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Extraskelatal Myxoid Chondrosarcoma with non-*EWSR1-NR4A3* Variant Fusions Correlate with Rhabdoid Phenotype and High Grade Morphology

Narasimhan P Agaram¹, Lei Zhang¹, Yun-Shao Sung¹, Samuel Singer², and Cristina R Antonescu¹

¹Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY

²Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY

Abstract

Extraskelatal myxoid chondrosarcomas (EMC) are rare soft tissue sarcomas with distinctive histology and uncertain histogenesis, characterized by *EWSR1-NR4A3* fusion in 75% of the cases. A smaller proportion of cases show *NR4A3* fused to other gene partners including *TAF15*, *TCF12* and *TFG*. The impact of various gene fusions on morphology and outcome has not been previously evaluated. We investigated 26 consecutive EMCs and correlated the genetic findings with morphology and clinical outcome. There were 5 females and 21 males (median age of 49.5 years). Mean size of the tumors was 11 cm. FISH analysis showed *EWSR1-NR4A3* gene fusion in 16 (62%) cases; *TAF15-NR4A3* gene fusion in 7 (27%) cases and *TCF12-NR4A3* gene fusion in one (4%) case. Two cases showed only *NR4A3* gene rearrangements. Morphologically, most *EWSR1*-rearranged tumors (10 of 16) showed low cellularity, minimal cytologic atypia and low mitotic counts. In contrast, 80% of EMCs with variant (non-*EWSR1*) *NR4A3* gene fusions (*TAF15*, *TCF12*) had high grade morphology with increased cellularity, proliferation and cytologic atypia, showing a plasmacytoid / rhabdoid morphology in half the cases. Follow-up showed that only 1 of 16 patients with *EWSR1*-rearranged tumors died of disease, in contrast to 3 of 7 (43%) *TAF15*-rearranged tumors. In conclusion, EMCs with variant *NR4A3* gene fusions show a higher incidence of rhabdoid phenotype, high grade morphology and, a more aggressive outcome compared to the *EWSR1-NR4A3* positive tumors. Furthermore, FISH assay for *NR4A3*, along with *EWSR1*, may be an additional ancillary test to confirm diagnosis of EMCs.

Keywords

NR4A3; CHN; TAF15; EWSR1; TCF12; extraskelatal myxoid chondrosarcoma

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Correspondence: Narasimhan P. Agaram, MBBS, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY, 10065 (agaramn@mskcc.org) or Cristina R Antonescu, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065 (antonesc@mskcc.org).

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INTRODUCTION

Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma, occurring with predilection in the deep soft tissue of the limbs in middle-aged adults. [1] Despite having often an indolent course, EMC has a propensity for late recurrence and metastasis, particularly to the lungs.[2] Most tumors show a distinctive morphology, composed of monotonous oval to short spindled cells arranged in cords or reticular pattern, in a diffusely myxoid extracellular matrix.[1] However, in a subset of cases the tumors are more cellular, with a predominant epithelioid or rhabdoid phenotype and higher degree of cytologic atypia. This latter appearance in particular is under-recognized and often misdiagnosed with a variety of other soft tissue tumors, including myoepithelial tumors (parachordoma), extra-axial chordoma, and myxofibrosarcoma, among others.

The genetic hallmark of EMC is a pathognomonic *NR4A3* gene rearrangement, which in most cases is being fused to *EWSR1* as a result of a t(9;22)(q22;q12). [3-6] However, in a smaller percentage of cases *NR4A3* is fused instead to alternative gene partners, including *TAF15*, *TCF12* and *TFG*. [7-10] Despite this rather comprehensive catalog of genetic abnormalities, most clinical labs have only limited molecular tests available to be applied in daily practice, such as *EWSR1* break-apart assay by FISH or the *EWSR1*-*NR4A3* fusion by RT-PCR. [11, 12] These tests typically are not informative for the 25-30% *EWSR1*-negative subset of EMC, which often remain mis-diagnosed. Thus, the purpose of our study is to investigate the prevalence of these genetic abnormalities in a series of consecutive EMCs with typical morphology, using a comprehensive panel of FISH probes covering the entire spectrum of reported fusions in EMC and to correlate the transcript variant to morphology and clinical outcome.

MATERIALS AND METHODS

Twenty-six cases of EMC were identified between 2000 and 2013 from the Department of Pathology files at Memorial Sloan-Kettering Cancer Center. The criteria for the selection included a typical morphology and available tissue for molecular studies. Hematoxylin and eosin sections and immunohistochemical stains performed at the time of diagnosis were reviewed. The gross and microscopic findings, including tumor size, anatomic location, tumor borders, surgical margins, cellularity, percentage of myxoid component, nuclear pleomorphism, mitoses (per 10 high power fields), and presence of necrosis were recorded. Clinical and follow-up data were obtained from the clinical database. The study was approved by the Institutional Review Board (IRB# 02-060 MSKCC).

Fluorescence in situ hybridization (FISH)

FISH on interphase nuclei from paraffin embedded 4-micron sections was performed applying custom probes using bacterial artificial chromosomes (BAC), covering and flanking *NR4A3* (*CHN*), *EWSR1*, *TAF15*, *TCF12*, *TFG* and *FUS* genes. (Supplementary Table 1) BAC clones were chosen according to USCS genome browser (<http://genome.uscs.edu>) and obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI; Oakland, CA; <http://bacpac.chori.org>). DNA from individual

BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution. The genomic location of each BAC set was verified by hybridizing them to normal metaphase chromosomes. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems). A positive score was interpreted when at least 20% of the nuclei showed a breakapart signal. Nuclei with incomplete set of signals were omitted from the score.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted in 3 cases with available frozen tumor tissue, using the Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The quality of RNA was assessed by RT-PCR for PGK housekeeping gene (as evidenced by the presence of a 247 bp amplified product). RT-PCR was performed by SuperScriptVR First-Strand Synthesis System (Invitrogen). Primers used for RT-PCR validation included: TAF15 exon 6 forward primer (5'-GAGCACCTTCCTATGACCAGCCAG-3') and CHN exon 3 reverse primer (5'-CGAACTCAAGCCTTCCTGCGTGTA-3'). Primers used to detect the *EWSR1-NR4A3* fusions are as follows: *EWSR1* exon 12 forward (5'-AAGGCGATGCCACAGTGTC-3') and *NR4A3* reverse A exon 3 (5'-CCTGGAGGGGAAGGGCTAT-3') for the type 1 *EWSR1-NR4A3* fusion (109 base pair product), and *EWSR1* exon 7 forward (5'-CGTAAGCTTTCCTACAGCCAAGCTCCAAGTC-3') and *NR4A3* reverse B exon 2 (5'-TCTCTCCAGCGGAGGCTGAGA-3') for the type 2 fusion (201 base pair product). The PCR products were analyzed by electrophoresis. The amplified PCR products were sequenced using the Sanger's method.

RESULTS

Clinical Features

Clinical features are summarized in Table 1. The study was comprised of twenty-six patients including 5 females and 21 men, with a median age of 49.5 yrs (range, 33-81 yrs). The anatomic location of the tumors included: thigh (14), elbow (1), buttock (2), back (1), arm (2), groin (1), perineum (1), pelvis (1), calf (1), foot (1) and inguinal (1). The samples available for molecular analyses originated from the primary lesions in 23 cases, from the local recurrences in 2 cases and from the lung metastasis in one case. The primary tumor of the case of the lung metastasis sample was located in the inguinal region. All patients were treated by surgical excision of the mass.

Pathologic Findings

The primary tumors ranged in size from 2-24 cm (mean, 11 cm). All tumors had well-circumscribed borders and involved the deep soft tissues. (Fig 2A) Microscopically, all tumors showed a multi-nodular and lobular growth pattern. The tumors were diffusely (100%) myxoid in 19 cases, predominantly myxoid (>80%) in 6 cases. Only one case showed scant myxoid matrix (10%). The tumor cells ranged from small to medium in size and from ovoid to epithelioid in shape. The tumor cells were arranged in cords and reticular

patterns in the predominantly myxoid areas (Fig 1A), while in tumors with increased cellularity throughout, the tumor cells were distributed in trabeculae and closely packed cords (Figs. 2B-E). Six cases showed an epithelioid / rhabdoid morphology (Figs 2F, 3B). Mitoses ranged from 0-1 to 10 per 10 high power fields. Necrosis, ranging from focal to 90% of the tumor, was seen in 9 cases.

Immunohistochemical stains were performed at diagnosis on 14 cases. Review of the IHC stains revealed no specific immunoprofile with most tumors (8 of 14) being negative for S100, EMA, pan-keratin, SMA, Desmin and CD34. Five cases showed focal scattered positivity for S100 protein. Only one case (EMC#17) was positive diffusely positive for S100 protein (Fig. 2 G) but was negative for other markers. This latter case occurred in the perineum and had a distinctive rhabdoid morphology (Fig. 2 F), reminiscent of a proximal type epithelioid sarcoma. However, INI1 (BAF47) expression was retained in this case as well as four additional cases tested (2 with *EWSR1-NR4A3* and 3 with *TAF15-NR4A3* fusions), including 4 cases with rhabdoid features.

FISH and Molecular findings

FISH for *NR4A3* and *EWSR1* was performed on all 26 tumors. All cases showed rearrangement of the *NR4A3* gene confirming the diagnosis of EMC (Fig. 1B, 3C). Rearrangements of the *EWSR1* and *NR4A3* genes were seen in 16 of 26 cases (62%) indicating a t(9;22) translocation (Fig. 1 B,C). FISH for *TAF15*, *TFG*, *TCF12* and *FUS* was performed on the tumors that were *NR4A3* positive and *EWSR1* negative. Rearrangements of both *NR4A3* and *TAF15* genes were identified in 7 cases (27%) indicating a t(9;17) translocation (Fig. 2I). Only one case was positive for *TCF12* gene rearrangement in keeping with a t(9;15) translocation (Fig. 3C). Two cases showed rearrangement of the *NR4A3* gene only. FISH for *TFG* and *FUS* gene rearrangements were negative in the three cases tested.

In EMC#22 with available frozen tissue, RT-PCR showed the fusion of exon 3 of *NR4A3* with exon 6 of the *TAF15* gene (Fig. 2H). In addition, RT-PCR for *EWSR1-NR4A3* gene fusion performed in two cases (EMC# 1 & 10) detected *EWSR1-NR4A3* type 1 gene fusion (exon 12 of *EWSR1* fused to exon 3 of *NR4A3*) in both the cases.

Correlation of the clinical features and morphology with the genetic findings

EMCs with *EWSR1-NR4A3* gene fusion—There were 16 tumors in this group, occurring in 11 males and 5 females. Eleven of the 16 tumors (69%) originated in the muscles of the thigh. These cases predominantly showed low cellularity (Fig. 1A) with only 4 of 16 cases (25%) showing moderate to high cellularity. Only 1 of the 4 cases with increased cellularity showed a rhabdoid / epithelioid phenotype. Mitotic counts for most cases were 0-1/ 10 HPFs with only 3 of 16 cases (19%) showing increased mitoses of more than or equal to 4 per 10 high power fields. Tumor necrosis ranging from focal to 40% was seen in 4 of 16 cases.

EMCs with variant non-*EWSR1 NR4A3* fusions—There were 10 tumors with *NR4A3* variant gene fusions all occurring in males and only 3 (30%) occurred in the thigh.

Morphologically, 8 of 10 cases (80%) showed moderate to increased cellularity with moderate to severe cytologic atypia (Figs. 2,3). The tumor cells showed a plasmacytoid / rhabdoid morphology in half (50%) of the cases (Figs. 2F and 3B). Eight of 10 cases showed increased mitotic activity of >4 per 10 high power fields with an average of 7 MF/ 10 HPFs. Tumor necrosis of 15% and 90% was seen in 2 of 10 cases, respectively.

Clinical Follow-up

Follow up information was available in all patients, ranging from 2-99 months (mean follow-up for survivors – 35 months). Four patients (15%) developed local recurrence (LR) and 9 cases (35%) developed distant recurrence / metastasis (DR). Local recurrences occurred from 3-36 months after the primary surgical resection, while metastases occurred at 2-70 months following original tumor resection. At the time of last follow-up, 14 patients had no evidence of disease (NED), 7 were alive with disease (AWD), 1 patient died of unknown cause (DUK) and 4 patients died of disease (DOD).

Of the 16 patients with tumors exhibiting *EWSR1-NR4A3* gene fusion, follow-up ranged from 2 to 96 months (mean follow-up – 43.3 months). LR and DR were seen in 1 (6%) and 6 (37%) patients, respectively. Only one of the 16 patients (6%) with this fusion variant DOD. Six patients are AWD with a mean follow-up of 60.8 months. Of the 4 cases with increased cellularity and mitotic activity, 3 were NED at last follow-up ranging from 2 – 11 months and 1 was AWD at a follow-up of 95 months.

Of the 7 patients with *TAF15-NR4A3* gene fusion-positive tumors, follow-up ranged from 2 to 99 months (mean follow-up – 21.7 months). Two (28%) patients each developed LR and DR. Three (43%) of the 7 patients with this fusion DOD at 7, 12 and 99 months. Among the 5 tumors with variant non-*EWSR1-NR4A3* fusions having an epithelioid/rhabdoid phenotype there was no distinct correlation with outcome, with 3 patients being NED at 3-30 months follow-up, one patient AWD at 40 months and one patient DOD at 7 months.

DISCUSSION

The terminology and histogenesis of extraskeletal myxoid chondrosarcoma (EMC) has been long controversial. Originally described as ‘chordoid tumor’ by Dr Stewart in 1948,[13] to reflect its resemblance to a chordoma and lack of definitive chondroid lineage, was later defined as EMC by Enzinger and Shiraki into a distinct clinicopathologic entity.[1] Despite its name, there is no convincing evidence of cartilaginous differentiation,[14] and previous studies have shown distinct genetic abnormalities between EMC and most skeletal chondrosarcomas with extensive myxoid changes.[12]

Patients with EMC follow a protracted clinical course with a relatively high incidence of distant spread, typically to the lung,[15] and poor response rates to most conventional chemotherapeutic agents.[16] In one of the larger clinicopathologic studies to date including 42 patients with EMC, the overall survival was 100% at 5 years and 69% at 10 years.[15] The correlation between histologic grade in EMC and behavior has been controversial. Most cases of EMC are predominantly myxoid, with low cellularity and classified as low grade tumors. Cellular forms of EMC have been reported, although the detailed criteria for truly

high-grade tumors are limited. Antonescu et al. and Lucas et al. reported that histologically high-grade EMCs showed adverse clinical outcomes, including higher metastatic rates.[12, 17] The study by Lucas et al pointed out specifically to a distinctive and striking histological feature in their study group of numerous large epithelioid cells, which they define as 'high grade EMC', with all four patients succumbing of disease in a relatively short interval.[17] In contrast, studies by Saleh et al and Jambhekar et al found no relation between cellularity and prognosis.[2, 18] Furthermore, Meis-Kindblom et al. showed that clinical parameters, but not histologic features, such as old age, large tumor size, and proximal tumor locations, were associated with decreased survival.[19] Oliveira et al. defined large tumor size, high cellularity, presence of anaplasia or rhabdoid features, high mitotic activity, and high Ki-67 expression as adverse prognostic factors.[20] In the study by Kawaguchi et al., neither the histologic grade nor clinical parameters including age, gender, tumor size, location, and depth of the tumor showed significant impact on disease-free, metastasis-free, or recurrence-free survival.[15] A significant caveat is that most of these prior studies analyzing clinical adverse factors in EMC are based on morphology alone. As the histologic overlap between EMC and other epithelioid and myxoid soft tissue neoplasms is noteworthy, especially at the high grade end of the spectrum, it is difficult to exclude that some of the tumors included in these series may represent alternative diagnoses. As our study includes only genetically confirmed EMC, it provides a unique platform to investigate the impact of the variability of fusion structure on morphologic traits and behavior, not previously analyzed in these clinicopathologic studies.

About two-thirds of EMC harbor the chromosomal reciprocal translocation t(9;22)(q22;q12),[3] resulting in the fusion of *EWSR1* gene to the nuclear receptor subfamily 4, group A, member 3 (*NR4A3*, *a.k.a. CHN, TEC and NOR1*).[4-6] An additional subset is characterized by a t(9;17)(q22;q11), resulting in the fusion of TATA binding protein-associated factor 15 (*TAF15*) to *NR4A3*. [7, 8] Rare cases with either (9;15)(q22;q21)[9] or t(3;9)(q12;q22)[10] have also been reported, in which *NR4A3* is fused to transcription factor 12 (*TCF12*) or TRK-fused gene (*TFG*), respectively. *TAF15* (a.k.a. *TAF2N*, *RBP56* and *YAF(II)68*), together with *EWSR1* and *FUS* belong to the TET multifunctional proteins that bind both RNA and DNA.[21] The N-terminal region of *EWSR1*, *FUS* and *TAF15* contains degenerate repeats of the SYGQ motif and mediates powerful transcriptional activation when fused to heterologous DNA-binding domains of a variety of transcription factors.[22] Although *FUS* may substitute *EWSR1* gene in a variety of translocation-associated sarcomas, EMC is the only tumor type to date in which *TAF15* substitutes the *EWSR1* for *NR4A3* fusions. Furthermore, the *NR4A3* gene fusions have not been described in other sarcoma subtypes and are considered pathognomonic for this disease. Both *EWSR1-NR4A3* and *TAF15-NR4A3* fusion proteins have been shown to be strong transcriptional activators. [23, 24]

Our study confirms that *EWSR1-NR4A3* fusion is the most common genetic event in EMC, occurring in 62% of cases with classic morphology. The morphologic appearance of this molecular subset showed predominantly low cellularity, minimal cytologic atypia and low mitotic activity, with 12 cases (75%) being classified as low grade and only one patient (6%) succumbing to the disease. In contrast, 27% of cases had a *TAF15-NR4A3* fusion with this

subset predominantly (71%) showing features of 'high grade morphology' as described in prior studies. Also, three of the 7 patients with *TAF15-NR4A3*-positive tumors died of disease. Interestingly, EMCs with non-*EWSR1* variant translocations seem to occur more frequently outside the common thigh anatomic location and are more often associated with a rhabdoid phenotype microscopically. Although epithelioid/rhabdoid morphology did not seem to correlate with clinical outcome, a confounding factor could be the short follow-up duration in the patients with variant gene fusions. Furthermore, the rates of distant recurrences appear to be quite similar regardless of the fusion type.

As the morphologic spectrum of EMC varies from a low grade myxoid tumor to a more solid high grade epithelioid neoplasm, so is the histologic overlap with other myxoid and epithelioid soft tissue tumors. Particularly difficult is the distinction with the recently described soft tissue myoepithelial tumors.[25] Although myoepithelial neoplasms are defined by their positivity for EMA/Keratin and S100, the lack of a consistent immunohistochemical profile in EMCs makes the distinction rather difficult based on morphology and immunohistochemistry alone. Furthermore, both these entities can show *EWSR1* gene rearrangements[25], adding to the challenge in diagnosis. In these difficult cases, FISH for the presence of a *NR4A3* gene rearrangement can confidently distinguish between these two tumor types.[11, 26]

Extra-axial or soft tissue chordomas (a.k.a. chordoma periphericum) also show similar morphologic features with EMC. Immunohistochemical reactivity for the notochordal specific protein T-Brachyury is extremely helpful in confirming a diagnosis of extra-axial chordomas over an EMC. Although predominantly an extra-skeletal disease, EMCs can involve the bone occasionally. Rare reports of such tumors with the *EWSR1-NR4A3* fusions have been reported (14). In osseous locations, differential diagnosis includes primary skeletal chondrosarcoma with secondary myxoid changes, which lack *EWSR1-NR4A3* fusions.[12]

The presence of EMC with predominantly rhabdoid phenotype has raised the possibility of a genetic relationship with the prototypical rhabdoid soft tissue tumor, the proximal epithelioid sarcoma. Latest reports of EMC showing loss of INI1 expression immunohistochemically further questioned this hypothesis.[14, 27] As such, we tested 5 *NR4A3*-fusion positive (2 with *EWSR1-NR4A3* and 3 with *TAF15-NR4A3* fusions) tumors with predominantly rhabdoid phenotype (4 of the 5 cases tested) for INI1 expression by immunohistochemistry and 2 cases (cases' 21 and 25) for *SMARCB1* deletions by FISH, however, none showed loss of protein expression or gene deletion (data not shown).

In summary, our study highlights the association between variant translocations and morphology in EMCs. EMCs with *TAF15-NR4A3* fusions appear to have 'high grade' histology. Although the findings suggest that EMCs with variant translocations may have a more aggressive behavior compared to EMCs with the more common *EWSR1*-related fusions, additional studies with longer follow-up data are required to confirm this finding. We have also emphasized the importance of applying genetic ancillary techniques for a more accurate classification of EMC from its morphologic mimics and to confirm a *NR4A3* gene rearrangement in challenging diagnoses. The presence of an *EWSR1* gene

rearrangement in this context of tumors with overlapping morphologies is not specific. In our opinion, FISH assay for *NR4A3* break-apart, alone or supplementing *EWSR1*, is more useful to differentiate EMC from its morphologic look-a-likes, especially in tumors with high grade, epithelioid morphology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. *EWSR1-NR4A3* positive EMC (EMC#2)

(A) Morphologic appearance showing a low grade myxoid lesion composed of bland ovoid cells arranged in a reticular pattern (H&E, 200x); FISH analysis shows break-apart signals (arrows) for *NR4A3* (*CHN*) gene (B) and *EWSR1* (C)(red, centromeric; green telomeric)

Figure 2. Pathologic spectrum of *TAF15-NR4A3* fusion positive tumors

(A) Gross appearance of a well-circumscribed tumor, involving soft tissue, with a myxoid to hemorrhagic cut surface (EMC#22); (B) Low magnification commonly show a distinctive lobular and multinodular growth pattern (EMC#23, 40x); Higher magnification showing a more solid and less myxoid appearance, with vague nest formation (C; EMC#22; H&E, 200x), undifferentiated morphology with monotonous small round cells in a vague myxoid stroma (D, EMC#21, H&E, 200x); solid and more anaplastic / high grade appearance (E, EMC#18, H&E, 200x); distinctive rhabdoid appearance, with epithelioid cells showing densely eosinophilic cytoplasm and eccentric nuclei, reminiscent of a plasmacytoid phenotype (F, EMC#17, H&E, 200x). (G) Strong S100 protein expression in the latter case with rhabdoid morphology, occurring in the perineum of a 63 year-old man (EMC#17, 200x); (H) RT-PCR identified the presence of a *TAF15-NR4A3* (*CHN*) fusion transcript. The encoded protein sequence is shown below; (I) FISH showing rearrangement (arrows) of *TAF15* gene (red, centromeric, green, telomeric).

Figure 3. *TCF12-NR4A3* rearranged high grade EMC (EMC#24)

(A) Low power showing nodular growth pattern (H&E, 40x); (B) higher power showing a solid growth of epithelioid cells with densely eosinophilic cytoplasm and vague rhabdoid morphology; prominent nucleoli are noted within a vesicular chromatin (H&E, 200x); FISH showing an unbalanced rearrangement (arrows) for *NR4A3* gene with loss of the centromeric part (red) (C) and a more complex pattern of rearrangement of the *TCF12* locus (D) with deletion of the centromeric part (red) as well as an additional break/intra-chromosomal inversion on the other chromosome 15 (green, telomeric, and red, centromeric, are apart rather than fused signals)

Clinicopathologic and cytogenetic findings and follow-up

Table 1

Case#	Age/Sex	Site	Size (cm)	Cellularity	Atypia [⊕]	Mitoses (/10 HPF)	Fluorescence In-situ hybridization (FISH)					LR/DR	Follow-up Status	Duration (months)
							NR4A3 (CHN)	EWSR1	TAF15	TCF12	TFG	FUS		
1	56 / M	elbow	2	low	minimal	0	+	+					NED	88
2	51 / F	thigh	18.5	low	minimal	0	+	+				LR	NED	56
3	81 / M	thigh	14	low	mild	0	+	+					DUK	96
4*	64 / M	thigh	16.5	moderate	moderate	4	+	+					NED	11
5	41 / F	thigh	14.8	low	minimal	0	+	+				DR	AWD	46
6	58 / F	thigh	24	low	mild	0	+	+				DR	AWD	41
7	38 / M	thigh	7	low	mild	1	+	+					NED	25
8*#	77 / M	back	6.5	high	moderate	10	+	+					NED	5
9	46 / M	lung	1	low	mild	1	+	+				DR	AWD	95
10	78 / M	thigh	11	low	mild	3	+	+				DR	DOD	4
11*#	68 / F	thigh	10.5	high	severe [⊕]	6	+	+					NED	2
12*	49 / M	thigh	12.5	low	mild	1	+	+					AWD	55
13	33 / M	thigh	15.2	moderate	moderate	1	+	+				DR	AWD	66
14	42 / M	arm	6	low	mild	0	+	+				DR	AWD	62
15	50 / M	thigh	11	low	mild	1	+	+					NED	7
16*	74 / M	calf	11	low	mild	0	+	+					NED	34
17#	63 / M	perineum	19	moderate	moderate [⊕]	5	+	-	+				NED	3
18	39 / M	thigh	4.5	low	mild	2	+	-	+				NED	18
19*	46 / M	buttock	16.5	high	moderate	9	+	-	+			LR/DR	DOD	12
20	40 / M	foot	7	low	mild	6	+	-	+			DR	DOD	99
21#	63 / M	groin	12	high	moderate [⊕]	12	+	-	+				NED	11
22*	46 / M	pelvis	12.2	high	severe [⊕]	5	+	-	+			LR	DOD	7
23*#	70 / M	buttock	12	high	moderate	10	+	-	+				NED	2
24	35 / M	thigh	2.7	high	severe [⊕]	10	+	-	-	+	-		NED	30
25*	41 / M	thigh	8.7	high	severe [⊕]	3	+	-	-	-	-	DR	AWD	40

Case#	Age/Sex	Site	Size (cm)	Cellularity	Atypia $\oplus$	Mitoses (/ 10 HPF)	Fluorescence In-situ hybridization (FISH)				LR/DR	Follow-up Status	Duration (months)
							NR4A3 (CHN)	EWSR1	TAF15	TCF12	TFG	FUS	
26	39 / M	arm	3.5	moderate	moderate	4	+	-	-	-	-	-	36

* Cases with necrosis;

Cases on which IN11 expression was tested;

$\oplus$ Cases with rhabdoid / plasmacytoid morphology;

LR – local recurrence; DR – distant recurrence; NED – No evidence of disease; AWD – Alive with disease; DUK – Died of unknown causes; DOD – died of disease.