

# ALK alterations in adult renal cell carcinoma: frequency, clinicopathologic features and outcome in a large series of consecutively treated patients

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Chromosomal rearrangements involving the anaplastic lymphoma kinase gene (*ALK*) at 2p23 result in fusion with various partner genes leading to aberrant production of oncogenic protein products in multiple tumor types. Recently, the *ALK* protein inhibitor crizotinib was shown to be an effective therapy in patients with *ALK*-rearranged non-small cell lung cancer. The goal of this study was to determine the frequency of *ALK* alterations in adult renal cell carcinoma (RCC) and define associated clinicopathologic features and outcome. RCCs from a cohort of 534 consecutive surgically treated adult patients were analyzed for alterations of *ALK* by fluorescence *in situ* hybridization. *ALK* rearrangements were identified in 2 of 534 (<1%) RCCs. Both showed similar histologic features and the patients had a poor outcome. *ALK* copy number gain was identified in 54 (10%) RCCs. In clear cell type RCC (CCRCC), *ALK* copy number gain was significantly associated with tumor size ( $P=0.02$ ) and nuclear grade ( $P<0.001$ ), and with a worse 10-year cancer-specific survival vs similar patients lacking *ALK* copy number gain ( $P=0.03$ ). *ALK* rearrangement is rare in adult RCC but may be associated with distinct histological features and poor outcome. Another potential mechanism to elevate *ALK* expression, increased *ALK* gene copy number, was observed in 10% of adult CCRCC, where it is associated with a higher tumor grade and poorer outcome. Additional studies are necessary to determine whether patients RCCs with *ALK* rearrangement and/or those with an increase in *ALK* copy number would benefit from *ALK* inhibitor treatment. *Modern Pathology* (2012) 25, 1516–1525; doi:10.1038/modpathol.2012.107; published online 29 June 2012

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Chromosomal rearrangements involving the anaplastic lymphoma kinase gene (*ALK*) at 2p23 were first recognized in anaplastic large cell lymphoma.<sup>1</sup> The characteristic translocation was shown to result in fusion of the 3' portion of *ALK* with the 5' portion of *NPM1* at 5q35 to produce a chimeric oncogene.<sup>2</sup> Recurrent rearrangements of *ALK* have since been described with numerous partner genes in anaplastic lymphoma and other hematolymphoid malignancies

as well as in solid tumors such as inflammatory myofibroblastic tumors, esophageal squamous cell carcinoma, breast carcinoma, colonic adenocarcinoma, lung adenocarcinoma and, recently, pediatric renal cell carcinoma (RCC).<sup>3–9</sup>

The *ALK* protein is a membrane-associated tyrosine kinase belonging to the insulin receptor superfamily. Outside of the embryonic state, *ALK* expression is normally confined to the central nervous system.<sup>2</sup> Chromosomal rearrangements resulting in *ALK* gene fusion lead to aberrant *ALK* activation by formation of chimeric protein products, which are expressed in a cytoplasmic and/or membranous distribution, depending on the fusion partner. The *ALK* fusion products have significant oncogenic activity.<sup>10–14</sup> Because of the expression

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pattern, ALK fusion products have become an enticing chemotherapeutic target. Recently, the ALK inhibitor crizotinib was shown to be an effective therapy in patients with *ALK*-rearranged non-small cell lung cancers.<sup>15–17</sup>

The present study involved screening RCCs from a large cohort of surgically treated adult patients from a single institution for *ALK* alterations. The goal was to define the frequency of *ALK* alterations, including rearrangements and copy number gain, in a comprehensive manner. Additionally, we wished to identify the associated clinical and pathological features as well as any relationship between patient outcome and *ALK* locus alteration.

## Materials and methods

### Patient Selection

The study was first approved by the Institutional Review Board. Using the Mayo Clinic Nephrectomy Registry, 647 patients treated with radical or partial nephrectomy for unilateral, sporadic, clear cell type RCC (CCRCC) or PRCC were identified. This included 334 patients with clear-cell RCC consecutively treated from 1990 to 1994 and 313 patients with PRCC consecutively treated from 1970 to 2002.

### Clinical and Pathological Features

Clinical features studied included age at surgery, gender, and symptoms at diagnosis. Patients with a palpable mass, abdominal discomfort, gross hematuria, acute onset varicocele, or constitutional symptoms including rash, sweats, weight loss, fatigue, early satiety, or anorexia were considered symptomatic at diagnosis. Pathologic features studied included histologic subtype, tumor size, the 2010 primary tumor, regional lymph node, and distant metastases classifications, nuclear grade, coagulative tumor necrosis, sarcomatoid differentiation, and multifocality. A urologic pathologist (JCC) reviewed the microscopic slides from all tumor specimens for the pathologic features of interest. Grade was based only on nucleolar prominence other than grade 4. Tumors with nucleoli readily visible at  $\times 100$  magnification, filling at least one field, were classified as grade 3. Tumors with nucleoli visible at  $\times 200$  magnification in one field were considered grade 2. Tumors with inconspicuous nucleoli were classified as grade 1. Tumors were considered grade 4 if sarcomatoid differentiation was present or the lesion contained markedly pleomorphic or multinucleate giant cells. SSIGN scores were calculated for patients with CCRCC.<sup>18</sup> Vital status for patients in the Nephrectomy Registry is updated yearly through a review of medical

records and correspondence with patients and their local physicians.

### Tissue Preparation and ALK FISH Analysis

Tissue microarrays (TMAs) were constructed using formalin-fixed, paraffin-embedded tumor samples from all 808 patients. For each tumor, a representative tissue block was selected based on review of the H&E sections, and four, 0.6 mm cores of tumor were taken from each patient block and placed in a recipient block. Non-neoplastic kidney tissue cores were also incorporated to serve as location markers and control tissues. From the TMAs, 5  $\mu$ m sections were cut and slides prepared for fluorescence *in situ* hybridization (FISH) analysis. Slides were hybridized with a commercially available dual-color break-apart *ALK* FISH probe set (Abbott Molecular, Abbott Park, IL, USA) according to the previously established protocols.

FISH patterns were established by assessment of 200 interphase tumor nuclei. If the tissue was not sufficient to analyze 200 nuclei or the signals were not interpretable, the tumor was removed from the study. Values for gain of intact *ALK* copies (denoted as F for fusion signal) were reported if at least 5% of the tumor nuclei showed that particular number of additional fusion signals. For example, a tumor was denoted as 2–4F that had 4F signals in 10 of 200 nuclei (5%), 3F signals in 30 of 200 nuclei (15%), and 2F signals in the remaining nuclei. Alternatively, a tumor with 2 of 200 nuclei (1%) showing 8F signals and the remaining nuclei having 3F signals was given a score of 3F. The 5% value was selected to reduce the fusion signal artifact due to nuclear overlap while maintaining sensitivity for the presence of additional *ALK* genes in a small subset of tumor cells due to tumor heterogeneity. Rearrangement of *ALK* was considered present if  $\geq 15\%$  of nuclei had a FISH signal pattern, indicating split of the *ALK* locus (1R1G1F). This 15% cutoff was based on clinical validation of the *ALK* probe for use on paraffin-embedded tissue sections in the Mayo cytogenetics laboratory.

For cases that showed *ALK* signal split, FISH was repeated on whole sections cut from the original paraffin-embedded tumor blocks to confirm the *ALK* rearrangement pattern and determine if intratumoral FISH signal heterogeneity was present.

In addition, whole sections of tumor were interrogated for rearrangements of *TFE3* by FISH using a homebrew dual color break-apart probe set. The *TFE3* probe set was constructed with bacterial artificial chromosomes selected using the University of California Santa Cruz Biotechnology Genome Browser and Database (<http://genome.ucsc.edu>, genome assembly hg18), and then obtained from Invitrogen (Carlsbad, CA, USA). DNA was isolated from bacterial cultures by a Plasmid Maxi Kit (QIAGEN, Valencia, CA, USA) and fluorescently

labeled via nick translation (Abbott Molecular, Des Plaines, IL, USA). Clone specificity was verified by PCR and by FISH on metaphases from normal male blood specimens. Once individually validated, the clones were combined for use as a probe set. The 3' portion of *TFE3* is detected by RP11-416B14 and RP11-1137J13 (273 kb total size) labeled in SpectrumGreen and the 5' portion of *TFE3* is covered by RP11-1037C20 and RP11-315L18 (416 kb total size) labeled in SpectrumOrange. For each tumor, 200 interphase nuclei were scored.

### Immunohistochemistry

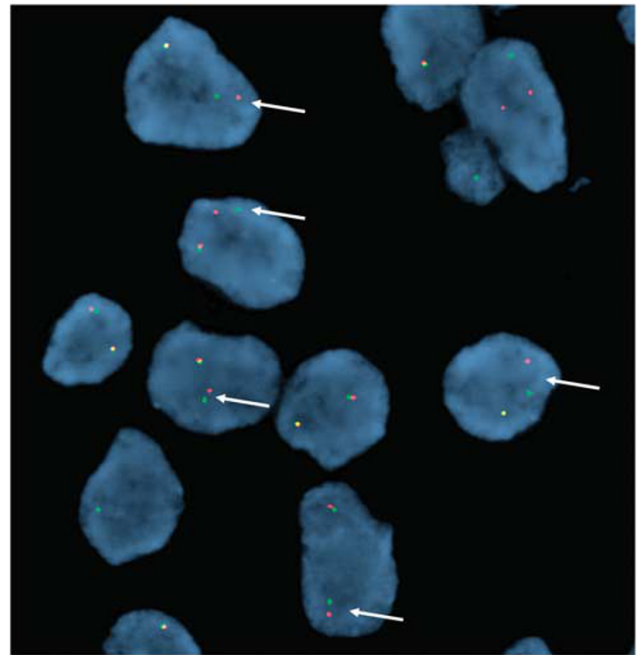
Immunohistochemistry was performed according to the manufacturer's protocol on formalin-fixed, paraffin-embedded tumor tissue from the two RCCs showing *ALK* rearrangement by FISH (patients 1 and 2) using the following antibodies directed against the following antigens: ALK protein/CD246 (Dako, Carpinteria, CA, USA); CD10 (Novocastra, Newcastle Upon Tyne, UK); keratin AE1/AE3 (Dako); CAM5.2 (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA); keratin 7 (Dako); HMB45 (Dako); Melan A (Dako); Napsin A (Novocastra); P504S (Biocare Medical, Concord, CA, USA); vimentin (Dako).

### Statistical Methods

Comparisons of clinical and pathological features were evaluated using Wilcoxon rank sum,  $\chi^2$ , and Fisher's exact tests. Overall and cancer-specific survivals were estimated using the Kaplan–Meier method and compared using log-rank tests. Associations with death from any cause and death from RCC were further evaluated using Cox proportional hazards regression models and summarized with hazard ratios and 95% confidence intervals (CIs). Statistical analyses were performed using the SAS software package (SAS Institute, Cary, NC, USA). All tests were two-sided and *P*-values <0.05 were considered statistically significant.

### Results

Of the 647 tumors studied, *ALK* FISH assessment was successful in 534 tumors consisting of 250 CCRCC and 284 PRCC. FISH analysis was unsuccessful in 113 tumors (84 CCRCC and 29 PRCC), primarily due to insufficient tumor tissue in the TMA resulting from loss of cores on sectioning. Among patients with PRCC, those with an unsuccessful assessment were less likely to have necrosis compared with those with successful assessments (24% vs 50%; *P* = 0.009). Within each subtype, none of the other clinical or pathological features studied were significantly different between those with successful and unsuccessful assessments of *ALK* alterations. Therefore, FISH success rate was not



**Figure 1** Interphase fluorescence *in situ* hybridization to identify the rearrangement of anaplastic lymphoma kinase (*ALK*) using a commercially available *ALK* break-apart probe (Abbott Molecular) on the tumor from patient 1. Abnormal tumor cells show one red (R), one green (G), and one fusion (F) signal pattern indicating one rearranged *ALK* gene (white arrows) and one intact *ALK* gene.

likely to have biased the sample population with regard to tumor features.

### *ALK* Rearrangement

Rearrangement of the *ALK* gene was identified in 2 of 534 RCCs. Both tumors were originally diagnosed as PRCC. By FISH, both showed a 1R1G1F signal pattern indicating one rearranged *ALK* locus and one intact *ALK* locus (Figure 1). Because of the small number of positive cases, further statistical analysis with regard to clinical features was not possible. However, clinical information on both the patients was available (Table 1). Both the patients had poor clinical outcomes progressing to death from disease in <5 years.

Histologically, both tumors with *ALK* rearrangement had very similar features. The tumor from patient 1 demonstrated a papillary architecture (Figure 2a) and was comprised predominantly by cells with abundant clear to slightly eosinophilic cytoplasm and nuclei with irregular contours and indistinct to small nucleoli. Psammoma bodies and foamy macrophages were present (Figure 2b and c). Coagulative necrosis was not observed. The tumor from patient 2 was, in some areas, nearly histologically identical to the tumor from patient 1, also showing papillary structures lined by cells with abundant clear to eosinophilic cytoplasm (Figure 2d). These areas had marked hemosiderin deposition and occasional psammoma bodies. Also



present were areas with papillary structures lined by cells with scant cytoplasm and small nuclei with inconspicuous nucleoli (Figure 2e). Cells in these areas lined the papillary structures in a single layer. In addition, there were areas showing a solid growth pattern where the tumors cells had primarily eosinophilic cytoplasm and the nuclei were larger with more prominent nucleoli (Figure 2f). The

transition between histologically distinct regions was abrupt (Figure 2g). This tumor also had significant coagulative necrosis.

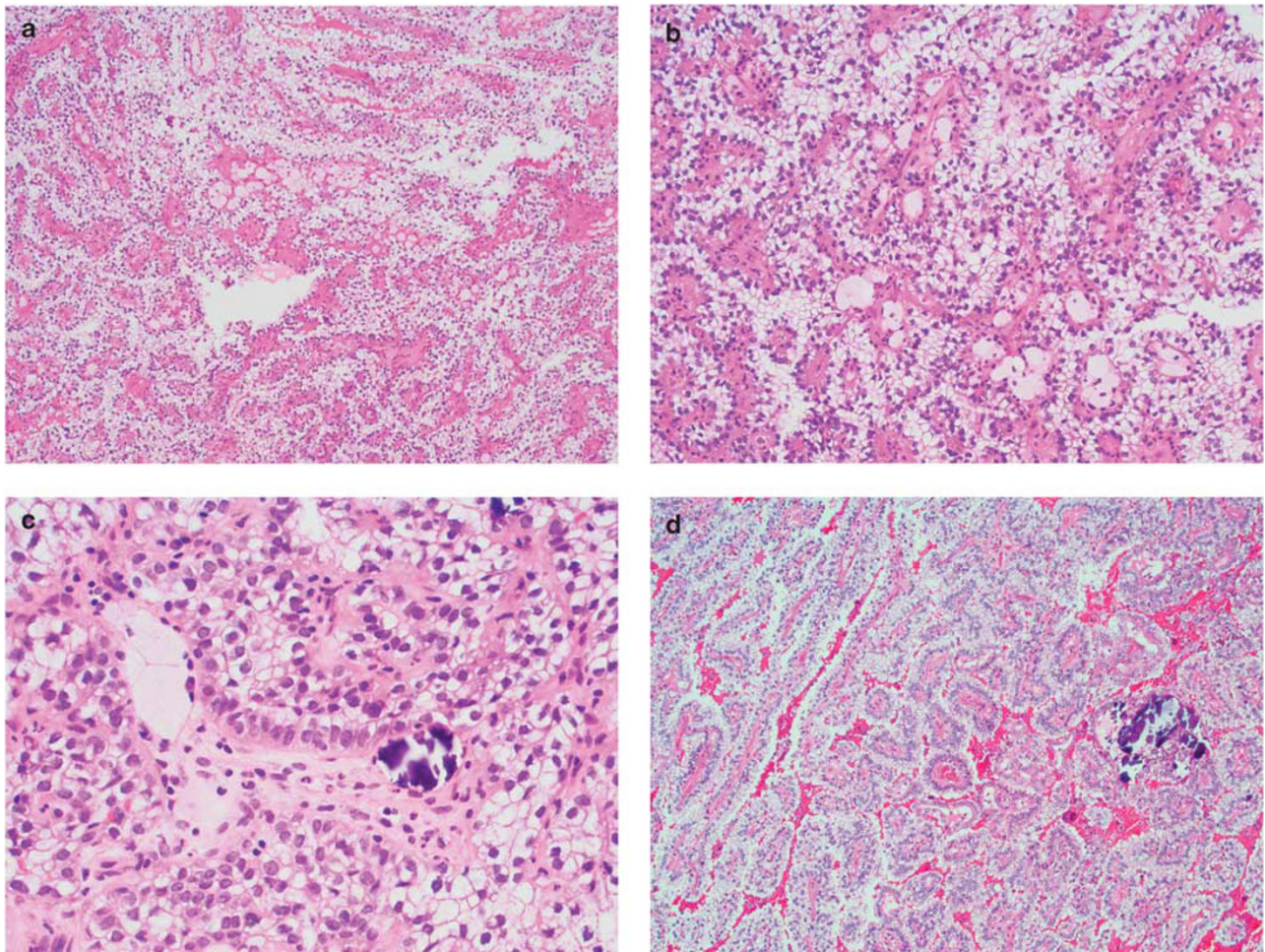
Confirmatory *ALK* FISH analysis on whole tumor sections showed a 1R1G1F signal pattern that was present uniformly throughout all cells in both tumors, even within both the histologically distinct regions of the tumor from patient 2. Immunohistochemistry

**Table 1** Clinical and pathological results for patients with *ALK*-rearranged renal cell carcinoma

Patient	Age	Gender	RCC subtype	Tumor size (cm)	pT <sup>a</sup>	pN <sup>a</sup>	M <sup>a</sup>	Grade	Necrosis	Sarcomatoid features	Multifocal	Years to death from RCC
1	61	Male	Papillary	5	1B	X	0	2	No	No	No	4
2	59	Male	Papillary	5.5	1B	X	0	3	Yes	No	No	1.4

Abbreviations: *ALK*, anaplastic lymphoma kinase; RCC, renal cell carcinoma, X, not assessed.

<sup>a</sup>Pathological staging based on the American Joint Committee on Cancer Staging, 7th edition, 2010.



**Figure 2** Histological features of renal cell carcinomas with anaplastic lymphoma kinase (*ALK*) rearrangements. The tumor from patient 1 showed a papillary architecture with papillae lined by cells with clear to eosinophilic cytoplasm and small nuclei with irregular contours and indistinct to small nucleoli (a). Foamy histiocytes were seen within fibrovascular cores of papillae (b) and psammoma bodies were present (c). The tumor from patient 2 primarily showed papillary structures and cells with clear to eosinophilic cytoplasm, similar to the features seen in the tumor from patient 1 (d). In addition, the tumor showed regions with papillae lined by small cuboidal cells with scant cytoplasm (e) as well as areas of solid growth, with cells showing primarily eosinophilic cytoplasm, larger nuclei, and more prominent nucleoli (f). The transition between areas with distinct morphology was abrupt (g), however rearrangement of *ALK* was uniform throughout the tumor.



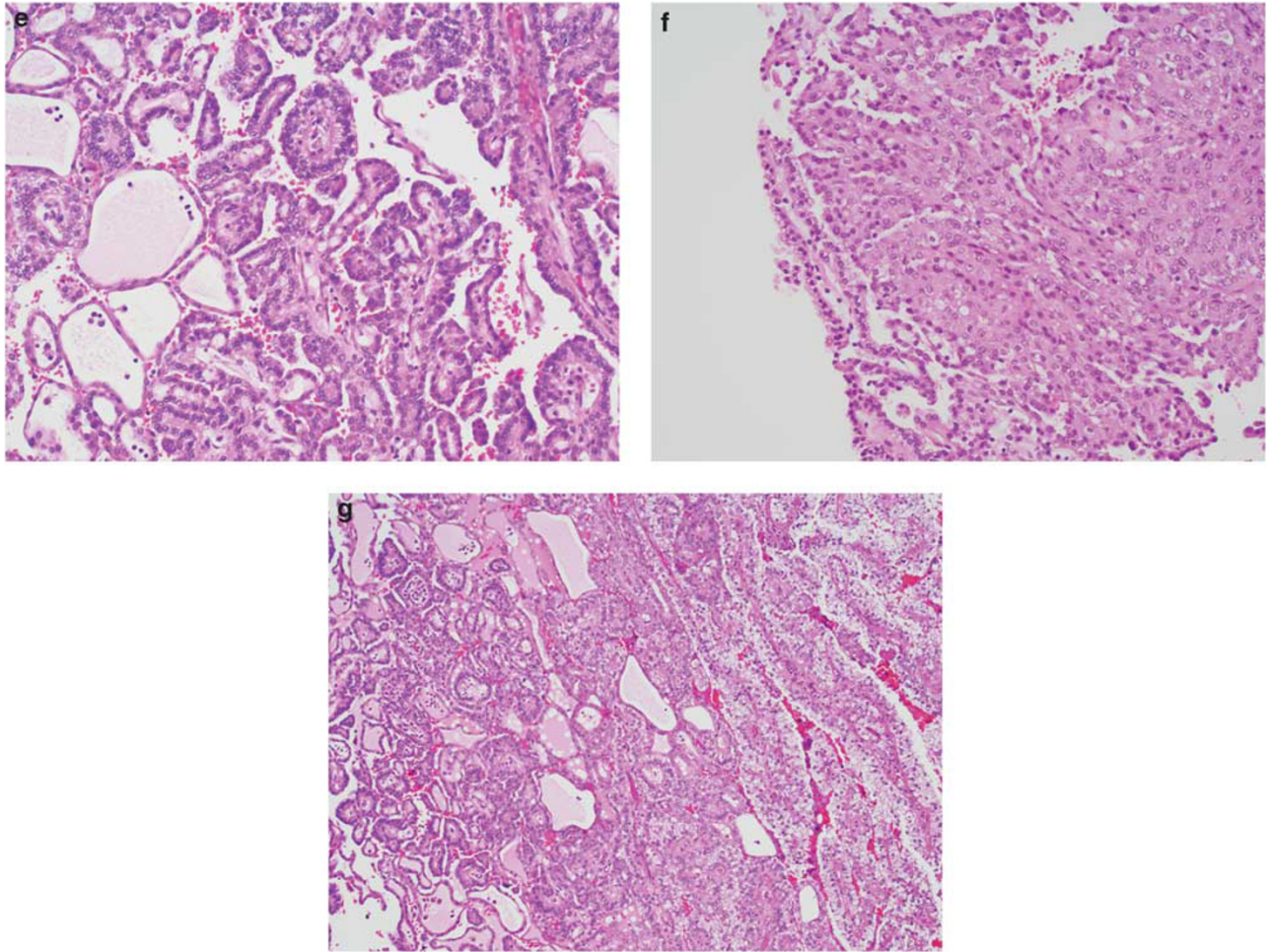


Figure 2 Continued.

demonstrated overexpression of ALK protein in both tumors, with weak cytoplasmic staining in the tumor from patient 1 and moderate to focally strong cytoplasmic staining in the tumor from patient 2. Both tumors also showed strong positivity for keratin 7, P504S, AE1/AE3, vimentin and CAM5.2, focal staining for CD10, and were negative for Napsin A, HMB45, and Melan A. Both tumors were negative for *TFE3* rearrangement by FISH.

#### ALK Copy Number Gains

ALK copy number was also assessed in 532 CCRCC and PRCC (*ALK*-rearranged tumors were excluded). Among 250 CCRCC, 146 (58%) demonstrated 2F signals (2 intact *ALK* loci) in  $\geq 95\%$  of nuclei, 62 (25%) had 3–4F signals present in  $\geq 5\%$  of nuclei, and 42 (17%) had  $\geq 5F$  signals (range 5F–11F; median 6F) in  $\geq 5\%$  of nuclei. Among the 282 PRCC, similar signals were identified in 211 (74%), 59 (21%), and 12 (4%), respectively. Of the PRCC with  $\geq 5F$  signals, the range of signals was 5F to 10F with a median of 6F. The distribution of ALK

copy number gains differed significantly between patients with CCRCC and PRCC ( $P < 0.001$ ).

#### Clear Cell Renal Cell Carcinoma

Comparisons of *ALK* gene copy number gains to clinical and pathological features for the 250 patients with CCRCC are summarized in Table 2. The presence of *ALK* gain by FISH was significantly associated with tumor size and nuclear grade. For example, only 36% of the patients with 2F signals in all nuclei had high-grade tumors compared with the higher rate of 47% of the patients with 3–4F signals in  $\geq 5\%$  of nuclei and 79% of the patients with  $\geq 5F$  signals in  $\geq 5\%$  of nuclei ( $P < 0.001$ ).

At last follow-up, 180 patients with CCRCC died at a mean of 7.0 years following surgery (median 6.4; range 0–20). Among the 70 patients still alive at last follow-up, the mean duration of follow-up was 15.9 years (median 16.8; range 0–21). Five patients died from unknown causes and could not be included in the analyses of cancer-specific survival. Of the remaining 245 patients, 90 died from RCC at a mean

**Table 2** Comparison of ALK copy number gain with clinical and pathological features for 250 patients with clear cell type renal cell carcinoma

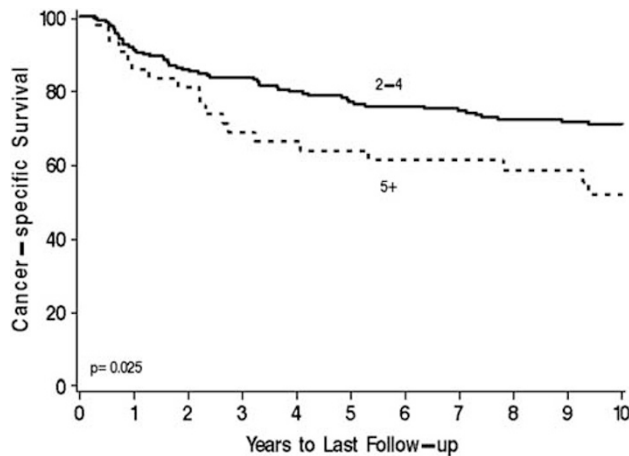
Feature	ALK copy number gain			P-value
	2F (N = 146)	3-4F (N = 62)	≥ 5F (N = 42)	
<i>Feature</i>		<i>Mean (median; range)</i>		
Age at surgery (years)	62.6 (64; 32-85)	62.0 (64; 35-84)	62.9 (66; 37-83)	0.85
Tumor size (cm; N = 249)	6.5 (5.8; 1.0-18.0)	6.9 (6.0; 1.0-22.0)	7.9 (6.8; 3.7-16.0)	0.017
<i>Gender</i>		<i>N (%)</i>		
Female	58 (40)	24 (39)	12 (29)	0.41
Male	88 (60)	38 (61)	30 (71)	
<i>Symptoms</i>				
Constitutional symptoms	97 (66)	42 (68)	33 (79)	0.32
	37 (25)	17 (27)	15 (36)	0.42
<i>2010 primary tumor classification (N = 249)</i>				
pT1a	48 (33)	13 (21)	2 (5)	0.020
pT1b	35 (24)	19 (31)	15 (36)	
pT2a	13 (9)	8 (13)	6 (14)	
pT2b	9 (6)	3 (5)	3 (7)	
pT3a	28 (19)	13 (21)	12 (29)	
pT3b	9 (6)	4 (6)	2 (5)	
pT3c	3 (2)	2 (3)	0	
pT4	0	0	2 (5)	
<i>2010 primary tumor classification (N = 249)</i>				
pT1, pT2	105 (72)	43 (69)	26 (62)	0.42
pT3, pT4	40 (28)	19 (31)	16 (38)	
<i>2010 regional lymph node involvement</i>				
pNX	110 (75)	52 (84)	26 (62)	0.13
pN0	30 (21)	8 (13)	14 (33)	
pN1	6 (4)	2 (3)	2 (5)	
<i>Distant metastases</i>				
M0	134 (92)	56 (90)	37 (88)	0.76
M1	12 (8)	6 (10)	5 (12)	
<i>Nuclear grade</i>				
1	19 (13)	3 (5)	1 (2)	<0.001
2	75 (51)	30 (48)	8 (19)	
3	40 (27)	28 (45)	23 (55)	
4	12 (8)	1 (2)	10 (24)	
<i>Nuclear grade</i>				
1, 2	94 (64)	33 (53)	9 (21)	<0.001
3, 4	52 (36)	29 (47)	33 (79)	
Coagulative tumor necrosis	39 (27)	15 (24)	18 (43)	0.08
Sarcomatoid differentiation	7 (5)	1 (2)	2 (5)	0.66
Multifocality	9 (6)	1 (2)	0	0.19
SSIGN scores	3.4 (2.5; 0-14)	3.6 (3; 0-11)	5.3 (5; 0-11)	<0.001

Abbreviation: ALK, anaplastic lymphoma kinase.

of 4.8 years following the surgery (median 3.0; range 0-18). Overall and cancer-specific survival rates (95% CI; number still at risk) at 10 years following surgery were 50% (44-57; 122) and 67% (62-74; 118), respectively.

Among the patients with 2F, 3-4F, and ≥5F signals, there were 99, 47, and 34 deaths, respectively, including 45, 23, and 22 deaths from RCC. Overall survival rates (95% CI; number still at risk) at 10 years following surgery for patients with 2F, 3-4F, and ≥5F signals were 52% (44-60; 73), 55%

(44-69; 33), and 38% (26-56; 16), respectively ( $P=0.35$ ). Cancer-specific survival rates (95% CI; number still at risk) at 10 years following surgery for patients with 2F, 3-4F, and ≥5F signals were 72% (64-80; 70), 69% (58-83; 32), and 52% (38-70; 16), respectively. Although the difference in cancer-specific survival among all three groups did not reach statistical significance ( $P=0.07$ ), the cancer-specific survival was significantly worse for patients with ≥5F signals compared with those with 2F and 3-4F signals combined. Specifically, the 10-year



**Figure 3** Cancer-specific survival by anaplastic lymphoma kinase (*ALK*) copy number gain for 250 patients with clear cell renal cell carcinoma (CCRCC) classified into two groups of 2–4 copies and  $\geq 5$  copies of *ALK*. Patients with tumors classified as having  $\geq 5$  copies compared with 2–4 copies had a significantly worse outcome ( $P=0.025$ ).

cancer-specific survival rate (95% CI; number still at risk) for patients in the combined 2–4F group was 71% (64–78; 102) compared with 52% (38–70; 16) for patients in the  $\geq 5$ F signal group ( $P=0.025$ ; Figure 3). This is also demonstrated by the hazard ratio for the association of  $\geq 5$ F signals with death from CCRCC, which was 1.72 (95% CI 1.07–2.79;  $P=0.025$ ), indicating that patients with a tumor showing  $\geq 5$  intact *ALK* signals were 72% more likely to die from RCC compared with patients whose tumor showed 2–4 *ALK* copies. However, this difference was no longer statistically significant after adjusting for nuclear grade (hazard ratio 0.87; 95% CI 0.54–1.43;  $P=0.59$ ). Similarly, after adjusting for the SSIGN score, the hazard ratio for the association of 5 + F signals with death from RCC was 1.31 (95% CI 0.81–2.13;  $P=0.27$ ).

### Papillary Renal Cell Carcinoma

Comparisons of *ALK* copy number gains with clinical and pathological features for the 282 patients with PRCC (after excluding the two patients with *ALK* rearrangement) are summarized in Table 3. *ALK* copy number gains were significantly associated with patient gender; 88 and 86% of patients in the 2F and 3–4F groups were male compared with 50% of patients with  $\geq 5$ F ( $P=0.001$ ).

At last follow-up, 162 patients with PRCC died at a mean of 7.8 years following surgery (median 6.0; range 0–35). Among the 120 patients still alive at last follow-up, the mean duration of follow-up was 12.6 years (median 11.9; range 0–39). Five patients died from unknown causes and could not be included in analyses of cancer-specific survival. Of the remaining 277 patients, 34 died from RCC at a mean of 3.4 years following surgery (median 2.7; range 0–17). Overall and cancer-specific survival

rates (95% CI; number still at risk) at 10 years following surgery were 60% (54–66; 135) and 86% (82–91; 133), respectively.

Among patients in the 2F, 3–4F, and  $\geq 5$ F tumor groups, there were 130, 25, and 7 deaths, respectively, including 28, 4, and 3 deaths from RCC. Overall survival rates (95% CI; number still at risk) at 10 years following surgery for patients in the 2F, 3–4F, and  $\geq 5$ F tumor groups were 58% (51–65; 99), 71% (60–84; 30), and 50% (28–88; 6), respectively ( $P=0.19$ ). Cancer-specific survival rates (95% CI; number still at risk) at 10 years following surgery for patients in the 2F, 3–4F, and  $\geq 5$ F tumor groups were 85% (79–90; 97), 93% (86–100; 30), and 80% (59–100; 6), respectively ( $P=0.33$ ). The 10-year cancer-specific survival rate (95% CI; number still at risk) for patients with 2–4 *ALK* signals was 87% (82–91; 127) compared with 80% (59–100; 6) for patients with  $\geq 5$  *ALK* signals ( $P=0.55$ ).

### Discussion

Based on recent clinical studies in lung adenocarcinoma, the *ALK* tyrosine kinase receptor is a promising target for directed therapy with crizotinib, an inhibitor of *ALK* and *ALK*-variant proteins resulting from rearrangement of the *ALK* locus.<sup>15–17</sup> Therefore, the identification of *ALK* rearrangements in other tumor types has gained importance owing to the potential for a similar treatment response.<sup>19</sup> Rearrangement of *ALK* has recently been reported in pediatric RCC.<sup>20,21</sup> However, to our knowledge, no study has yet studied *ALK* alterations in a large series of adult RCC. The goal of the present study was to comprehensively analyze alterations of the *ALK* locus, specifically rearrangements and copy number gain, in adult RCC to identify the frequency and nature of *ALK* alterations and to determine if there was a relationship between these alterations and clinical or pathological findings and/or clinical behavior. This was accomplished by screening tumors from a large population of adult patients consecutively treated by surgery at Mayo Clinic for either PRCC or CCRCC.

We identified rearrangement of the *ALK* locus in 2 of 534 adult RCC, representing <1% of all RCC and 2% of all PRCC studied, suggesting that *ALK*-rearranged tumors characterize a small proportion of adult RCC. Both patients with RCC harboring *ALK* rearrangements had a poor clinical outcome, dying from disease in <5 years, despite having a low stage at the time of original surgery, suggesting that these RCCs may be an aggressive subset. The tumors from both showed similar and distinctive histological features and were defined primarily by a papillary architecture populated by cells with clear to eosinophilic cytoplasm. By immunohistochemistry, these tumors demonstrated a phenotype consistent with PRCC, showing the expression of keratin 7 and P504S. Recent studies have also defined a group of



**Table 3** Comparison of ALK copy number gain with clinical and pathologic features for 282 patients with papillary type renal cell carcinoma

Feature	ALK copy number gain			P-value	
	2F (N = 211)	3-4F (N = 59)	≥ 5F (N = 12)		
		<i>Mean (median; range)</i>			
Age at surgery (years)	64.1 (66; 30-89)	62.2 (64; 34-82)	67.3 (65; 52-82)	0.45	
Tumor size (cm; N = 279)	5.5 (4.0; 0.6-24.5)	5.1 (3.8; 1.2-16.0)	5.4 (4.3; 2.5-10.0)	0.71	
<i>Gender</i>		<i>N (%)</i>			
Female	25 (12)	8 (14)	6 (50)	0.001	
Male	186 (88)	51 (86)	6 (50)		
Symptoms	117 (55)	28 (47)	7 (58)	0.53	
Constitutional symptoms	37 (18)	7 (12)	3 (25)	0.43	
<i>2010 primary tumor classification (N = 280)</i>					
pT1a	103 (49)	30 (51)	6 (60)	0.97	
pT1b	44 (21)	14 (24)	3 (25)		
pT2a	18 (9)	8 (14)	2 (17)		
pT2b	17 (8)	2 (3)	0		
pT3a	19 (9)	4 (7)	1 (8)		
pT3b	6 (3)	1 (2)	0		
pT3c	1 (<1)	0	0		
pT4	1 (<1)	0	0		
<i>2010 primary tumor classification (N = 280)</i>					
pT1, pT2	182 (87)	54 (92)	11 (92)		0.60
pT3, pT4	27 (13)	5 (8)	1 (8)		
<i>2010 regional lymph node involvement</i>					
pNX	184 (87)	50 (85)	10 (83)	0.80	
pN0	16 (8)	6 (10)	1 (8)		
pN1	11 (5)	3 (5)	1 (8)		
<i>Distant metastases</i>					
M0	205 (97)	56 (95)	12 (100)	0.61	
M1	6 (3)	3 (5)	0		
<i>Nuclear grade</i>					
1	3 (1)	0	0	0.18	
2	129 (61)	33 (56)	4 (33)		
3	74 (35)	26 (44)	7 (58)		
4	5 (2)	0	1 (8)		
<i>Nuclear grade</i>					
1, 2	132 (63)	33 (56)	4 (33)	0.10	
3, 4	79 (37)	26 (44)	8 (67)		
Coagulative tumor necrosis	101 (48)	29 (49)	10 (83)	0.06	
Sarcomatoid differentiation	4 (2)	0	1 (8)	0.18	
Multifocality	24 (11)	5 (8)	1 (8)	0.79	

Abbreviations: ALK, anaplastic lymphoma kinase.

RCC with architectural features of PRCC but comprised largely of cells with clear cytoplasm similar to our ALK-rearranged RCCs.<sup>22</sup> A subset of these PRCC harbor either *TFE3* or *TFEB* translocations. However, both tumors with ALK rearrangements in this study were tested for concurrent rearrangements of *TFE3* by FISH and were negative. We did not test for rearrangements of *TFEB*, and therefore a concurrent alteration of this locus cannot be excluded. Also, recent studies have identified a subset of RCCs with similar histological features that show overlap with periepthelioid cell tumors.<sup>23</sup> Although the two tumors in this series showed little overlap with the

features of PEComa, we also performed immunohistochemistry for Melan A and HMB45, which were negative in both tumors. It will be important to identify and study additional patients with RCCs harboring ALK rearrangements to determine if tumors with ALK rearrangement represent a more aggressive subset of RCC and to further define a relationship between ALK rearrangement and histological features.

The presence of ALK rearrangement in a certain subset of adult RCCs may be quite significant, given the possibility that such tumors may be sensitive to therapy with the ALK inhibitor criztuzimab. This treatment option becomes even more significant if



ALK rearrangement is indeed associated with a more aggressive subset of RCC. The frequency of ALK-rearranged RCC appears very small, making general screening of adult RCCs unrealistic. It may be that the histological features can be used to identify RCCs with a higher likelihood of harboring ALK rearrangements and therefore substantially diminish the number of tumors that need to be tested. Additionally, as both ALK-rearranged tumors showed overexpression of ALK, immunohistochemistry may be utilized in tumors with suspicious morphology to identify RCCs that may respond to ALK inhibitors, with subsequent FISH testing providing confirmation of ALK rearrangement.

The presence of additional copies of the ALK locus is a frequent finding in lung adenocarcinoma; however, the significance of this finding has yet to be determined. In the current study, our goal was to comprehensively study alterations of the ALK locus in adult RCC. Therefore, we also evaluated tumors for gain of ALK copy number. Each tumor (excluding the two tumors with ALK rearrangement) was defined by the highest ALK copy number present in at least 5% of tumor cells. This 5% cut point was chosen to account for intratumoral heterogeneity with regard to ALK copy number and was based in part on criteria used for HER2 testing in breast cancer.<sup>24</sup> Tumors were assigned to one of three copy number groups: the 2F (normal copy number) group, the 3–4F group, or the  $\geq 5F$  group. The behavior of tumors in the normal copy number group should not be influenced by ALK. Similarly, the presence of one or two additional ALK copies (three or four in total) is not likely to be a functionally important gain of ALK, probably reflecting either trisomy of chromosome 2 or tetraploidization as may be seen in dividing cells. However, the presence of  $\geq 5$  copies of ALK suggests that additional gene copies may have a functional role in oncogenesis. This concept has been established for HER2 testing in breast cancer and MYCN testing in neuroblastoma, although the cutoffs used in our current study vary somewhat from those that define ‘copy number gain’ or ‘amplification’ in HER2 and NMYC testing.<sup>25,26</sup>

We identified 54 RCCs with ALK copy number gain ( $\geq 5$  copies). We also identified an association between the presence of  $\geq 5$  copies of ALK and a poorer cancer-specific survival in patients with CCRCC but not in PRCC. The presence of increased ALK copy number was also significantly associated with prognostic factors including increased tumor size and nuclear grade in CCRCC. However, when adjusted for nuclear grade, the association did not remain significant. This may reflect a role of ALK in tumor progression, which is reflected as a higher tumor nuclear grade. However, it is more likely that this association simply reflects the association between larger nuclear size (reflected in nuclear grade) and an overall increase in chromosome copy number unrelated to the function of

ALK *per se*. Further study is needed to determine if additional intact copies of ALK have a pathogenic role in RCC, and consequently whether patients with such tumors can benefit from directed ALK inhibitor therapy.

In conclusion, we present, what is to our knowledge, the first study to characterize ALK rearrangements and copy number gain in a large series of adult patients treated consecutively for RCC. Our findings indicate that ALK rearrangement is a rare finding in adult RCC but may be associated with distinct histological features and a poor outcome. Patients with RCC harboring ALK rearrangements may benefit from ALK inhibitor therapy and based on the present findings, this subset of tumors may be identifiable based histological and immunophenotypic features.

## Disclosure/conflict of interest

The authors declare no conflict of interest.

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