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**Title:**
Tumor Heterogeneity of *Fibroblast Growth Factor Receptor 3 (FGFR3)* Mutations in Invasive Bladder Cancer: Implications for Peri-Operative anti-FGFR3 Treatment

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ABSTRACT

**Background:** FGFR3 is an actionable target in bladder cancer. Preclinical studies show that anti-FGFR3 treatment slows down tumor-growth suggesting that this tyrosine kinase receptor is a candidate for personalized bladder cancer treatment, particularly in patients with mutated FGFR3. We addressed tumor heterogeneity in a large multi-center, multi-lab study, as this may have significant impact on therapeutic response.

**Patients and methods:** We evaluated possible FGFR3 heterogeneity by the PCR-SNaPshot method in the superficial and deep compartments of tumors obtained by trans-urethral resection (TUR, n=61) and in radical cystectomy (RC, n=614) specimens and corresponding cancer-positive lymph nodes (LN+, n=201).

**Results:** We found FGFR3 mutations in 13/34 (38%) T1 and 8/27 (30%) ≥T2-TUR samples with 100% concordance between superficial and deeper parts in T1-TUR samples. Of eight FGFR3 mutant ≥T2-TUR samples, only four (50%) displayed the mutation in the deeper part. We found 67/614 (11%) FGFR3 mutations in RC specimens. FGFR3 mutation was associated with pN0 (p<0.001) at RC. In 10/201 (5%) LN+ a FGFR3 mutation was found, all concordant with the corresponding RC specimen. In the remaining 191 cases, RC and LN+ were both wild type.

**Conclusions:** FGFR3 mutation status seems promising to guide decision making on adjuvant anti-FGFR3 therapy as it appeared homogeneous in RC and LN+. Based on the results of TUR, the deep part of the tumor needs to be assessed if neoadjuvant anti-FGFR3 treatment is considered. We conclude that studies on the heterogeneity of actionable molecular targets should precede clinical trials with these drugs in the peri-operative setting.
Key words: FGFR3, mutations, heterogeneity, bladder, cancer, targeted therapy

Key message:
FGFR3 is a major potential actionable target in urothelial bladder cancer (BC). We found that FGFR3 mutations appeared conserved in primary BC and corresponding lymph-node metastases. We also showed that the deep part of the tumor needs to be assessed if neoadjuvant anti-FGFR3 treatment is considered. This suggests that personalized anti-FGFR3 therapy may improve BC treatment in the peri-operative setting.
INTRODUCTION

Radical Cystectomy (RC) has been the gold standard for treatment of invasive, non-metastatic, urothelial carcinoma of the bladder (UCB) for more than 50 years. Despite major surgery, five-year survival still only ranges from ±75% in pT2N0 to ±25% in pN+ UCB (1,2). Peri-operative (neoadjuvant and adjuvant) platinum-based combination chemotherapy has only marginally (5-7% overall survival benefit for neoadjuvant chemotherapy) improved patient’s prognosis (3-5). Consequently, better systemic treatment is urgently needed to improve clinical outcomes for invasive UCB.

Activating oncogenic mutations of FGFR3 were identified more than 10 years ago in UCB (6). Interestingly, FGFR3 mutations were predominantly found in genetically stable UCB with a favorable prognosis (7). Moreover, FGFR3 and TP53 mutations rarely coincide and FGFR3 mutations are, even in advanced UCB, most of the time accompanied by fewer molecular alterations than FGFR3 wild-type tumors (7-10). This indicates that FGFR3 is also a major potential actionable target in a subgroup of advanced UCB (9-11). Furthermore, preclinical in-vitro and in-vivo data show that anti-FGFR3 therapy slows down tumor growth, especially in FGFR3-mutated tumors (12). However, the heterogeneity of FGFR3 status within a tumor or a patient has not been adequately addressed and may negatively impact therapeutic response (11).

We report a large multi-center, multi-lab study investigating the heterogeneity of the FGFR3 mutations in invasive UCB. We analyzed paired samples (superficial and deep compartments of the same lesion) of primary trans-urethral resection (TUR) of 61 patients. We also analyzed paired samples from RC and positive lymph nodes (LN+) of 614 patients who were treated for cN0M0-UCB without prior systemic
chemotherapy and/or radiotherapy. FGFR3 expression was also analyzed by IHC in a subgroup of patients.
MATERIALS AND METHODS

Study populations

Three cohorts of patients with UCB were established to study the heterogeneity of FGFR3 mutation status in UCB. In total, 10 different hospitals were involved in the treatment of the patients and molecular analyses were done in 4 different laboratories.

1. Cohort of transurethral resection (TUR):

To evaluate intra-tumor FGFR3 mutation heterogeneity, we studied a cohort of 61 patients who underwent a primary TUR for UCB. All tumors were primary UCB. The procedures were performed in 2 hospitals (Toronto; \( n=26 \) and Leeds; \( n=35 \)) between 1993 and 2006. Mean age at diagnosis was 70.3 years (SD 8.3 years); 15/61 patients were female. All TUR specimens contained muscle as assessed by pathology review (TvdK and PH). For each case, a superficial and deep part of the same tumor specimen were separately dissected from the tissue-block or blank slides for DNA isolation and subsequent FGFR3 mutation analysis. All DNA-samples of the 61 TURs were analyzed in both labs (Toronto and Leeds) and the results were the same. An additional 4 TUR-cases, in which multiple parts of the same superficial (\( n=3 \)) or invasive (\( n=1 \)) areas were available, were analyzed in Toronto.

2. Cohorts of radical cystectomy

The second (International) cohort included 494 patients treated with radical cystectomy (RC) including a pelvic lymph-node dissection for cN0M0 (staged with at least abdominal CT and chest X-ray) UCB in 4 hospitals in Amsterdam, the Netherlands (\( n=204 \)); Toronto, Canada (\( n=104 \)); Dallas, TX, USA (\( n=132 \)) and Turku, Finland (\( n=54 \). A previous diagnosis of non-invasive UCB was allowed. Mean age at RC was 65.1 years (SD 10.8 years); 121/494 patients were female. Patients were
treated between 1986 and 2012 by RC without prior neo-adjuvant chemotherapy or pelvic radiation. Of these patients, 83/494 (17%) received adjuvant chemotherapy. Pathology review was done by JdJ, JS (Amsterdam) and TvdK (Toronto, Dallas, Turku). Node samples were available for reliable FGFR3 analysis in 117/155 pN+ cases. The lab in Amsterdam analyzed the 204 RC-cases from Amsterdam and the 290 RC-cases from Toronto, Dallas and Turku were all analyzed in Toronto.

In the third (French) cohort, 120 cN0M0 UCB patients treated in 5 French hospitals for locally advanced pT3/pT4 \((n=100)\) and/or pN+ \((n=99)\) UCB were identified. All these patients were treated by RC including a pelvic lymph-node dissection and adjuvant platinum-based chemotherapy between 2000 and 2009 at the Henri Mondor hospital, Créteil \((n=36)\); the Gustave Roussy institute, Villejuif \((n=28)\); the Curie institute, Paris \((n=7)\); the Claudius Regaud institute, Toulouse \((n=28)\) and Bergonié institute, Bordeaux \((n=21)\). Mean age at RC was 62.1 years (SD 9.1 years); 16/120 patients were female. A previous diagnosis of non-invasive UCB was allowed. None of the patients had prior neo-adjuvant chemotherapy or pelvic radiation. Central pathology review was done by YA. Node samples were available for reliable FGFR3 analysis in 84/99 pN+ cases. The lab in Créteil analyzed all the RC-cases of the French cohort.

**Clinicopathological data collection**

The clinico-pathological characteristics, treatment and follow-up data were retrospectively collected. Tumors were staged according to the 2009 TNM classification (13) and graded according to WHO criteria. Local ethics committees and/or translational research boards approved the three experimental protocols and,
if applicable, patients provided written informed consent for central collection of their tissue specimens and clinical data for research purposes.

**Tissue (TUR & RC) specimens and DNA extraction**

Hematoxylin and eosin slides served as templates for the manual macro-dissection procedure on the formalin fixed, paraffin embedded tissue-block or blank slides. The dissected samples contained a minimum of 70% tumor cells, as assessed by histological examination. DNA was extracted from the tissues according to the manufacturer’s protocols using the DNeasy® Tissue Kit in the TUR and international RC cohorts. In the French RC cohort, the Maxwell® 16 FFPE Plus LEV DNA Purification Kit and an automated Maxwell® platform (Promega®) were used for DNA isolation.

**FGFR3 mutation analysis**

FGFR3 mutation analysis was done using the PCR-SNaPshot method in all labs. Details of this method were reported previously (14,15). Briefly, 3 regions (exons 7, 10 and 15) frequently mutated and representing at least 99% of activating oncogenic FGFR3 mutations in UCB, were simultaneously amplified by PCR. After removing excess primers and deoxynucleotides, specific SNaPshot primers were annealed to the PCR products, separated by capillary electrophoresis and analyzed in an automatic sequencer (Prism® 3100 genetic analyser). With this PCR-SNaPshot method, a total of 11 known oncogenic FGFR3 mutations can be detected. The codon numbering refers to the cDNA open reading frame of the FGFR3b isoform expressed in epithelia (6).
FGFR3 expression analysis

FGFR3 expression could be studied with IHC in 357/494 cystectomy specimens and in 72/117 paired RC/LN+ from the International cohort (a subset from Amsterdam, Toronto and Turku). Standard TMA technology was used in both labs (16). The available cases were routinely processed with a monoclonal antibody against FGFR3 (FGFR3 B9, Santa Cruz, CA). Positive and negative controls were included in each run. Slides were assessed by BvR and TvdK (Toronto) and by BVR and JS (Amsterdam). A semi-quantitative scoring system was used: 0, negative; 1, faint/normal; 2, moderate positivity; 3, strong positivity. FGFR3 overexpression was defined by a score of 2 or 3 as previously described (15,17,18).

Statistics

SPSS®, version 20 was used for data documentation and analysis. Chi-square statistics were used to analyze possible associations between FGFR3 status and pathological variables. Statistical significance was set at $p<0.05$. 
RESULTS

Within the TUR cohort, *FGFR3* mutations were detected in 13/34 T1 and 8/27 ≥T2 UCB, respectively. Comparing paired superficial and deep parts, no discordance was found within the T1-TUR samples (Figure1A), whereas discordance was observed in half of the cases within the ≥T2-TUR samples with only 4/8 *FGFR3* mutations in the invasive area (Figure1B). In another 4 TUR cases (one with mutation), multiple samples from same area (3 multiple superficial, 1 multiple invasive areas) were analyzed as a control experiment. We found no difference among these samples.

Within the RC cohort, *FGFR3* status was known for 614 RC of which 254 (41%) were pN+. Of the 254 LN+ cases, *FGFR3* status was available for 201 (79%) paired RC/LN+ samples. In the 614 cystectomies, 67 (11%) *FGFR3* mutations were detected, of which 54 were pN0 (Table1). Suppl. Table1 shows the distribution of mutations for the International and French RC cohorts, respectively. In suppl. Table2, the types of *FGFR3* mutations, with S249C (67%) as the most frequent one, are listed. Table2 shows the clinico-pathological characteristics of the 13 patients with a *FGFR3* mutation and pN+ UCB. In the 201 paired RC/LN+ samples, the same *FGFR3* mutation was detected in the cystectomy and LN+ specimen (Figure1C). Discordance between the 201 paired samples was not observed (specificity: 100%). The presence of a *FGFR3* mutation was associated with lower pT-stage (*p*<0.001) and pN0 (*p*<0.001) at RC (Table1).

Finally, FGFR3 expression was studied with immunohistochemistry (IHC) in 357/614 cystectomy specimens (Table3a). In 280 RC, FGFR3 expression was normal and no mutation was found. We found 70 RC with overexpression of whom 37 had a mutation. In 7 cases, we found a FGFR3 mutation with normal expression
at IHC (Table3a). IHC samples were available for 72/201 paired RC/LN+ cases (Table3b). FGFR3 expression was concordant in 64/72 (89%) cystectomy and LN+ specimens.
DISCUSSION

In metastatic UCB, several targeted therapies have been evaluated as second-line treatment (19) but none of them has made it into the clinical practice so far. Although development of effective inhibitors (including anti-FGFR3 treatment) still is at an early stage, FGFR3 is a very promising actionable target in UCB (9-12,19). Comparable to other malignancies, targeted therapy has shown significant activity in only a minority of UCB-patients (10-12,19). Reasons for this limited activity may include the diverse genomic landscape of UCB (10), the absence of molecular tumor-analysis before test-drug administration (12) and lack of adequate studies addressing intra-tumor/patient heterogeneity of potential actionable targets (11). Considering cN0M0 patients in the peri-operative setting, molecular tumor analysis and heterogeneity assessment are pivotal before administering a drug against an actionable target. To our knowledge, the present study is the first to address tumor-heterogeneity for the peri-operative setting in UCB with TUR and RC/LN+ specimens.

FGFR3 activation mostly occurs via oncogenic mutations (6-12), occasionally by rearrangements (10,20) and also via over-expression by other mechanisms such as copy-number gain (10,15,17). Less is known about FGFR3 intra-tumor/patient heterogeneity in UCB (21). The main purpose of our multi-center, multi-lab study was to address this heterogeneity for the peri-operative setting of invasive UCB. Previous small, single center, single lab studies have shown an approximately 80% concordance in multiple synchronous and metachronous non-invasive UCB (17,21). Furthermore, recent important preclinical work provided a cellular and genetic basis for this diversity in UCB (22). In our study on TUR samples, we showed that FGFR3 mutation status may differ between the superficial and invasive part of one tumor. So far, only one previous study reported on FGFR3 heterogeneity in superficial and
deep invasive parts at TUR (17). Within 18 mutated UCB, 9 had the same mutation in the two compartments, 8 had mutation only in the most superficial area and one had different mutations in the two parts. However, the authors were not sure that samples were from the same lesion in the bladder. In the present TUR-series, the same tumor was analyzed. It was notable that we found 4 cases with a FGFR3 mutation in the superficial part but not in the deep part of the same ≥T2 tumor. Conversely, we did not observe a difference in FGFR3 status in 201 RC and LN+ samples of our RC cohort. Therefore, it is likely that, at RC, the deep part of the tumor has been analyzed and that the superficial part was already removed by the preceding TUR. The mutation frequency at RC (11%) also corresponded to the mutation frequency of the deep part of the ≥T2-TUR cohort (15%). The frequency of FGFR3 mutations (12%) in the TCGA-cohort of 131 high-grade muscle-invasive UCB (mostly cystectomy specimens) was also comparable to our cohort. This implies that the deep part of the tumor at TUR needs to be assessed if neoadjuvant anti-FGFR3 treatment is considered.

Our study showed that, if a mutated clone progresses in MI-UCB, the FGFR3 mutation is conserved in the invasive compartment and also in the metastatic node despite the notion that not all the lesions in the RC cohorts were primary (first diagnosis) UCB. We also reported that the FGFR3 mutation was associated with lower T-stage and pN0 at RC. Others have already reported that FGFR3 mutations are also in MI-UCB most of the time not accompanied by many other molecular alterations (8,10). Taken together, all these findings suggest that anti-FGFR3 treatment may have significant clinical impact in the peri-operative setting for a relative small subgroup of MI-UCB patients.
FGFR3 expression is another way to explore FGFR3 activity. Turo et al. (18) recently reported a heterogeneity study using FGFR3 expression by IHC without FGFR3 mutation evaluation. In their cohort, paired RC/LN+ samples were available for IHC analysis in 106/150 pN+-UCB and concordance was found in 79/106 (75%) cases. We here reported IHC-concordance in 64/72 (89%). Previous IHC studies showed that approximately 40% of invasive FGFR3 wild-type tumors overexpress FGFR3 suggesting an alternative mechanism to activate FGFR3 (10,15,17). In our RC-series, only 10% of wild-type cases showed overexpression (Table3). One of the reasons for this lower percentage might be that we analyzed RC specimens and consequently deeper parts of the tumor than in the previous studies. Nevertheless, we can’t exclude that a small subset of patients with wild-type tumors may still benefit from anti-FGFR3 treatment. On the other hand, we showed that FGFR3 mutation analysis was extremely robust across 4 labs. IHC is likely more prone to observer variability than FGFR3 mutation analysis making it less appropriate to assess FGFR3 heterogeneity within a tumor or metastases of a patient. Future study should focus on how to combine FGFR3 mutation, translocation and copy-number status with FGFR3-IHC to guide optimal personalized anti-cancer treatment.

In conclusion, we found that FGFR3 mutations appeared conserved in primary bladder cancer and corresponding lymph-node metastases. Hence, anti-FGFR3 treatment may have significant clinical impact in the adjuvant setting. We also showed that the deep part of the tumor needs to be assessed if neoadjuvant anti-FGFR3 treatment is considered. Our data on tumor heterogeneity suggest that personalized anti-FGFR3 therapy may improve bladder cancer treatment for a relatively small, well-selected subgroup of invasive UCB patients. Studies on the
heterogeneity of actionable molecular targets should precede clinical trials with these
drugs in the peri-operative setting.
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The Regional Ethics Board of the University Health Network, Toronto (02-0515-C and 08-0263-T) gave approval. The Translational Research Board of the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital (CFMPB 160) approved the study. Approval was obtained from the Leeds East Research Ethics Committee. The Regional Ethics Board of Ile-de-France IX (Comité de protection des Personnes – Ile-de-France IX, Créteil) approved the study (11-052).

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Disclosure Statement:

The authors have declared no conflicts of interest.
REFERENCES

FIGURE LEGEND

Legend Figure 1

Figure 1 shows the distributions of FGFR3 mutations in the superficial and deep compartments of the 61 (34 T1 and 27 ≥T2) patients included in the trans-urethral resection (TUR) cohort and in the 614 radical cystectomy patients with 201 paired cystectomies and metastatic nodes available.

A: The 13 mutated cases in 34 paired T1-TUR samples are displayed. Both parts (superficial and deep) were wild type in 21 cases.

B: The 8 mutated cases in paired ≥T2-TUR samples are displayed. Both parts (superficial and deep) were wild type in 19 cases.

C: The 10 mutated cases in paired cystectomies and metastatic nodes are displayed. The cystectomy and metastatic node were both wild type in 191 cases.
Figure 1C
Table 1 – The distribution of samples according to the primary tumors pathologic pT-stage and FGFR3 mutation status among either N0 or N+ cases in the radical cystectomy cohort. FGFR3 mutations were associated with lower pT-stage ($p<0.001$) and pN0 ($p<0.001$) at radical cystectomy.

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Table 2 - Clinical and pathological characteristics of patients with pN+ UC and a FGFR3 mutation detected in cystectomy and/or positive lymph node. In 3 cases, the node sample was not available.

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<th>Follow-up (yr.)</th>
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<td>Yes</td>
<td>DM</td>
<td>Dead</td>
<td>1.9</td>
<td>DOD</td>
<td>R248C</td>
<td>T,N</td>
</tr>
<tr>
<td>VCA023</td>
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<td>M</td>
<td>UC</td>
<td>pT3aN2</td>
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<td>Yes</td>
<td>No</td>
<td>-</td>
<td>Alive</td>
<td>2</td>
<td>FOD</td>
<td>S249C</td>
<td>T,N</td>
</tr>
<tr>
<td>VCA045</td>
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<td>M</td>
<td>UC</td>
<td>pT3bN1</td>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>DM</td>
<td>Dead</td>
<td>3.6</td>
<td>DOD</td>
<td>S249C</td>
<td>T,N</td>
</tr>
<tr>
<td>VCA047</td>
<td>56</td>
<td>M</td>
<td>UC</td>
<td>pT4aN1</td>
<td>3</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>Alive</td>
<td>11</td>
<td>FOD</td>
<td>S249C</td>
<td>T,N</td>
</tr>
<tr>
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<td>M</td>
<td>UC</td>
<td>pT4aN2</td>
<td>3</td>
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<td>Yes</td>
<td>DM</td>
<td>Dead</td>
<td>2.6</td>
<td>DOD</td>
<td>S249C</td>
<td>T,N</td>
</tr>
</tbody>
</table>

AC: Adjuvant Chemotherapy; M: Male; F: Female; UC: Urothelial Carcinoma; UC+SCC: Urothelial carcinoma with squamous differentiation; T: Tumor; N: Node; DM: Distant Metastasis; FOD: Free of Disease; DOD: Dead of Disease.
**Table 3a** - FGFR3 expression and FGFR3 mutation (cystectomy specimens) in a subset of 357/494 cases from the international radical cystectomy cohort.

<table>
<thead>
<tr>
<th>FGFR3 mutation in cystectomy</th>
<th>FGFR3 expression in cystectomy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Normal</td>
<td>280</td>
</tr>
<tr>
<td>Mutated</td>
<td>Over-expression</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>287</td>
</tr>
</tbody>
</table>

**Table 3b** - FGFR3 expression in cystectomy and corresponding metastatic lymph nodes in a subset of 72/117 pN+ cases from the international radical cystectomy cohort.

<table>
<thead>
<tr>
<th>FGFR3 expression in positive node</th>
<th>FGFR3 expression in cystectomy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>57</td>
</tr>
<tr>
<td>Over-expression</td>
<td>Over-expression</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>61</td>
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</tbody>
</table>
Supplemental Table 1 - Distribution (frequencies) of samples according to the primary tumors pathologic stage and FGFR3 mutation status among either N0 or N+ cases in the International (A) and French (B) cohorts. Please note that adjuvant chemotherapy was given to all the patients in the French cohort and to 83/494 (17%) patients in the international cohort.

**A**

<table>
<thead>
<tr>
<th></th>
<th>pTa, pT1, pTis</th>
<th>pT2</th>
<th>pT3</th>
<th>pT4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>47</td>
<td>93</td>
<td>106</td>
<td>40</td>
<td>286</td>
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<tr>
<td>Mutated</td>
<td>23</td>
<td>11</td>
<td>14</td>
<td>5</td>
<td>53</td>
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<tr>
<td>N+</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
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<td>29</td>
<td>81</td>
<td>34</td>
<td>146</td>
</tr>
<tr>
<td>Mutated</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>133</td>
<td>205</td>
<td>84</td>
<td>494</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th></th>
<th>pTa, pT1, pTis</th>
<th>pT2</th>
<th>pT3</th>
<th>pT4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
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<td>8</td>
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</tr>
<tr>
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<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>3</td>
<td>17</td>
<td>46</td>
<td>29</td>
<td>95</td>
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<tr>
<td>Mutated</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Total</td>
<td>3</td>
<td>17</td>
<td>60</td>
<td>45</td>
<td>120</td>
</tr>
</tbody>
</table>
**Supplemental Table 2** - *FGFR3* mutation type in 67 mutated radical cystectomy samples. The *FGFR3* mutations G372C, A393E, K652M, K652T, K652E and K652Q were not detected in this radical cystectomy series. Of note, the types of *FGFR3* mutations in the TUR series (*n*=21) can be derived from Figure 1.

<table>
<thead>
<tr>
<th>FGFR3 mutation type</th>
<th>Mutations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R248C</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>S249C</td>
<td>45 (67%)</td>
</tr>
<tr>
<td>S373C</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Y375C</td>
<td>11 (16%)</td>
</tr>
<tr>
<td>G382R</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>67 (100%)</strong></td>
</tr>
</tbody>
</table>
