Mod Pathol 36 (2023) 100160

MODERN PATHOLOGY

USCAP UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY
Creating a Better Pathologist

Journal homepage: https://modernpathology.org/

Research Article

Extraenteric Malignant Gastrointestinal Neuroectodermal Tumor—A Clinicopathologic and Molecular Genetic Study of 11 Cases

Veronica Ulici[a], Jason L. Hornick[b], Jessica L. Davis[c], Swati Mehrotra[d], Jeanne M. Meis[e], Kevin C. Halling[a], Christopher D.M. Fletcher[b], Erica Kao[f], Andrew L. Folpe[a,*]

[a] *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota;* [b] *Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts;* [c] *Department of Pathology, University of Indiana, Indianapolis, Indiana;* [d] *Department of Pathology and Laboratory Medicine, Loyola University Medical Center, Maywood, Illinois;* [e] *Department of Anatomical Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas;* [f] *Department of Pathology, Brooke Army Medical Center, San Antonio, Texas*

ARTICLE INFO

Article history:
Received 20 January 2023
Revised 28 February 2023
Accepted 12 March 2023
Available online 17 March 2023

Keywords:
sarcoma
soft-tissue neoplasm
malignant gastrointestinal neuroectodermal tumor
clear cell sarcoma
immunohistochemistry
molecular genetics

ABSTRACT

Malignant gastrointestinal neuroectodermal tumors (MGNETs), also known as "gastrointestinal clear cell sarcoma–like tumors", are very rare, aggressive sarcomas characterized by enteric location, distinctive pathologic features, and *EWSR1/FUS::ATF1/CREB1* fusions. Despite identical genetics, the clinicopathologic features of MGNET are otherwise quite different from those of clear cell sarcoma of soft parts. Only exceptional extraenteric MGNET (E-MGNET) has been reported. We report a series of 11 E-MGNETs, the largest to date. Cases diagnosed with MGNET and occurring in nonintestinal locations were retrieved. A clinical follow-up was obtained. The tumors occurred in 3 men and 8 women (range, 14-70 years of age; median, 33 years) and involved the soft tissues of the neck (3), shoulder (1), buttock (2), orbit (1), tongue/parapharyngeal space (1), urinary bladder (1), and falciform ligament/liver (1). Tumors showed morphologic features of enteric MGNET (small, relatively uniform, round to ovoid cells with round, regular nuclei containing small nucleoli growing in multinodular and vaguely lobular patterns, with solid, pseudoalveolar, and pseudopapillary architecture). Immunohistochemical results were S100 protein (11/11), SOX10 (11/11), synaptophysin (3/10), CD56 (7/9), CD117 (3/9), DOG1 (0/4), ALK (4/8), chromogranin A (0/10), HMB-45 (0/11), Melan-A (0/11), tyrosinase (0/4), and MiTF (0/11). Next-generation sequencing results were *EWSR1::ATF1* (7 cases), *EWSR1::CREB1* (3 cases), and *EWSR1::PBX1* (1 case). The *EWSR1::PBX1*-positive tumor was similar to other cases, including osteoclast-like giant cells, and negative for myoepithelial markers. A clinical follow-up (range, 10-70 months; median, 34 months) showed 4 patients dead of disease (10.5, 12, 25, and 64 months after diagnosis), 1 patient alive with extensive metastases (43 months after diagnosis), 1 patient alive with persistent local disease (11 months after diagnosis), and 4 alive without disease (10, 47, 53, and 70 months after diagnosis). One case is too recent for the follow-up. The clinicopathologic and molecular genetic features of rare E-MGNET are essentially identical to those occurring in intestinal locations. Otherwise, typical E-MGNET may harbor *EWSR1::PBX1*, a finding previously unreported in this tumor type. As in enteric locations, the behavior of E-MGNET is aggressive, with metastases and/or death from disease in at least 50% of patients. E-MGNET should be distinguished from clear cell sarcoma of soft parts and other tumors with similar fusions. ALK expression appears to be a common feature of tumors harboring *EWSR1/FUS::ATF1/CREB1* fusion but is unlikely to predict the therapeutic response to ALK inhibition. Future advances in our understanding of these unusual tumors will hopefully lead to improved nomenclature.

* Corresponding author.
E-mail address: folpe.andrew@mayo.edu (A.L. Folpe).

0893-3952/$ - see front matter
https://doi.org/10.1016/j.modpat.2023.100160

Introduction

Malignant gastrointestinal neuroectodermal tumors (MGNETs),[1] also known as "gastrointestinal clear cell sarcoma–like tumors,"[2] are rare, aggressive sarcomas characterized by enteric location, distinctive morphologic and immunohistochemical features, and gene fusions involving *EWSR1* or *FUS* with either *CREB1* or *ATF1*.[2-4] Although Zambrano et al[5] are generally credited for the recognition of this tumor as a distinctive entity (termed "osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts [CCS]"), an identical case had been reported some years previously by Alpers and Beckstead[6] as a "malignant neuroendocrine tumor of the jejunum with osteoclast-like giant cells." MGNET and CCS share *EWSR1/FUS::ATF1/CREB1* fusions and S100 protein/SOX10 coexpression; however, their morphologic features are different, and the expression of melanocytic markers (eg, HMB-45, Melan-A, tyrosinase, and MiTF) is seen in CCS but not in MGNET.[2-4] For these reasons, the term "MGNET" is preferable to "clear cell sarcoma–like tumor."

Although MGNET was originally believed to be unique to the gastrointestinal tract, a small number of similar tumors occurring in nonenteric locations have recently been reported.[7-14] Herein, we report our experience with 11 extraenteric MGNETs (E-MGNETs).

Materials and Methods

Case Accrual

The institutional review boards of the participating institutions approved this study. All available slides and blocks for 11 cases previously diagnosed as "MGNET" and occurring in nonenteric locations were retrieved from our archives for the period 2006 to the present. Clinical information, including the follow-up, was obtained from contributing pathologists, clinicians, and electronic medical records.

Immunohistochemistry

At the Mayo Clinic, immunohistochemistry (IHC) was performed on 4-μm-thick formalin-fixed paraffin-embedded (FFPE) whole-tissue sections using the following antibodies: wide-spectrum keratins (1:100 dilution; AE1/AE3; Agilent Dako), S100 protein (1:750; polyclonal; Leica), SOX10 (predilute; SP267; Cell Marque), synaptophysin (1:50; 27G12; Leica), chromogranin A (predilute; LK2H10; Ventana Roche), CD117 (1:200; YR145; Cell Marque), (1:100; D5F3; Cell Signaling), CD56 (predilute; MRQ-42; Cell Marque), Melan-A (1:50; A103; Agilent Dako), HMB-45 (1:400; HMB-45; Agilent Dako), DOG1 (predilute; 244R18; Cell Marque), and MiTF (1:100; D5; Agilent Dako). Ultra Cell Conditioning Solution (Ultra CC1) was used as a pretreatment step for all antibodies. OptiView DAB IHC Detection Kit or UltraView Detection System (Ventana Roche) was used for all antibodies.

At the Brigham and Women's Hospital, IHC was performed on 4-μm-thick FFPE whole-tissue sections using the following antibodies: keratins (1:200 dilution; AE1/AE3; Agilent Dako), S100 protein (1:1000; EP32; Cell Marque), SOX10 (1:1500; polyclonal; Cell Marque), synaptophysin (1:100; 27G12; Leica), chromogranin (1:8000; LK2H10; Thermo Fisher), CD117 (1:150; polyclonal; Agilent Dako), and ALK (1:200; 5A4; Leica). Pressure cooker antigen retrieval (Target Retrieval Solution; pH 6.1 citrate buffer; Agilent Dako) was used for SOX10, chromogranin, and ALK. Protease antigen retrieval was used for AE1/AE3. No antigen retrieval was used for S100 protein, synaptophysin, or CD117. The EnVision+ Detection System (Agilent Dako) was used for all antibodies except for *ALK*, for which the Novolink Polymer Detection System (Leica) was used.

Next-Generation Sequencing

Next-generation sequencing (NGS) was performed using the Mayo Clinic Sarcoma Targeted Gene Fusion/Rearrangement Panel. This is a targeted, custom-designed, amplicon-based panel that uses Qiagen's QIAseq chemistry and uses single primer extension target enrichment and unique molecular identifier technology to identify fusions in 138 genes. RNA was extracted from FFPE unstained slides using the Qiagen miRNeasy FFPE Kit. RNA samples were converted to double-stranded cDNA, end-repaired, and A-tailed. The cDNA was then ligated with a unique molecular identifier and separate sample index. Adapter-ligated cDNA molecules were subject to limited target enrichment using single primer extension. Universal(PCR) is carried out to amplify the library and to add a second sample index. The final library was then sequenced on an Illumina MiSeq sequencer. Sarcoma Targeted Gene Fusion/Rearrangement Panel NGS data were analyzed using SeekFusion, an internal bioinformatic pipeline that uses a combination of traditional alignment and *de novo* assembly-based approaches.[15]

Results

Clinical Features

Table 1 summarizes the clinicopathologic features of the studied cases. The tumors occurred in 3 men and 7 women (median, 33 years; range, 14-70 years) and involved somatic soft-tissue locations (Fig. 1A), including the neck (3) shoulder (1), buttock (1), orbit (1), tongue/parapharyngeal space (1), urinary bladder (1) and liver/falciform ligament (1). The tumors ranged from 3.7 to 10 cm in size (median, 5.5 cm).

Clinical follow-up (10 patients; range, 10-70 months; median, 34 months) showed 4 patients dead of disease (11, 12, 25, and 64 months after diagnosis), 1 patient alive with extensive metastases (43 months after diagnosis), 1 patient alive with persistent local disease while undergoing radiotherapy (11 months after diagnosis), and 4 alive without disease (10, 47, 53, and 70 months after diagnosis). There were local recurrences in 5 patients (4, 10, 25, 34, and 57 months after diagnosis). Four patients developed distant metastases (4, 11, 17, and 36 months after diagnosis); metastatic sites included the bone (3 events), lung (2 events), liver (2 events), lymph nodes (1 event), pleura/pericardium (1 event), peritoneum (1 event), and adrenal gland (1 event). One case is too recent for a meaningful follow-up.

Nine patients were known to have undergone surgical resection of their tumors. Of these, 3 tumors were reported to have been excised with negative margins, 3 were described as having "very close" or "positive" margins, and 1 was

Table 1
Clinicopathologic and Molecular Genetic Features

Case	Age (y)/Sex	Location/Size	IHC Results	Molecular Genetic Results	Therapy	Local recurrence	Metastasis	Outcome	Other
1	70/F	Chest wall/NA	Positive: S100 protein, SOX10, CD56, CD117, and ALK Negative: Keratins, HMB45, Melan-A, tyrosinase, MiTF, synaptophysin, and chromogranin	*EWSR1* exon 7::*CREB1* exon 6	Resection of primary tumors and recurrence	Yes: 10 mo	Pleura, pericardium, diaphragm, bone, liver: 11 mo	DOD at 25 mo	History of breast and thyroid cancer
2	36/M	Bladder/NA	Positive: S100 protein, SOX10, synaptophysin, and CD56 Negative: Keratins, HMB-45, Melan-A, chromogranin A	*EWSR1* exon 7::*ATF1* exon 5	Cystectomy; chemo/radiotherapy for recurrent and metastatic disease	Yes: 34 mo	Multiple bones, lungs: 36 mo	AWD at 43 mo; lost to follow-up at 45 mo	
3	14/M	The right neck/3.7 cm	Positive: S100 protein, SOX10, synaptophysin, and CD56 Negative: Keratins, HMB-45, Melan-A, MiTF, chromogranin A, CD117, DOG1, and ALK	*EWSR1* exon 8::*ATF1* exon 4	Resection with positive margin, radiotherapy	No: persistent local disease, undergoing radiotherapy	No	AWD at 11 mo	
4	29/F	Right shoulder/NA	Positive: S100 protein, CD56, ALK Negative: Keratins, HMB-45, Melan-A, tyrosinase, synaptophysin, MiTF, chromogranin A, CD117, and DOG1	*EWSR1::ATF1*	NA	NA	NA	DOD at 12 mo	
5	30/M	The right neck/NA	Positive: S100 protein, SOX10, synaptophysin, and CD56 Negative: Keratins, HMB-45, Melan-A, MiTF, chromogranin A, CD117	*EWSR1* exon 8::*ATF1* exon 4	Marginal resectio, radiotherapy	Yes: 57 mo	No	ANED at 70 mo	
6	48/F	Left buttock/NA	Positive: S100 protein, SOX10, CD56, and ALK Negative: Keratins, HMB-45, tyrosinase, MiTF, Melan-A, chromogranin A, synaptophysin, CD117, and DOG1	*EWSR1* exon 8::*PBX1* exon 5	Resection with negative margins	NA	NA	ANED at 53 mo	
7	48/F	The right neck/5.5 cm	Positive: S100 protein, SOX10 Negative: Keratins, synaptophysin, MiTF, HMB45, Melan-A, synaptophysin, chromogranin A, CD117, and ALK	*EWSR1* exon 7::*ATF1* exon 5	Marginal resection	No	No	ANED at 10 mo	
8	30/F	Liver and falciform ligament/10 cm	Positive: S100 protein, SOX10 Negative: Keratins, HMB45, Melan-A, MiTF, chromogranin A, synaptophysin, CD56, CD117, DOG1, and ALK	*EWSR1* exon 7::*CREB1* exon 6	Hepatic lobectomy and cholecystectomy	Ye, 4 mo	Extensive peritoneal and omental spread, intra-abdominal lymph nodes: 4 mo	DOD at 11 mo	
9	29/F	Left orbit/NA	Positive: S100 protein, SOX10, CD56, and CD117 Negative: Keratins, synaptophysin, chromogranin A, ALK	*EWSR1* exon 10::*ATF1* exon 3	Resection of primary tumor and recurrence: radiotherapy	Yes: 25 mo	Lung, liver, adrenal, multiple bones: 17 mo	DOD at 64 mo	
10	45/F	Tongue/parapharyngeal space >5 cm	Positive: S100 protein, SOX10, CD117, and ALK Negative: Keratins, Melan-A, MiTF, synaptophysin, chromogranin A, and CD56	*EWSR1* exon 7::*CREB1* exon 6	"Piecemeal" excision	No	No	ANED at 47 mo	Nasopharyngeal carcinoma was treated with radiation> 20 y prior
11	40/F	Left gluteus	Positive: S100 protein, SOX10 Negative: HMB45, Melan-A, tyrosinase, MiTF, and BRAFv600E	*EWSR1* exon 8:: *ATF1* exon 4	Incisional biopsy	Recent case	Recent case	Recent case	Recent case

ANED, alive with no evidence of disease; AWD, alive with disease; DOD, dead of disease; F, female; IHC, immunohistochemistry; LTFU, lost to follow-up; M, male; NA, not available.

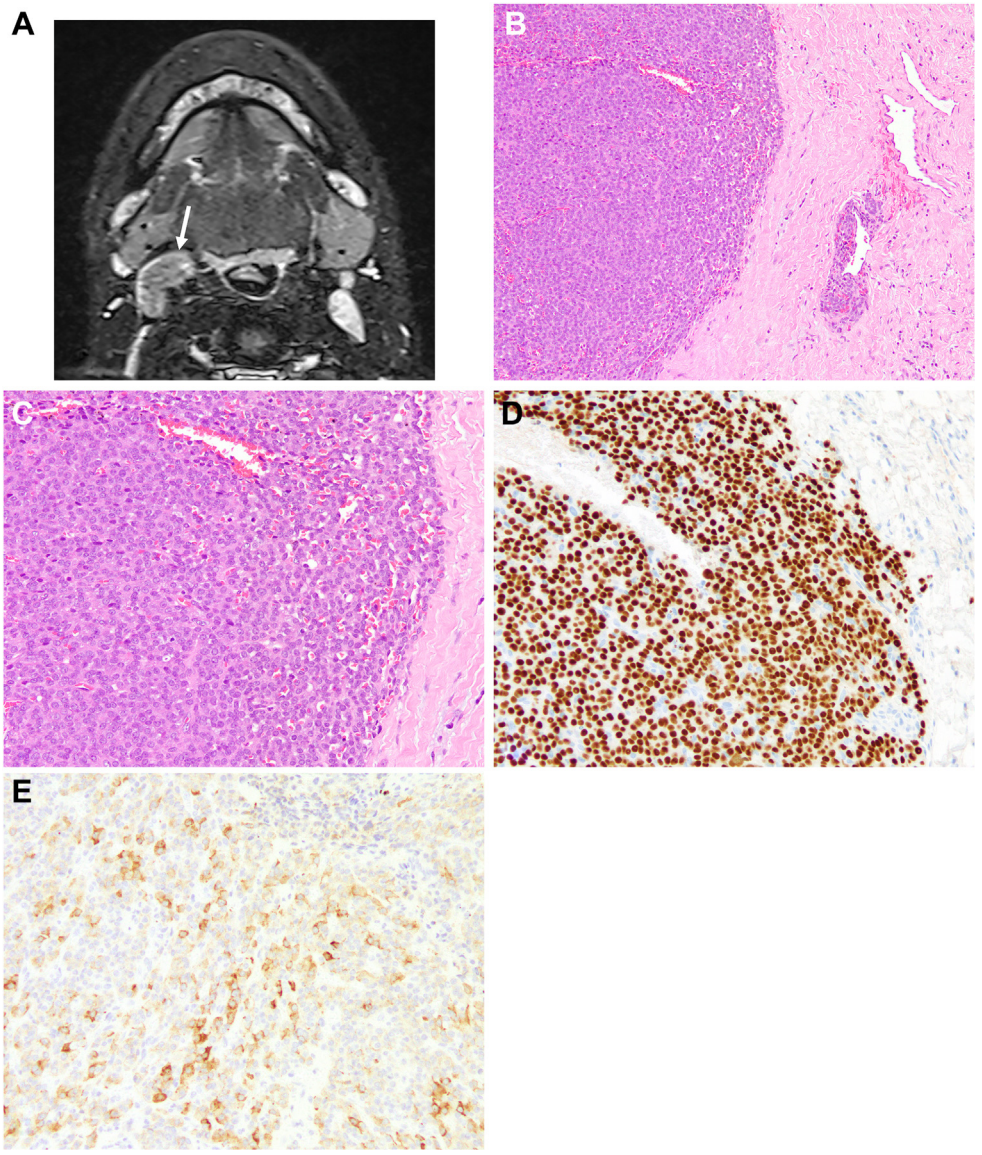

Figure 1.
Extraenteric malignant gastrointestinal neuroectodermal tumor involving the soft tissues of the neck in a 14-year-old boy (case 3). (A) Axial T2-weighted, fat-saturated magnetic resonance image demonstrated a mass within the upper right neck posterior to the submandibular gland, with heterogeneous but predominantly intermediate T2 signal (arrow). (B) The tumor grew in a multinodular, lobular manner. Note separate focus of subendothelial tumor. (C) The lesional cells were small, uniform, and ovoid, with small nucleoli. (D) Diffuse SOX10 and S100 protein (not shown) expression was present. (E) Subsets of tumor cells expressed synaptophysin.

removed in a "piecemeal" manner. An incisional biopsy was performed in 1 case. In 2 cases, the margin status was unknown. Four patients were known to have received adjuvant therapy, with 3 receiving radiotherapy alone and 1 receiving radiotherapy and chemotherapy. One patient whose mass occurred in the neck was known to have received radiotherapy for nasopharyngeal carcinoma >20 years previously; whether the present tumor arose in the irradiated field is unknown. Another patient whose tumor involved the posterior chest wall had a history of carcinomas of the breast and thyroid gland; details about the treatment of those tumors were not available. The patient whose primary tumor involved the liver and falciform ligament was not known to have a history of a primary enteric tumor, nor was one subsequently discovered.

Morphologic, Immunohistochemical, and Molecular Genetic Findings

Figures 1 to 5 illustrate the representative morphologic and immunohistochemical features of cases 2, 7, 4, 6, and 9, respectively. The morphologic features of the 11 E-MGNETs were essentially identical to those of their enteric counterparts. The masses grew in multinodular and vaguely lobular patterns (Figs. 1B, 2A, 4A), infiltrated into the surrounding soft tissues, and displayed solid, pseudopapillary, and pseudoalveolar architecture (Figs. 2B-D, 4B-D, 5A). In all but 1 case, the neoplastic cells were small, uniform, and round to ovoid, with a modest amount of lightly eosinophilic cytoplasm, round nuclei with irregularly dispersed chromatin, and small to inapparent nucleoli (Figs. 1C, 2E, 4E, 4F, 5B). One case (case 4) consisted of larger, epithelioid

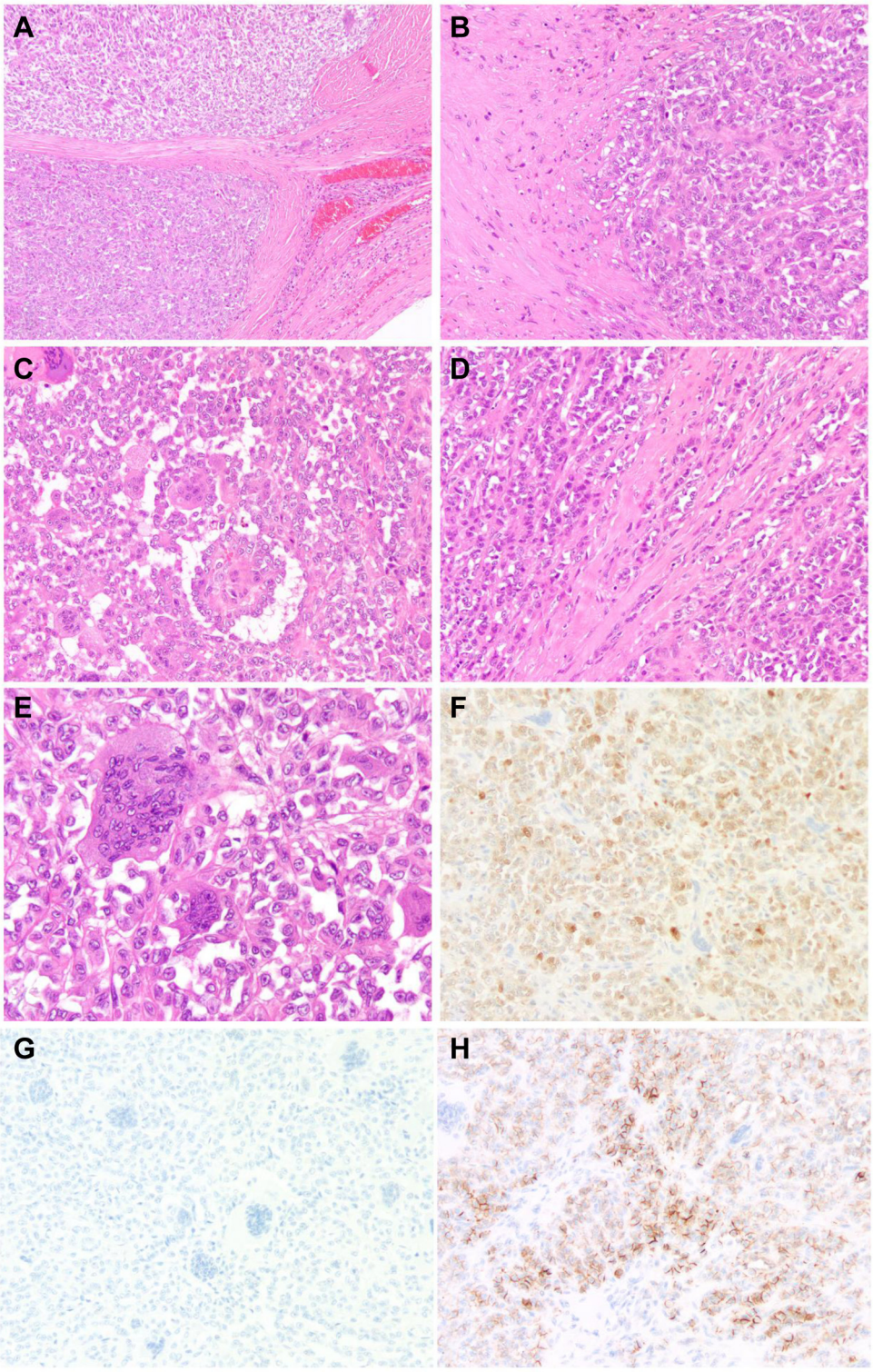

Figure 2.
Extraenteric malignant gastrointestinal neuroectodermal tumor (E-MGNET) of the neck of a 48-year-old woman (case 7). (A) Multiple nodules of tumor infiltrated into the surrounding soft tissues. (B) This tumor showed a variety of characteristic architectural patterns seen in E-MGNET, including solid areas (shown here), (C) pseudopapillary formations, and (D) pseudoalveolar or pseudovascular growth. (E) When present, osteoclast-like giant cells are a distinctive feature of E-MGNET, not present in clear cell sarcoma of soft parts. (F) This tumor expressed S100 protein and SOX 10 (not shown) and (G) lacked expression of MiTF and other melanocytic markers (not shown) and (H) was CD56 positive.

cells with eosinophilic to occasionally clear cytoplasm and exhibited greater nuclear variability, with coarse chromatin and large nucleoli (Fig. 3A-D). This case, also notable for a striking chronic lymphoplasmacytic inflammatory cell infiltrate, harbored

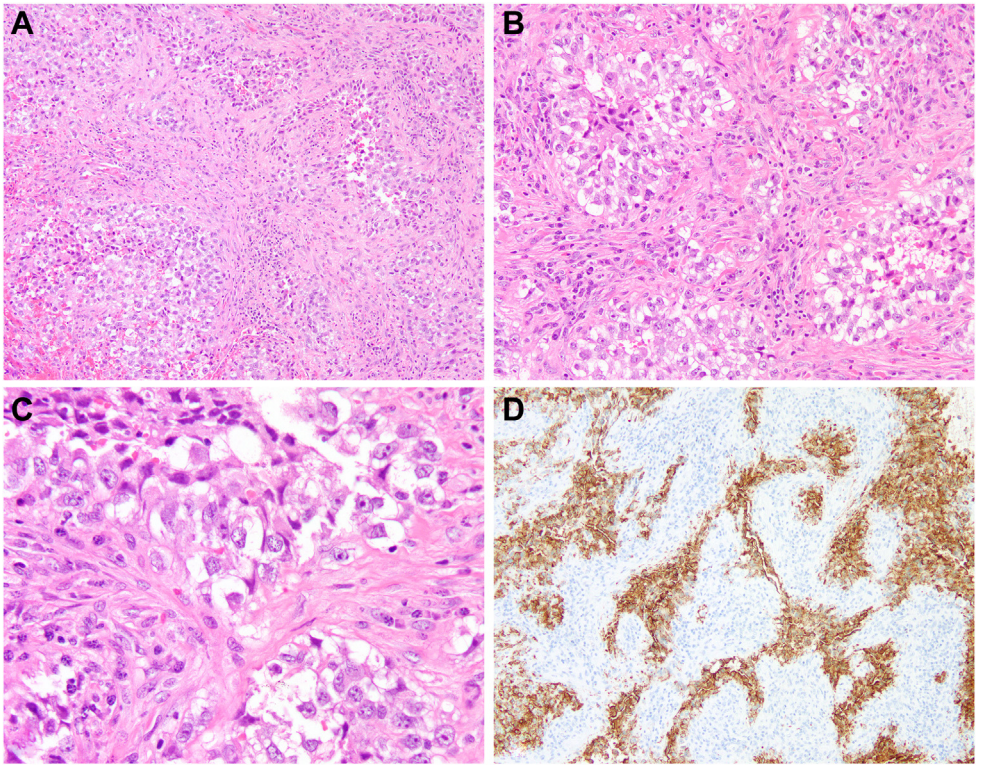

Figure 3.
"Large cell" extraenteric malignant gastrointestinal neuroectodermal tumor (E-MGNET) presenting in the shoulder of a 29-year-old woman (case 4). (A) This tumor was composed of nests of moderately variable cells with eosinophilic to clear cytoplasm in a fibrotic, inflamed background. (B) Nest of malignant cells separated by fibrous septa containing chronic inflammatory cells. (C) Although this tumor displayed "large cell" morphology, its nuclear features were like those of more conventional E-MGNET. (D) In addition to expressing markers typical of E-MGNET, this tumor showed diffuse ALK expression.

EWSR1::ATF1. The mitotic activity was easily identified in all cases, and tumor cell necrosis was frequently present. Osteoclast-like giant cells were present in 3 of 10 cases, including 1 with known *EWSR1::PBX1* fusion (case 6) (Figs. 2E, 4E). None of the cases displayed neoplastic wreath-like giant cells, melanin pigment, or pagetoid involvement of the mucosa.

By IHC, the tumors were uniformly positive for both S100 protein (11/11) and SOX10 (11/11) (Figs. 1D, 2F) and more variably positive for synaptophysin (3/10) (Fig. 1E), CD56 (7/9) (Fig. 2H), CD117 (3/9), and ALK (4/8) (Fig. 3D). Chromogranin A was negative in all tested cases (0/10). All cases were negative for HMB-45 (0/11), Melan-A (0/11), tyrosinase (0/4), MiTF (0/11), and DOG1 (0/4). Other markers, performed in individual cases, including desmin, EMA, CD99, BRAFv600E, and WT1, were negative or noncontributory.

NGS identified *EWSR1::ATF1* in 7 cases, *EWSR1::CREB1* in 3 cases, and *EWSR1::PBX1* in 1 case. There was no relationship between the fusion subtype and morphology or immunophenotype. The *EWSR1::PBX1*-positive tumor was morphologically identical to the other cases and negative for markers of myoepithelial differentiation, including keratins (PAN-K/MNF116 and CAM5.2), epithelial membrane antigen, glial fibrillary acidic protein, and p63.

Discussion

Inclusive of the present series, 19 cases of E-MGNET have been reported (Tables 1 and 2). These tumors most often occur in middle-aged adults, although they have been reported in adolescents and in the elderly (median patient age, 44 years; range, 14-82 years). Women are affected somewhat more often than men (13 women and 6 men). Cases have been reported in a wide variety of anatomical locations, with the tongue (4/19; 21%) and neck (3/19; 16%) representing the most common sites of disease. A clinical follow-up, available for 17 of 19 (90%) patients (median duration, 16 months; range, 4-70 months) showed 4 patients are dead of disease (24%), 6 patients are alive with metastatic or unresectable local disease (35%), and 5 patients are free of disease (29%). Although the follow-up duration for patients reported to be disease free is relatively short (median, 26 months; range, 4-70 months), it is longer than that for the overall cohort.

The clinicopathologic and molecular genetic features of E-MGNET are comparable to those of their more common enteric counterparts. Green et al,[3] in a comprehensive review of the previously published cases of enteric MGNET from 2018, documented death from disease in 12 of 58 (21%) patients, with metastases in 27 (47%) patients. MGNET has generally been thought of as more aggressive than CCS as MGNET has a shorter time to first metastasis (<1 month for MGNET vs 15 months for CCS) and a shorter median survival (9.5 months for MGNET vs 28 months for CCS).[3] However, the overall percentages of patients eventually dying from disease (55%) and suffering from metastases (63%) are higher for CCS. Longer-term follow-up in additional cases of MGNET will be revealed if it is in fact more aggressive than CCS.

As noted in Tables 1 and 2, the most common fusion event in E-MGNET is *EWSR1::ATF1* (12/19; 63%), followed by *EWSR1::CREB1* (5/19; 26%); isolated tumors harbor *EWSR1::CREM*[14] or *EWSR1::PBX1*. *EWSR1::CREB1* fusions were originally thought to be more common in MGNET[16]; however, *EWSR1::ATF1* fusions have

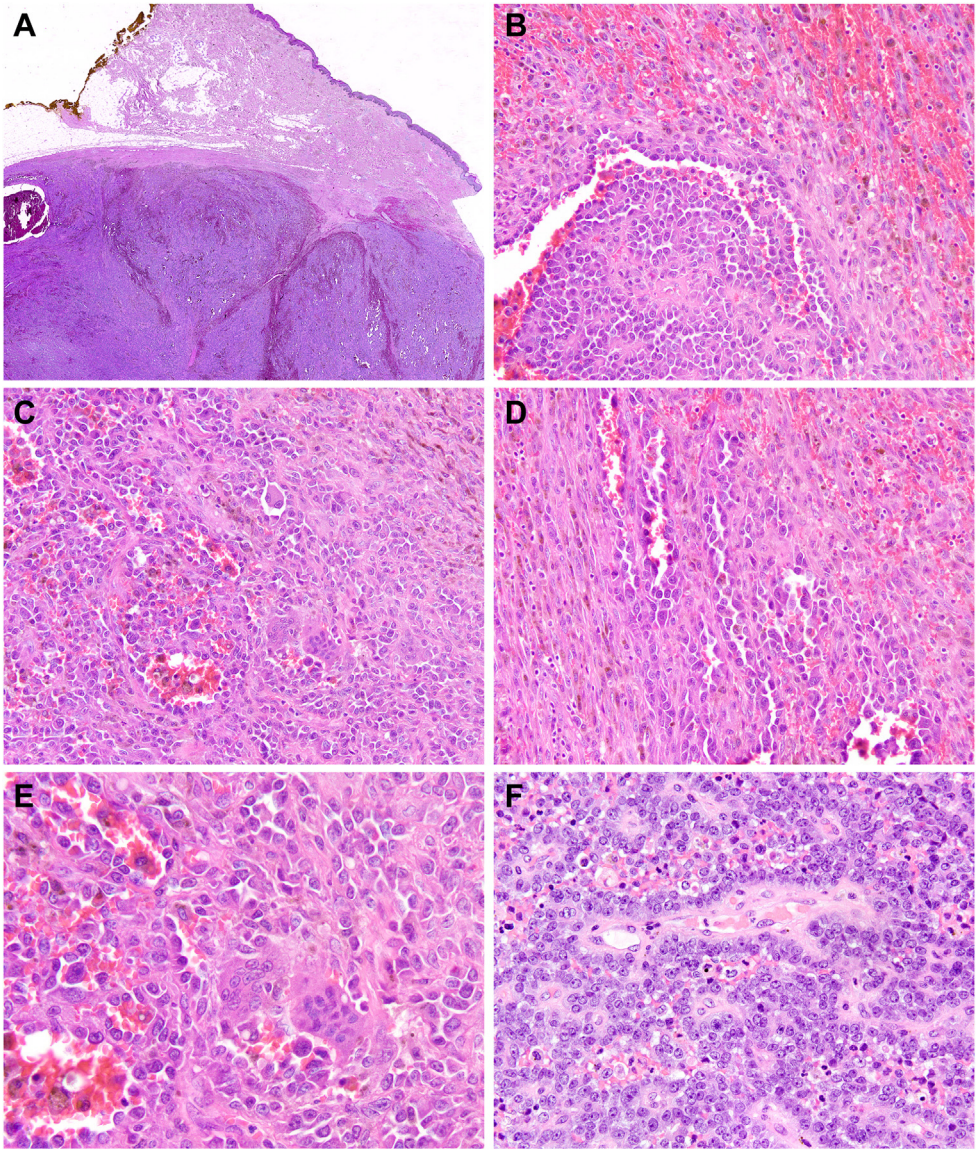

Figure 4.
Extraenteric malignant gastrointestinal neuroectodermal tumor arising in the subcutis of the buttock in a 48-year-old woman (case 6). (A) Although this tumor harbored a unique fusion event, *EWSR1::PBX1*, it was morphologically identical to other extraenteric malignant gastrointestinal neuroectodermal tumor. (B) Pseudovascular, (C) pseudopapillary, and (D) pseudoalveolar areas were present, with surrounding desmoplasia. (E) The neoplastic cells were small, ovoid, and monotonous; osteoclast-like giant cells were seen. (F) The pulmonary metastases showed identical morphologic features.

been found to outnumber *EWSR1::CREB1* fusions by roughly 2:1.[3] In CCS, *EWSR1::ATF1* is far more common than is *EWSR1::CREB1*, accounting for 94% of cases in a large Japanese series.[17] *EWSR1::PBX1* fusions have not been previously reported in MGNET but are seen in rare soft-tissue myoepithelial tumors, typically showing low-grade spindle cell morphology and stromal sclerosis.[18,19] The morphologic and immunohistochemical features of the *EWSR1::PBX1*-positive E-MGNET in the present series were identical to those of MGNET harboring more common fusion types and distinctly different from myoepithelial tumors with this same fusion. Furthermore, this tumor lacked expression of myoepithelial markers (eg, keratin, EMA, GFAP, and p63). In addition to a single *bona fide* E-MGNET,[14] *EWSR1::CREM* fusions have been reported in exceptional CCS, some myxoid angiomatoid fibrous histiocytomas, undifferentiated intra-abdominal spindle cell sarcomas, and a small group of keratin-positive, epithelioid malignant neoplasms of the abdominal cavity.[20,21] Fusions involving *EWSR1* (or *FUS*) and *ATF1* or *CREB1* are also characteristic of angiomatoid fibrous histiocytomas, unusual myxoid neoplasms of the lung and central nervous system, and clear cell salivary gland carcinomas.[22] Expression of ALK protein, noted in a subset of tested cases, seems to be a relatively common feature of tumors harboring *EWSR1* rearrangements.[23,24] Although the absence of *ALK* gene rearrangements suggests that E-MGNET is unlikely to be responsive to ALK inhibition, there are anecdotal reports of partial response or durable stable disease in patients with MGNET treated with multitargeted tyrosine kinase inhibitors.[25,26] Whether these responsive tumors were ALK positive is unknown.

Based on the provided photomicrographs and microscopic descriptions, a small number of cases reported as E-MGNET seem more likely to represent conventional CCS occurring in an unusual location and lacking expression of HMB-45 and/or Melan-A. CCS

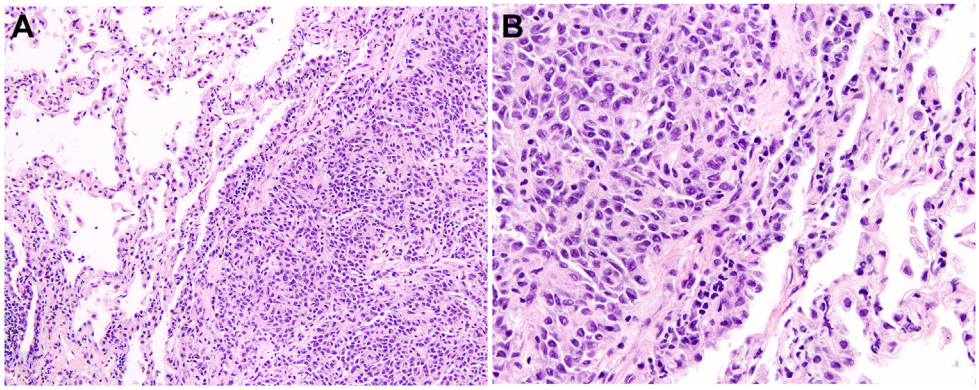

Figure 5.
The primary tumor for this extraenteric malignant gastrointestinal neuroectodermal tumor occurred in the orbit of a 29-year-old woman (case 9). (A) Seventeen months after presentation, the patient developed multiple metastatic lesions, including pulmonary metastasis. (B) The metastatic lesions showed identical morphologic features to those of her primary tumor and the other extraenteric malignant gastrointestinal neuroectodermal tumor.

rarely shows very limited or even absent expression of these melanocytic markers, as is well known to occur in conventional malignant melanoma.[27] These include cases reported by Allanson et al,[28] Çomunoğlu et al,[29] and one of the 2 cases recently reported by Kuo et al[8] (case 1). We have omitted these cases from Table 2.

The chief differential diagnostic consideration for E-MGNET is CCS. As noted above, E-MGNET and CCS share identical genetic findings, and thus, molecular genetic testing is not helpful for this distinction. IHC is of greater value as melanocyte-specific markers (eg, HMB-45, Melan-A, tyrosinase, and MiTF) are not expressed by E-MGNET. We would caution, however, that the *absence* of melanocyte marker expression does not necessarily exclude CCS, as noted above. Expression of neuroendocrine markers (eg, synaptophysin and CD56) does not favor E-MGNET over CCS as expression of both markers is common in CCS[17] and in conventional melanoma.[30] Ultimately, morphology remains, for the time being, the "gold standard" for the distinction of E-MGNET from CCS. In almost all instances, E-MGNETs are characterized by small, relatively uniform, round to ovoid cells with a small amount of lightly eosinophilic cytoplasm and round, regular nuclei containing small nucleoli growing in multinodular and vaguely lobular patterns, with solid, pseudoalveolar, and pseudopapillary architecture. Very rare cases may consist of larger, epithelioid cells or have oncocytic features.[31] When present, osteoclast-like giant cells are a highly characteristic feature of E-MGNET, not present in CCS. CCS is a quite different appearing tumor consisting of compact nests or fascicles of spindled to epithelioid cells with a larger amount of lightly eosinophilic to clear cytoplasm and prominent macronucleoli surrounded by hyalinized collagen. Wreath-like neoplastic giant cells, but not osteoclast-like giant cells, are often present in CCS. Pagetoid spread into adjacent mucosa may be seen in CCS,[32] but is not a feature of E-MGNET, in our experience. Gene expression profiling studies comparing MGNET and CCS have shown activation of the *MiTF* pathway in CCS but not in MGNET,[16,33] and for this reason, we also recommend IHC for MiTF.

The differential diagnosis of E-MGNETs also includes a variety of other soft-tissue sarcomas. Classical E-MGNETs are most likely to be confused with primitive round cell sarcomas, including Ewing sarcoma, CIC-rearranged sarcoma, and BCOR-altered sarcomas. Among these, diffuse expression of S100 protein and SOX10 is seen only in E-MGNETs, with strong expression of CD99/NKX2.2, WT1/ETV4, and BCOR protein characterizing Ewing sarcoma, *CIC*-rearranged sarcoma, and *BCOR*-altered sarcomas, respectively. E-MGNETs with large cell morphology might be confused with epithelioid sarcoma or sclerosing epithelioid fibrosarcoma, but they lack the keratin expression and SMARCB1 loss that typify the former and the MUC4 expression seen in the latter. When present, oncocytic changes in E-MGNETs may suggest malignant granular cell tumors, which will also show diffuse S100 protein and SOX10 expression. Malignant granular cell tumors, however, almost always arise in association with conventional granular cell tumors and most often display spindle cell morphology, reminiscent of malignant peripheral nerve sheath tumors. Intra-abdominal and retroperitoneal E-MGNETs may also be confused with gastrointestinal stromal tumors (GIST), especially when CD117 positive. However, the morphologic features of GIST are generally quite different from those of E-MGNETs; DOG1 expression is almost always present, and SOX10 expression is not seen. Ultimately, molecular genetic analysis may be the most

Table 2
Previously reported extraenteric malignant gastrointestinal neuroectodermal tumors

Reference	Age (y)/sex	Location/size (cm)	Molecular genetic findings	Outcome
Kraft et al (2013)[7]	82/F	Tongue/2.0	*EWSR1::ATF1*	AWD at 7 mo
Zheng et al (2019)[12]	40/M	Left bronchus/1.5	*EWSR1::ATF1*	AWD at 24 mo
Breton et al (2019)[13]	44/F	Tongue/4.1	*EWSR1::CREB1*	Not provided
Li et al (2021)[9]	62/M	Right atrium and right ventricle/3.0 and 4.7	*EWSR1::ATF1*	DOD at 20 mo
Yang et al (2021)[11]	47/F	Right thigh/6.0	*EWSR1::ATF1*	ANED at 11 mo
Sbaraglia et al (2021)[10]	62/F	Tongue/3.3	*EWSR1::CREB1*	ANED at 4 mo
Kuo et al (2022)[8]	56/M	Skull base/4.5	*EWSR1::ATF1*	AWD at 32 mo
Sugimoto et al (2022)[14]	38/F	Retroperitoneum/7.0	*EWSR1::CREM*	AWD at 7 mo

ANED, alive with no evidence of disease; AWD, alive with disease; DOD, dead of disease; F, female; M, male.

definitive way of resolving these differential diagnoses as only E-MGNET harbors *EWSR1::ATF1/CREB1* fusions, among these possibilities.

In summary, we have reported the clinicopathologic and molecular genetic features of 11 E-MGNETs, the largest series to date. The pathologic features of these very rare tumors are essentially identical to those of their enteric counterparts and quite different from those of CCS, the chief differential diagnostic consideration. As in more common enteric locations, E-MGNETs behave as aggressive sarcomas, often with metastatic disease and adverse patient outcomes. Although it is difficult to come up with a wholly satisfactory name for these distinctive, rare sarcomas, we believe our proposed terminology "E-MGNET" to clearly link these lesions with identical enteric tumors and to echo the accepted terminology for GIST presenting outside the gastrointestinal tract, "E-GIST." Future advances in our understanding of these unusual tumors will hopefully lead to improved nomenclature and treatment options.

Acknowledgments

The authors gratefully acknowledge the following pathologists who kindly provided case material and clinical follow-up information, when available: Dr. P Helseth (Minneapolis, MN), Dr. R. Gupta (Camperdown, Australia), Dr. D. Sauer (Portland, OR), Dr. R. Thomas (Fort Hood, TX), and Dr. W. Zhang (Dallas, TX).

Author Contributions

All authors participated in study design, data collection, and manuscript editing.

Data Availability

Data are available from the corresponding author upon reasonable request.

Funding

The authors report no relevant disclosure sources of funding.

Declaration of Competing Interest

The authors report no relevant conflicts of interest.

Ethics Approval and Consent to Participate

The institutional review boards of the participating institutions approved this study. Informed consent was waived by the institutional review boards of the particpating institutions.

References

1. Stockman DL, Miettinen M, Suster S, et al. Malignant gastrointestinal neuroectodermal tumor: clinicopathologic, immunohistochemical, ultrastructural, and molecular analysis of 16 cases with a reappraisal of clear cell sarcoma-like tumors of the gastrointestinal tract. *Am J Surg Pathol*. 2012;36(6):857–868.
2. Wang J, Thway K. Clear cell sarcoma-like tumor of the gastrointestinal tract: an evolving entity. *Arch Pathol Lab Med*. 2015;139(3):407–412.
3. Green C, Spagnolo DV, Robbins PD, Fermoyle S, Wong DD. Clear cell sarcoma of the gastrointestinal tract and malignant gastrointestinal neuroectodermal tumour: distinct or related entities? A review. *Pathology*. 2018;50(5):490–498.
4. Kosemehmetoglu K, Folpe AL. Clear cell sarcoma of tendons and aponeuroses, and osteoclast-rich tumour of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: a review and update. *J Clin Pathol*. 2010;63(5):416–423.
5. Zambrano E, Reyes-Mugica M, Franchi A, Rosai J. An osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: reports of 6 cases of a GIST simulator. *Int J Surg Pathol*. 2003;11(2):75–81.
6. Alpers CE, Beckstead JH. Malignant neuroendocrine tumor of the jejunum with osteoclast-like giant cells. Enzyme histochemistry distinguishes tumor cells from giant cells. *Am J Surg Pathol*. 1985;9(1):57–64.
7. Kraft S, Antonescu CR, Rosenberg AE, Deschler DG, Nielsen GP. Primary clear cell sarcoma of the tongue. *Arch Pathol Lab Med*. 2013;137(11):1680–1683.
8. Kuo CT, Kao YC, Huang HY, Hsiao CH, Lee JC. Malignant gastrointestinal neuroectodermal tumor in head and neck: two challenging cases with diverse morphology and different considerations for differential diagnosis. *Virchows Arch*. 2022;481(1):131–136.
9. Li Z, Pu X, He L, et al. Malignant gastrointestinal neuroectodermal tumor in the right heart: a report of an extremely rare case presenting with a cardiac mass. *Front Cardiovasc Med*. 2021;8:702215.
10. Sbaraglia M, Zanatta L, Toffolatti L, et al. Clear cell sarcoma-like/malignant gastrointestinal neuroectodermal tumor of the tongue: a clinicopathologic and molecular case report. *Virchows Arch*. 2021;478(6):1203–1207.
11. Yang Y, Chen Y, Chen S, Han A. Malignant gastrointestinal neuroectodermal tumour in soft tissue. *Pathology*. 2021;53(2):276–278.
12. Zheng Q, Chen H, Li Y. Primary gastrointestinal-type clear cell sarcoma-like tumor of the bronchus: a hitherto unreported bronchial tumor. *J Thorac Oncol*. 2019;14(9):e202–e205.
13. Breton S, Dubois M, Geay JF, et al. [Clear cell sarcoma or gastrointestinal neuroectodermal tumor (GNET) of the tongue? Case report and review of the literature of an extremely rare tumor localization]. *Ann Pathol*. 2019;39(2):167–171.
14. Sugimoto A, Yoshizawa A, Yoshida A, et al. Retroperitoneal malignant extra-gastrointestinal neuroectodermal tumor with EWSR1::CREM fusion and IL-6-related systemic inflammatory symptoms: a case report. *Virchows Arch*. 2022. https://doi.org/10.1007/s00428-022-03442-0
15. Balan J, Jenkinson G, Nair A, et al. SeekFusion—a clinically validated fusion transcript detection pipeline for PCR-based next-generation sequencing of RNA. *Front Genet*. 2021;12:739054.
16. Antonescu CR, Nafa K, Segal NH, Dal Cin P, Ladanyi M. EWS-CREB1: a recurrent variant fusion in clear cell sarcoma—association with gastrointestinal location and absence of melanocytic differentiation. *Clin Cancer Res*. 2006;12(18):5356–5362.
17. Hisaoka M, Ishida T, Kuo TT, et al. Clear cell sarcoma of soft tissue: a clinicopathologic, immunohistochemical, and molecular analysis of 33 cases. *Am J Surg Pathol*. 2008;32(3):452–460.
18. Suurmeijer AJH, Dickson BC, Swanson D, et al. A morphologic and molecular reappraisal of myoepithelial tumors of soft tissue, bone, and viscera with EWSR1 and FUS gene rearrangements. *Genes Chromosomes Cancer*. 2020;59(6):348–356.
19. Brandal P, Panagopoulos I, Bjerkehagen B, et al. Detection of a t(1;22)(q23;q12) translocation leading to an EWSR1-PBX1 fusion gene in a myoepithelioma. *Genes Chromosomes Cancer*. 2008;47(7):558–564.
20. Yoshida A, Wakai S, Ryo E, et al. Expanding the phenotypic spectrum of mesenchymal tumors harboring the EWSR1-CREM fusion. *Am J Surg Pathol*. 2019;43(12):1622–1630.
21. Shibayama T, Shimoi T, Mori T, et al. Cytokeratin-positive malignant tumor in the abdomen with EWSR1/FUS-CREB fusion: a clinicopathologic study of 8 cases. *Am J Surg Pathol*. 2022;46(1):134–146.
22. Thway K, Fisher C. Mesenchymal tumors with EWSR1 gene rearrangements. *Surg Pathol Clin*. 2019;12(1):165–190.
23. Agaimy A, Stoehr R, Otto M, et al. Intra-abdominal EWSR1/FUS-CREM-rearranged malignant epithelioid neoplasms: two cases of an emerging aggressive entity with emphasis on misleading immunophenotype. *Virchows Arch*. 2022;480(2):481–486.
24. Agaram NP, Zhang L, Sung YS, et al. Expanding the spectrum of intraosseous rhabdomyosarcoma: correlation between 2 distinct gene fusions and phenotype. *Am J Surg Pathol*. 2019;43(5):695–702.
25. Subbiah V, Holmes O, Gowen K, et al. Activity of c-Met/ALK inhibitor crizotinib and multi-kinase VEGF inhibitor pazopanib in metastatic gastrointestinal neuroectodermal tumor harboring EWSR1-CREB1 fusion. *Oncology*. 2016;91(6):348–353.
26. Kandler T, Cortez E, Clinton L, et al. A case series of metastatic malignant gastrointestinal neuroectodermal tumors and comprehensive genomic profiling analysis of 20 cases. *Curr Oncol*. 2022;29(2):1279–1297.
27. Goldblum JR, Folpe AL, Weiss SW. *Enzinger & Weiss's Soft Tissue Tumors*. 7th ed. Elsevier; 2019.

28. Allanson BM, Weber MA, Jackett LA, et al. Oral malignant gastrointestinal neuroectodermal tumour with junctional component mimicking mucosal melanoma. *Pathology*. 2018;50(6):648–653.
29. Çomunoğlu N, Dervişoğlu S, Elçin B, Tekant G, Apak H. Malignant extra-gastrointestinal neuroectodermal tumor located at right cervical region. *Open J Pathol*. 2015;5(5):125–128.
30. Romano RC, Carter JM, Folpe AL. Aberrant intermediate filament and synaptophysin expression is a frequent event in malignant melanoma: an immunohistochemical study of 73 cases. *Mod Pathol*. 2015;28(8):1033–1042.
31. Boland JM, Folpe AL. Oncocytic variant of malignant gastrointestinal neuroectodermal tumor: a potential diagnostic pitfall. *Hum Pathol*. 2016;57:13–16.
32. Shawa H, Dahle S, Schulman JM. Compound clear cell sarcoma: a case report with ulceration and pagetoid scatter. *Am J Dermatopathol*. 2022;44(9):687–690.
33. Vanoli F, Meskauskaite B, Herviou L, et al. Generation of human embryonic stem cell models to exploit the EWSR1-CREB fusion promiscuity as a common pathway of transformation in human tumors. *Oncogene*. 2021;40(32):5095–5104.