeutics, X-Biotech (C/A, SAB), CureMatch Inc., IDbyDNA (OI), CureMatch (co-founder and board member), CureMetrix (board member). **Scott Lippman** indicated no financial relationships.

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Author Contributions

Conception/design: D.L.J., S.Z.M., R.K. Provision of study material or patients: S.Z.M., J.S.R., S.L., S.M.A. Collection and/or assembly of data: D.L.J., S.Z.M., J.S.R., S.M.A., R.K. Data analysis and interpretation: D.L.J., S.Z.M., J.S.R., R.K. Manuscript writing: D.L.J., S.Z.M., R.K. Final approval of manuscript: All authors.

Data Availability

The data underlying this article were provided by Foundation Medicine under licence, by permission. Data will be shared on request to the corresponding author with the permission of Foundation Medicine.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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