Published in final edited form as:

Am J Surg Pathol. 2012 October ; 36(10): 1516–1526. doi:10.1097/PAS.0b013e3182613d8f.

Molecular Confirmation of t(6;11)(p21;q12) Renal Cell Carcinoma in Archival Paraffin-embedded Material Using a Break-apart *TFEB* FISH Assay Expands its Clinicopathologic Spectrum

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Abstract

A subset of renal cell carcinomas (RCCs) is characterized by t(6;11)(p21;q12), which results in fusion of the untranslated *Alpha* (*MALAT1*) gene to the *TFEB* gene. Only 21 genetically confirmed cases of t(6;11) RCCs have been reported. This neoplasm typically demonstrates a distinctive biphasic morphology, comprising larger epithelioid cells and smaller cells clustered around basement membrane material; however, the full spectrum of its morphologic appearances is not known. The t(6;11) RCCs differ from most conventional RCCs in that they consistently express melanocytic immunohistochemical (IHC) markers such as HMB45 and Melan A and the cysteine protease cathepsin K but are often negative for epithelial markers such as cytokeratins. *TFEB* IHC has been proven to be useful to confirm the diagnosis of t(6;11) RCCs in archival material, because native *TFEB* is upregulated through promoter substitution by the gene fusion. However, IHC is highly fixation dependent and has been proven to be particularly difficult for *TFEB*. A validated fluorescence in situ hybridization (FISH) assay for molecular confirmation of the t(6;11) RCC in archival formalin-fixed, paraffin-embedded material has not been previously reported. We report herein the development of a break-apart *TFEB* FISH assay for the diagnosis of t(6;11)(p21;q12) RCCs. We validated the assay on 4 genetically confirmed cases and 76 relevant expected negative control cases and used the assay to report 8 new cases that expand the clinicopathologic spectrum of t(6;11) RCCs. An additional previously reported *TFEB* IHC-positive case was confirmed by *TFEB* FISH in 46-year-old archival material. In conclusion, *TFEB* FISH is a robust, clinically validated assay that can confirm the diagnosis of t(6;11) RCC in

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Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website, www.ajsp.com.

Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

archival material and should allow a more comprehensive clinicopathologic delineation of this recently recognized neoplastic entity.

Keywords

TFEB; TFE3; FISH; translocation; renal cell carcinoma

The past decade has witnessed the characterization of a subset of renal cell carcinomas (RCCs) that have chromosomal translocations resulting in gene fusions involving members of the MiT subfamily of transcription factors. The best known members of this subset are the Xp11 translocation RCCs, which were recognized by the World Health Organization in 2004.¹ These neoplasms comprise the majority of pediatric RCCs and a smaller percentage of adult RCCs and classically feature a papillary architecture lined by clear cells with extensive psammomatous calcification.^{2–8} Xp11 translocation RCCs are characterized by gene fusions involving the *TFE3* transcription factor gene that maps to this locus; at least 5 different fusion partners for *TFE3* have been identified to date.^{2,3,5,9,10}

A less well-known member of the translocation RCC family is the subset of RCCs characterized by t(6;11)(p21;q12), which results in fusion of the untranslated *Alpha* (*MALAT1*) gene on 11q12 to the related *TFEB* gene on 6p21.^{11–14} Only 21 genetically confirmed cases of t(6;11) RCCs have been reported.^{5,11–23} This neoplasm typically demonstrates a distinctive biphasic morphology, comprising larger epithelioid cells and smaller cells clustered around basement membrane material; however, the full spectrum of its morphologic appearances is not known. The t(6;11) RCCs differ from most conventional RCCs in that they consistently express melanocytic immunohistochemical (IHC) markers such as HMB45, Melan A, and the cysteine protease cathepsin K^{24,25} but are either negative or only focally positive for epithelial markers such as cytokeratins.^{11,12} On the basis of clinical, pathologic, and genetic similarities between the t(6;11) RCCs and the Xp11 translocation RCCs, we have proposed that these 2 neoplasms be classified together under the broader category of “MiT family translocation RCC.”¹²

Molecular confirmation of a diagnosis of a translocation RCC is relatively simple if fresh tissue is available for either cytogenetics or reverse transcriptase polymerase chain reaction assay using primers from the genes known to be involved in the gene fusion. However, in many cases, only archival, formalin-fixed, paraffin-embedded material is available. For the Xp11 translocation RCCs and t(6;11) RCCs, IHC for TFE3 and TFEB, respectively, have proven to be useful for confirming the diagnosis in archival material.^{6,12} This is because both TFE3 fusion proteins and native TFEB are upregulated by promoter substitution by the gene fusions in these 2 RCCs relative to the level of expression of the respective native proteins. However, IHC is highly fixation dependent and has proven to be particularly difficult for TFE3 and TFEB for several reasons. These include the scarcity of genetically confirmed positive controls and the fact that the assays are optimally performed by overnight incubation, which is difficult to automate.²⁶ Recently, break-apart fluorescence in situ hybridization (FISH) assays for *TFE3* gene fusions were developed for archival material^{27–29} and have allowed the expansion of the morphologic spectrum of the Xp11

translocation RCCs.³⁰ A validated FISH assay for molecular confirmation of t(6;11) RCC has not been reported previously.

We report herein the development of a break-apart *TFEB* FISH assay for the diagnosis of t(6;11)(p21;q12) RCCs. We validated the assay on 4 genetically confirmed cases and 76 pertinent negative control cases, confirmed the presence of a *TFEB* gene rearrangement in a previously reported *TFEB* IHC-positive case *from 46 years ago*, and used the assay to report 8 new cases that expanded the clinicopathologic spectrum of t(6;11) RCCs.

MATERIALS AND METHODS

Institutional Review Board Approval

This study was approved by the institutional review board of The Johns Hopkins Medical Institution.

Cases

The known positive cases consisted of 4 cytogenetically confirmed t(6;11)(p21;q12) RCCs. Cases expected to be negative (expected negative control) included 22 clear cell (conventional) RCCs, 10 papillary RCCs, 16 Xp11 translocation RCCs, and 13 chromophobe RCCs. Aside from the Xp11 translocation RCCs, all of these expected negative control cases were typical examples of their respective entities, diagnosed on hematoxylin and eosin-stained sections. The Xp11 translocation RCCs were all confirmed to harbor *TFE3* gene rearrangements by FISH.³⁰ All of the expected negative control cases were negative for *TFEB* by IHC. We also studied 15 perivascular cell neoplasms (PEComas), including 3 arising in the soft tissue, 2 arising in the uterus, and 10 angiomyolipomas arising in the kidney (5 of which were predominantly epithelioid). In addition, we examined 8 previously unreported test cases that we suspected to be t(6;11)(p21;q12) RCCs on the basis of a combination of young patient age, morphologic features (biphasic large cell/small cell population), and IHC profile (Melan A and cathepsin K immunoreactivity, in some cases *TFEB* immunoreactivity, and absence of *TFE3* immunoreactivity) and 1 previously reported case verified only by *TFEB* IHC.¹⁶ IHC analysis was performed as previously described.⁴

Fluorescence In Situ Hybridization

FISH was performed with a probe consisting of 2 contigs that flank the *TFEB* gene on 6p21. The distal contig consists of 3 BAC/PAC clones (RP11-298J23, RP5-973N23, and RP11-533020) labeled with Spectrum Orange, and the proximal contig consists of 2 BAC/PAC clones (RP1-149M18 and RP11-328M4) labeled with Spectrum Green, from BacPac Resources at CHORI (Children's Hospital Oakland Research Institution), designed for research use. Sections of 5 μ m thickness were deparaffinized, dehydrated, and pretreated in a VP 2000 processor (Abbott Molecular), followed by subsequent treatment with pepsin. The probe mixture was applied, and the slides were incubated in a humidified atmosphere (Thermobrite, Abbott Molecular) at 80°C for 8 minutes to denature the probe and target DNA simultaneously, followed by hybridization overnight at 37°C. The slides were then washed in 2 $\times$ SSC/0.3% NP-40 for 2 minutes at 72°C. The nuclei were counterstained with

4,6-diamidino- 2-phenylindole. After hybridization, all slides were maintained at 4°C in the dark. FISH signals were assessed using a Zeiss Axioskop microscope.

Scoring was performed by 6 experienced technicians in The Johns Hopkins Cytogenetics Laboratory. These technicians are designated as A to F in the Supplemental Tables 1 to 3, which list raw scoring data for the positive controls, for 40 expected negative controls, and for test cases. Each observer who scored a tumor scored 60 to 100 nuclei. These included nuclei with the following signals: the expected normal 2 fusion signals reflecting intact *TFEB* alleles; 1 fusion signal; 3 fusion signals; 1 red, 1 green, and 1 fusion signal; 1 red and 1 green signal; 1 green and 1 fusion signal; 1 red and 1 fusion signal; 4 fusion signals; and any other combination rarely observed. The signal pattern of 1 red, 1 green, and 1 intact fusion was the most typical positive pattern for *TFEB* rearrangement. To avoid false positives due to nuclear truncation, nuclei with only a single color were not included in the scoring. To be scored as a split, the signals had to be separated by a signal diameter >1. This was based on the distance between the 2 contigs when the probe was designed and is also considered a standard criterion for most break-apart FISH assays.

To determine a cutoff for a positive result, we examined 12 cases of normal kidney tissue and cases of clear cell RCCs, all of which were negative for rearrangement. The binomial distribution cutoff was calculated using the β inverse function from Microsoft Excel with a 95% confidence level.³¹ Using this method, for a case with 120 scored nuclei to be positive, a cutoff of >15.8% of tumor nuclei with split signals was set (see the Results section).

As shown in Supplemental Tables 1 to 3, all positive control cases were scored by 2 to 6 observers, each of whom scored 100 nuclei. Expected negative control cases were scored by 2 observers, each of whom scored 60 to 100 nuclei. Three test cases were scored by 6 observers, each of whom scored 100 nuclei. Four other test cases were scored by 2 observers, each of whom scored 60 nuclei. One test case was scored by only 1 observer, as the slide broke after viewing. This case was included as its result was clear-cut. The number of observers and cells scored is not identical among the tumors partly because of laboratory staffing. More importantly, after our initial experience with the assay, we found that the results were so clear-cut that interobserver variability was not an issue, and fewer observers were thus needed (ie, 2 rather than 6). The percentage of nuclei scored as split by each observer was tabulated, and the percentages were averaged among the case observers to give the final mean score for each case.

RESULTS

The clinicopathologic features of the positive control cases are summarized in Table 1, as well as the mean percentage of split *TFEB* FISH signals. All 4 genetically confirmed t(6;11) (p21, q12) RCCs demonstrated a high frequency of split *TFEB* FISH signals (mean of mean scores=53.2%, range=31.7% to 73.5%) (Supplemental Table 1, <http://links.lww.com/PAS/A130>). One other cytogenetically confirmed case was considered a test failure, as signals could not be detected in the formalin-fixed, paraffin-embedded tissue, likely because of processing artifacts. In contrast, none of the 76 evaluable negative control cases (22 clear cell RCCs, 10 papillary RCCs, 13 chromophobe RCCs, 16 Xp11 translocation RCCs, and 15

PEComas) demonstrated a high frequency of split *TFEB* FISH signals (mean of mean scores=1.75%, range=0% to 4.2%) (Supplemental Table 2, <http://links.lww.com/PAS/A131>). Five cases, all clear cell RCCs, were test failures as the FISH probe signals could not be detected.

All 9 cases that we suspected to be t(6;11)(p21;q12) RCCs proved to show *TFEB* split signals by FISH, thereby confirming the diagnosis genetically (mean of mean scores=51.4%, range=35.8% to 79.2%) (Fig. 1) (Supplemental Table 3, <http://links.lww.com/PAS/A132>). These cases included 1 previously published case, which was resected 46 years before the *TFEB* FISH assay was applied.¹⁶ This case had been previously shown to be immunoreactive for TFEB and demonstrated the typical biphasic morphology of the t(6;11) RCCs. The other 8 “test cases” in this study have not been published previously and showed features that expand the clinicopathologic spectrum of these neoplasms (Table 2). For example, test case 2 was extensively hyalinized and ossified and contained only small clusters of epithelioid clear cells without a second population of smaller cells embedded in its thick fibrous wall (Fig. 2). Test case 6 was predominantly oncocytic with a papillary architecture (Fig. 3). Two cases (test cases 4 and 8) were extensively cystic, with 1 case (test case 4) having areas that resembled oncocytoma and clear cell RCCs (Fig. 4). Two other neoplasms (test cases 5 and 6) presented with liver metastasis at diagnosis (Fig. 5). One other case (test case 7) arose in a patient with end-stage renal disease. Whereas 6 of these cases demonstrated a second population of smaller cells, in 3 of them the small cell population was only focal. Only 2 cases demonstrated hyalinized basement membrane material, and in none of the cases did the smaller cells cluster around it as is the conventional appearance of t(6;11) RCCs. We also noted that Melan A labeling was consistently more diffuse than labeling with HMB45, which was completely negative in 4 cases. Moreover, of greater diagnostic importance, only 2 of these 8 test cases (cases 2 and 8) demonstrated convincing nuclear TFEB labeling by IHC. In 3 of the other cases, there was focal labeling of questionable significance at the periphery of the lesion but absence of labeling in the interior, suggesting fixation dependence of TFEB labeling (Fig. 5). One of the other cases was completely negative by TFEB IHC, whereas 2 others were weak and equivocal. Hence, the *TFEB* FISH assay resolves cases that could not be proven to be t(6;11) RCC by TFEB IHC.

Parenthetically, all 6 of 6 test cases examined showed nuclear labeling for PAX8, confirming renal origin of the neoplasms and that the tissue fixation permitted nuclear labeling by IHC for some proteins. Sufficient unstained slides from cases 1 and 7 were not available for PAX8 IHC studies.

The clinicopathologic features of the 8 new genetically confirmed cases of t(6;11) RCCs identified in this study, as well as those previously published in the literature, are presented in Table 3. The current study raises the number of genetically confirmed cases from 21 to 30, including the previously published TFEB IHC-positive case mentioned above.

DISCUSSION

We report herein the development of a break-apart FISH assay to detect *TFEB* gene rearrangements that can genetically confirm the diagnosis of t(6;11)(p21;q12) RCCs in archival material. On the basis of clinicopathologic, IHC, and molecular similarities to the Xp11 translocation RCCs, t(6;11) RCCs have been postulated to be part of a family of translocation RCCs harboring gene rearrangements involving the MiT transcription factor family. However, the clinicopathologic features of t(6;11) RCCs have not been well delineated, in large part because only a small number (21) of genetically confirmed cases have been described. This barrier can be overcome if the diagnosis can be confirmed genetically in archival formalin-fixed, paraffin-embedded material. IHC for TFEB is an excellent surrogate for *TFEB* gene rearrangements; however, it suffers from many of the well-known limitations of IHC, including fixation dependence and lot-to-lot variation of the polyclonal anti-body utilized. Moreover, it is possible that there are other mechanisms of *TFEB* protein expression that have not been described yet, similar to the recent description of amplification of *TFE3* as a mechanism of low-level overexpression of TFE3 protein by IHC.³² *TFEB* FISH overcomes both of these limitations, although it should be noted that FISH was unsuccessful in a small number of cases (approximately 5%) in this series. Hence, FISH is also fixation dependent (although to a lesser degree than IHC), and we still recommend procurement of fresh tissue for cytogenetics and potential molecular diagnostic assays whenever the diagnosis of translocation RCC is suspected. Nevertheless, the ability to detect a *TFEB* rearrangement by FISH in a case resected 46 years earlier speaks of the robustness of the *TFEB* FISH assay.

The differential diagnosis of the t(6;11) RCC is broad and includes PEComas, Xp11 translocation RCCs, and conventional clear cell and papillary RCCs. The differential diagnosis with PEComa is particularly difficult, as both t(6;11) RCCs and PEComa label for melanocytic markers HMB45 and Melan A but not for S100 protein; in addition, they label for cathepsin K, are typically cytokeratin negative, and feature epithelioid neoplastic cells with clear to eosinophilic cytoplasm. In fact, we know of 2 cases published in the literature in which a t(6;11) RCC was initially mistaken for a renal PEComa.^{12,22} In addition, Antic et al³³ have reported in abstract form that PEComas express TFEB by IHC, although this has not been our experience. TFEB FISH analysis can resolve this differential diagnosis, as we have shown herein that PEComas are negative for *TFEB* gene rearrangement. In addition, we have found that PEComas almost never label for the renal transcription factor PAX8 (19 of 20 negative, 1 focal positive), whereas this study and our other unpublished results show that the majority of t(6;11) RCCs are PAX8 positive, supporting renal tubular differentiation (N. Smith and P. Argani, unpublished data, 2012). t(6;11) RCCs overlap significantly with the Xp11 translocation RCCs, such that we now regard these as 2 members of a larger family of MiT translocation renal carcinomas. Both frequently affect young patients, frequently have epithelioid neoplastic cells with a second population of smaller cells, and are frequently labeled by IHC for melanocytic markers and for cathepsin K. IHC and FISH for TFE3/TFEB can ultimately make this distinction. As our study shows, not all cases of t(6;11) RCCs contain a characteristic small cell component, and many areas of these neoplasms can be indistinguishable from conventional clear cell RCCs or papillary RCCs.

FISH and IHC for TFEB can easily resolve these differential diagnoses, but the diagnosis of t(6;11) RCCs must be suspected before these assays are considered.

In summary, our study adds 8 new cases of t(6;11) RCCs to the literature (Table 3). We confirm the malignant potential of these neoplasms, as 2 presented with liver metastasis. We expand the morphologic spectrum to include extensive hyalinization, oncocyoma-like morphology, oncocytic papillary morphology, and absence of a small cell component. Importantly, most of these cases were either negative or equivocal for TFEB by IHC, illustrating the added value of the TFEB FISH assay. The absence of TFEB rearrangements in PEComas supports our view that t(6;11) RCCs are distinct from angiomyolipomas/PEComas and are best classified as RCCs. Utilization of this validated TFEB FISH assay in archival material should permit a more complete delineation of the clinicopathologic spectrum of this distinctive neoplasm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Selected TFEB FISH images from 2 positive test cases. Both of these neoplasms demonstrated split telomeric and centromeric TFEB FISH signals (isolated red and green dots) indicative of a TFEB gene rearrangement, in addition to the expected red-green fusion signal, which indicates an intact TFEB gene.

FIGURE 2.

Case 2: this neoplasm was extensively hyalinized (A), with only small clusters of neoplastic epithelium, which mimicked clear cell RCC (B). The neoplasm demonstrated diffuse nuclear labeling for PAX8 (C) and cytoplasmic labeling for Melan A (D). The neoplasm demonstrated nuclear and cytoplasmic labeling for TFEB (E) and was diffuse positive for cathepsin K (F). This case was proven to harbor a TFEB gene rearrangement by FISH.

FIGURE 3.

Case 6: this neoplasm demonstrated a predominant papillary oncocyctic growth pattern, with only rare clusters of small cells on hematoxylin and eosin stains (A and B). The neoplastic cells demonstrated nuclear labeling for PAX8 (C) and diffuse immunoreactivity for Melan A (D) and patchy immunoreactivity for cathepsin K (E). This neoplasm was almost completely immunonegative for TFEB (F) but was proven by FISH to harbor a TFEB gene rearrangement.

FIGURE 4.

Case 4: this neoplasm demonstrated variable morphology, including areas resembling oncocytoma (A, right; B; C, top) and other areas that resembled clear cell RCC (A, left; C, bottom). Both areas demonstrated diffuse nuclear labeling for PAX8 (D), demonstrated cytoplasmic labeling for Melan A (E), and showed focal labeling for cathepsin K (F). A minority of the neoplasm (< 5%) demonstrated a more conventional morphology for t(6;11) RCCs, featuring larger clear cells and smaller cells (G). The neoplasm demonstrated focal moderate immunolabeling for TFEB at its periphery (H), where it intermingled with native renal tubules. This case was proven to harbor a TFEB gene rearrangement by FISH.

FIGURE 5.

Case 5: this neoplasm demonstrated a solid nested growth pattern, with clusters of somewhat smaller cells located centrally and larger polygonal cells located at the periphery of the nests (A). The neoplasm demonstrated diffuse cytoplasmic labeling for both Melan A (B) and cathepsin K (C) and also labeled for PAX8 (not shown). This neoplasm demonstrated a gradient of TFEB immunoreactivity, which was most prominent at the periphery of the lesion. (D, top of the figure). The central portion of the neoplasm did not show TFEB labeling (E), but the peripheral portion demonstrated moderate nuclear labeling for TFEB (F). This neoplasm demonstrated TFEB gene rearrangement by FISH.

TABLE 1

Positive Controls

Case #	Reference	Age/Sex	TFEB IHC	Average % TFEB Split Signals	
<i>Previously published genetically confirmed t(6;11) RCCs (positive controls)</i>					
1	Argani et al ¹² , case 7	33/M	+	31.6	
2	Argani et al ⁵ , case 3	6/F	+	66.75	
3	Argani et al ¹² , case 4	20/F	+	41	
4	Zhong et al ²³	20/M	+	73.5	
<i>Previously published case confirmed by TFEB IHC only</i>					
Case #	Reference	Age/Sex	TFEB IHC	Year of Resection	Average % Split Signals
1	Geller et al ¹⁶	Unknown	+	1965	49

TABLE 2

Test Cases Now Proven to Have TFEB Rearrangement

Case #	Age/ Sex	Size/Stage	Distinctive Features of Case	TFEB IHC	Average % TFEB Split Signals	Melan A/ HMB45	Small Cells/BM
1	14/F	NA	None	+/- (weak with gradient)	41.6	+/-focal	+/-
2	37/M	4 cm	Extensively hyalinized/ ossified	+	51.8	+/focal	-/-
3	3/F	2 cm/pT1NX,	Mimic clear cell RCCs	+/- (weak)	40	+/-	+/+
4	58/F	3 cm/pT1NX	Cystic oncocytoma like	+/- (weak with gradient)	35.8	+/focal	Rare/-
5	42/M	27 cm/pT3MI (liver metastasis)	None	+/- (focal moderate with gradient)	56.7	+/-	+/-
6	60/M	14.5 cm/pT3bN0 MI (liver metastasis, IVC thrombus)	Eosinophilic papillary	+/- (focal weak)	55	+/-	Rare/-
7	34/M	1.8 cm	ESRD; extensive psammoma bodies	-	50.8	+/-	-/-
8	25/M	15 cm	Cystic; pericellular hyalinization	+	79.2	+/focal	Rare/focal

BM indicates basement membrane material; ESRD, end-stage renal disease; IVC, inferior vena cava; NA, not available.

TABLE 3

Genetically Confirmed t(6;11) RCC Cases in the Literature

Case #	References	Age	Sex	Size (cm)	Stage	Follow-up
1	Argani et al ¹¹ case 1 Argani et al ¹² case 1 Davis et al ¹³ case 2	18	M	7	pT2NXMX	NED 18 mo
2	Argani et al ¹¹ case 2 Argani et al ¹² case 2 Martignoni, ²⁵ case 11	10	M	12.2	pT2NXMX	NED 26 mo
3	Argani et al ¹² case 5 Kuiper et al ¹⁴ case 3 Martignoni et al ²⁵ case 17	14	F	4.5	pT1NXMX	NA
4	Kuiper et al ¹⁴ case 1	42	F	NA	NA	NA
5	Kuiper et al ¹⁴ case 2	17	F	NA	NA	NA
6	Argani et al ¹² case 3 Davis et al ¹³ case 1	18	F	2.8	pT1NXMX	NED 18 mo
7	Argani et al ¹² case 4 Martignoni et al ²⁵ case 15	20	F	9.5	pT2NXMX	NED 30 mo
8	Argani et al ¹² case 6 Geller et al ¹⁶ case 9 Martignoni et al ²⁵ case 16	9	F	2.0	pT1NXMX	NED 42 mo
9	Argani et al ¹² case 7 Martignoni et al ²⁵ case 14	33	M	6.0	pT1NXMX	NA
10	Martignoni et al ¹⁸	42	F	NA	NA	DOD 3 y
11	Martignoni et al ¹⁸ Martignoni et al ²⁵ Pecciarini et al ¹⁹ Pettersson et al ²⁰ case 4	54	F	7	pT2NXMX	NED 36 mo
12	Argani et al ⁵ case 3 Martignoni et al ²⁵ case 12	6	F	5	pT1NXMX	NED 3 mo

Case #	References	Age	Sex	Size (cm)	Stage	Follow-up
13	Camparo et al ¹⁵ case 30	34	F	15	pT2N0M0	NED 50 mo
14	Camparo et al ¹⁵ case 31	36	M	20	pT3bN2M0	DOD 3 mo
16	Suarez-Vilela et al ²¹	22	M	10	pT2NXMX	NED/NA
17	Zhan et al ²²	26	M	4.3	pT1NXMX	NED 6 mo
18	Inamura et al ¹⁷ case 1	57	M	10.2	pT2NXMX	NED 8 mo
19	Inamura et al ¹⁷ case 2	37	M	NA	NA	DOD 120 mo
20	Inamura et al ¹⁷ case 3	47	M	3.2	pT1NXMX	NED 12 mo
15	Zhong et al ²⁹	20	M	19	pT2NXMX	NA
21	Petersson et al ²⁰ case 2	24	F	14	pT2NXMX	NED 3 y
22	Geller et al ¹⁶	NA	NA	NA	NA	NA
23	This study, case 1	14	F	NA	NA	NA
24	This study, case 2	37	M	4	pT1NXMX	NA
25	This study, case 3	3	F	2	pT1NXMX	NA
26	This study, case 4	58	F	3	pT1NXMX	NA
27	This study, case 5	42	M	27	pT3NXM1	NA
28	This study, case 6	60	M	14.5	pT3bN0M1	NA
29	This study, case 7	34	M	1.8	pT1NXMX	NA
30	This study, case 8	25	M	15	pT2NXMX	NA

DOD indicates dead of disease; NA, not available; NED, no evidence of disease.