

cytoplasm and larger nuclei with more prominent nucleoli.

Such findings are in contrast to those reported by both cases with *VCL/ALK* fusion. The right kidney tumor from Marino-Enriquez *et al*<sup>3</sup> had a focal reticular growth pattern, prominent lymphoplasmacytic infiltrate, stromal desmoplasia and solid sheet of polygonal-to-spindle cells with vesicular nuclei and abundant eosinophilic cytoplasm with intracytoplasmic lumina. The immunohistochemistry profile included expression of EMA and the cytokeratins MNF116, AE1/AE3 and CAM5.2, as well as maintaining nuclear INI-1 expression. The final pathological diagnosis was renal medullary carcinoma (RMC). In the study by Debelenko *et al*,<sup>2</sup> the initial needle-core biopsy finding was RMC of sickle cell trait. Analysis of a nephrectomy specimen 5 months later was described as having solid and diffuse sheet-like growth of large polygonal-to-round tumor cells with abundant and vaguely granular eosinophilic cytoplasm characterized by intracytoplasmic lumina and moderately pleomorphic clear nuclei and small nucleoli, occasional grooves and rare vacuoles. Immunohistochemistry demonstrated tumor cell expression of EMA, the cytokeratins AE1/AE3, CAM5.2 and CK7, as well as INI-1 expression, whereas CD10, renal antigen, S-100, HMB45 and WT1 were negative. The final pathological diagnosis was renal cell carcinoma of indeterminate subtype.

It is important to note that RMC often demonstrates significant variation in the microscopic appearance. Considering this, combined with the overlap in patient demographics, pathological features and especially the new genetic results from these two published cases, it suggests that the tumor from the Debelenko *et al*<sup>2</sup> study may be within the RMC spectrum.

Supplementary Information accompanies the paper on Modern Pathology website (<http://www.nature.com/modpathol>)

## Reply to ‘Distinct ALK-rearranged and VCL-negative papillary renal cell carcinoma variants in two adults without sickle cell trait’

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**To the editor:** ALK activation by chromosomal rearrangement in renal cell carcinoma (RCC) was first demonstrated by two independent pediatric pathology studies 2 years ago.<sup>1,2</sup> These reports coincided with the successful results of the clinical trial that tested targeted therapy with crizotinib in ALK-positive lung cancer.<sup>3</sup> Consequently, two

In summary, *ALK* rearrangement in papillary renal cell carcinoma cases in older adults may represent a distinct pathological entity with poorer outcome. Our cases did not have *VCL* as a partner and *TFE3* was not disrupted. This is in contrast to all previously described categories, including kidney tumors with *VCL/ALK* fusion in young black males with sickle cell trait. Such distinction of *ALK*-rearranged pathological entities is of particular importance because of the potential benefit from *ALK* inhibitor therapy in these patients.

### Disclosure/conflict of interest

The authors declare no conflict of interest.

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- 3 Marino-Enriquez A, Ou WB, Weldon CB, *et al*. ALK rearrangement in sickle cell trait-associated renal medullary carcinoma. *Genes Chrom Cancer* 2011;50: 146–153.

recently published large series of adult RCCs cumulatively identified four new *ALK*-rearranged tumors.<sup>4,5</sup> Current data, combined from four independent studies during 2010–2012, include six *ALK*-rearranged tumors in a representative cohort of 884 RCCs of main morphologies arising in pediatric and adult patients of diverse ethnicities (Table 1).

**Table 1** Clinicopathologic characteristics and fusion partners of ALK-rearranged renal cell carcinomas

Study	Number of cases studied	ALK-rearranged cases						
		#	Patient's age and sex	Patient's ethnicity	Stage of disease	Tumor morphology	Fusion partner	Follow-up
Debelenko <i>et al</i> <sup>1</sup>	6	1	16 Years, M	African-American	III	Unclassified <sup>a</sup>	VCL	9 Months, NED
Mariño-Enríquez <i>et al</i> <sup>2</sup>	1	1	6 Years, M	African-American	II	Medullary <sup>a</sup>	VCL	21 Months, NED
Sugawara <i>et al</i> <sup>4</sup>	343	2	36 Years, F	Japanese	I	Unclassified	TPM3	2 Years, NED
			53 Years, F	Japanese	I	Papillary type 2A	EML4	3 Years, NED
Sukov <i>et al</i> <sup>5</sup>	534	2	61 Years, M	Unknown	I	Papillary	Unknown, not VCL	4 Years, DOD
			59 Years, M	Unknown	I	Papillary	Unknown, not VCL	1.4 Years, DOD
Total	884	6 (0.7%)						

DOD, died of disease; F, female; M, male; NED, no evidence of disease.

<sup>a</sup>Retrospective analysis indicates similar morphology of the tumors.

The overall frequency of *ALK* rearrangement in RCC is low (approximately 0.7%) and no rearrangement has been detected in 505 cases of clear-cell RCC.<sup>4,5</sup> Further studies should clarify whether the molecular mechanism of clear-cell RCC governed by loss of *VHL* and *ALK* activation are mutually exclusive. Hereditary and some sporadic papillary RCCs are driven by *MET* tyrosine kinase,<sup>6</sup> and testing of both kinases in papillary RCCs appears to be rational especially because crizotinib combines anti-*ALK* and anti-*MET* activities.

Sukov *et al*<sup>5</sup> found substantial proportions of both clear-cell and papillary RCCs with increased *ALK* gene copy numbers that correlated with poorer outcome in the clear-cell subtype. *ALK* amplifications with activating point mutations are known to occur in neuroblastomas;<sup>7</sup> it would be interesting to explore a possibility of this mechanism in RCCs with *ALK* amplifications.

All authors paid close attention to morphologic and clinical features of *ALK*-positive RCCs and the handful of cases shows several trends. Thus, both pediatric cases affected children of African-American descent carrying the sickle cell trait; the tumors were arising in the renal medulla and exhibited similar morphologies. Mariño-Enríquez *et al*<sup>2</sup> classified their tumor as renal medullary carcinoma (RMC); we<sup>1</sup> did not label ours as RMC because it lacked extreme clinical aggressiveness characteristic of RMC.<sup>8,9</sup> Retention of *INI1* by immunohistochemistry was also not in favor of RMC.<sup>10</sup> In a retrospect, both pediatric cases shared all clinicopathologic characteristics and the same *VCL-ALK* fusion. A consensus should be reached whether they belong to the least aggressive part of the RMC spectrum or favorable clinical course and intact *INI1* are significant enough to distinguish them from RMCs.

Three of four adult cases had papillary morphology. The case of Sugawara *et al*<sup>4</sup> was classified as

papillary type 2A and cases of Sukov *et al*<sup>5</sup> as papillary with clear eosinophilic cytoplasm; all three tumors were positive for CK7. The *ALK* fusion partner of the papillary type 2A RCC of Sugawara *et al*<sup>4</sup> was *EML4*, implicated in most of the cases of pulmonary *ALK*-associated carcinoma; fusion partner(s) of cases of Sukov *et al*<sup>5</sup> are not known, but *VCL*, participating in pediatric *ALK*-positive RCCs, was excluded. The 4th adult *ALK*-positive RCC had different variant morphology and *TPM3* as the *ALK* fusion partner.<sup>4</sup>

Thus, five of six *ALK*-positive RCCs cluster in two groups: two *VCL-ALK*-positive pediatric tumors with characteristic histology (solid growth, abundant eosinophilic cytoplasm, intracytoplasmic lumina) and three adult tumors with papillary architecture and other than *VCL-ALK* fusion partner(s). Morphologic spectrum of *ALK*-rearranged tumors, however, is not limited to these two groups. The common feature of all *ALK*-positive RCCs is eosinophilic cytoplasm mentioned in all studies,<sup>1,2,4,5</sup> but this characteristic is hardly specific. It appears that prediction of *ALK* rearrangement cannot be accurately drawn from tumor histology and at this time it would be impractical to recognize *ALK*-positive RCC as a separate pathologic subtype. However, the broader availability of new generation technologies may change the current tumor classification faster than we think and *ALK*-positive carcinomas, or *ALK*omas, will find their niche.

The overall 5-year survival of renal cancer is 70.6%; however, 17% of patients have distant metastatic disease and in this group the 5-year survival is only 11.6%.<sup>11</sup> Thus, although the frequency of *ALK* rearrangement in RCC is low, patients with distant metastatic disease can be tested and, if positive, tried with crizotinib or other *ALK* inhibitors to determine whether targeted therapies are as effective in RCC as they proved in pulmonary adenocarcinoma.

## Disclosure/conflict of interest

The authors declare no conflict of interest.

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## HER-2 intratumoral heterogeneity

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**To the editor:** We read with great interest the paper published by Seol H *et al*<sup>1</sup> concerning HER-2 intratumoral heterogeneity (IH) in breast cancer.

Nowadays most pathologists involved in HER-2 characterization in breast cancer more or less frequently have to deal with cases of IH in the everyday routine.

While reading this article, we asked ourselves the following question: ‘which could be the best way to write the report for the clinicians?’.

At the present time, our Lab-FISH report shows synthetically if the tumour sample is found to be amplified or unamplified; however, if focal-amplified neoplastic clones are seen, in accordance with what has been recently established in an Italian consensus conference,<sup>2</sup> the percentage of cells with overexpression of the protein or gene amplification (Her-2/CEP-17 ratio >2 or number of HER-2 copies >6 or HER-2 clusters), even if less than 10%, should be added in the report.

So far we have noted however that one ‘final’ question most clinicians frequently ask to us is: ‘how should I consider it amplified or non-amplified?’.

This is likely to be due to the legislation regulating the administration of the drug (<http://www.agenziafarmaco.gov.it/content/trastuzumab>) according to which ‘Herceptin should be used only in patients whose tumours show HER-2 overexpression or

HER-2 gene amplification as determined by an accurate and validated test’.

The same situation occurs for gastric and gastroesophageal junction adenocarcinomas for which the law provides the following: ‘Trastuzumab should be administered only to patients with metastatic gastric cancer whose primary tumours exhibit HER-2 overexpression, defined as IHC 3+ or IHC 2+ together with a positive SISH or FISH result’.

So they both only refer to HER-2 protein overexpression and/or gene amplification and make no mention at all of a possible IH.

We believe that a reasonable way to document HER-2 IH could be to attach the FISH count results to the report and to close the report with a final interpretation of the results obtained. In agreement with Albarracín *et al*,<sup>3</sup> we think that an analytical report completed with a critical evaluation of the results about HER-2 genetic heterogeneity (GH) should be worldwide promoted.

In accordance with the recently published guidelines,<sup>4,5</sup> FISH report should describe: (a) the number of cells analysed; (b) the HER-2 gene copy number per nucleus; (c) the CEP17 copy number per nucleus; (d) the HER-2/CEP17 ratio for each nucleus; (e) the overall average ratio and the s.d.; (f) the number and the percentage of cells, if any, with ratio >2.2, and finally (g) the average ratio in this group of cells.