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Ewing Sarcoma/Peripheral Primitive Neuroectodermal Tumor and Related Tumors

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Abstract

Ewing sarcoma/peripheral primitive neuroectodermal tumor (EWS/pPNET) and other tumors with EWS gene rearrangements encompass a malignant and intermediate neoplasm with a broad anatomic distribution and a wide age range but a predilection for soft tissue in children, adolescents, and young adults. The overlapping histologic, immunohistochemical and cytogenetic and molecular genetic features create diagnostic challenges despite significant clinical and prognostic differences. Ewing sarcoma is the 3rd most common sarcoma in children and adolescents, and desmoplastic small round cell tumor is a rare neoplasm that occurs more often in older children, adolescents, and young adults. Pathologic examination is complemented by immunohistochemistry, cytogenetics, and molecular genetics. This article reviews the clinicopathologic features of EWS/pPNET and desmoplastic small round cell tumor in the spectrum of tumors with EWS gene rearrangements. Other tumors with different histopathologic features and an EWS gene rearrangement are discussed elsewhere in this volume.

Keywords

desmoplastic small round cell tumor; EWS gene rearrangement; EWS/pPNET; Ewing sarcoma; peripheral primitive neuroectodermal tumor

INTRODUCTION

The neoplasms discussed in this review share genetic abnormalities related to the *EWS* gene on chromosome 22q12, whose identification has been the source of insights into the role that this gene plays in oncogenesis. Although detailed attention will be given to Ewing sarcoma/peripheral primitive neuroectodermal tumor (EWS/pPNET) and desmoplastic small round

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cell tumor (DSRCT), several other entities with *EWS* translocations are briefly reviewed and are discussed more extensively elsewhere in this volume (Table 1).

EWING SARCOMA/PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOR

Almost 90 years ago, Ewing reported a malignant osseous neoplasm in a young patient and subsequently described additional similar cases, thus establishing the entity as Ewing sarcoma, which has been the source of continued speculation about its histogenesis from an endothelial cell, a primitive mesenchymal skin cell, and many other possible progenitors [1–10]. The existence of the entity was doubted by Willis, who felt that cases of Ewing tumor actually represented incompletely investigated instances of neuroblastoma or lymphoma [11].

Prior to Ewing's observations, Stout was one of the 1st to describe a soft tissue tumor involving a peripheral nerve whose histologic features resembled a neuroblastoma and was later designated neuroepithelioma [12]. Some 50–60 years later, these initial observations were expanded to include extraosseous EWS, malignant small cell tumor of the chest wall (Askin tumor), and neuroectodermal tumor of bone [13–15]. In addition to locations in bone, peripheral nerve, and soft tissue, EWS is now recognized in the kidney and a variety of other visceral and nonvisceral sites [16–18].

It is generally recognized that EWS and pPNET represent one and the same neoplastic process, as demonstrated by the overlapping morphologic features, including ultrastructure, immunophenotype, and a set of molecular genetic abnormalities [9,10,19–21]. This neoplasm is currently referred to as EWS/pPNET, the preferred terminology of the World Health Organization classification of soft tissue and bone tumors [22] or, alternatively, the Ewing family of tumors. The histogenesis remains uncertain, but the tumor cells most likely are derived from primitive mesenchymal cells with the potential for limited neural differentiation [23,24]. The unifying criterion for inclusion in the Ewing family of tumors is the presence of a nonrandom translocation, most commonly t(11;22)(q24;q12), which juxtaposes a portion of the *EWS* gene on chromosome 22q with the *FLI-1* gene on 11q [25,26]. A variety of other cytogenetic abnormalities have been identified that involve related families of genes [27].

Ewing sarcoma/peripheral primitive neuroectodermal tumor is rare, with approximately 300 new cases per year of both osseous and extraosseous tumors in the United States, but it is the 3rd most common primary malignant neoplasm of bone and soft tissues in children, after osteosarcoma and rhabdomyosarcoma, respectively [28]. Most extraosseous EWS/pPNET present in the 2nd decade of life, but infrequent cases are seen in early childhood and older adults [15,29–38]. There are some unexplained ethnic and racial differences in the occurrence of soft tissue and osseous tumors [39]. Overall, there is a male predilection. Extraskelatal tumors constitute 20%–40% of all EWS/pPNET and most often are found in the soft tissues of the trunk and extremities, followed in frequency by head and neck, retroperitoneum, and other sites, especially those associated with skeletal muscle. Rarer still are organ-based sites, including the female genital tract, adrenal gland, and kidney [13,18,31–33,36,38,40–48]. Pain and tenderness are often presenting complaints in the cases

of bone and soft tissue tumors alike [43,44], together with paresthesias, weakness, or loss of function, mainly when nerves are involved [12,49,50]. Fever may be present, suggestive of an infection, most commonly acute osteomyelitis. Periosteal changes may be detected on radiographic imaging in the absence of osseous involvement [13,29]. Familial cases have been reported, and extraosseous EWS/pPNET may rarely occur as a 2nd malignant neoplasm [51,52]. Ethnic and racial differences in the occurrence of soft tissue and osseous tumors have been described [39]. The tumor may be disseminated on presentation without an obvious primary site [53].

Extraosseous EWS/pPNET is often large at presentation, ranging from 1 to 40 cm in diameter and frequently larger than 10 cm [33,38,44,46,54,55]. The tumors are grossly circumscribed or infiltrating, multinodular, friable, or lobulated, with some combination of whitish-tan, mucoid, hemorrhagic, cystic, or necrotic areas (Figs. 1–4). An apparent capsule or pseudocapsule is often identified in the resected tumor. The gross features are altered in most cases because chemotherapy is administered prior to resection. In some resected specimens, it may be difficult to identify any residual gross tumor. The adequacy of margins of resection is an important determination in the resection specimen because it affects the decision about radiotherapy [56–59]. Positive or proximate surgical margins are generally managed by radiotherapy.

Extraskelatal EWS/pPNET is morphologically identical to its osseous counterpart, including some of the variant patterns [13,60–62]. Classic (typical) EWS/pPNET makes up 60%–70% of cases, with its patterns of formless sheets and lobules of uniform round to oval cells measuring approximately 20 μ m in diameter (Figs. 5–8). Spindle cell areas may be present (Fig. 9) [63]. The nuclei are round to oval, containing finely dispersed chromatin and 1–3 small nucleoli [13,20,64]. The cytoplasm is clear, finely vacuolated, or amphophilic, with a variably prominent cytoplasmic rim that reflects the state of the tumor before fixation and rapidity of fixation. Those tumors with well-preserved neoplastic cells with clear to vacuolated cytoplasm often are strongly periodic acid–Schiff stain positive, with elimination after diastase digestion. Little or no extracellular stroma is seen between adjacent tumor cells, whereas aggregates or lobules are often surrounded by a collagenous matrix. Mitoses are present in variable numbers and are inconspicuous in some cases. Two cell types are frequently seen, consisting of a majority of lighter staining cells and smaller numbers of more darkly staining cells, the significance of which is unknown; this light cell–dark cell pattern is useful in the evaluation of neoplasms despite its nonspecificity. Cytoplasmic glycogen is also characteristic but nonspecific (Fig. 10) [65,66]. The tumors of the classical form and other types may invade the surrounding stroma in strands of 1–2-cell thickness separated by fibrous tissue or thick-walled blood vessels, an arrangement termed the “filigree pattern” (Fig. 11) [67]. The infiltrating tumor cells may acquire a septal pattern with central discohesive tumor cells, similar to alveolar rhabdomyosarcoma.

The so-called atypical or large cell variant is composed, as the name implies, of larger, more pleomorphic cells than those of the classic type, measuring approximately 40 μ m in diameter. The nuclei are more irregular and often indented and frequently contain conspicuous nucleoli. The cytoplasm is more abundant, eosinophilic, and glycogen rich. Spindle cell areas are common, as are epithelioid or clear cells. A mucoid background

similar to embryonal rhabdomyosarcoma may be seen on occasion. Mitotic activity is increased [60,62,68–70]. With modern treatment modalities, the prognosis is similar to that of the classic form [60,62,68–70].

Apparent neural differentiation may be present in some extraosseous EWS/pPNET [12,15,36,44,54,55]. A round cell or slightly spindled cell morphology is accompanied by nested or trabecular profiles [43,55]. In other cases, a complex lobular pattern with necrosis, nuclear crowding or overlapping, mitotic figures, indistinct cell borders, cellular atypia, and pleomorphism are present; these tumors have a resemblance to high-grade neuroendocrine carcinoma in adults. Neural differentiation is most convincing in those tumors with Homer-Wright rosettes similar to those seen in neuroblastoma, vaguely formed rosettes, and perivascular pseudorosettes (Figs. 12–14) [14,36,38]. Less often seen is the presence of eosinophilic fibrillary processes corresponding to neuropil, with or without rosette formation; this network of cytoplasmic processes is demonstrated by electron microscopy. Rare ganglion cells may be seen [36,44]. Immature elongated primitive neural canals are features of the medulloepithelioma variant [71]. Other uncommon variants include the adamantinoma-like variant, resembling classic adamantinoma of bone, but with the EWS/pPNET translocation [62,72,73]. Vascular or pelioid patterns have been recognized for many years and probably account for Ewing's early consideration of a malignant endothelioma [54,74]. A sclerosing pattern with a resemblance to sclerosing epithelioid fibrosarcoma or sclerosing rhabdomyosarcoma is accompanied by abundant collagenous stroma surrounding the tumor cells [62].

The evaluation of residual tumor in the resected specimen in a manner similar to the post-treatment osteosarcoma [75,76] has been the subject of several studies [77–80]. According to these studies, a complete cross-section of the tumor at its greatest diameter should be submitted for microscopic examination. Each slide from this area should be evaluated for viable and nonviable tumor as a percent necrosis or fibrosis in the same fashion as osteosarcoma. In addition to coagulative necrosis, an alveolar pattern may occur (Fig. 15), in which tumor cells are arranged around spaces or areas of cellular dropout, with formation of rosette-like structures [81].

The pathologic diagnosis of EWS/pPNET may be challenging and must be considered in the context of differential diagnosis, but the microscopic features in most cases are provocative enough to employ the appropriate ancillary studies. Electron microscopy, widely utilized before the advent of immunohistochemistry, demonstrates a spectrum of differentiation, ranging from primitive undifferentiated cells with abundant cytoplasmic glycogen, few mitochondria and free ribosomes, poorly developed Golgi apparatus, and rough endoplasmic reticulum (Fig. 16) [61] to overt neuroectodermal differentiation, with microtubules, neurosecretory granules, and/or neuritic processes, especially in tumor areas showing rosette formation by light microscopy (Fig. 17) [82–84]. Rudimentary or well-formed cell-cell junctions and cytoplasmic tonofilaments are present in a small proportion of EWS/pPNET with epithelial differentiation [85,86].

The most widely applied ancillary technique today is immunohistochemistry to confirm the diagnosis of EWS/pPNET and to exclude the other similar-appearing round cell neoplasms

(Table 2). Fluorescent in situ hybridization (FISH) for the *EWS* gene break apart has also emerged as an important adjunct and may be essential in an equivocal case. The great majority of EWS/pPNET express MIC2 (CD99) and FLI-1. MIC2, a 32-kDa membrane glycoprotein, is encoded by a pseudoautosomal gene on the X and Y chromosomes involved in cell adhesion, migration, and apoptosis [87–89]. CD99 epitopes are recognized by the monoclonal antibodies 12E7, HBA71, O13, and HO36–1.1 with diffuse membrane staining (Fig. 18). Unfortunately, the same pattern of expression is also observed in other neoplasms, such as synovial sarcoma and malignant rhabdoid tumor. Several other small round cell tumors may display weak or focal staining, but neuroblastoma is generally negative [90]. Vascular endothelial cells also typically react with antibodies to MIC2.

Fli-1, a member of the erythroblastosis virus-associated transforming sequences family of DNA-binding transcription factors, is expressed in endothelial and hematopoietic cells and is involved in cellular proliferation and tumorigenesis [91]. Strong nuclear staining is detected in 90%–100% of EWS/pPNET, but its expression is dependent on the polyclonal or monoclonal nature of the antibodies (Fig. 19). FLI-1 monoclonal antibody is less specific than a polyclonal agent, because it detects an antigen that can be expressed in a wide spectrum of tumors, including vascular tumors, Merkel cell carcinoma, and malignant melanoma [62,70,92–95].

Caveolin-1, a membrane protein involved in tumor development and metastatic progression, is a target of *EWS/FLI-1* [96]. This protein has been identified in more than 95% of EWS/pPNET and may be helpful in the differential diagnosis from other small round cell tumors; however, the protein is not specific, because it is expressed by several types of carcinomas and sarcomas [70–96].

Neuroectodermal differentiation is reflected by variable immunopositivity for antigens such as neuron-specific enolase, PGP 9.5, S-100, CD56, CD57 (also termed Leu-7 or HNK1), synaptophysin (Fig. 20), and, less frequently, neurofilament protein [36,97,98].

The same epithelial differentiation has been documented by electron microscopy and is also manifested by focal positive staining for broad-spectrum cytokeratin in 20%–30% of EWS/pPNET [62,99,100]. The pattern is either dot like or membranous-cytoplasmic. When cytokeratin is positive, it is usually restricted to a minority of tumor cells, unlike the diffuse staining reaction for vimentin. Variable expression of tight junction proteins (claudin-1, zonula occludens-1, and occludin) or the desmosomal protein desmoplakin parallels the presence of poorly formed tight junctions as seen by electron microscopy. Adamantinoma-like EWS/pPNET expresses high-molecular weight cytokeratins 1, 5, 10, and 14/15. The expression of the desmosomal proteins desmoplakin and desmoglein reflects the more complex epithelial differentiation associated with the formation of complete desmosomes [72,86].

Muscle markers are generally not expressed in EWS/pPNET, although desmin expression has been reported in sporadic cases that are morphologically typical but lack genetic confirmation [62,101,102]. Desmin and myogenin expression in an apparent EWS/pPNET,

even in conjunction with characteristic genetic findings, should raise the possibility of ectomesenchymoma [103] (discussed elsewhere in this volume).

The EWS/pNET-specific fusion transcripts are detectable by cytogenetics, reverse transcription polymerase chain reaction (RT-PCR), or FISH techniques available in molecular pathology laboratories. Standard cytogenetics can be informative, especially in those tumors with variant translocations (see below). However, in vitro tumor cell growth is required, which is not always successful. In most diagnostic settings, RT-PCR and FISH are performed for the detection of genetic rearrangements. However, detection of variant fusions by RT-PCR requires use of multiple primers and can be labor intensive. Fluorescent in situ hybridization assay with the commercial *EWS* “break-apart” probe is very useful except for those tumors with *FUS* rearrangements. There is also the potential problem of differentiating EWS/pNET from other tumors with overlapping histology and *EWS* rearrangements [27]. Furthermore, because of the growing number of variant fusions in EWS/pNET, standard molecular testing may be negative. Therefore, negative molecular testing should not exclude the diagnosis of EWS/pNET in the presence of typical histopathologic features and after vigorous exclusion of other tumors with a similar histology. In some of the cases, the default diagnosis may read “undifferentiated round cell sarcoma with features of EWS/pNET.”

The spectrum of differential diagnosis of EWS/pNET includes several other entities that share with EWS/pNET the classic small round cell morphology or the fusion of the *EWS* gene with different partner genes (Table 2). In most cases, histology and an appropriate immunohistochemical panel allow the distinction of EWS/pNET from alveolar rhabdomyosarcoma, neuroblastoma, and other entities in the differential diagnosis. The strong diffuse membrane staining for CD99 in biopsies from poorly differentiated synovial sarcoma, lymphoblastic lymphoma, and malignant rhabdoid tumor may create a diagnostic dilemma or pitfall [104–106]. Rhabdomyosarcoma at times may express CD99 in a membrane pattern or in a cytoplasmic pattern that can show a distinctive paranuclear dot-like positivity. Cytokeratin positivity can be useful when considering other round cell neoplasms because most others are negative except in poorly differentiated or monophasic synovial sarcomas. However, rhabdoid tumor is frequently cytokeratin positive. Positive immunostaining for TdT in lymphoblastic lymphoma, or myogenin in alveolar rhabdomyosarcoma, and lack of INI-1 staining in rhabdoid tumors (and, when appropriate, cytogenetic and molecular studies) generally distinguish these tumors from EWS/pNET. Serum catecholamine levels provide a valuable feature distinguishing EWS/pNET from neuroblastoma, in addition to the age and clinical differences in most cases [36,54,107–110].

Cutaneous EWS/pNET may mimic Merkel cell carcinoma, a tumor that more typically occurs in elder adults. Cytokeratins, CD99, FLI-1, and neuroendocrine markers can be expressed in both lesions. However, CK20 is selectively positive in Merkel cell carcinoma [111–113]. *NUT* carcinoma, a recently described entity, was originally reported in young women in a midline site in the head and neck or upper respiratory tract and lung. These tumors are characterized by the *BRD4-NUT* fusion transcript. Positive nuclear immunostaining for NUT protein is associated with the translocation and is diagnostic of *NUT* carcinoma. These neoplasms also express p63 and cytokeratins [114,115].

A search for the most common translocations involving the *EWS* gene may be helpful in small biopsies from spindle cell neoplasms, such as infantile fibrosarcoma, malignant peripheral nerve sheath tumors with EWS/pPNET-like features, or a predominantly blastemal Wilms tumor [116,117]. Among the round cell neoplasms with the *EWS* translocation, DSRCT may mimic the adamantinoma-like variant of EWS/pPNET. Reverse transcription polymerase chain reaction for the appropriate breakpoint fusions may be necessary for the differential diagnosis [62]. Clear cell sarcoma of soft parts, myxoid/round cell liposarcoma, and myoepithelial carcinoma of soft parts have sufficiently characteristic morphologic and immunohistochemical features that these tumors should pose little difficulty in most cases [118,119]. Angiomatoid fibrous histiocytoma has a typical morphology with a nodular pattern, peripheral lymphatic follicles, and/or pseudovascular spaces containing red cells, but a small biopsy can be problematic when the cellular areas are strongly positive for CD99 [120]. Also, FISH analysis with an *EWS* breakpoint region probe may be misleading because it is positive in both EWS/pPNET and angiomatoid fibrous histiocytoma. Extraskelatal myxoid chondrosarcoma may be difficult to distinguish from EWS/pPNET in highly cellular areas that lack myxoid stroma.

The specific chromosomal translocations of EWS/pPNET constitute its unifying genetic trait (Table 3). These translocations generally lead to an in-frame fusion of the *EWS* gene on chromosome 22 to a gene of the *ETS* family of transcription factors. The most frequent translocation, t(11;22) (q24;q12) fuses *EWS* (EWSR1) to *FLI1* (Fig. 21) [121–123]. The *EWS/FLI1* fusion is encountered in 85% of EWS/pPNET. Approximately 10% of EWS/pPNET exhibit a 2nd translocation, t(21;22) (q22;q12) (Fig. 22), which fuses *EWS* to *ERG* [124,125]. The remaining 5% of EWS/pPNET exhibit variant translocations (Table 3) [126]. Both *EWS/FLI1* and *EWS/ERG* gene fusions are heterogeneous because of variability in genomic breakpoint locations leading to *EWS/FLI1* transcripts with a variety of exons from the *EWS* and *FLI1* or *ERG* genes [124]. The 2 most frequently observed in-frame fusions are the type 1 between *EWS* exon 7 and *FLI1* exon 6 and the type 2 between *EWS* exon 7 and *FLI1* exon 5 [123], which occur in approximately 60% and 25% of the cases, respectively [127].

Rare EWS/pPNET have fusions involving the *EWS* homolog *FUS* (*TLS*) of the *TET* family, with t(16;21) (p11;q24) and *FUS-ERG* fusion [128] and with t(2;16) (q35;p11) and *FUS-FEV* fusion [129]. Tumors that are like EWS/pPNET may have fusions in which *EWS* is juxtaposed to genes encoding non-*ETS* transcription factors, such as *SP3* and *ZNF278*, *POU5F1*, *NEATc2*, and *SMARCA5* [126,130–133]. *CIC-DUX4*, a completely novel fusion unrelated to *EWS* and *ETS* family, has been described as well [126]. Most tumors with unusual fusions are extraskelatal (92%), often exhibit atypical histology, such as spindle cell and/or pleomorphic areas, and are negative or only focally positive for CD99. The relation of these tumors to other EWS/pPNET family members remains to be determined.

EWS/FLI-1 and the other *EWS/ETS* fusions have been classified as oncogenes because they can transform immortalized murine NIH3T3 cells [134–136]. These fusion transcripts when introduced into murine mesenchymal progenitor or primary bone-derived cells give rise to poorly differentiated EWS-like tumors in mouse xenograft models [23,137]. However, not all cells are permissive to tumor formation upon expression of *EWS/FLI-1*. For example,

primary human or mouse fibroblasts transfected with *EWS/FLI-1* undergo growth arrest and apoptosis, which require complementary oncogenic events for stable expression of *EWS/FLI-1* and transformation [138,139]. Human mesenchymal progenitor cells form colonies in vitro and exhibit a *EWS/pPNET*-like gene signature, although they do not form tumors in mice [140,141]. Other studies have shown that *EWS/FLI-1* induces a cancer stem cell population in pediatric, but not adult human, mesenchymal stem cells by direct repression of microRNA-145 [142]. These data suggest that *EWS/FLI-1* is able to induce genetic reprogramming in human cells without help from other cofactors, but only in a permissive cellular context.

EWS/ETS fusion proteins contain the amino terminal transactivation domain of *EWS* fused to the carboxyl terminal DNA-binding domain of the corresponding *ETS* partner and are more potent transcriptional activators than the parent genes [143]. Several studies have shown that *EWS/FLI-1* exerts both activating and repressive effects on gene expression and that both effects are important for *EWS/pPNET* oncogenesis [144,145]. A molecular function map constructed from whole genome gene expression profiling analyses showed that *EWS/FLI-1* upregulated genes are associated with cell cycle regulation, proliferation, and response to DNA damage, whereas repressed genes are associated with differentiation and cell communication [146]. *EWS/FLI-1* regulates its target genes by binding to consensus *ETS* motifs [143,147,148] or specific microsatellite sequences [149,150] and also by mechanisms not involving DNA binding and possibly related to RNA processing [145].

Early studies showed that the *EWS/FLI-1* fusion transcript blocks differentiation of pluripotent murine mesenchymal progenitor cells [151], in agreement with recent data showing that *EWS/FLI-1* silencing restores a pluripotent mesenchymal cell phenotype in *EWS/pPNET* cells [24]. These data would suggest that *EWS/pPNET* cells have mesenchymal stem cell properties enhanced by loss of *EWS/FLI-1* expression and therefore originate from mesenchymal stem cells. Other recent studies have shown that ectopic expression of *EWS/FLI-1* in human fibroblasts and rhabdomyosarcoma cells induces neuronal and epithelial differentiation and upregulates genes involved in neural crest development, supporting the notion that the neural features in *EWS/pPNET* may merely be the result of *EWS/FLI-1* expression and not signify origin from a neural progenitor cell [152,153]. Interestingly, 2 studies comparing gene expression profiles of *EWS/pPNET* tissues with a wide variety of normal human cells and tissues reached different conclusions; 1 study showed significant similarities of *EWS/pPNET* cells to endothelial and neural cells [154] and the other study demonstrated similarity to mesenchymal stem cells [146]. It has recently been proposed that a mesenchymal and neural crest origin of *EWS/pPNET* may not be mutually exclusive [155], because human neural crest stem cells derived from embryonic stem cells retain their mesenchymal lineage plasticity [156], and that neuroepithelium-derived mesenchymal stem cells are present in the mouse embryonic trunk and bone marrow [157].

In addition to the *EWS-ETS* rearrangements, other genetic changes have been described in *EWS/pPNET*, including numerical and structural chromosomal aberrations in approximately 80% of cases [158,159]. Among the most common aberrations are gains of chromosomes 8 and 12 seen in 50% and 30% of cases, respectively, gains of chromosomes 2 and 1q, an

unbalanced translocation t(1;16), and deletions of the short arm of chromosome 1. The latter has been associated with adverse prognosis in localized EWS/pPNET [160]. Mutations in *p53* and deletions in *p16^{INK4a}/p14^{ARF}* (*CDKN2A*) are present in 10% and 25%, respectively, of EWS/pPNET and are associated with a poor prognosis [161]. Meta-analysis of 6 studies that assessed *p16^{INK4a}* status and 2-year survival rates revealed that this deletion was present in 20%–25% of cases and connoted a poor outcome regardless of tumor stage [162]. Another study showed association of poor event-free survival with high levels of *p16/p14^{ARF}* mRNA and not with *CDKN2A* deletion [163]. It has been hypothesized that *p16^{INK4a}/p14^{ARF}* alterations are late events leading to inactivation of the *RB* and *p53* pathways and cell cycle progression. The *CDKN2A* (*p16^{INK4a}/p14^{ARF}*) locus maps to chromosome 9p21.3, and its deletion is usually detected by FISH, which may fail to detect small microdeletions in some EWS/pPNET [164].

The management of osseous and extraosseous EWS/pPNET is the same in most centers [42,56,165]. After biopsy, chemotherapy generally includes some variations in active agents, including vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide. Definitive surgery, radiation therapy, or a combination of the 2 follows the completion of induction chemotherapy, depending on tumor location, size, and response to chemotherapy. Radiation therapy is applicable in those cases of an unresectable tumor or those that prove to have margins of resection involved or closely approached by tumor. Treatment intensification for high-risk patients may consist of high-dose chemotherapy followed by stem cell transplant. The current 5-year event-free survival rate for nonmetastatic EWS/pPNET ranges from 60% to 75% [42,56,166]. Metastases at presentation diminish the 5-year relapse-free survival rate to approximately 20%. Tumors in excess of 8 cm and an axial location, features that correlate with each other to some extent, are adverse prognostic features [42,56,166]. Patients with metastases or recurrence on therapy generally fare poorly regardless of therapeutic approach.

Superficial, cutaneous EWS/pPNET have a more favorable clinical course, which is related in part to small size [167].

Age at diagnosis is now recognized as a prognostic factor, with younger patients generally having an improved outcome. The relationship between neural differentiation and prognosis has been controversial, with earlier reports concluding that this feature was associated with a poor prognosis, but that no longer appears to be the case [80,165,168–171]. Patients with extraosseous EWS did not have a different outcome from those of osseous EWS in a recent Children's Oncology Group study [56].

The so-called type 1 *EWS*-fusion transcript was thought to be associated with a more favorable outcome, but that is no longer thought to be the case [172,173]. Any differences were explained by the more intensive therapeutic approaches, which have eliminated differences in clinical outcomes. The degree of response to chemotherapy in osseous EWS/pPNET has been correlated with outcome in a number of studies [77–80]. Data regarding a similar correlation in extraosseous EWS are not available to date.

EWS/ETS fusions are tumor specific and essential for the viability and growth of *EWS/pPNET* cells in culture and in xenograft murine models, as shown by RNA interference experiments [174–178], and they are considered ideal therapeutic targets. The translation of these findings into clinical trials is challenged by the absence of a useful siRNA delivery system.

DESMOPLASTIC SMALL ROUND CELL TUMOR

The DSRCT is an aggressive neoplasm with overlapping histologic, immunophenotypic, and molecular genetic features with *EWS/pPNET* but has its own distinctive attributes [179–182]. This tumor was originally described as multiple intra-abdominal mass lesions, but it is now recognized in the pleura [179,183–185], as well as the kidney, ovary, scrotum, meninges, bone, scalp, paranasal sinuses, pancreas, and parotid gland [186–196]. There is a male predilection (M:F = 5:1). The clinical presentation occurs in the 2nd and 3rd decades and is rare in early childhood and later adulthood. Abdominal pain and multiple abdominal masses without a single organ origin are the usual presenting findings (Fig. 23) [190,197,198].

One and usually more solid masses have the appearance of implants on the mesentery, omentum, on the surface of the liver, and in the pelvis. In some cases, the implants are more restricted. A dominant organ-based tumor is unusual. Some variation in the distribution and appearance of the tumor(s) is site dependent. The mass(es) has a smooth bosselated surface and is firm except in the presence of extensive necrosis. A grayish, glistening surface is present in cut surface (Figs. 24,25). More often than not, the DSRCT is submitted for diagnosis as several tissue fragments or needle cores. As one examines these biopsies, some sense of the clinical presentation is important in the formulation of the differential diagnosis. Variably sized nests and larger lobules of malignant small cells are separated by a dense stroma (Fig. 26); this pattern is recapitulated in other types of malignant round cell tumors, including rhabdomyosarcoma, blastemal Wilms tumor, malignant rhabdoid tumor, *EWS/pPNET*, hypercalcemic small cell tumor of the ovary, and neuroblastoma. Trabecular profiles and formless sheets of malignant small cells are other patterns (Figs. 27,28). Central necrosis and calcification may be seen in larger nests. The cells are round with round to oval or slightly angulated with nuclei that have finely granular chromatin and inconspicuous nucleoli (Fig. 29). Apoptosis and mitotic figures are frequent. Little cytoplasm is present, and cell borders are indistinct. However, the malignant cells may have a rhabdoid or signet ring appearance, and glandular formations have been described [179,181–183,189,190,193,199–201]. Tightly juxtaposed undifferentiated small cells with sparse cytoplasmic organelles, tight junctions, desmosomes, and bundles of intermediate filaments forming typical paranuclear inclusions are some of the ultrastructural features. Dendritic-like processes containing microtubules and dense core granules have been observed. Microfilaments and Z-band-like material suggestive of smooth or skeletal muscle differentiation are lacking [184,202–204].

Desmoplastic small round cell tumor is more phenotypically diverse than *EWS/pPNET*, with the coexpression of vimentin, cytokeratins (AE1-AE3 and CAM 5.2) (Fig. 30), epithelial membrane antigen (Fig. 31), and muscle and neural markers [184,203]. Desmin is detected

in 80%–90% of cases (Fig. 32), with a dot-like pattern corresponding to paranuclear bundles of intermediate filaments. Smooth muscle actin and myoglobin are rarely positive; myogenin and MyoD are negative [184,190,193,202,203]. Neuron-specific enolase and CD57 are the most commonly expressed neural markers, in 70% and 65% of cases, respectively (Fig. 33). Rare cases display scattered synaptophysin-positive cells [184,193,202,203]. CD99 is expressed in 20%–55% of DSRCT with a diffuse membranous pattern similar to EWS/pPNET [190,203]. Positive immunostaining for WT-1 is routinely found (Fig. 34).

Two genes, *EWS* and *WT-1*, are fusion partners of DSRCT and result in the signature translocation t(11;22)(p13;q12); the N-terminal domain of the *EWS* gene on chromosome 22 is fused to the C-terminal domain of the Wilms tumor suppressor (*WT-1*) gene on chromosome 11 [205,206]. The resulting in-frame fusion includes the transactivation domain of *EWS* and the zinc-finger DNA-binding domain of *WT-1* [190]. The presence of this specific and sensitive translocation is found in more than 90% of cases and also accounts for the aberrant nuclear expression of *WT-1* in the tumor cells. Occasional negative cases have been attributed to technical problems but could also be true negative cases, or cases with as yet unknown fusions [193,207,208]. For example, a chest wall tumor exhibiting small round cell morphology and desmin positivity but lacking desmoplasia was reported with a fusion transcript between *EWS* and another zing finger family gene, *ZNF278* [130]. Whether this tumor represents DSRCT with a variant fusion is currently unclear. The most frequent *EWS/WT-1* fusion consists of the 1st 7 exons of the *EWS* and the last 3 exons of the *WT-1* gene. Three additional variant fusions have been reported with different breakpoint locations within the *EWS* gene. These fusions contain in-frame junctions of exons 8, 9, or 10 of *EWS* to exon 8 of *WT-1* [207,209–211]. The participating portion of *WT-1* gene in all *EWS/WT-1* fusions is consistent and includes exons 8–10 [190]. The only exception occurred in 1 case, which expressed 2 novel *EWS/WT-1* fusions, both without exons 9 and 10 of *WT-1* and 1 with additional exons 3–7 of *WT-1* [212]. Subpopulations of variant fusions have been detected by RT-PCR in addition to the main fusion transcript in some DSRCT, but they usually show a low level of expression [209,211]. *EWS/WT-1* fusions can be detected by RT-PCR in fresh or frozen tumor tissue [207] but also in paraffin-embedded tissues, and thus RT-PCR is useful in the diagnosis of DSRCT [213]. However, the combination of characteristic immunophenotype (vimentin, cytokeratin, desmin, and WT-1) and an *EWS* breakpoint by FISH should establish the diagnosis of DSRCT. More than 90% of DSRCT show nuclear staining using antibodies recognizing the C-terminal region of *WT-1*, and most tumors are nonreactive with antibodies recognizing the N-terminal region of *WT-1*, which is lost in the fusion protein EWS/WT-1. A reverse staining pattern for *WT-1*, negative for the C-terminus and positive with the N-terminus antibody, has been reported in association with a rare fusion transcript characterized by deletion of *WT1* exons 9 and 10 [212,214].

The differential diagnosis of DSRCT includes a restricted group of round cell tumors with the coexpression of epithelial and myogenic markers. In those exceptional EWS/pPNET with desmin expression [62,102], WT-1 staining and/or molecular confirmation by RT-PCR will lead to the correct diagnosis. Malignant rhabdoid tumors may occasionally show a predominant small round cell morphology, but its occurrence in infants and young children

and the lack of nuclear INI-1 and WT-1 staining are the diagnostic features [215]. Nuclear positivity for myogenin discriminates from DSRCT.

Aggressive surgery, chemotherapy, radiotherapy, and stem cell transplant have resulted in a 3-year survival rate of approximately 50%, but long-term survival rates remain poor [198,202,203,216–222].

Because of its predominant location in mesothelium-lined cavities and its immunohistochemical expression of mesenchymal and epithelial markers and WT-1, it was originally thought that the DSRCT was a primitive neoplasm of mesothelial/submesothelial cells. Despite the appeal of this hypothesis, the recognition of this tumor in sites remote from mesothelial cells has dampened this notion [202]. The polyphenotypic profile of DSRCT suggests an origin from a multipotential cell, but unlike EWS/pPNET, no permissive cell type has been identified yet in which EWS/WT-1 can induce the characteristic phenotype of DSRCT.

The EWS/WT-1 fusion protein contains the transactivation domain of EWS and the zinc finger DNA-binding domain of WT-1; for this reason, it is expected to function as an aberrant transcription factor with target specificity defined by the *WT-1* gene. However, although WT-1 is a repressor, EWS/WT-1 is an activator of transcription [223]. A higher DNA-binding affinity was found for the EWS/WT-1 fusion protein than for WT-1 alone [224]. Unlike other EWS-related chromosomal translocations, there are 2 isoforms of the EWS/WT-1 fusion, which are defined by the insertion or deletion of 3 amino acids in the region between zinc fingers 3 and 4 of WT-1. The 2 isoforms are generated by alternative splicing and have different oncogenic properties. For example, only the isoform with the deletion of amino acids exhibits DNA binding and transforming activity in vitro [225]. The function of the EWS/WT-1 fusion protein can also be altered by phosphorylation. EWS/WT-1 can be phosphorylated in vivo and in vitro, and its DNA-binding ability is abolished by phosphorylation. Therefore, it has been postulated that a variable phosphorylation status of *EWS/WT-1* may be responsible for the observed differential ability of EWS/WT-1 to regulate its targets in various cell types [226]. Promoter sequence studies have identified many genes as potential EWS/WT-1 targets [223]. Early growth response 1 (*EGR-1*) [211], insulin-like growth-factor receptor (*IGFR1*) [227], platelet-derived growth factor-A (*PDGFA*) [228], and equilibrative nucleoside transporter 4 (*ENT4*) [229] are genes whose promoters are activated by EWS/WT-1. However, only *PDGFA* and *ENT4* are overexpressed in DSRCT. Because *PDGFA* is a chemoattractant for fibroblasts [230], it has been suggested that its induction in DSRCT may contribute to the fibrotic stromal response [228]. The connective tissue growth factor CCN2 is yet another overexpressed protein in DSRCT and may have a role in the stromal reaction [231].

Development of targeted therapies in DSRCT is limited by its rarity, but experimental data have suggested potential therapeutic targets that are under clinical investigation [232–236].

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Figure 1.
Ewing sarcoma/peripheral primitive neuroectodermal tumor of soft tissue of arm shows a firm white and yellow mass with fine trabeculae.

Figure 2.
Ewing sarcoma/peripheral primitive neuroectodermal tumor of the chest wall shows a tumor on the inner surface of the rib with abundant hemorrhage and necrosis.

Figure 3. Ewing sarcoma/peripheral primitive neuroectodermal tumor of the mesentery shows a white and red focally hemorrhagic necrotic mass.

Figure 4.
Ewing sarcoma/peripheral primitive neuroectodermal tumor of the kidney shows extensive hemorrhage and necrosis within lobules of a firm white tumor.

Figure 5.
Ewing sarcoma/peripheral primitive neuroectodermal tumor touch imprint for cytology.
Nuclear features are well preserved, hematoxylin and eosin (H&E), ×600.

Figure 6.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with sheets of uniform cells,
H&E, $\times 200$.

Figure 7.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with uniform cells in sheets and delicate nuclear chromatin, hematoxylin and eosin (H&E), $\times 600$.

Figure 8.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with light and dark cells, H&E,
×600.

Figure 9.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with spindle cells, H&E, ×600.

Figure 10.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with cytoplasmic glycogen,
periodic acid-Schiff, $\times 400$.

Figure 11.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with filigree pattern, H&E,
×200.

Figure 12.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with neuropil, H&E, ×400.

Figure 13.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with poorly formed rosettes,
hematoxylin and eosin (H&E), $\times 200$.

Figure 14.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with neuropil and poorly formed rosettes.

Figure 15. Ewing sarcoma/peripheral primitive neuroectodermal tumor after chemotherapy with a pattern simulating alveolar rhabdomyosarcoma, H&E, ×200.

Figure 16.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with abundant cytoplasmic glycogen, electron microscopy.

Figure 17.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with cluster of membrane-bound granules in cell process, electron microscopy.

Figure 18.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with uniform CD99 immunostaining in a membranous pattern, CD99, ×400.

Figure 19.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with diffuse nuclear FLI-1 immunostaining, $\times 400$.

Figure 20.
Ewing sarcoma/peripheral primitive neuroectodermal tumor with synaptophysin staining in a dot-like cytoplasmic pattern.

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Figure 21.

Ewing sarcoma/peripheral primitive neuroectodermal tumor karyotype with translocation between chromosomes 11 and 22.

22 46, XY, t (7;22) (p21;q 11.2)

Figure 22.

Ewing sarcoma/peripheral primitive neuroectodermal tumor karyotype with translocation between chromosomes 7 and 22.

Figure 23.
Ewing sarcoma/peripheral primitive neuroectodermal tumor computed tomographic study of multifocal abdominal DSRCT with multiple liver nodules.

Figure 24.
Intra-abdominal mass with fibrous bands separating nests of tumor cells. Photograph courtesy of William L. Gerald, M.D.

Figure 25.
Desmoplastic small round cell tumor with hemorrhagic and necrotic abdominal mass.

Figure 26.
Desmoplastic small round cell tumor with sheets of round cells with little cytoplasm, hematoxylin and eosin (H&E), $\times 400$.

Figure 27.
Desmoplastic small round cell tumor with geographic pattern of tumor cells and intervening fibrotic tissue, H&E, $\times 200$.

Figure 28.
Desmoplastic small round cell tumor with strands of tumor cells of varying width, separated by fibrotic stroma, H&E, $\times 400$.

Figure 29.
Cytospin preparation of desmoplastic small round cell tumor cells with fine nuclear chromatin and inconspicuous nucleoli, H&E, $\times 600$.

Figure 30.
Desmoplastic small round cell tumor with diffuse cytokeratin immunostaining, cytokeratin AE1/AE3, $\times 400$.

Figure 31.
Desmoplastic small round cell tumor with epithelial membrane antigen immunostaining,
×400.

Figure 32.
Desmoplastic small round cell tumor with desmin immunostaining, $\times 400$.

Figure 33.
Desmoplastic small round cell tumor with focal positive neuron-specific enolase immunostaining, $\times 600$.

Figure 34.
Desmoplastic small round cell tumor with diffuse WT1 immunostaining, $\times 400\times$.

Table 1.

Soft tissue tumors other than Ewing sarcoma/peripheral primitive neuroectodermal tumor carrying *EWS* translocations

	Translocation	Gene fusion	Immunophenotype
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12)	<i>EWSR1/ATF1</i>	Desmin, EMA, MSA
Clear cell sarcoma	t(12;22)(q13;q12)	<i>EWSR1/ATF1</i>	S100, HMB45, MART.1, MITF-1
Desmoplastic round cell tumor	t(11;22)(p13;q12)	<i>EWSR1/WT1</i>	Coexpression of Keratin, EMA, desmin, and neural markers (NSE, S-100, CD57), WT1
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	<i>EWSR1/NR4A3</i>	S-100 <20%
Myxoid/round cell liposarcoma	t(12;22)(q13;q12)	<i>EWSR1/DDIT3</i>	S-100
Myoepithelial carcinoma	t(19;22)(q13;q12)	<i>EWSR1/ZNF444</i>	P63, S100, Keratin, SMA

Table 2.

Differential diagnosis of Ewing sarcoma/peripheral primitive neuroectodermal tumor from other small round cell tumors lacking EWS translocations

Tumor	Histology	Immunohistochemistry	Molecular genetics
Ewing sarcoma/peripheral primitive neuroectodermal tumor	Sheets of small round cells Salt and pepper pattern	CD99+, NSE+/-, CK +/-, CAV +, Flil+	t(11;22) and others (see Table 3)
Alveolar rhabdomyosarcoma	Alveolar, solid pattern Wreath-like cells Rhabdomyoblasts	Desmin, myogenin, MSA+ CD99-/+	t(2;13);t(1;13)
Lymphoblastic lymphoma		TdT+, CD45-/+ CD99+ (B,T lineage markers variously expressed)	
Synovial sarcoma poorly differentiated	Oval cells, cellular molding Focal whorled pattern	CD99+, EMA+	t(X;18)
Small cell osteosarcoma	Osteoid	CD99-	No specific translocation
Mesenchymal chondrosarcoma	Chondroid		No specific translocation; der(13;21) (q10;q10) in 2 cases
NUT carcinoma	Focal keratinization (may be absent)	P63+, ker+, EMA+, NUT (nuclear)+	BRD4 (or BRD3)-NUT transcript
Neuroblastoma	Abundant neuropil, ganglion cell differentiation Cells with coarse granular chromatin	NB84a+, CD99-	nMYC amplification, del 1p (in a subset)
Undifferentiated sarcoma		No specific lineage markers	CIC-DUX4 transcript in a subset

Table 3.

Translocations and fusion gene products in Ewing sarcoma

Translocation	<i>EWS/FUS</i> to <i>ETS</i> proteins	<i>EWS</i> to non- <i>ETS</i> proteins	Non- <i>EWS</i> to non- <i>ETS</i> proteins
t(11;22)(q24;q12)	<i>EWS/FLI1</i>		
t(21;22)(q22;q12)	<i>EWS/ERG</i>		
t(7;22)(p22;q12)	<i>EWS/ETV1</i>		
t(17;22)(q12;q12)	<i>EWS/ETV4(EIAF)</i>		
t(2;22)(q33;q12)	<i>EWS/FEV</i>		
t(16;21)(p11;24)	<i>FUS-ERG</i>		
t(2;16)(q35;p11)	<i>FUS-FEV</i>		
t(2;22)(q31;q12)		<i>EWS/SP3</i>	
t(6;22)(p21;q12)		<i>EWS/POU5F1</i>	
t(20;22)(q13;q12)		<i>EWS/NFATc2</i>	
t(4;22)(q31;q12)		<i>EWS/SMARCA5</i>	
t(4;19)(q35;q13)			<i>CIC-DUX4</i>