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Original Article

Primary intracranial Ewing's sarcoma with unusual features

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Abstract: Pediatric primary "small round blue cell" tumors in the CNS represent several entities, some more common than others. Ewing sarcoma/peripheral primitive neuroectodermal tumor (ES/pPNET) is rare and must be distinguished from other tumors such as medulloblastoma [1, 2], atypical rhabdoid/teratoid tumor, ependymomal tumors, metastatic sarcomas, hematologic malignancies, and other mimics. Although therapy for ES/pPNET is effective, it brings severe side effects, including cardiac toxicity, making correct recognition important [3]. As small blue cell tumors look similar, diagnosis often depends on special stains, immunohistochemistry, and molecular techniques. While the combination of membranous immunohistochemical reactivity for CD99 with cytoplasmic glycogen provides effective screening, demonstration of characteristic translocations of *EWSR1* (chromosome 22) or *FUS* (chromosome 16) by fluorescent *in situ* hybridization (FISH) can confirm the diagnosis. We are reporting three primary ES/pPNET of the CNS, two of which occurred in children. While the adult case demonstrates the classic histopathology, the two pediatric cases have histopathology that significantly deviates from the usual. One is suggestive of a primary sarcoma, and the other mimics an ependymoma, but all three cases are confirmed with FISH. These observations suggest that primary ES in the CNS may have histology different from the classic morphology and a high index of suspicion should be maintained in order to make the correct diagnosis. A search of the literature suggests that these tumors are most frequently seen in children and young adults. Imaging often shows a supratentorial enhancing mass that touches the leptomeninges. Survival over three years is good but long term prognosis is unknown [3, 4].

Keywords: Ewing sarcoma, peripheral primitive neuroectodermal tumor, diagnosis

Introduction

The so-called "small round blue cell" tumor group of the central nervous system (CNS) in the pediatric population is a consortium of primary tumors and occasional metastatic lesions. These tumors share the common feature of densely packed "small blue cells" or embryonal appearing small tumor cells with hyperchromatic nuclei. On many occasions, the tumor cells are solidly packed without a specific histologic pattern. As they tend to appear similar in histopathology, the use of special stains, immunohistochemistry, and molecular studies are necessary for accurate classification. Also common to this family of tumors are signature molecular derangements which provide important diagnostic aids and also hint at further insights on the mechanisms of tumorigenesis of these tumors. Astute histopathologic observation and a high index of suspicion, however, still form the first line of defense in diagnosis.

While medulloblastoma is the most commonly seen "small blue cell" tumor in the pediatric age group, the other commonly seen entities include central primitive neuroectodermal tumors (cPNET), most often arising as supratentorial and spinal cord tumors, and some atypical teratoid/rhabdoid tumor. Less common entities include ependymoblastoma, embryonal tumor with abundant neuropils and true rosettes (ETANTR), metastatic neuroblastoma, pineoblastomas, lymphoma, and metastatic sarcomas.

Table 1. Primary extraosseous intracranial Ewing's Sarcoma

Case	Age/Sex	Location and Imaging characteristics	CD99/ t(11:22)	Treatment & Outcome	Author/Year
1	2Y11M/F	Imaging: Frontal lobe; intra-axial, enhancing, solid with cystic changes. ICDL: Yes	+/+	Treatment: Treated with surgery, radiation therapy, and chemotherapy with vincristine, cyclosporine, and doxorubicin. Extracranial metastasis: none CSFD: none Outcome: Developed cardiac failure and required a cardiac transplantation. Remained disease free after 5 years.	Current Case 1
2	2Y4M/M	Imaging: Frontal parietal lobe; intra-axial, enhancing, predominantly cystic. ICDL: Yes	+/+	Treatment: Treated with surgery and chemotherapy alone as the patient is under 3 years of age. Extracranial metastasis: none CSFD: none Outcome: The patient is disease free 18 months after diagnosis.	Current Case 2
3	61Y/M	Imaging: Right frontal temporal; enhancing cystic mass in right temporal lobe, 6.2 x 5.1 cm. ICDL: Uncertain, likely predominantly an intra-axial mass that probably approximate the dura when the size and location of this tumor are considered.	+/+	Treatment: Treated with surgery alone. Extracranial metastasis: none CSFD: none Outcome: The surgery was successful but the patient fail to follow up after the surgery.	Current Case 3
4	8Y/F	Imaging: Tentorium cerebelli with broad tentorial attachment; extra-axial, enhancing, solid. ICDL: Yes	+/+	Treatment: Treated with surgery, further treatment information not given in the original publication (may be the same as case 6 below) Extracranial metastasis: None CSFD: None Outcome: No information in the original publication	Pekala JS, 2006 [5]
5	7Y/F	Imaging: Right frontal closely approximate anterior falx cerebri; intra-axial, enhancing, solid. ICDL: Yes	+/+	Treatment: Treated with partial surgical resection; further treatment information not given in the original publication (May be the same as case 8 below) Extracranial metastasis: Left lung base CSFD: No information in the original publication Outcome: No information in the original publication	Pekala JS, 2006 [5]
6	7Y/F	Imaging: Anterior cranial fossa; intra-axial, enhancing, solid. ICDL: Yes	+/+	Treatment: Treated with surgery, chemotherapy with vincristine, cyclophosphamide, and doxorubicin, alternating with ifosfamide and etoposide, and radiation. Extracranial metastasis: None CSFD: None Outcome: No definitive information in the original publication, but tolerated 48 weeks of chemotherapy and radiation without significant side effects.	Kazmi SA, 2007 [6]
7	8Y/F	Imaging: Tentorium cerebelli compressing cerebellar vermis and quadrigerminal plate; extra-axial, enhancing, solid. ICDL: Yes	+/+	Treatment: Treated with surgery, chemotherapy with vincristine, cyclophosphamide, doxorubicin, alternating with ifosfamide and etoposide, and 56 Gy radiation. Extracranial metastasis: None CSFD: None Outcome: Disease-free and alive at 2 years from diagnosis	Mazur MA, 2005 [7]
8	7Y/F	Imaging: Frontal lobe, Intra-axial, enhancing, solid. ICDL: Yes	+/+	Treatment: Treated with surgical resection of the brain and lung masses, with chemotherapy identical to case 7 above, with future radiation therapy planned at the time of publication Extracranial metastasis: lung CSFD: Suspicious cells in CSF Outcome: stable disease still present at the time of publication	Mazur MA, 2005 [7]

9	4Y/M	Imaging: Posterior fossa, midline, enhancing, with supratentorial hydrocephalus ICDL: No	-/+	Treatment: Treated with surgery, chemotherapy with vincristine, cyclophosphamide, and epirubicin initially. Received radiation and chemotherapy with ifosfamide, cyclophosphamide, and epirubicin after progression. Extracranial metastasis: None CSFD: Yes Outcome: Follow up CT scan after an unspecified period of time, following the first treatment round, showed progression of the leptomeningeal spread.	Jay V, 1996 [8]
10	30Y/F	Imaging: Anterior fossa; extra-axial, presented as a meningioma, enhancing. ICDL: Yes	+/+	Treatment: Initial disease treated with total surgical resection. Chemotherapy with Adriamycin, vincristine, and cyclophosphamide and 50 Gy radiation therapy after recurrence Extracranial metastasis: None CSFD: None Outcome: Died of disease after 10 years; one local and one distant recurrence	Papotti M, 1998 [9]
11	6Y/M	Imaging: Anterior fossa; extra-axial, with cystic change, enhancing. ICDL: Yes	+/+	Treatment: Treated with surgical resection, and chemotherapy and radiation were recommended. Extracranial metastasis: None CSFD: No specific information in the original publication Outcome: No information in the original publication.	Antunes NL, 2001 [10]
12	17Y/M	Imaging: Right anterior fossa, right CPA recurrence; dural based, enhancing. ICDL: Yes	+/+	Treatment: Treated with surgery and radiation at first, then chemotherapy with cisplatin, ifosfamide, and etoposide, along with radiation after the recurrence Extracranial metastasis: None CSFD: None Outcome: Recurred after 8 years, disease free 12 months after recurrence.	Dedeurwaerdere F, 2002 [11]
13	12Y/M	Imaging: Right anterior fossa; dural based, enhancing. ICDL: Yes	+/+	Treatment: Treated with surgical resection, chemotherapy with intrathecal methotrexate, carboplatin, and VP16,and radiation Extracranial metastasis: None CSFD: None Outcome: Alive after 27 months	Dedeurwaerdere F, 2002 [11]
14	50Y/F	Imaging: Right temporal convexity; dural based. ICDL: Yes	+/+	Treatment: Treated with surgical resection, but no other treatment specifically mentioned in the original publication Extracranial metastasis: None CSFD: No information in the original publication Outcome: Alive after one year	D'Antonio et al., 2004 [12]
15	21Y/M	Imaging: Right occipital lobe, parafalcine; meningioma like dural based mass on MRI. ICDL: Yes	+/+	Treatment: Treated with partial resection and "adjuvant therapy" unspecified in the original publication, without complete regression of disease on imaging. Recurrence treated with chemotherapy with cyclophosphamide and topotecan, as well as radiation. Extracranial metastasis: Yes. Cervical and thoracic vertebrae CSFD: None Outcome: Recurred after 18 months, alive after 21 months.	Mobley et al., 2006 [13]
16	8Y/F	Imaging: Tentorium; enhancing, compressing cerebellar vermis and quadrigeminal plate. ICDL: Yes	+/+	Treatment: see above Extracranial metastasis: None CSFD: None Outcome: Alive after 2 years	Mazur et al. 2005 [7] Pekala et al., 2006 [5]

17	48Y/F	Imaging: Left cavernous sinus; T1 enhancing, extending into medial temporal lobe and posterior fossa ICDL: uncertain, may be arising from bone.	+/+	Treatment: Treated with surgical resection, radiation, and chemotherapy with vincristine, cyclophosphamide, and doxorubicin, alternating with etoposide and ifosfamide Extracranial metastasis: None CSFD: None Outcome: Stable disease at 14 months	Attabib et al., 2006 [14]
18	3Y/M	Imaging: Tentorium ICDL: Yes	+/+	Treatment: Treated with surgical resection, chemotherapy with vincristine, cyclophosphamide, and Adriamycin, alternating with ifosfamide and etoposide, and radiation (45 Gy) Extracranial metastasis: None CSFD: None Outcome: Disease free after 18 months	Navarro et al., 2007 [15]
19	56Y/F	Imaging: Right temporal convexity; large cystic lesion adhered to the dura ICDL: Yes	+/+	Treatment: Treated with surgical resection and no further adjuvant treatment Extracranial metastasis: No specific information in the original publication, but none mentioned CSFD: No specific information in the original publication, but none mentioned Outcome: Disease free after 18 months	Mellai et al., 2010 [16]
20	11Y/F	Imaging: Left temproparietal, solid mass, enhancing ICDL: Yes. There is also with temporal bone destruction	+/+	Treatment: Treated with surgical resection, chemotherapy with vincristine, doxorubicin, and cyclophosphamide, alternating with etoposide and ifosfamide, and radiation (56Gy) Extracranial metastasis: None CSFD: No specific information in the original publication Outcome: Disease free at 36 weeks.	Choudhury et al., 2011 [17]
21	17Y/F	Imaging: Frontal-parietal ICDL: Yes, with infiltration of the superior sagittal sinus	+/?	Treatment: Treated with surgical resection and 6000 cGy radiation Extracranial metastasis: None CSFD: No specific information in the original publication, but workup was said to be negative Outcome: Disease free and maintaining good grades at 24 months	Bunyaratavej K, 2005 [18]
22	17Y/M	Imaging: Temporal ICDL: No specific information in the original publication	+/?	Treatment: Treated with surgical resection and 6000 cGy radiation Extracranial metastasis: None CSFD: No specific information in the original publication, but workup was said to be negative Outcome: Disease free and doing well in school at 12 months	Bunyaratavej K, 2005 [18]
23	25Y/M	Imaging: Temporal fossa; extra-axial, associated with a subdural hematoma secondary to trauma ICDL: Yes	+/?	Treatment: Treated with surgical resection, radiation, and chemotherapy with ifosfamide, Adriamycin, cyclophosphamide, vincristine, and dactinomycin. Extracranial metastasis: None CSFD: None Outcome: Disease free at 19 months	Stechschulte SU, 1994 [19]
24	5Y/M	Imaging: Middle and posterior fossa and tentorium cerebelli; extra-axial, enhancing. ICDL: Yes	+/?	Treatment: Treated with surgical resection and intrathecal methotrexate, while other chemotherapy was refused. Extracranial metastasis: None CSFD: None Outcome: Disease free at 7 years	Katayama Y, 1999 [20]
25	5M/M	Imaging: Frontal cranial base with extension to ethmoid sinus and orbit, involving falx cerebri; axial status unclear, enhancing. ICDL: Yes	+/?	Treatment: Treated with surgical resection Extracranial metastasis: None CSFD: None Outcome: Died 8 days after surgery of DIC and renal failure	Niwa J, 2001 [21]

26	67Y/F	Cerebellar pontine angle ICDL: None, but interwoven with CN VIII	+/?	Treatment: Treated with surgical resection and palliative radiation Extracranial metastasis: None CSFD: No specific information in the original publication Outcome: Patient had no progression of symptoms for 13 months, followed by rapid decline and death	Simmons MA, 2001 [22]

ICDL: Involvement or close approximation with dura or leptomeninges on imaging. CSFD: CSF dissemination.

Table 2. Antibodies used in this study

Antibody	Manufacturer	Species	Clonality	Concentration
CD5 (SP19)	Cell Marque (Rocklin, CA)	rabbit	monoclonal	predilute
CD3	Cell Marque (Rocklin, CA)	rabbit	polyclonal	predilute
CD20 (L26)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
CD43 (L60)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
CD45RO (UCHL-1)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
CD79a (JCB117)	Cell Marque (Rocklin, CA)	mouse	monoclonal	predilute
CD1a (MTB1)	Cell Marque (Rocklin, CA)	mouse	monoclonal	predilute
ALK	Dako (Carpinteria, CA)	mouse	monoclonal	1:50
CD15	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
Synaptophysin	Ventana (Tucson, AZ)	rabbit	monoclonal	predilute
Vimentin (3B4)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
S100	Ventana (Tucson, AZ)	rabbit	polyclonal	predilute
CD15	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
CD99 (H036-1.1)	Cell Marque (Rocklin, CA)	mouse	monoclonal	predilute
Chromogranin (LK2H10)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
EMA (E29)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
CD45/LCA (RP2/18)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute
BAF47	BD Biosciences (San Jose, CA)	mouse	monoclonal	1:250
Ki-67	Ventana (Tucson, AZ)	rabbit	monoclonal	predilute
GFAP (E672Y)	Cell Marque (Rocklin, CA)	rabbit	monoclonal	predilute
Neu-N A60	Millipore (Darmstadt, Germany)	mouse	monoclonal	1:100
Neurofilament	Invitrogen (Grand Island, NY)	mouse	monoclonal	1:100
Pan-cytokeratin (AE1/AE3/PCK26)	Ventana (Tucson, AZ)	mouse	monoclonal	predilute

Primary extraosseous, intradural and/or intramedullary Ewing's sarcoma/peripheral primitive neuroectodermal tumor (ES/pPNET) of the brain is a rather uncommon offender. A search of the literature reveals 19 published cases (**Table 1**) [5-22], with the earliest published in 1994 [8]. Although there are few described cases, recent publications have begun to make tentative statements regarding common imaging findings/preferred locations, prognosis and appropriate treatment regimens as the numbers increase [3]. Although chemotherapy for ES/pPNET is rather effective, it also comes with significant side effects including cardiac failure due to cardiac toxicity, which can become serious enough to warrant transplantation. Therefore, the diagnosis must be made with accuracy in order to justify implementing this risky treatment regimen.

Herein, we are reporting three cases confirmed by fluorescent *in situ* hybridization. Electron microscopy is performed in two of the three cases. We also conducted a review of literature.

Materials and methods

A total of three cases of intracranial ES/pPNET were found in the archival material in the past 10 years from the Department of Pathology of the University of Oklahoma Health Sciences Center (OUHSC), Oklahoma City, OK and Albany Medical College, Albany, NY.

Immunohistochemistry was performed using a Ventana Benchmark autostainer (Ventana, Tucson, AZ) with varied antigen retrieval protocols as recommended by the manufacturer. Antibodies and concentrations used, as well as manufacturer data, are listed in **Table 2** below. Fluorescence in situ hybridization (FISH) assays were performed utilizing LSI EWSR1 (22q12), dual-color, break-apart rearrangement probe, which was purchased from a commercial source (Abbott Molecular Inc., Des Plaines, IL, USA). A total of 200 interphase cells were analyzed for each case and the digital images carrying specific hybridization signals were processed using the software CytoVision Software

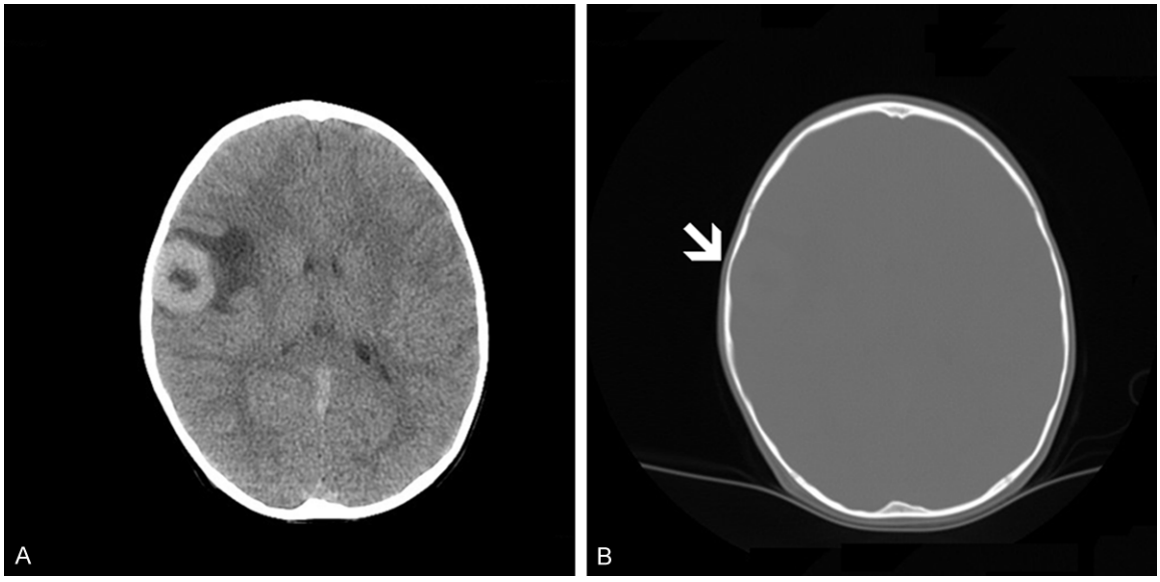

Figure 1. CT image of Case 1 demonstrates a round lesion with peritumoral vasogenic edema (A). Remodeling of the bone overlying the tumor is also noted (B).

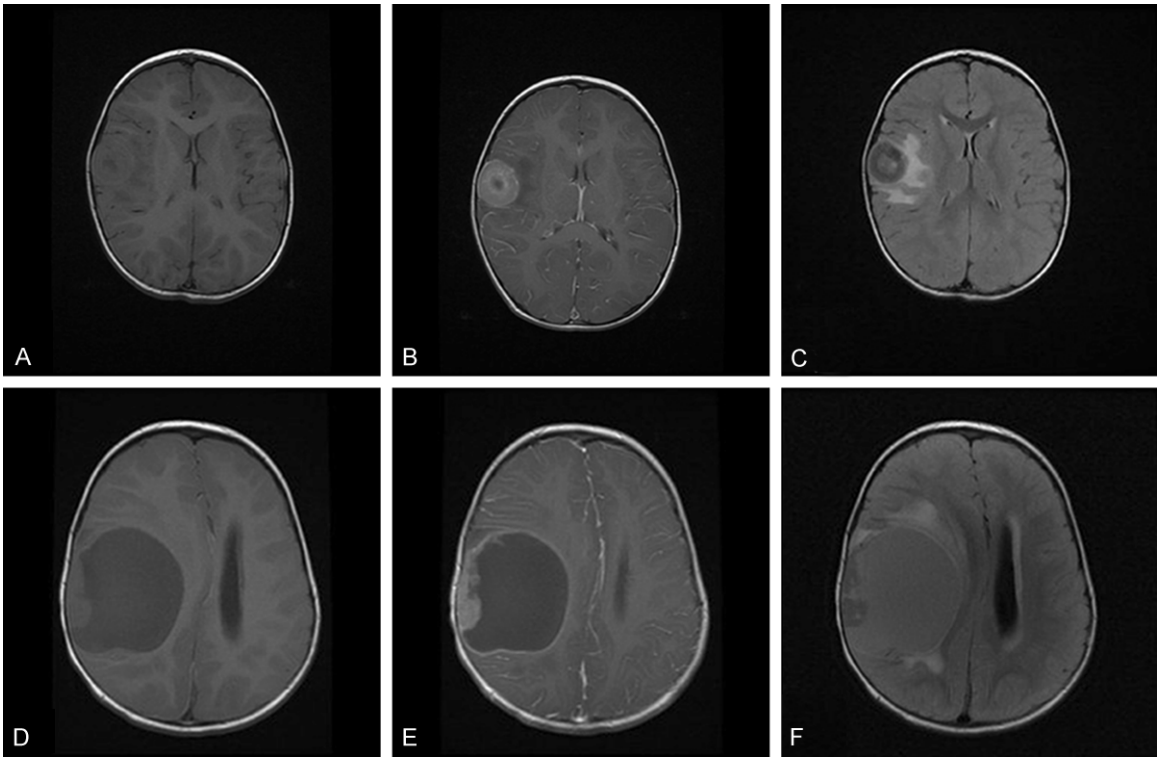

Figure 2. The tumor of Case 1 appears as a well demarcated right frontal lobe mass which is slightly hyperintense to grey matter and slightly hypointense to white matter on non-contrast T1 weighted imaging (A). The lesion enhanced with contrast (B). A centrally located necrotic nidus is present. FLAIR imaging demonstrates a moderate amount of edema around the tumor (C). The tumor of case 2 appears as a well demarcated primarily cystic lesion (D) causing midline shift. It has a mural nodule that is isointense to grey matter and hypointense to white matter. Enhancement of the solid component is present (E). FLAIR imaging demonstrates a moderate amount of edema around the tumor (F).

Version Number 7 (Applied Spectral Imaging, Genetix, USA).

The two cases from OUHSC had immunohistochemistry, electron microscopy, and fluores-

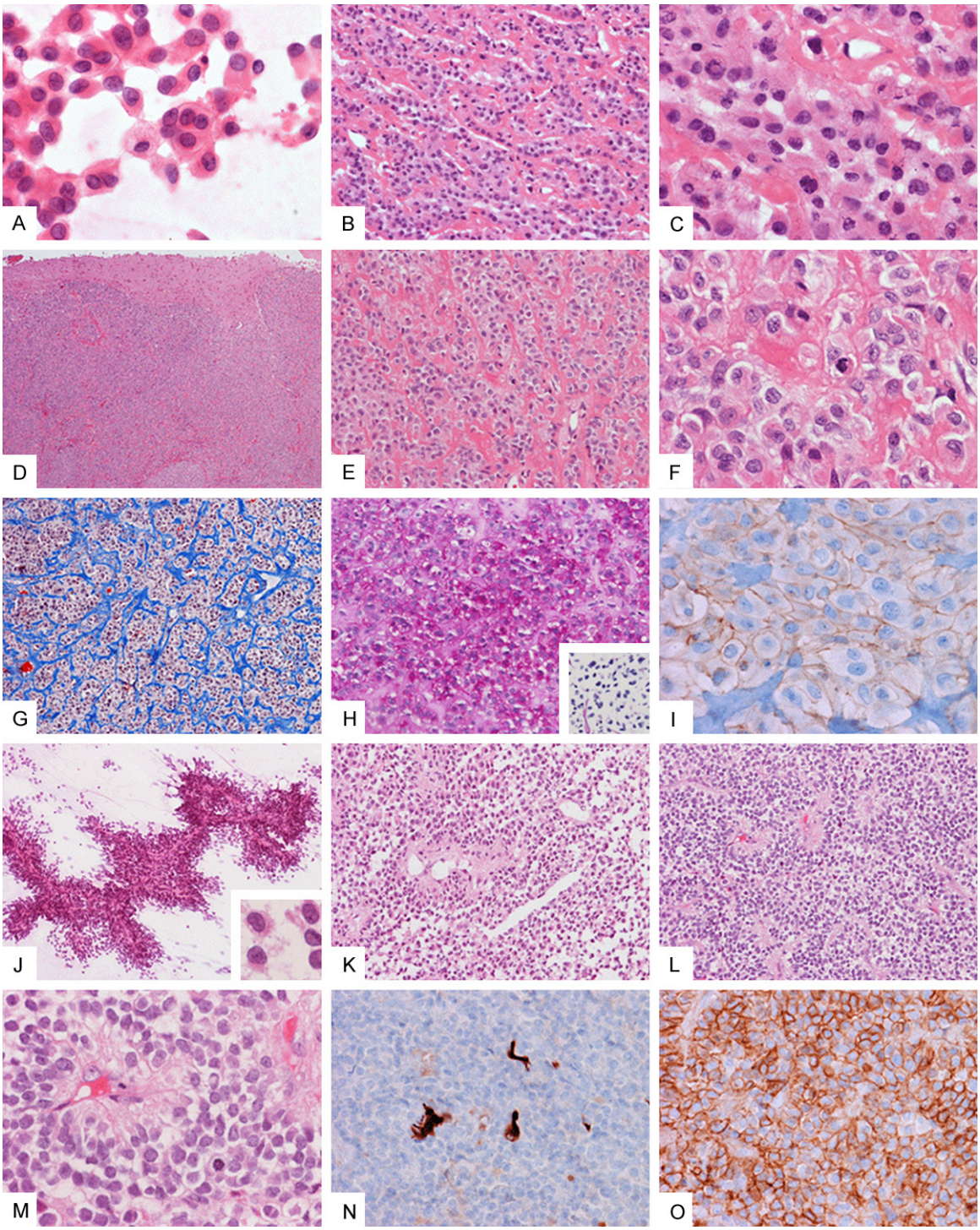

Figure 3. The tumor of Case 1 is composed of rather monotonous neoplastic cells (A) with a moderate amount of cytoplasm and round to oval nuclei that are best demonstrated in this cytologic preparation. Note that some fine cytoplasmic vacuoles that correspond to cytoplasmic glycogen are present. On frozen sections (B and C), monotonous neoplastic cells admixed with collagenous fibers are present. These features are well demonstrated in permanent sections (D to F). Note that the tumor cells have some clearing of cytoplasm. The fibrotic background is well demonstrated by trichrome stain (G). The tumor cells contains substantial amount of glycogen that can be demonstrated by PAS stain (H) and digested by diastase (inset in H). Immunohistochemistry for CD99 (I) demonstrates a membranous pattern of staining. The tumor of Case #2 has a papillary pattern of arrangement in cytologic preparation (J). The tumor cells are also rather monotonous and contain fine cytoplasmic vacuoles. In comparison to case #1, the tumor cells have less cytoplasm (inset in J). Frozen section demonstrated solid sheets of tumor with clear cell

features and vague arrangement suggestive of perivascular arrangement of tumor cells. Permanent sections (L and M) demonstrate clear cell features and perivascular arrangement. Immunohistochemistry for glial fibrillary acidic protein (GFAP) demonstrates only scant positive cells (N). Immunohistochemistry for CD99 (O) demonstrates extensive membranous pattern of immunoreactivity. Original magnification in (A), (C), (F), (I), Inset in (J), (M) are 60x; in (B), (E), (G), (H), inset in (H), (K), (L) are 20 x in (D) is 4 x; in (N) and (O) are 40 x.

cent *in situ* hybridization studies at the time of diagnosis. FISH study of the case from Albany Medical College was performed later in OUHSC.

Results

Case 1

Case history: The patient was a 35 month-old girl with no significant past medical, surgical, or birth history. She was brought to her local emergency department after an episode of lip and tongue smacking, left facial twitching, and impaired coordination. A second identical episode occurred in the waiting room of the emergency department. These were characterized as simple partial seizures and follow-up was recommended. She was brought three days later for further workup. No episodes occurred in the meantime. Physical examination, including careful fundic examination revealed no abnormalities at this time.

Imaging studies: Computerized tomography (CT) without contrast enhancement demonstrated a circumscribed right frontal lobe hyperdense mass with central cavitation and surrounding vasogenic edema (**Figure 1**). There was minimal remodeling of the adjacent inner table of the skull. Magnetic resonance imaging (MRI) with and without contrast enhancement showed an intra-axial circumscribed cortically based posterolateral right frontal lobe mass (3.0 x 2.5 x 2.5 cm) with central cavitation (**Figure 2A-C**). The mass was slightly hyperintense to grey matter and slightly hypointense to white matter on non-contrast axial T1 weighted imaging (**Figure 2A**). This lesion was hypointense to grey matter and isointense to hypointense to white matter on non-contrast T2-weighted imaging (not shown). Axial T1 weighted imaging with contrast enhancement revealed diffuse enhancement of the solid portion of the mass, providing a concentric ring pattern (**Figure 2B**). A moderate amount of peritumoral vasogenic edema was present as shown on axial non-contrast FLAIR imaging (**Figure 2C**). The mass exerted minimal adjacent mass effect with no midline shift or herniation.

Gross pathology: A gross total resection was performed which yielded a rubbery, spherical, pink-tan tumor mass 2.4 cm in greatest dimension. The cut surface of this mass was homogeneous creamy white with poorly defined, small, opaque yellow areas. A centrally located irregularly shaped cavity at 1.3 cm in greatest dimension and without content was noted.

Histopathology: Intra-operative smeared cytologic preparation demonstrated a rather monotonous population of tumor cells of moderate size, with centrally located oval to round nuclei without nucleoli. Small cytoplasmic vacuoles were often found at the periphery and the cell membrane was distinct (**Figure 3A**). On frozen section, the tumor was composed of solid sheets to cords of tumor cells that were separated by collagenous fibers (**Figure 3B**). The tumor cells often, but not always, separated from each other (**Figure 3C**). An intraoperative diagnosis of "cellular neoplasm" was given, with further diagnosis deferred for examination of permanent sections.

Histopathology of the permanent sections mirrors those of the frozen sections. The tumor cells were rather monotonous and separated by collagen fibrous septa. The tumor has a pushing interface with the surrounding brain parenchyma (**Figure 3D**). The cytoplasmic vacuolation was more prominent and many cells had clear cell morphology (**Figure 3E**, **3F**). Mitotic figures were frequent. Foci of necrosis were present but there was large geographic necrosis.

Immunohistochemistry and special stain: The collagenous septa were best demonstrated by Masson's trichrome stain (**Figure 3G**). The tumor contains a substantial amount of digestible glycogen as demonstrated by periodic acid Schiff (PAS) stain with and without diastase (**Figure 3H**). Immunohistochemistry demonstrated a widespread membranous staining pattern for CD99 (**Figure 3I**). The tumor cells were positive for BAF47 and vimentin, and negative for glial fibrillary acidic protein (GFAP), neurofilament proteins, synaptophysin, chro-

mogranin, CD1a, Pancytokeratin (AE1/AE3), Cam 5.2, LCA (CD45), CD3, CD5, CD20, CD43, CD45RO, CD79a, CD15, ALK, and epithelial membrane antigen (EMA). Immunohistochemistry for Ki67 demonstrated a high labeling index.

Electron microscopy: showed findings supportive of the light microscopy diagnosis. The tumor is highly cellular with common grooved nuclei and a moderate amount of cytoplasm with a small number of organelles and a substantial amount of intermediate filament. There is little glycogen accumulation.

Molecular pathology: FISH demonstrated 150 out of 200 cells with a breakpoint in the 3' EWSR1 gene, though not at the common breakpoint in intron 4 of the gene. The remaining 50 cells had a normal hybridization pattern.

Follow up: After gross total resection, the patient was subsequently treated with radiation therapy and chemotherapy with vincristine, cyclosporine, and doxorubicin. As a complication of therapy, she developed cardiac failure and required heart transplant, but she had no further events, remained tumor free, and was generally well 6 years after the initial diagnosis.

Case 2

Case History: The patient was a 28 month old boy with no significant past medical or surgical history, born healthy at term, who presented to the emergency department our institution with a two week history of increasing number of falls. One day prior to presentation, he had developed noticeable weakness on the left side, particularly of the left upper extremity, for which he was assessed at the emergency department of another institution for possible fracture but discharged when no fractures were noted. His primary care physician requested urgent evaluation at our institution. Physical examination revealed a preference for movement of the right side and no voluntary movement of the left upper extremity, particularly hand grip. The remaining findings of the neurologic examination were intact and no papillary edema was noted.

Imaging studies: MRI of the brain with and without contrast enhancement demonstrated a 6.8 x 6.5 x 6.5 cm circumscribed mixed solid and cystic intra-axial right frontal parietal lesion (**Figure 2D-F**). Pre-contrast T1 weighted axial imaging revealed a primarily cystic mass, with T1 hypointense cyst fluid contents. The cyst fluid was hyperintense on T2 weighted imaging (not shown). A solid tumor component (5.1 x 4.4 x 1.2 cm) was along the lateral aspect of the inner cyst wall; this solid component was isointense to grey matter and hypointense to white matter (**Figure 2D**); this solid tumor component was mildly hypointense on T2 weighted imaging (not shown). This solid tumor component showed diffuse strong enhancement; the remainder of the cyst wall lining also circumferentially enhanced (**Figure 2E**, axial T1 weighted image with contrast). Axial non-contrast FLAIR imaging revealed peritumoral white matter vasogenic edema (**Figure 2F**). Significant mass effect was present with 1 cm of right to left midline shift; no herniation was identified.

Gross pathology: Gross total resection was performed and yielded multiple fragments of pink-tan soft tissue with an aggregate size of 2.0 x 2.0 x 0.2 cm and small papillary excrescences.

Histopathology: Intraoperative cytotogic preparation demonstrated a tumor with features suggestive of a papillary architecture (**Figure 3J**). High magnification demonstrated cells with a small amount of cytoplasm and cytoplasmic vacuoles of variable size (F**igure 3J**, inset). Frozen sections revealed a rather non-cohesive tumor with monotonous small tumor cells and vague tendency of perivascular palisading arrangement (**Figure 3K**). The tumor cells also have some clear cell features. An intraoperative diagnosis of "neuroepithelial tumor possibly ependymoma" was given.

Histology of the permanent sections echoed the findings on the frozen sections. The tumor was composed of rather monotonous tumor cells with small amount of cytoplasm and centrally located oval to round nuclei (**Figure 3L**). Cytoplasmic clearing leading to clear cell like morphology was common. Mitotic figures were present but no atypical mitoses were noted. The tumor cells also demonstrated frequent perivascular arrangement of tumor cells with small perivascuolar rosette. The overall histopathology suggested an eppendymoma (**Figure 3M**). No necrosis was readily seen.

Immunohistochemistry and special stain: Immunohistochemistry for GFAP demonstrated scant positive tumor cells (**Figure 3N**). There

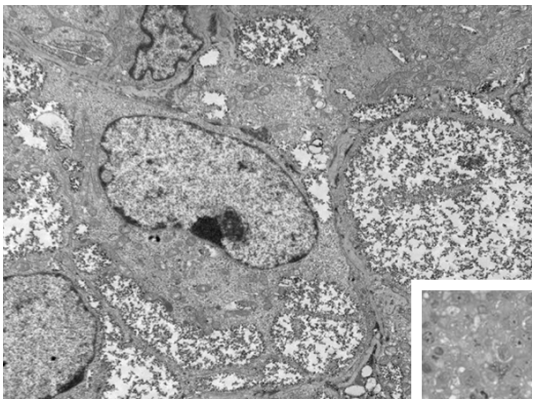

Figure 4. The tumor cells have small amount of cytoplasm and clusters of glycogen are present in the cytoplasm. A prominent nucleolus is noted in many tumor cells. The glycogen particles also lead to the formation of fine cytoplasmic vacuoles which can be well appreciated with the semithin sections stained with toluidine blue (inset). Original magnification is 3000 x, and for the insert is 40 x.

was a widespread membranous pattern of positive immunoreactivity for CD99 (**Figure 30**). The tumor cells were also focally positive for synaptophysin and epithelial membrane antigen (EMA). Immunohistochemistry also demonstrated strong nuclear immunoreactivity for BAF47, and neurofilament proteins.

Molecular pathology: FISH demonstrated a classic rearrangement of the 3' EWSR1 gene using a breakapart probe.

Electron microscopy: The tumor was composed of a monophasic tumor cell population of small neoplastic cells. The features included prominent nucleoli, abundant glycogen, numerous lysosomal granules, cytoplasmic filaments, and basement membrane surrounding clusters of cells (**Figure 4**). The ultrastructures were compatible with ES/pPNET and also atypical teratoid/rhabdoid tumor. Although the abundance of glycogen would also be consistent with clear cell ependymoma but more specific features of ependymal differentiation were not observed.

Follow up: The patient remains well and tumor free 21 months after the initial diagnosis, and has completed chemotherapy and radiation therapy at the time of writing. As the patient was under 3 years old, radiation therapy was withheld initially, but was undertaken after his third birthday. Since completion of therapy, he has been admitted once for new onset of seizures and has had a gastric tube placed due to poor oral intake and failure to thrive, but continues to develop and maintain his near-normal neurologic baseline.

Case 3

Case History: The patient was a 61 year-old male who presented primarily with vague complaints of fatigue and weakness for two months along with depressed mood, poor concentration, decreased appetite, decreased psychomotor energy and anhedonia. He developed slight slurring of speech and occasional word-finding difficulty later. Two days prior to admission he started having left-sided facial droop and left sided weakness. The patient was referred to the emergency room to exclude acute ischemic event. Imaging revealed a large right cerebral mass. His past medical history was significant for triple artery bypass, hyperlipidemia, arthritis, benign prostatic hyperplasia, and reflux. He was a former smoker and worked as a carpenter before retiring. His family history was significant for Parkinson's disease in his father, cardiovascular accident and hypertension in his mother. There was no history of malignancy or diabetes in his family.

On admission, the patient was afebrile, with stable vital signs and oriented to time, place and person. Pupils were equal, round and reactive bilaterally. Tongue was in midline. He had slight left-sided seventh nerve palsy. Otherwise he had full strength in all extremities and had no other focal neurologic symptoms. His laboratory results were unremarkable.

Imaging studies: A cystic mass, 6.2 x 5.1 cm is demonstrated in the right temporal lobe with vasogenic edema and ring enhancement. The mass was causing significant herniation. Further details could not be obtained and the relationship of this tumor with the dura was not clear. However, the original report did not use words such as "dural based lesion" which made this tumor most likely a predominantly intra-axial mass with contact or approximation with the dura when the size and location (temporal lobe) are considered.

Gross pathology: The patient underwent a right frontal temporal craniotomy with evacuation of cyst and biopsy. The procedure yielded a small biopsy specimen that is 0.4 x 0.3 x 0.3 cm.

Histopathology: The lesion was that of a densely packed small blue cell tumor with hyperchro-

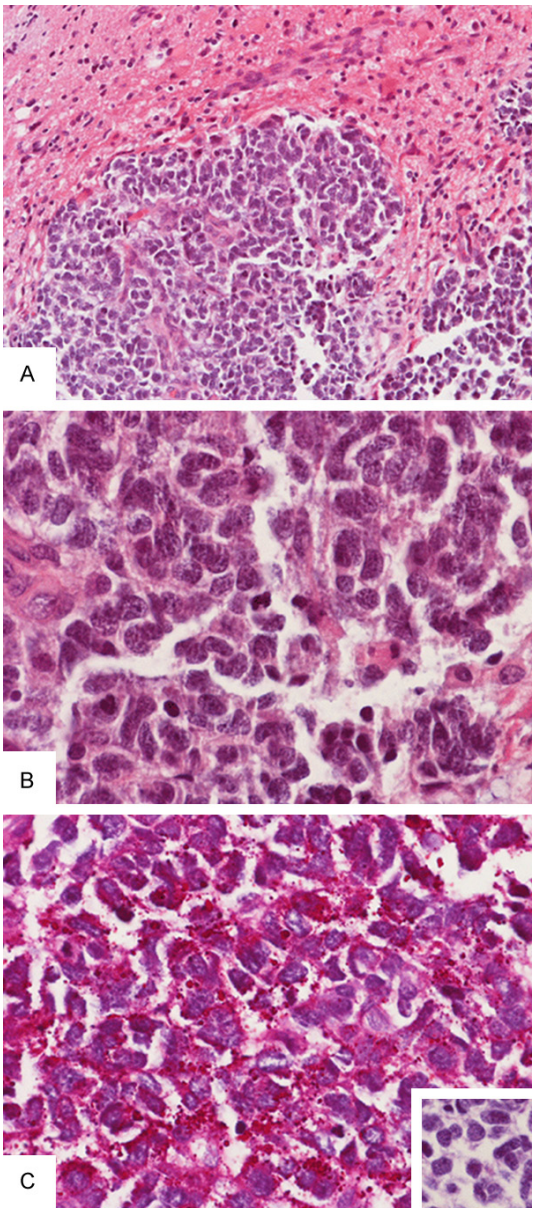

Figure 5. The tumor of Case 3 is composed of an undifferentiated and necrotic small blue cell tumor that has a well demarcated interface with the surrounding brain parenchyma (A). The tumor cells have minimal amount of cytoplasm and hyperchromatic small nuclei (B). The overall features are similar to that of ES/pPNET arising in extracranial locations. Digestible glycogen is well demonstrated by PAS stain without (C) and with diastase pretreatment (inset). Original magnification for (A) is 20 x, for (B), (C), and inset is 60 x.

matic nuclei and minimal amount of cytoplasm. Necrosis was also present.

Immunohistochemistry and special stain: A membranous immunohistochemistry for CD99 and digestible glycogen were demonstrated by immunohistochemistry and periodic acid Schiff stain with and without diastase digestion (PAS ± D) (**Figure 5**). The tumor cells are also weakly reactive for synaptophysin and CD57. The overall histology was in agreement with classic ES/pPNET that would be seen arising in other parts of the body.

Molecular pathology: Repeated attempts with FISH failed to show appropriate signal for evaluation likely due to the older age of this specimen.

Follow-up: A whole body scan to exclude a metastatic ES/pPNET was performed and was negative. The patient tolerated the surgical procedure well and was discharged; follow up MRI showed good evacuation of the cyst. The patient lost to follow up shortly after the initial follow up after surgery.

Discussion

ES/pPNET is an uncommon small blue cell tumor, primarily of bone, found most typically in children and young adults. Though the most common sites are in long bones, extraosseous Ewing sarcoma is a familiar entity in the soft tissues and other part of the body. Intracranial primary ES/pPNET is rare and, they are nearly always a dural based tumor. Only two cases of primary ES/pPNET of the CNS have been reported in the English literature that are without doubt neither dural based or involving the spinal cord (Table, Cases 9 and 26, with uncertainty in cases 3 and 17). ES/pPNET is more likely to arise from the cranial bone with intracranial extension. Metastasis of ES/pPNET to the brain in cases with an extracranial primary is described to occur in up to approximately 5% of cases in some series [23]. It is important to define these cases, as proper treatment and accurate prognostic information is the key to good outcome.

Primary ES/pPNET is uncommon and we could only identify 3 cases from our two institutes in the past 5 years. Two of our cases occurred in young children and the third in an adult. The adult case has classic histology and immunohistochemical profile for ES/pPNET arising from other parts of the body, but the histopathology of the two pediatric cases is quite distinctive from the classic histopathology. Case 1 is com-

posed of islands of round polygonal cells with a moderate amount of cytoplasm and some clearing of cytoplasm. The neoplastic cell islands are separated by fibrous septa. Case 2 has histopathology closely resembling ependymomas. Both cases deviate from the classic small blue cell tumor pattern of ES/pPNET arising from other parts of the body. Also common to the three cases are relatively large tumor size, supratentorial location, a variable extent of cystic change, predominantly intra-axial tumor with focal contact or approximation with leptomeninges and/or dura, tumor enhancement, negative or weak immunoreactivity for synaptophysin, and good outcome after therapy. In contrast, most of the previously described cases are dural based or with substantial involvement of the dura. Imaging features suggestive of ES/pPNET were well characterized by Pekala et al. [5] in their review. Consistent MR features noted by them include a single well-circumscribed lesion, lobularity, intense diffuse enhancement with contrast, and a dural attachment. These features are noted in our three current cases.

The list of differential diagnoses for ES/pPNET in the CNS is broad and includes multiple entities. In particular, it can be a challenge to morphologically distinguish them from other common intracranial small blue cell tumors such as medulloblastoma and, less commonly, supratentorial PNET. Many other entities, including atypical teratoid/rhabdoid tumor (AT/RT), ependymoma, metastatic neuroblastoma, hematopoietic malignancies, and other sarcomas like rhabdomyosarcoma, must be considered. The unusual histology presented in the two current pediatric cases definitely makes such distinction more challenging. In our experience, a definitive membranous pattern of immunoreactivity with CD99 and demonstrable digestible glycogen with PAS±D stain form a good combination for initial screening. However, the diagnosis must be confirmed by FISH.

Ewing sarcoma/pPNET is characterized by a classic set of translocations between the *EWSR1* gene on chromosome 22 and one of the ETS family of proto-oncogenes, most notably *FLI1* on chromosome 11, but also including *ETV1* on chromosome 7 and *ERG* on chromosome 21. FISH assays using EWS break-apart probes have been reported to have 91-100% sensitivity and specificity, though RT-PCR can also be used, particularly to identify the partner gene for *EWSR1*. Occasional cases can be noted to have a translocation in the *FUS* gene on chromosome 16 by FISH, or with other abnormalities such as p53 or p16 mutations.

Of the common chimeric transcripts produced from *EWSR1* and *FLI1*, a translocation between intron 7 of *EWSR1* and intron 6 of *FLI1* is the most common, known as a Type 1 transcript. The second most common, a Type 2 transcript, involves intron 5 of *FLI1*. These fusion transcripts act as transcription factors in an unregulated manner, targeting numerous genes involved in angiogenesis, cell signaling, cell cycle regulation, metabolism, and post-translational mRNA splicing and modification. They, along with downstream mechanisms, are enticing targets for molecular therapy.

Immunohistochemistry is rather useful in distinguishing ES/pPNET from tumors that may have similar morphology. Medulloblastoma is usually strongly positive for synaptophysin. In addition, an array of changes characteristic of medulloblastoma [24] can be used for differentiation. Embryonal tumor with multilayered rosettes is a newly recognized family which includes the formerly known ependymoblastoma, medulloepithelioma, and embryonal tumor with abundant neuropil and true rosettes. This family of tumor also shares a common genetic aberration of amplification of a focus on chromosome 19q13.41 [25] that lead to a fusion between TTYH1 with the C19MC microRNA cluster [26] and amplification of C19MC microRNA cluster. Atypical teratoid/rhabdoid tumor typically contains rhabdoid cells which could be easily recognized. Negative immunoreactivity for BAF47, deletion of chromosome 22q, or demonstration of point mutation of INI1 gene is sufficient to distinguish this tumor from ES/pPNET [27-29].

With so few cases reported, there has been little evidence on which to base hypotheses about prognostic factors in Ewing sarcoma/pPNET in the CNS, and there is no risk stratification system. Ibrahim et al, in their recent review, proposed some possible prognostic factors, some of which are applicable to Ewing sarcoma in a more broad sense. Specifically, age greater than 17 years, inaccessibility of the tumor for surgical resection, incomplete resec-

tion, multifocality, and tumor genetic factors (e.g. Type 1 fusion gene) appear to have negative prognostic implications.

While cPNET and ES/pPNET are both treated with surgical resection, chemotherapy, and radiation, the regimens used differ greatly, and response to therapy is driven by different factors. It is for this reason that these must be carefully differentiated, and this can often be accomplished through use of special stains, IHC, and molecular methods.

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Disclosure of conflict of interest

None.

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