

Research Article

EWSR1::POU2AF3(COLCA2) Sarcoma: An Aggressive, Polyphenotypic Sarcoma With a Head and Neck Predilection

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ABSTRACT

EWSR1::POU2AF3 (COLCA2) sarcomas are a recently identified group of undifferentiated round/spindle cell neoplasms with a predilection for the head and neck region. Herein, we report our experience with 8 cases, occurring in 5 men and 3 women (age range, 37–74 years; median, 60 years). Tumors involved the head/neck (4 cases), and one each the thigh, thoracic wall, fibula, and lung. Seven patients received multimodal therapy; 1 patient was treated only with surgery. Clinical follow-up (8 patients; range, 4–122 months; median, 32 months) showed 5 patients with metastases (often multifocal, with a latency ranging from 7 to 119 months), and 3 of them also with local recurrence. The median local recurrence-free and metastasis-free survival rates were 24 months and 29 months, respectively. Of the 8 patients, 1 died of an unknown cause, 4 were alive with metastatic disease, 1 was alive with unresectable local disease, and 2 were without disease. The tumors were composed of 2 morphologic subgroups: (1) relatively bland tumors consisting of spindled to stellate cells with varying cellularity and fibromyxoid stroma (2 cases) and (2) overtly malignant tumors composed of nests of “neuroendocrine-appearing” round cells surrounded by spindled cells (6 cases). Individual cases in the second group showed glandular, osteogenic, or rhabdomyoblastic differentiation. Immunohistochemical results included CD56 (4/4 cases), GFAP (5/8), SATB2 (4/6), keratin (AE1/AE3) (5/8), and S100 protein (4/7). RNA sequencing identified *EWSR1::POU2AF3* gene fusion in all cases. *EWSR1* gene rearrangement was confirmed by fluorescence in situ hybridization in 5 cases. Our findings confirm the head/neck predilection and

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aggressive clinical behavior of *EWSR1::POU2AF3* sarcomas and widen the morphologic spectrum of these rare lesions to include relatively bland spindle cell tumors and tumors with divergent differentiation.

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Introduction

With the widespread application by pathologists of sophisticated molecular genetic techniques such as next-generation sequencing, an ever-increasing number of new, distinctive entities are now recognized within the group of undifferentiated round and/or spindle cell sarcomas. One of the most recently identified categories comprises aggressive, histologically undifferentiated spindle cell/round cell sarcomas harboring *EWSR1/FUS::POU2AF3(COLCA2)* gene fusions, first reported by Agaimy et al¹ in a series of 5 sinonasal tumors and more recently reported by Hiemenz et al² in a series of 6 cases predominantly (but not exclusively) involving soft tissue and bone locations in the head/neck. Herein, we present our experience with 8 additional examples of *EWSR1/FUS::POU2AF3* sarcoma, further expanding the clinical, morphologic, and immunohistochemical spectrum of these rare malignant neoplasms.

Materials and Methods

Review of our collective consultation archives identified 8 cases known to harbor *EWSR1/FUS::POU2AF3* fusions. Clinical information was obtained from the medical records. Statistical analysis, including survival analyses, was performed with Bluesky Statistics software (version 7.40, BlueSky Statistics, LLC).

Immunohistochemistry for each case was performed at several different laboratories based on the original differential diagnostic considerations (staining protocols and antibody sources are available upon request). Some markers (ie, GFAP, SATB2, and neuroendocrine markers) were added in selected cases for the purposes of this study. In cases 1 and 6, both the primary and recurrent or metastatic tumors, respectively, were available for analysis. RNA sequencing in cases 1, 3, 4, 6, and 7 was performed using various customized versions of FusionPlex assay (Archer, Inc). Case 2 was analyzed using TruSight RNA Fusion panel (Illumina Inc), while DNA and RNA sequencing in case 8 was performed using the TruSight Oncology 500 kit (Illumina Inc). Cases 5 and 6 were sequenced using a targeted, custom-designed, amplicon-based panel using QIAGEN's QIAseq chemistry. In 6 cases, an *EWSR1* fluorescence in situ hybridization break-apart probe was used for confirmation of gene rearrangement. All methods have been described in more detail previously.³⁻⁷ Molecular data from case 5 have been previously reported.⁷

Results

Clinical Findings

The clinical features are summarized in Table 1. There were 5 male and 3 female patients aged 37 to 74 years (median, 60 years; mean, 57 years). The head and neck area was involved in 4 cases (frontal bone, nasopharynx, and 2 cases in the sinonasal area); 1 case each affected the thigh, thoracic wall, fibula, and lung.

Imaging studies were available for 4 patients (cases 1-3 and 8). Computed tomography (CT) scan in case 1 revealed a tumor

involving left ethmoidal and frontal sinuses with a soft tissue component and with destruction of bilateral cribriform plates and left sphenoidal ala. Magnetic resonance imaging revealed a T1 isointense and T2 hyperintense mass with contrast enhancement and diffusion restriction involving the left ethmoid sinus, left sphenoid wing, and bilateral frontal sinuses. CT scan in case 2 showed a lytic tumor in the right frontal bone (Fig. 1D), while an infiltrative, variably calcified lesion of soft tissue density involving the right nasal cavity and frontal, ethmoid, and maxillary sinuses was found in case 3 (Fig. 2A). The initial tumor in case 8 affected the left part of the thoracic wall around fifth rib with lytic bone involvement on CT scan.

All patients underwent surgical resection of their primary tumors with R0, R1, or R2 margins (for details, see Table 1). One patient received neoadjuvant chemotherapy/radiotherapy; adjuvant chemotherapy and radiation therapy were administered to 4 and 6 patients, respectively. One patient (case 6) was treated with surgery only. Clinical follow-up (8 patients; range, 4-122 months; median, 32 months; mean, 39 months) showed 5 patients with metastases (unconfirmed by biopsy in case 1), and 3 of them also exhibited local recurrence. Of the 8 patients, 1 died of an unknown cause, 4 were alive with metastatic disease, 1 was alive with unresectable local disease, and 2 were without disease. The metastases occurred with a latency ranging from 7 to 119 months, were often multifocal, and involved bone (n = 3) and pleura, base of the skull and lymph nodes (1 case each). The median local recurrence-free and metastasis-free survival rates were 24 months and 29 months, respectively.

Submitting diagnostic considerations from referring pathologists included Ewing-like sarcoma (2×), osteosarcoma (2×), "spindle cell sarcoma," ossifying fibromyxoid tumor (OFMT), desmoplastic fibroma, intranodal palisaded myofibroblastoma, atypical carcinoid, synovial sarcoma, myoepithelial tumor, and neuroendocrine carcinoma.

Morphologic Findings

Microscopically, 6 cases grew in an infiltrative fashion into surrounding soft tissue and bone, whereas cases 5 and 6 appeared relatively well circumscribed. Although all tumors consisted of relatively uniform spindled to round cells, 2 distinct morphologic groups could be discerned. The first of these, illustrated by cases 1 and 2, displayed variable cellularity and consisted of relatively bland, spindled to stellate cells in a fibromyxoid stroma; nuclear atypia was mild to moderate, mitotic activity was low, not exceeding 2 mitoses per 10 high-power fields, and necrosis was absent (Fig. 1). In case 1, the primary tumor showed predominantly low cellularity (Fig. 1A), with only a small focus having more cellular, round cell features (Fig. 1B); the recurrent tumor consisted chiefly of more cellular spindled proliferation (Fig. 1C). Similarly, case 2 was predominantly hypocellular (Fig. 1E), with scattered small to medium-sized nests having a more cellular, round cell appearance (Fig. 1E-G). The initial tumor in case 1 had a striking arborizing capillary vasculature (Fig. 1A), whereas small hyalinized vessels predominated in the recurrence and in case 2 (Fig. 1E).

Table 1Clinical features of *EWSR1::POU2AF3* tumors

Case no.	Age (y)/sex	Tumor site	Size	Prior diagnosis	Treatment	Local recurrence/distant mets (mo after diagnosis)	Status at the last follow-up	Overall survival (mo)
1	65/M	Sinonasal area (destructive lesion of ethmoidal and frontal sinuses)	2.9	Ossifying fibromyxoid tumor	Resection (R2) and adjuvant RT	Recurrence and lytic lesion in the left ilium on PET-CT suspicious for mets (not biopsied) in 24 mo	Died of an unknown cause	24
2	60/F	Frontal bone (lytic bone lesion)	5.2 × 4.5 × 2.5	Desmoplastic fibroma, Spindle cell sarcoma grade 2, dedifferentiated osteosarcoma	Resection (no residual disease on CT); CHT initiated after mets detected	Multiple lytic mets in bilateral frontal bones and at the base of the skull 7 mo after surgery	AWD	7
3	43/F	Sinonasal area (nasal cavity, frontal, ethmoid, and maxillary sinuses)	NA	Osteosarcoma	Functional endoscopic sinus resection (R1), adjuvant proton RT	No	AWD	4
4	46/M	Nasopharynx	NA	Ewing(-like) sarcoma; synovial sarcoma, myoepithelial tumor	Resection (R0), adjuvant RT, and CHT	Multifocal bone mets (119)	AWD	122
5	72/M	L thigh	3.9	Intranodal palisaded myofibroblastoma	Resection (R0); adjuvant CHT and RT	No	NED	33.5
6	60/M	Lung (intraparenchymal)	4.2	Atypical carcinoid	Resection (R0), mets resected	Local recurrence/multifocal pleural mets (22 and 38)	AWD	41.5
7	74/F	R proximal fibula (bone)	NA	Ewing(-like) sarcoma	Resection (R0) neoadjuvant and adjuvant RT and CHT	No	NED	53.5
8	37/M	Thoracic wall (soft tissue lesion with adjacent lytic rib involvement; no other tumor located elsewhere)	NA	Neuroendocrine carcinoma	Resection (R1) with RT (60 Gy) and CHT (EP); mets treated with CHT (VAC)	Multifocal locoregional recurrence with pleural, pericardial, diaphragmatic and lymph node mets found in 24 mo	AWD	30

AWD, alive with disease; CHT, chemotherapy; CT, computed tomography; EP, cisplatin/etoposide; F, female; L, left; M, male; mets, metastases; NA, not available; NED, no evidence of disease; PET, positron emission tomography; R, right; RT, radiotherapy; VAC, vincristine-D-actinomycin-cyclophosphamide.

The second, larger subgroup consisted predominantly of highly cellular sheets and fascicles of overtly malignant-appearing spindled and round cells, with somewhat biphasic architecture (Figs. 2-5). Mitotic activity ranged from 4 to 24 mitoses per 10 high-power fields, and small areas of necrosis were identified in 2 cases. Stroma was relatively scarce in cases 3, 4, 6, and 7 (Fig. 3B), whereas cases 4 and 5 contained foci with abundant myxoid stroma, in which the tumor cells grew in cord-like, pseudopapillary, pseudoglandular, and cribriform formations (Fig. 3A, C, E). Relatively hypocellular zones with hyalinized collagen were variably present (Figs. 3D and 5A-C). Some cases contained numerous hyalinized small vessels (Fig. 3A, B), whereas others had an inconspicuous vasculature.

Perhaps, the most distinctive feature of this second subgroup (found in 4 cases) was the presence of nests or loose dispersed sheets of round cells with basophilic nuclei with finely stippled chromatin (“neuroendocrine”), sometimes with peripheral palisading, surrounded by zones with more spindled cells having lighter and more evenly dispersed chromatin (Figs. 3D; 4B, C, G; and 5A-C). One prominent pinpoint nucleolus was typically present in both types of cells (Figs. 2D and 5C).

Unusual features, noted in individual cases, included keratin 7–positive glands containing Alcian blue–positive mucin (case 8) (Fig. 5D), clear cells (cases 7 and 8) (Fig. 4H), osteogenic differentiation with production of osteoid matrix (case 3) (Fig. 2E), and rhabdomyoblastic differentiation including

rhabdomyoblasts with visible cross-striations (case 6) (Fig. 4D). Interestingly, rhabdomyoblasts were absent in the metastasis from this tumor.

Scanned whole slide images for most cases are available online at the hyperlinks listed in [Supplementary File S1](#). No correlation was found between any of the histologic features studied and the clinical course of the disease.

Immunohistochemical Findings

The immunohistochemical results are summarized in [Table 2](#). The most commonly expressed markers were GFAP (5/8 cases; 63%) (Fig. 5F), SATB2 (4/6 cases; 67%) (Fig. 2F and 4F), keratins (AE1/AE3) (5/8 cases; 63%) (Fig. 5G), and S100 protein (4/7 cases; 57%) (Fig. 5E). In general, expression of these markers was more pronounced in the round cell nodules, compared with the spindled cells (Figs. 4F and 5E-G). Expression of CD56 (4/4 cases) (Fig. 3F), synaptophysin, chromogranin, and INSM1 (collectively 3/7; 43%) also tended to be restricted to these round cell areas (Fig. 5H, I). As expected, more robust SATB2 expression was seen in case 3 with overt osteoblastic differentiation as compared with undifferentiated spindle cell zones in other cases (Figs. 2F and 4F). In case 6, markers of rhabdomyoblastic differentiation (eg, desmin, myogenin, and MyoD1) were seen in differentiating rhabdomyoblasts as well as in morphologically undifferentiated cells (Fig. 4E). More limited expression of these markers was present in the metastasis as well. A wide variety of other markers, tested on

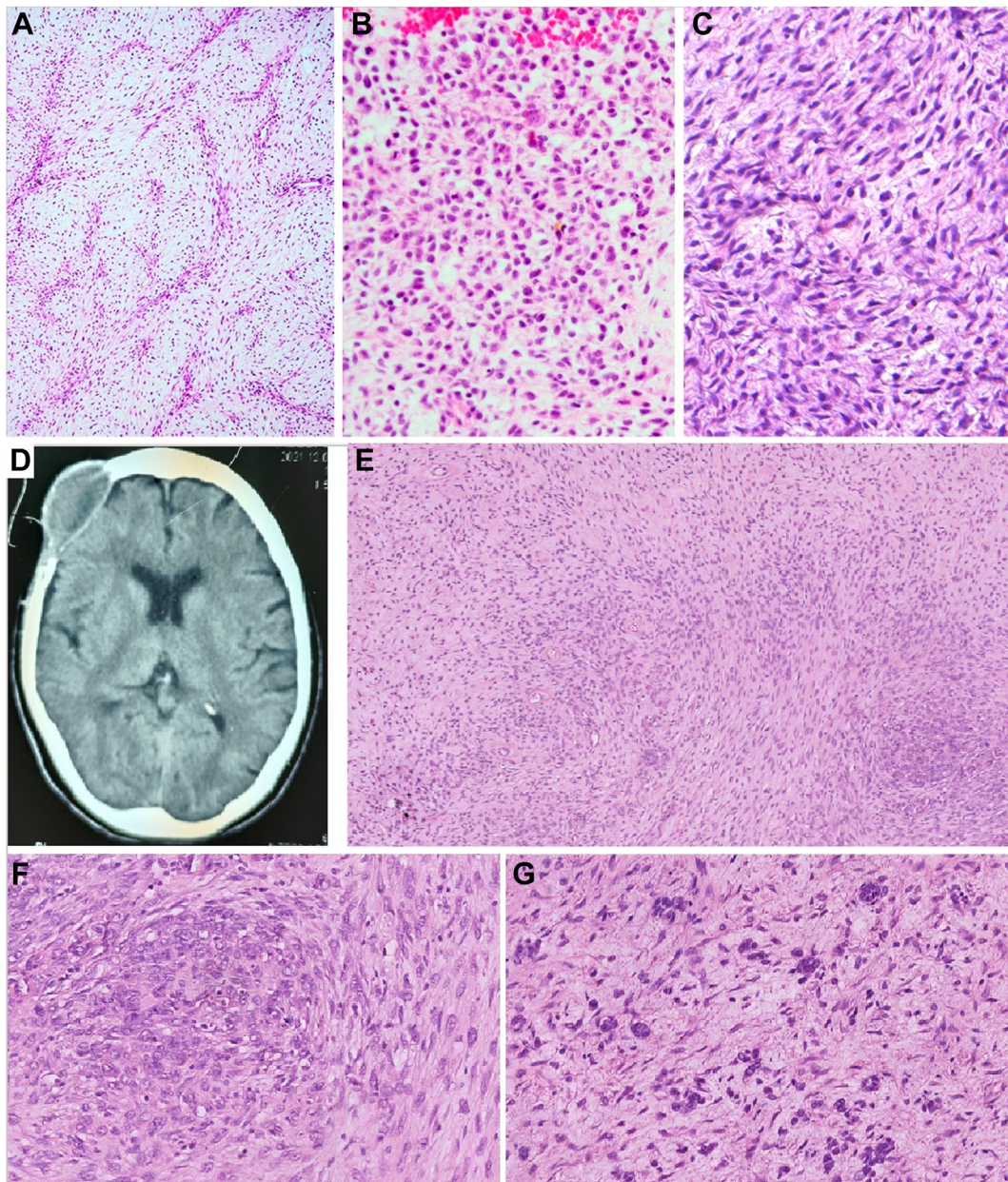


Figure 1.

(A-C) Case 1. The tumor in case 1 consisted of spindle to stellate cells with mild to moderate atypia and fibromyxoid stroma. (A) The primary tumor showed a striking arborizing vasculature and predominantly low cellularity, (B) with only a small focus having more cellular, round cell features. (C) The recurrent tumor consisted chiefly of more cellular spindled proliferation. (D-G) Case 2. (D) Computed tomography scan in case 2 showed a lytic tumor in the right frontal bone. This case was also hypocellular with scattered small-to medium-sized nests having a more cellular, round cell appearance. (E) Small, hyalinized vessels predominated. (F) High-power view of one of the larger nests with more round cell appearance. (G) High-power view of one of the small round cell aggregates.

an ad hoc basis in individual cases, were negative or noncontributory (listed in Table 2).

Molecular Genetic Findings

The molecular genetic data are summarized in Table 2. Fusions with genomic breakpoints in exons 9, 10, 14, 15, and 16 of *EWSR1* were noted across the cohort, while breakpoints in only exon 2 of *POU2AF3* gene were detected. Case 2 showed 3 coexisting breakpoint-defined subclones affecting adjacent exons 14 to 16 of *EWSR1*. Notably, the *EWSR1* genomic breakpoints in all 4 sinonasal

cases were located in exons 14 to 16, while all extrasinonasal cases had breakpoints in exons 9 or 10. In cases 1 to 3, 5, 7, and 8, a break of *EWSR1* was confirmed by fluorescence in situ hybridization. In case 8, DNA sequencing did not detect any pathogenic mutations, and the tumor mutation burden was low (3.9/Mb). No case harbored *FUS* rearrangement.

Discussion

Including the original description by Agaimy et al,¹ followed by the series by Hiemenz et al,² and our current cases, a total of 19

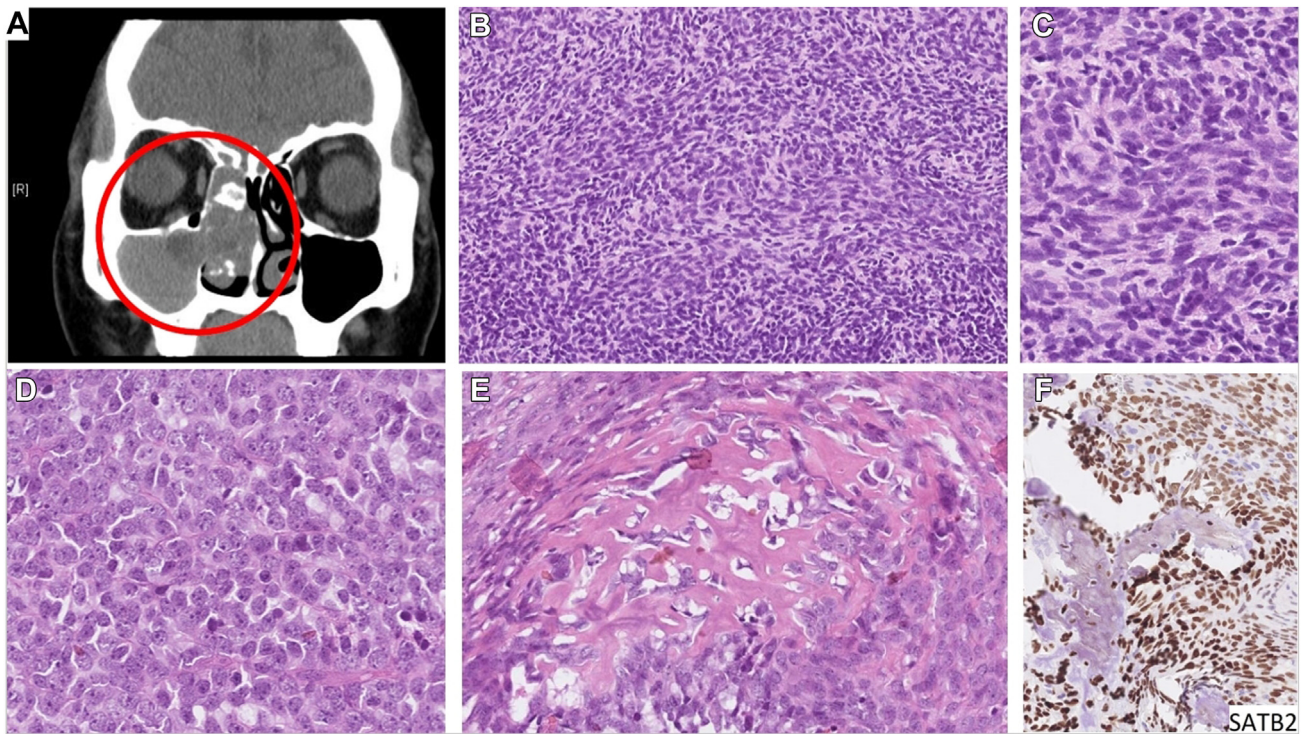


Figure 2.

Case 3. (A) Computed tomography scan revealed an infiltrative lesion of soft tissue density (red circle), involving the right nasal cavity and frontal, ethmoid, and maxillary sinuses with multifocal calcifications. (B, C) The tumor consisted of highly cellular sheets and fascicles of spindled cells and occasional round cells with little intervening stroma. (D) Foci with round cells featuring more basophilic nuclei with finely stippled chromatin and typically 1 prominent pinpoint nucleolus. (E) Some of the tumor cells showed osteogenic differentiation with production of osteoid matrix. (F) In line with the observed osteogenic properties, the tumor cells showed a diffuse strong expression of SATB2.

neoplasms carrying the *EWSR1/FUS::POU2AF3* (formerly *COLCA2*) fusion have been well documented (clinicopathological features of all cases are summarized in the [Supplementary Table S1](#)). A single case mentioned in one fusion detection methodological study (included in the current study as case 5) lacked any clinical or anatomical data.⁷

Clinically, these tumors show a striking predilection for the head and neck area (13/19 cases), particularly for the sinonasal tract (9/19 cases), but they may occur at other sites, including soft tissue and bone. They affect predominantly middle-aged to older adults without sex predilection. *EWSR1/POU2AF3*-rearranged neoplasms reported so far mostly behaved as high-grade sarcomas irrespective of their morphology. Half of the published cases (9/18) developed metastases (as late as 10 and 23 years after diagnosis, hence warranting a lifelong follow-up) with or without recurrence and additional 3 patients experienced either recurrence or progressive tumor growth. The 5-year local recurrence-free survival and metastasis-free survival were 48% and 54%, respectively, after combining the available clinical data from these 3 studies. While only 1 of 18 patients died of disseminated disease, most remaining patients were alive with disease despite multimodal therapy. However, 5 patients were without evidence of disease at the last follow-up, and all 4 with available treatment information received a multimodal therapy.

The previously emphasized morphologic features, including the presence of undifferentiated round and spindled cells with fine chromatin and prominent nucleoli^{1,2} and frequent biphasic arrangement of spindled and round/epithelioid cells, often mimicking synovial sarcoma,² were observed in our cases as well. The reported cases did not exhibit any consistent morphologic evidence of epithelial, mesenchymal, or other specific lines of

differentiation, apart from single cases in our study exhibiting glandular, osteoblastic, and rhabdomyoblastic differentiation, respectively. However, they seem to display an unusual immunophenotype with frequent expression of markers associated with epithelial (pankeratins and/or EMA expression) or neuroendocrine differentiation.^{1,2}

New findings in our study include the presence of 2 morphologically different subgroups. The first consisted of relatively uncharacteristic low-grade-appearing spindle cell tumors with mild to moderate atypia and variable cellularity with occasional foci showing round cells, while the second encompassed hypercellular neoplasms with predominantly high-grade round and spindle cell morphology. A very characteristic and frequent microscopic feature of this latter group was the presence of distinctly biphasic arrangement of the tumor cells with the formation of round cell nests on a predominantly spindle cell background reminiscent of biphasic synovial sarcoma but lacking true gland formation (apart from a single case). The round cells often contained more basophilic staining nuclei with more stippled chromatin, imparting a neuroendocrine appearance. Notably, it was these round cells that preferentially showed expression of keratins and the various neuroendocrine markers we tested. Novel findings also included the identification of true glands with mucin production and rhabdomyoblastic and osteogenic differentiation found in one case each.

The exact nosology of these neoplasms remains to be further delineated. Data from these 19 reported cases are in line with a sarcoma showing polyphenotypic (ambiguous) immunoprofile. Their morphologic heterogeneity precludes putting them into any of the defined epithelial or mesenchymal neoplastic categories despite their focal overlap with basaloid epithelial or

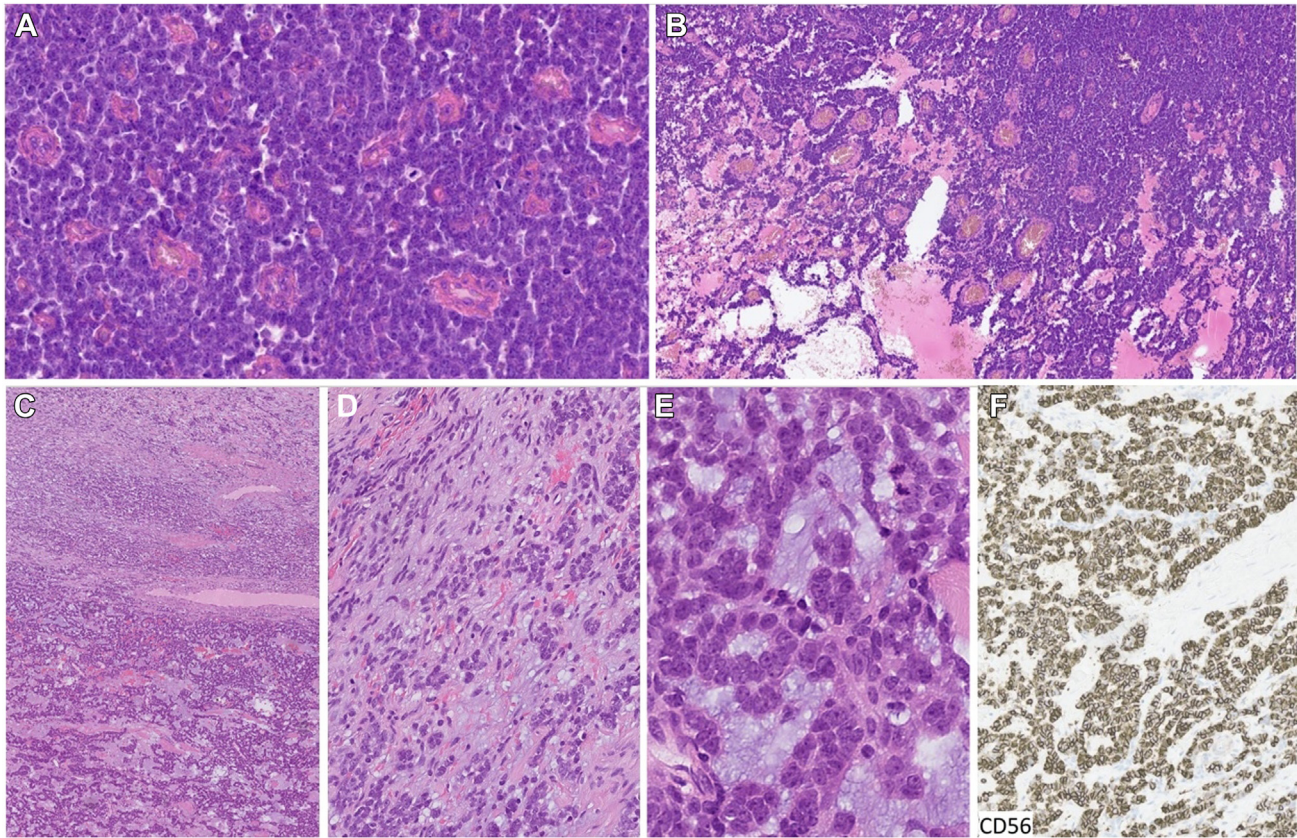


Figure 3.

(A, B) Case 4. This case consisted purely of nests or loose dispersed sheets of round cells with basophilic nuclei with finely stippled neuroendocrine-like chromatin. It contained (A) abundant small hyalinized vessels and (B) areas with myxoid stromal matrix, which imparted a cord-like and pseudopapillary architecture. (C-F) Case 5. (C) Case 5 showed a prominent biphasic arrangement consisting of predominantly spindle cell periphery (top), whereas toward the center (bottom), it contained round cells with neuroendocrine features with cord-like, pseudopapillary, and pseudoglandular arrangement within a myxoid stroma. (D) Transitional areas with round cells within the predominantly spindle cell background occasioning a resemblance to similar areas in case 2. (E) High-power view of round cells with neuroendocrine features forming pseudoglandular structures. (F) Diffuse strong expression of CD56.

neuroendocrine neoplasms in a subset of cases. While some may superficially mimic biphasic synovial sarcomas based on subtle biphasic arrangements of plump rounded and spindled cells, their morphology (variable spindle and round cell features) and polyphenotypic immunoprofile (variable expression of epithelial, rhabdomyogenic, and neurogenic markers such as S100, GFAP, and others) are partially reminiscent of the recently reported category of *EWSR1::PATZ1* fusion sarcomas.⁸ Their capacity for multilineage immunophenotypic differentiation is in line with derangement of cellular differentiation pathways downstream of the fusion transcript.

The observed morphologic differences between both subgroups obviously raise the question of whether all tumors presented in this study belong to the same entity or represent 2 fundamentally different neoplasms sharing the same genetic event, the *EWSR1/FUS::POU2AF3* fusion. We believe that several cases in our study offered a strong clue in support of a single entity with a wide morphologic spectrum. Notably, the significant variation in cell shape and cellularity within the same tumors and between primary tumors and their recurrences suggests the presence of a bland-looking spindled morphology at one end and a highly cellular undifferentiated round cell morphology at the other end of the spectrum with a lot of transitional morphologies on the spectrum in-between. A similar morphologic spectrum of morphologically low-grade—appearing

sarcomas with low cellularity on one end and frankly high-grade neoplasms with significantly different appearance on the other is commonplace in several other soft tissue entities such as low-grade fibromyxoid sarcoma (LGFS)/sclerosing epithelioid fibrosarcoma, low-grade/high-grade myxoid liposarcoma, or more recently within the spectrum of *EWSR1::PATZ1*-rearranged sarcomas.^{8–10}

The differential diagnosis of these tumors is defined by the predominant pattern seen in a given case and is also influenced by the anatomical site and immunohistochemical findings. The low-grade spindle cell tumors might be reminiscent of other spindle cell sarcomas with fibromyxoid stroma such as OFMT and LGFS. Features arguing against the diagnosis of OFMT were the wide infiltrative growth and lack of pseudocapsule and ossification (in the vast majority of cases).¹¹ The absence of abrupt transitions between collagenized and myxoid stroma and the lack of MUC4 expression (caveat: only 2 cases tested) argue against LGFS.¹² Low-grade myxofibrosarcoma or, in case of a primary bone tumor, various forms of osteosarcoma may enter the differential as well (see later discussion). The high-grade round and spindle cell tumors may be difficult to distinguish from Ewing sarcoma, CIC-rearranged sarcoma, and other sarcomas with this morphology which can be achieved using histology; immunohistochemistry; and, if necessary, molecular methods.¹³ While *EWSR1/FUS::POU2AF3* sarcomas can be added to a long list of mimickers of

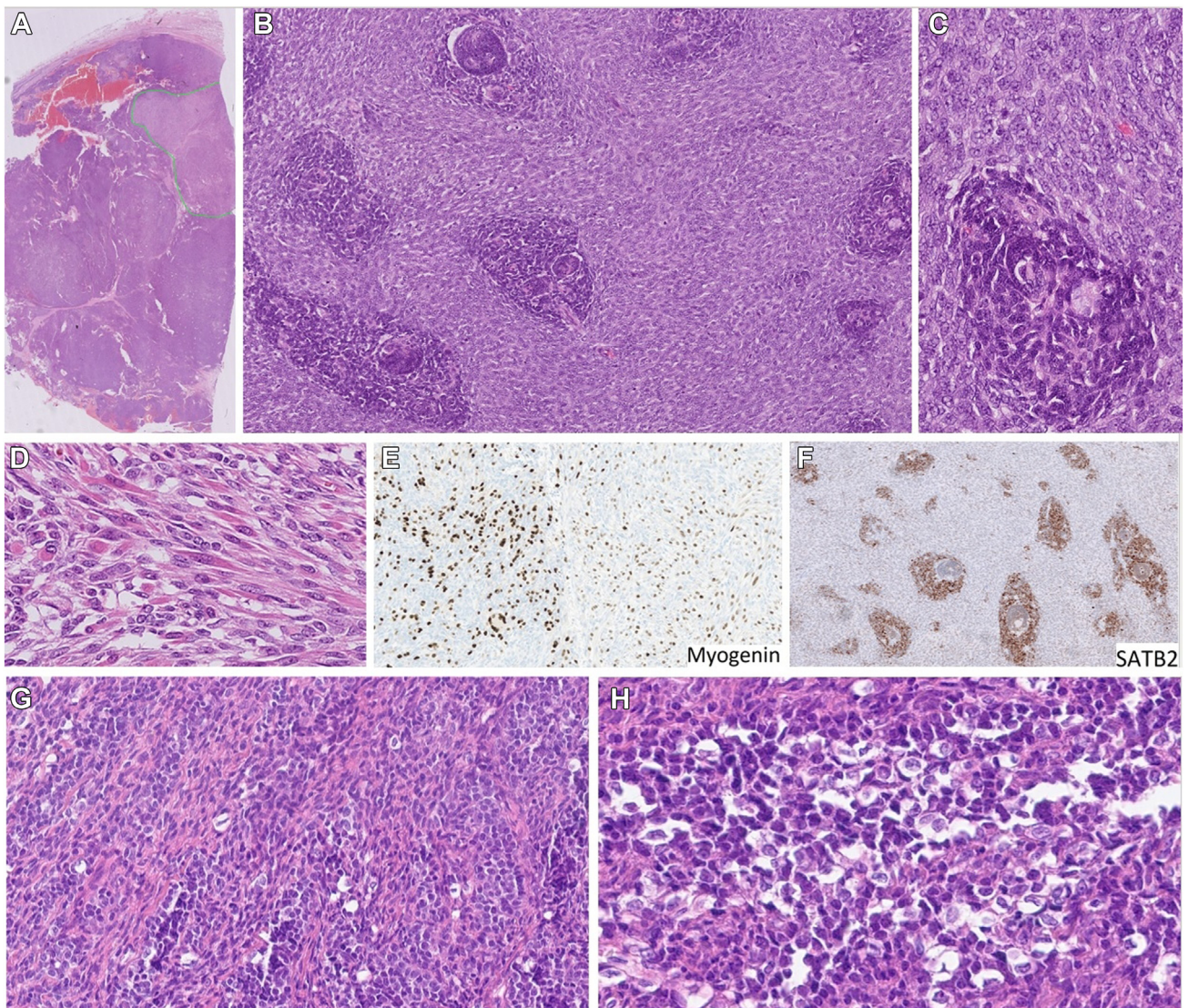


Figure 4.

(A-F) Case 6. (A) Case 6 contained a sharply demarcated rhabdomyoblastic nodule (green line). (B, C) Most parts showed a biphasic arrangement and consisted of round cell nests with predominantly neuroendocrine nuclear features on a background of more ovoid to spindle cells having lighter chromatin. (D) High-power view of the focus containing strap cells and rhabdomyoblasts with cross-striations. (E) Markers of rhabdomyoblastic differentiation including myogenin were positive throughout the tumor. (F) SATB2 expression was found almost exclusively in the round cell nests. (G, H) Case 7. (G) The tumor also showed a biphasic arrangement, with most parts of the tumor consisting of more loosely dispersed sheets of round cells with neuroendocrine features admixed with spindle cells. (H) Numerous clear cells were present.

neuroendocrine neoplasms,¹⁴ the latter typically exhibit more diffuse expression of epithelial and neuroendocrine markers (typically coexpression of synaptophysin, chromogranin, and/or INSM1), and in most cases, a primary lesion can be identified clinically. As already emphasized by Hiemenz et al.,² these tumors are in many aspects highly reminiscent of synovial sarcoma, particularly of those with predominantly spindle cell morphology, and a confident distinction purely on morphologic grounds is unreliable. However, with the use of novel highly specific and sensitive immunohistochemical markers that are becoming available at many institutions, synovial sarcoma can most likely be excluded (caveat: only 1 case tested for SS18::SSX antibody).¹⁵ Nevertheless, while we believe that, when present, some of the more recurrent histopathological features of *EWSR1/FUS::POU2AF3* tumors may raise suspicion for the diagnosis, high-throughput genetic assays such as RNA sequencing represent

the only way to reliably diagnose these tumors at this point. In addition, some cases lack the more characteristic histologic traits, and they cannot be distinguished from morphologically highly overlapping differential diagnostic entities that lack specific immunohistochemical markers such as low-grade myxofibrosarcoma, osteosarcoma, or various myoepithelial or basaloid epithelial neoplasms without appropriate molecular studies.

Regarding the molecular background, among the 19 reported cases, 17 carried the *EWSR1::POU2AF3* fusion, while 2 cases had *FUS* as the 5' fusion partner instead of *EWSR1*. While the number of published cases is low and the following observation might thus be merely coincidental, it is interesting to note that in 14 of 17 cases with *EWSR1::POU2AF3* fusion, the locations of genomic breakpoints seemed to follow an anatomical site-dependent pattern. Namely, 9 of 12 head and neck cases had genomic breakpoints located in exons 12 to 17 and only 3 cases in exons 9

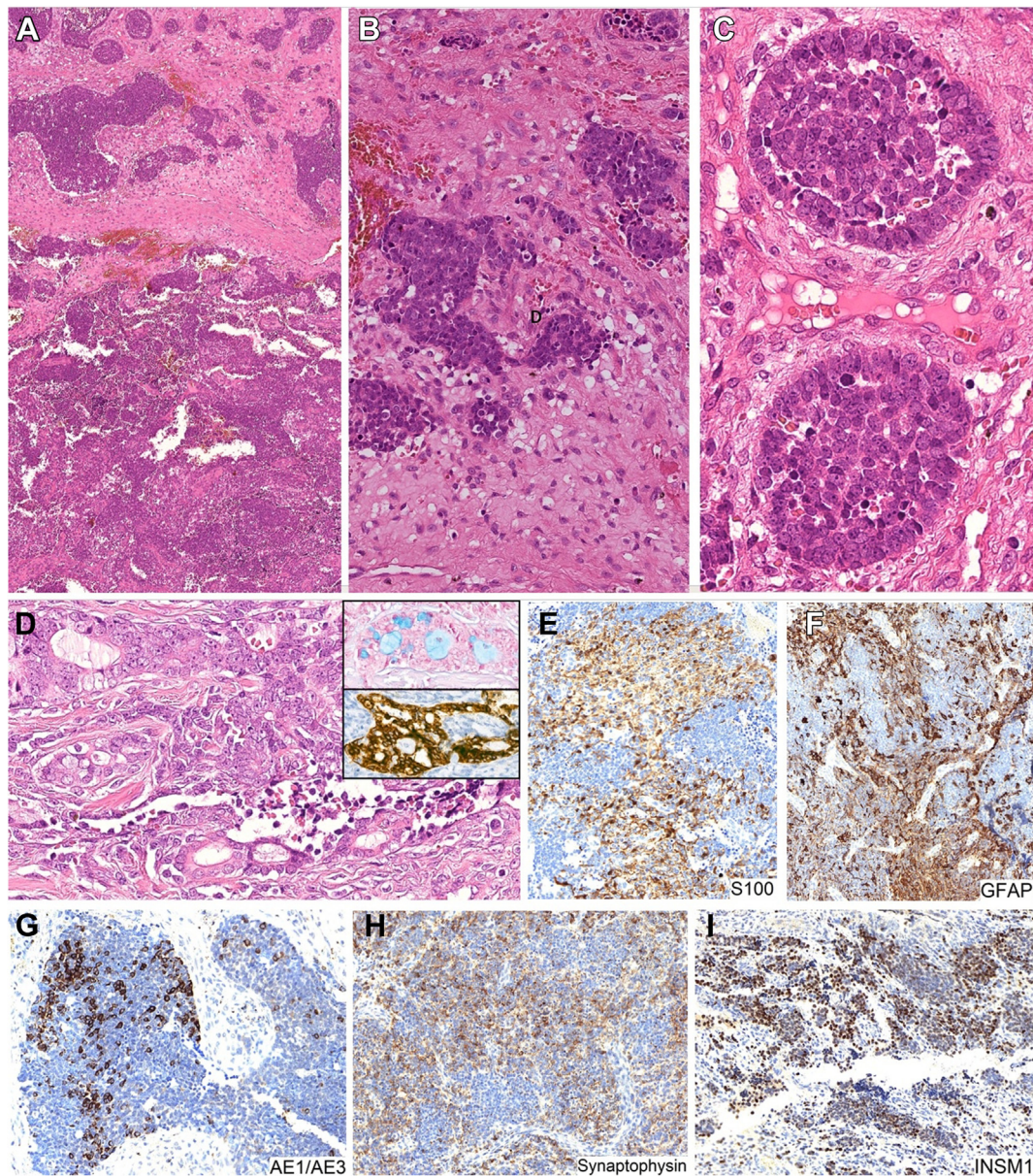


Figure 5.

Case 8. (A) Cellular stroma-poor areas (bottom) alternated with more hyalinized spindle cell regions containing scattered round cell nests (top). (B) Nests of round cells with neuroendocrine features on the hypocellular background featuring spindle to stellate shaped cells with little atypia were reminiscent of similar areas in cases 1 and 2. (C) Peripheral palisading of the round cells was present in some nests. (D) A small focus with keratin 7-positive glands (lower inset) containing Alcian blue-positive mucin (upper inset). (E-I) Multifocal expression of S100, GFAP, AE1/AE3, synaptophysin, and INSM1 preferentially found within the round cell areas.

or 10 of *EWSR1* gene. In contrast, all 5 cases located outside the head and neck area had breakpoints localized in exons 9 or 10. Exons 12 to 14 contain a central RNA recognition motif (RRM), which is involved in RNA and DNA binding (Fig. 6). Similar to Ewing sarcoma and many other *EWSR1*-rearranged tumors that usually have genomic breakpoints located in exons 6 to 8, the RRM is lost in non-head and neck cases.¹⁶⁻¹⁸ In contrast, most of the reported head and neck cases retained the RRM domain. The binding of regulatory nucleic acids or ribonucleoproteins may potentially affect the function of the fusion protein. A difference in the pathogenesis of the translocation, including the specific microenvironment of the head and neck area, might potentially be causative, while the exact significance and molecular basis remain unclear.

POU class 2 homeobox associating factor 3 (POU2AF3) gene [formerly named *colorectal cancer associated 2 (COLCA2)*] located on chromosome 11q23.1 has been associated with increased colorectal cancer risk in a genome-wide association study. The pattern of expression of the POU2AF3 protein in neoplastic, stromal, and immune cells in patients with higher-risk alleles for colorectal cancer suggested a tumor suppressor role.¹⁹ However, the exact mechanism through which the POU2AF3 protein is deregulated remains unclear.¹ Moreover, POU2AF3 codes for a transcriptional coactivator protein that through its OCA domain (Fig. 6) binds with the transcription factor POU2F3, which is critical for the development of tuft cells, a rare chemosensory lineage that coordinates immune and neural responses to foreign pathogens in mucosal tissues.

Table 2
Histologic, immunohistochemical, and molecular features of *EWSR1::POU2AF3* tumors

Case no.	Mits/10 HPF and necrosis	AE1/AE3	EMA	S100	GFAP	NE markers (all available)	SAT-B2	Other positive IHC	Other negative IHC	<i>EWSR1</i> breakpoint	<i>POU2AF3</i> breakpoint
1	2/10 Absent	–	–	–	–	ND	–	SMA+; CD10+	Desmin; SOX10; MUC4, CDX2	Ex 14	Ex 2
2	2/10 Absent	+	ND	–	–	SYN–	++	ND	MUC4; ALK; NUT; Pan-Trk; STAT6; Desmin; SOX10; SMA; Myo-D1; Myogenin; CD34; Beta-Catenin, CDX2	Ex 14-16	Ex 2
3	6/10 Present (10%)	–	–	–	+	SYN– CD56+	+++	ND	CD99; NKX2.2; SOX10; H3k27me3 retained; SS18::SSX; TLE1; Desmin; myogenin; STAT6	Ex 15	Ex 2
4	10/10 Present (10%)	++	+	+	++	SYN– CHROMO– CD56+	ND	CD99+++	Desmin; Myogenin; SMA	EX 14	Ex 2
5	9/10 Absent	–	–	++	++	SYN+ CHROMO– CD56+++	+	SMA+	OSCAR; Desmin; MyoD1; myogenin; SOX10; TLE1; BRAF; CD34; panTRK; CD99; CDX2	EX 9	Ex 2
6	4/10 Absent	++	–	+	+	SYN+ CHROMO+ CD56+++	++	Desmin/MyoD1/ Myogenin ++; SMA+; membranous CD99+; MyoD1+; Myogenin+	BCOR; WT1; TLE1, CDX2; SOX10	EX 9	Ex 2
7	8/10 Absent	++	–	ND	–	SYN– CHROMO–	ND	ND	Desmin; Myogenin; SMA; CD99	EX 9	Ex 2
8	24/10 Absent	+	+	++	++	SYN++ CHROMO++ INSM1+	–	CK5+; CK7+; SMA+	CD99; SOX10; TTF1; CDX2; CD34; p63; CK20; MC polyomavirus; calponin; WT1; ERG; CyclinD1 and ALK; Rb and INI1 retained; TP53 wt	EX 10	Ex 2
		5/8 (63%)	2/7 (29%)	4/7 (57%)	5/8 (63%)	CD56: 4/4 Any other: 3/7 (43%)					

CHROMO, chromogranin; Ex, exon; IHC, immunohistochemistry; mits/10 HPF, mitoses per 10 high-power fields; ND, not done; NE, neuroendocrine; SMA, smooth muscle actin; SYN, synaptophysin.

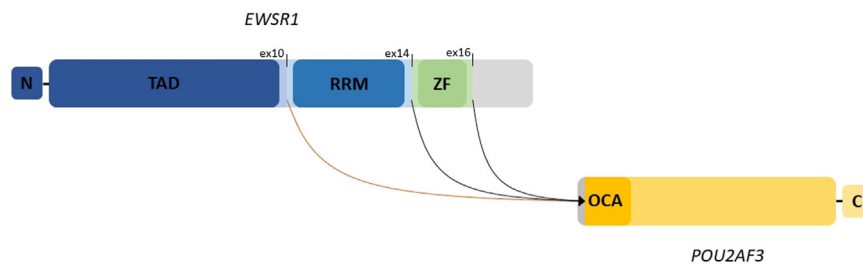


Figure 6.

Schematic depiction of the *EWSR1::POU2AF3* fusion. Dark blue, blue, green, and yellow parts of the respective genes represent the regions that are retained in the fusion products, while the gray parts are lost. In non-head and neck cases, only the transactivation domain (TAD) (dark blue) of *EWSR1* is retained. In contrast, the head and neck cases contain both TAD and the RNA recognition motif (RRM) (blue) of *EWSR1*; in addition, some head and neck cases also retain the RanBP2 type zinc finger (ZF) (green). The OCA domain of *POU2AF3* (yellow), which enables binding to POU domain-containing transcription factors, is almost completely retained in the fusion gene. ex, exon.

Recent studies have also revealed tuft-cell-like human tumors, particularly as a variant of small cell lung cancer.²⁰

In conclusion, we reported 8 additional cases of *EWSR1::POU2AF3* sarcomas, further defining their clinicopathological features and, for the first time, emphasizing their potential for heterologous differentiation. Many cases exhibit relatively characteristic biphasic morphologic features, whereas others may show epithelial, rhabdomyosarcomatous, or osteosarcomatous differentiation or present as nondescript spindle and/or round cell sarcomas. Although further studies are needed to confirm whether all tumors with *EWSR1::POU2AF3* fusion represent a single entity, we believe that some of the partially overlapping clinicopathological features in our cases lend support to this notion. Because most of these tumors follow an aggressive clinical course and, in some cases, their low-grade morphology may be deceiving, the use of high-throughput molecular methods is recommended to achieve early detection, enabling optimal treatment.

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Author Contributions

O.K., C.A.D., J.T.-M., and Michael Michal designed the study, analyzed the data, and drafted and edited the manuscript. A.A. and A.L.F. edited the manuscript. A.M.O. performed the statistical analysis. All other authors contributed cases, provided clinical information, or performed molecular testing. All authors approved the final version of the manuscript.

Data Availability

Data that support the findings of this study are available from the corresponding author on reasonable request.

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Declaration of Competing Interest

The authors have no conflict of interest to disclose.

Ethics Approval and Consent to Participate

The study was conducted following the rules set by the Faculty Hospital in Pilsen Ethics Committee. Informed consent was not required for the study.

Supplementary Material

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