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LETTER TO EDITOR

Primitive Neuroectodermal Tumor of Ovary in a Young Lady, Confirmed with Molecular and Cytogenetic Results—A Rare Case Report with a Diagnostic and Therapeutic Challenge

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Introduction

Primitive neuroectodermal tumor (PNET) is a small round cell tumor of neuroectodermal origin. It is the most differentiated form of PNET/Ewing's family of tumors (EFT) [1]. It is the second most common sarcoma among children and usually occurs in the bone and soft tissues [2]. Ewing's sarcoma/PNET is characterized by a t (11; 22) (q24; q12) chromosomal translocation that leads to formation of a chimeric transcript *EWS-FLI1* in 85% cases, presence of which confirms its diagnosis, especially at non-conventional sites [3]. It has been uncommonly documented at sites other than musculoskeletal system, such as kidneys [4]. PNET has been rarely documented in the female genital system, including ovary, with only few cases confirmed by molecular and / or molecular cytogenetic analysis [5–12].

Herein, we present an uncommon case of PNET involving ovary in a young lady, who presented with a pelvic mass. The diagnostic and therapeutic implications are discussed herewith.

Case Report

A 28-yr-old nulliparous lady, with no co-morbidities, presented with history of a left-sided pelvic mass of two months duration, for which she underwent an excision, elsewhere. She had a normal menstrual history. On clinical examination, her general condition was good. There was no pallor, lymphadenopathy, organomegaly or any other identifiable palpable mass. A healthy transverse suprapubic scar reminiscent of previous excision, was noted.

She underwent a pre-operative computed tomography (CT) scan abdomen and pelvis, followed by an exploratory laparotomy and a surgical excision, thereafter. Excised tissue in form of paraffin-embedded blocks and haematoxylin (H&E) stained slides, along with smears from the peritoneal fluid were submitted at our hospital for pathological review. Subsequently, paraffin blocks were subjected to molecular analysis and fluorescent in-situ hybridization (FISH), wherein diagnosis of PNET was confirmed.

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The patient was subjected to chemotherapy. Positive emission tomography (PET)-CT after one and half year of completion of chemotherapy, showed residual tumor mass, for which she underwent repeat exploratory laparotomy, followed by radical tumor excision. The tumor was pathologically reassessed.

Laboratory Investigations

Her serum coelomic antigen (CA) 125 was level at the time of initial surgery was elevated (70.3 units/ml. Normal range=0–35 units/ml). Other tumor marker levels, including α-foetoprotein, beta-HCG, carcino-embyonic antigen (CEA) and CA 15.3 were within normal limits.

Radiological Findings

Initial preoperative CT scan abdomen and pelvis showed a heterogeneous, left-sided ovarian mass measuring 12×11×10 cm. (Fig. 1).

Post-chemotherapy, positive emission tomography (PET)-CT showed a Fluorodeoxyglucose (FDG) avid soft tissue mass measuring 4×3 cm (SUV max 6) in left side of pelvis, indicative of local disease recurrence. A non FDG avid cyst was noted in the right ovary measuring 3.8 cm×3.3 cm×3.3 cm.

Pathological Findings

Papanicolaou stained smears from the peritoneal fluid showed lymphocytes and polymorphs, and clusters of ovoid to spindle cells on a hemorrhagic background.

Haematoxylin and eosin (H&E) stained biopsy slides from the initial tumor excision showed a malignant round cell tumor with focal cystic change. A focus of a cystic ovarian follicle was noted within the tumor vicinity. Differential diagnoses of a poorly differentiated sex-cord stromal tumor and a PNET were considered. On immunohistochemistry (IHC), tumor cells showed strong immunoreactivity with vimentin and negativity with cytokeratin (CK), calretinin, desmin synaptophysin and WT1. Mic2/CD99 staining was initially focally, weakly positive. Diagnosis a poorly differentiated sex-cord stromal tumor of the ovary was favored, over a PNET (non-metastatic). Further, molecular and molecular cytogenetic studies were recommended.

Post-chemotherapy excisions included tumor masses from pelvic tumor, along with suspected tumor deposits in the bowel, left pelvic wall, left fallopian tube, abdominal wall and omentum.

Grossly, the pelvic tumor mass, submitted in from of multiple grey-brown tissue bits, measured 10×9×4 cm, and displayed grey-white cut surface.

Microscopy from the tumor mass and from other suspected areas confirmed malignant round cell tumor with

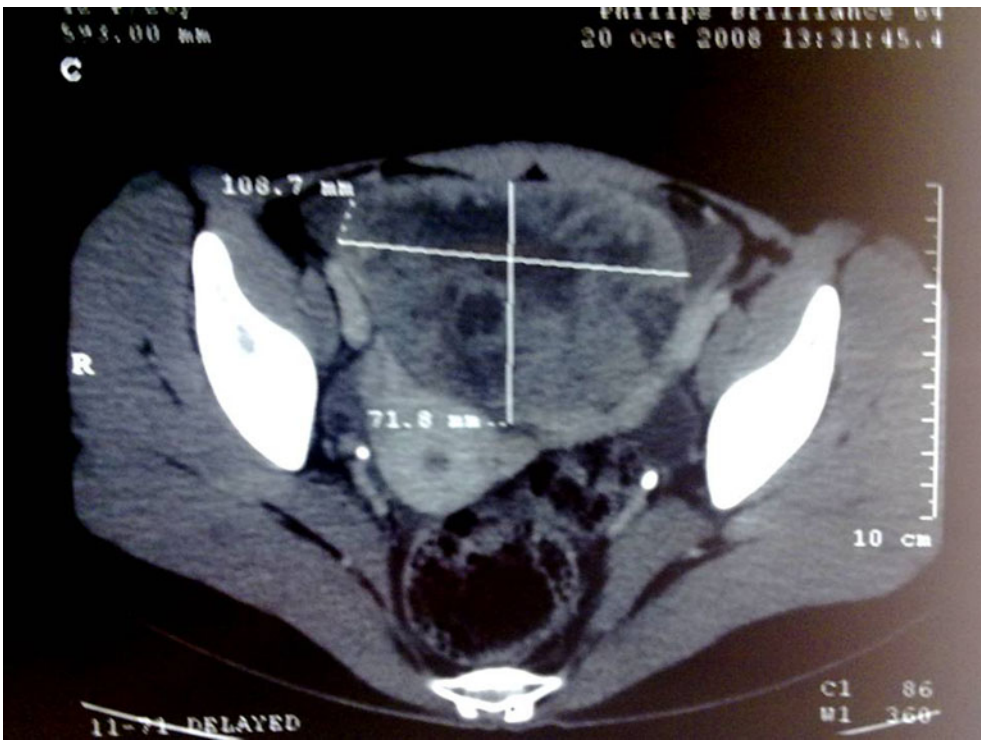

Fig. 1 Initial pre-operative computed tomography (CT) scan showing a discrete heterogeneous mass in the pelvis

numerous mitoses and prominent nucleolization within tumor cells. Repeat IHC showed diffuse membranous MIC2 positivity, and diffuse intranuclear Fli-1 positivity with synaptophysin, Melan-A, inbibin and calretinin negativity, within tumor cells. Diagnosis of PNET was offered. (Figs. 2 and 3). Further, molecular cytogenetic analysis was performed.

Molecular Analysis

RNA Isolation and Reverse Transcription Polymerase Chain Reaction (PCR)

Total RNA was isolated from formalin-fixed paraffin embedded tissue sections (FFPE tissue) using Recover All Total Nucleic Acid Isolation kit (Ambion, USA). Extracted RNA was treated with RNase-free DNase I before cDNA preparation. RNA was then reverse transcribed into cDNA using Superscript First strand synthesis system (Invitrogen). Briefly, five hundred nanogram of total RNA was reverse transcribed into cDNA using random hexamers at 42°C for 50 min followed by 70°C for 15 min. Synthesized cDNA was treated with RNase H for 20 min at 37°C to remove the RNA-DNA hybrids. Two μl from the reaction was PCR amplified using EWS 22.3 forward primer (5′-TCC TAC AGC CAA GCT CCA AGT C-3′) and FLI1 11.3 reverse primer (5′-ACT CCC CGT TGG TCC CCT CC-3′) in a 20 μl reaction volume containing 10 pmol each of the forward and reverse primer, 10 μl 2x PCR master mix (Qiagen, Germany). PCR conditions were as follows: 35 cycles of 94°C for 30 s, 65°C for 1 min and 72°C for 1 min. Amplified PCR products were checked in 10% polyacrylamide gel and stained with silver nitrate. Two positive controls (EWS-FLI1 type-I and type-II PCR product cloned into pTZ57R/T vector) and one water only (no cDNA) negative control were included. To check the quality and integrity of the cDNA, FOXO1A was amplified as a housekeeping gene (FKH-F: 5′ CAT CCC CTT CTC CAA GAT CA 3′; FKH-R: 5′ GCT GCC AAG AAG AAA GCA TC 3′).

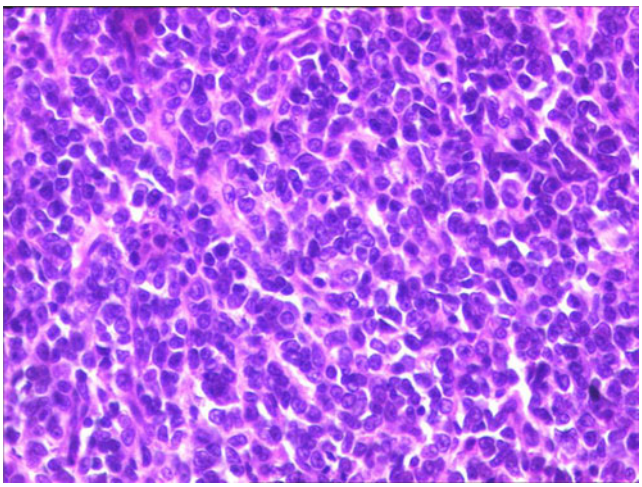

Fig. 2 Histopathological findings of ovarian PNET. Malignant round cell tumor with cells displaying vesicular chromatin. H&E x 400

Molecular analysis showed positive results for type 1 *EWS-FLI1* with two bands, at 394 bp and 202 bp location. *EWS-WT1* was negative. (Fig. 4).

Molecular cytogenetic analysis by fluorescence in-situ hybridization (FISH) study with LSI break apart, dual color EWSR1 probe (Vysis Abbott GMBH) was performed on 4 μ thick paraffin-embedded tissue sections. Processed sections were finally stained with 4′-6-Diamidino-2-phenylindole (DAPI) and examined under a fluorescent microscope (Carl Zeiss, Axio Imager Z1, Germany), using AxioCam MRc5 camera and Axio vision Rel 4.5 software. Tumor sample was considered positive if more than 15% of 100 cells analyzed showed rearrangement/'break-apart" [13]. A total of 100 cells were scored for analysis. Nearly 50% tumor cell nuclei showed rearrangement/break-apart, in form of single orange and green signals, in contrast to fused orange and green signals that indicate intact gene (Fig. 5).

Treatment and Management

After initial surgical excision, with unremarkable immediate, post-operative abdominal CT findings, patient underwent chemotherapy, as per EFT-2001 protocol (Appendix 1). In view of young age, complete tumor excision with negative surgical margins and no significant pelvic lymphadenopathy, radiotherapy was not offered.

One and half year after completion of chemotherapy, during when the patient was asymptomatic, she underwent repeat radical excision of the recurrent pelvic mass, including extensive involvement that was identified on follow-up radiological imaging. Histopathology and IHC confirmed recurrent PNET. In view of her poor general condition, she could not be subjected to further chemotherapy. Eventually, she died due to progressive disease.

Discussion

Peripheral PNETs constitute as 1% of all sarcomas [1]. The Ewing's family of tumors (EFT) is an aggressive childhood cancer that includes Ewing's sarcoma, Askin's tumor of thoraco-pulmonary region and peripheral PNET. Nearly one-fourths of patients with Ewing's sarcoma/PNET have detectable metastases at the time of diagnosis, most commonly to lungs, followed by bone and bone marrow [2].

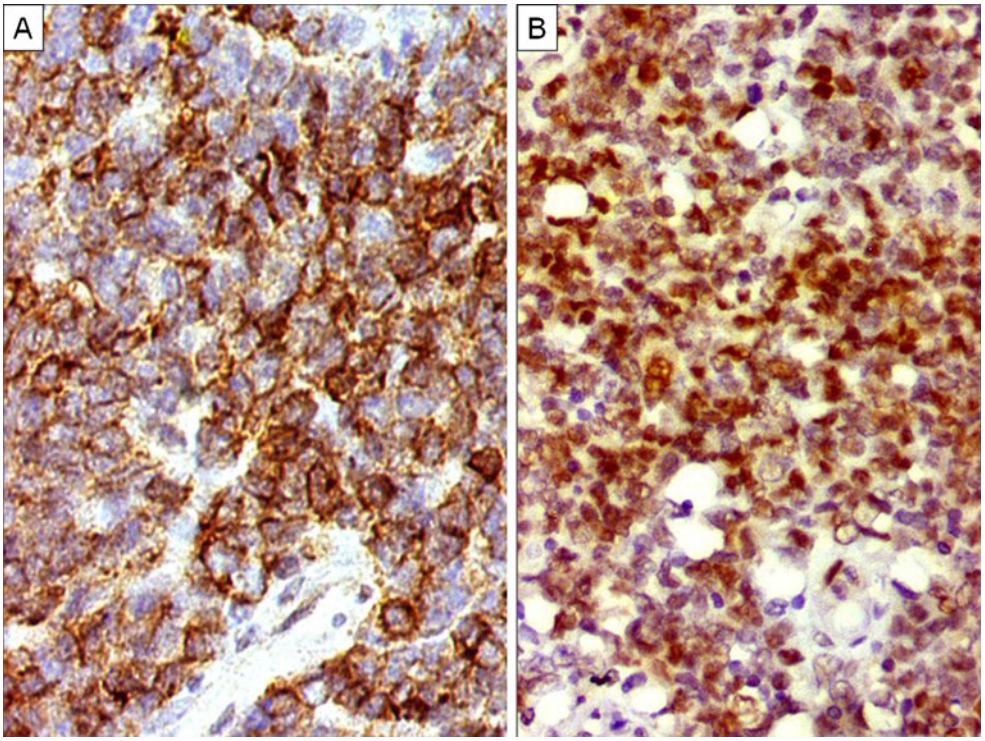

Fig. 3 Immunohistochemical findings. A. Membranous MIC2/CD99 positivity. DAB x 400. C. Nuclear positivity with FLI1. DAB x 400

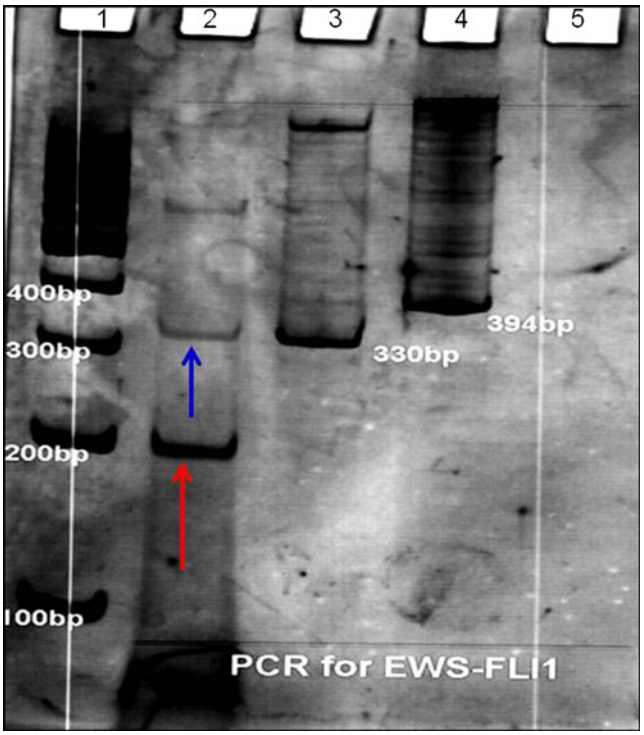

Fig. 4 Polymerase chain reaction (PCR) analysis of EWS-FLI1 translocation using EWS and FLI1 primers. **a** Reactions were subjected to electrophoresis on a 10% polyacrylamide gel and stained with silver nitrate. Lane 1: the DNA size markers in base pairs (bp); Lane 2: PCR run was performed with cDNA from test sample displaying weak band (*blue arrow*) at 330 bp. Another strong band observed at 202 bp (*red arrow*); Lane 3: Positive control DNA (pTZ57R/T-EWS/FLI1-330 bp); Lane4: Positive control DNA (pTZ57R/T-EWS/FLI1-394 bp); Lane5: PCR amplification without DNA template to rule out contamination

Although most PNETs occur at the musculoskeletal sites, some cases have been documented at unconventional sites [4]. Female genital tract, including ovary forms an extremely uncommon site for this tumor [5–12]. A range of neuroectodermal tumors, including PNETs, have been identified and described within the ovary [14]. Among the documented cases of ovarian PNETs, only rare cases have been objectively confirmed with molecular and or cytogenetic analysis [7, 8, 12]. The present case of a young lady diagnosed with PNET ovary, was well within the described age range of 13–31 years, for this tumor, in the established literature [6, 7, 10, 15].

On histopathology, presence of round cells with focal cyst formation led to differential diagnoses of small cell carcinoma with hypercalcemia, neuroendocrine carcinoma, PNET, a desmoplastic small round cell tumor (DSRCT) and a poorly differentiated sex-cord stromal tumor. On immunohistochemistry, diffuse, membranous positivity for MIC2 protein (CD99) and negativity for epithelial markers led to consideration of PNET/Ewing's sarcoma family, over a small cell carcinoma. Even though MIC2 is a useful IHC marker for substantiating a diagnosis of PNET, it can be positive in other tumors, including a DSRCT and a sex-cord stromal tumor that were close differentials [16, 17]. Negative expression for epithelial markers like CK, EMA, along with negativity with desmin and WT1, made diagnosis of a DSRCT, less likely. Further, Fli1 positivity helped in reinforcing this diagnosis in the histopathological context. Positive staining with CD56 (neural cell adhesion molecule) led to consideration of neuroendocrine carcinoma, although the

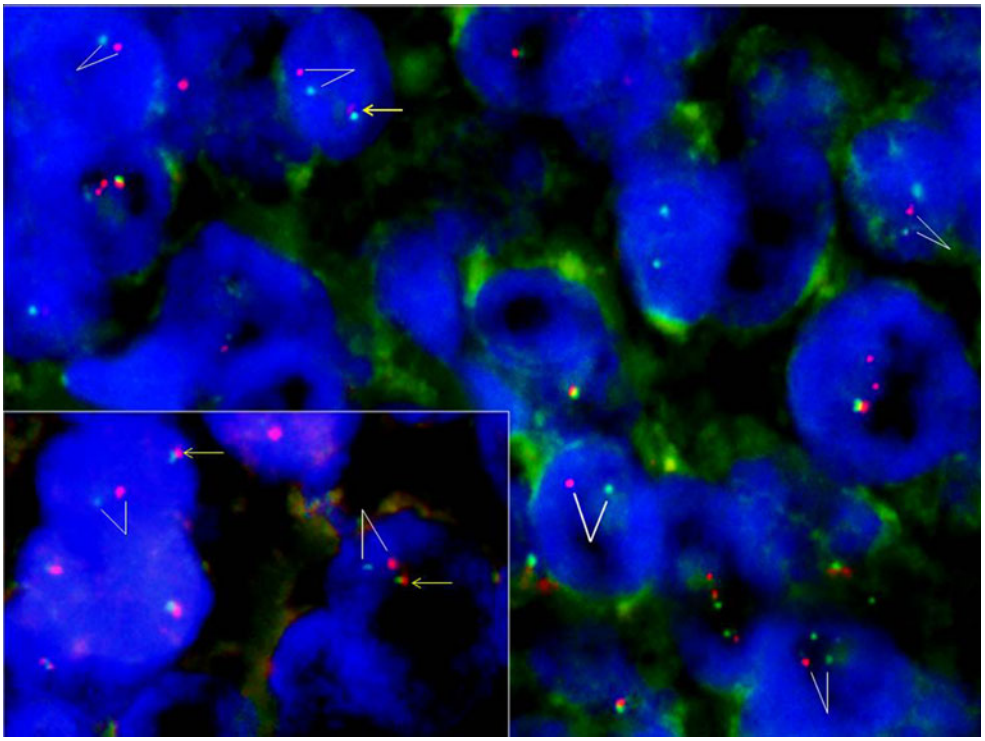

Fig. 5 Fluorescent in-situ hybridization results for *EWSR1* gene rearrangement. EWSR1 gene rearrangement noted as a break-apart, resulting in separate orange and green signals (*double arrows*), in contrast to single fused orange and green signals (single, pointed arrow) within same cells, and double copies in a few, representing intact gene copy. DAP1X1000. **Inset** showing other cells within tumor displaying *EWSR1* gene rearrangement in another tumor area. DAP1X1000

tumor showed MIC2 positivity. Therefore, for a more objective diagnosis, molecular analysis was performed that showed negativity for *EWS-WT1*, thereby ruled out a DSRCT, and positivity for *EWS-FLI1*. It is noteworthy that *EWS-FLI1* fusion transcript occurs in several forms. The type 1 transcript is the most common (60%) of the cases and is created by the fusion of the *EWS* exon 7 to *Fli1* exon 6. The type 2 translocation results from *EWS* exons 7 to *Fli1* exon 5 and is seen in 25% cases. Few studies have indicated that there is a better outcome with type 1 translocation [18]. Apart from classical translocations, Ewing's/PNET family of tumors also show variant transcripts with retained *EWSR1* rearrangement, but with other fusions genes, such as *ERG* [19]. Presence of two bands of type1 *EWS-FLI1*, on RT-PCR propelled us to further confirm the diagnosis with FISH that showed unequivocal *EWSR1* rearrangement, thereby confirming diagnosis of PNET. However, due to unavailability of the respective fusion probes, we could not further test the other fusion transcripts. Nonetheless, this case exemplifies application of more than one ancillary technique for enhancing objectivity in exact identification of sarcomas occurring at uncommon sites.

The value of accurate diagnosis has therapeutic relevance. Even though there was no residual mass immediately after initial excision, our patient was subjected to full course of chemotherapy, according to EFT 2001 protocol. Despite adjuvant treatment, she relapsed with extensive disease that was identified on follow-up radiological imaging. In earlier reported cases of ovarian PNETs, patients have had similar aggressive clinical disease course [6, 10, 11, 15]. An aggressive clinical course in cases of ovarian PNETs can be attributed to the disease location that lacks barriers and gives a chance to the tumor for spread and recurrences. The present case did not respond to chemotherapy that led to a further 'down-hill' course.

Lawlor et al [6] reported a primary ovarian PNET with extensive metastasis in a young girl, who underwent an incomplete surgical resection, followed by aggressive multi-agent chemotherapy. Complete clinical remission was observed, followed by autologous stem cell transplant. However, the patient died after a short interval of follow up. Kim et al [10] documented another ovarian PNET that metastasized to pelvis and para-aorta lymph nodes. The patient received chemotherapy, including taxol/carboplatin, pelvic cavity radiotherapy, followed by vincristine/actinomycin, cyclophosphamide/doxorubicin (VACA). She died after 10 months due to septic shock. Ateser et al [11] reported an eventual death in a pregnant female with an ovarian PNET, as a result of progressive disease, despite chemotherapy. Anfinan et al [15] , in another similar to the present one, reported death in a patient with an ovarian PNET, who developed recurrence after initial chemotherapy and failed to respond to second line chemotherapy. Recent studies suggest that EWS/FLI1 antagonists have been found to inhibit cell growth of the tumor of Ewing's sarcoma / PNET family *in-vitro* [20–22]. Therefore, the correct diagnosis of pPNET with *EWS/FLI1* chimeric gene becomes essential.

Our case has been presented in view of its rarity, associated with diagnostic and therapeutic challenge. Documentation of

more such cases with treatment and follow-up details would shed "light" on its existing literature, prognosis and would propel further research for identifying further treatment for this aggressive tumor at this unusual site.

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Conflict of Interest None

Appendix 1

Table 1 B. Ewing's Family of Tumor EFT-2001 protocol

Week	0	1	2	3	4	5	6	7	8
	V	V	V	V	V	V	V	V	V
	I			I			A		A
	E			E			C		C
Cycle	1			2			3		4

Maintenance: 2 cycles of VIE followed by alternate cycles of VAC and VCD (total 8 cycles)

V: Vincristine, I: Ifosfamide, E: Etoposide, A: Doxorubicin, C: Cyclophosphamide

References

1. Enzinger FM, Weiss SW (1995) Primitive neuroectodermal tumors and related lesions. In: Enzinger FM, Weiss SW (eds) Soft tissue tumors, 3rd edn. Mosby-Year Book, St Louis, pp 929–964
2. Grier HE (1997) The Ewing family of tumors: Ewing's sarcoma and primitive neuroectodermal tumors. Pediatr Clin North Am 44:991–1004
3. Aurias A, Rimbaut C, Buffe D, Dubousett MA (1983) Chromosomal translocations in Ewing's sacoma. New Eng J Med 309:496–497
4. Marley EF, Liapis H, Humphrey PA (1997) Primitive neuroectodermal tumor of the kidney-another enigma: a pathologic, immunohistochemical, and molecular diagnostic study. Am J Surg Pathol 21:354–359
5. Rekhi B, Qureshi S, Basak R, Menon S, Desai S, Medhi S, Kurkure P, Maheshwair AM, Jambhekar NA (2010) A primary vaginal Ewing's sarcoma/Primitive neuroectodermal tumor (PNET) in a young girl with t (11; 22) (q24; q12) (EWSR1-FLI1) positive results: a case report. J Med Case Report 4:88
6. Lawlor ER, Murphy JI, Sorensen PH, Fryer CJ (1997) Metastatic primitive neuroectodermal tumour of the ovary: successful treatment with mega-dose chemotherapy followed by peripheral blood progenitor cell rescue. Med Pediatr Oncol 29:308–312
7. Kawauchi S, Fukuda T, Miyamoto S, Yoshioka J, Shirahama S, Saito T, Tsukamoto N (1998) Peripheral primitive neuroectodermal tumor of the ovary confirmed by CD99 immunostaining, karyotypic analysis, and RT-PCR for EWS/FLI-1 chimeric mRNA. Am J Surg Pathol 22:1417–1422
8. Shigeto K, Toshiro F, Shingo M, Jun-Ichi Y, Syuya S, Toshiaki S (1998) Peripheral primitive neuroectodermal tumor of the ovary confirmed by CD99 immunostaining, karyotypic analysis and RT-PCR for EWS/FLI-1 chimeric mRNA. Am J Surg Pathol 22:1417–1422
9. Rangan A, Lobo FD, Rao AA (2003) Primary primitive neuroectodermal tumor of the ovary: a case report. Indian J Pathol Microbiol 46:58–59
10. Kim KJ, Jang BW, Lee SK, Kim BK, Nam SL (2004) A case of peripheral primitive neuroectodermal tumor of the ovary. Int J Gynecol Cancer 14:370–372
11. Ateser G, Yildiz O, Leblebici C, Mandel NM, Unal F, Turna H, Arikan I, Colcaki D (2007) Metastatic primitive neuroectodermal tumor of the ovary in pregnancy. Int J Gynecol Cancer 17:266–269
12. Chow SN, Lin MC, Shen J, Wang S, Jong YJ, Chien CH (2004) Analysis of chromosome abnormalities by comparative genomic hybridization in malignant peripheral primitive neuroectodermal tumor of the ovary. Gynecol Oncol 92:752–760
13. Bridge RS, Rajaram V, Dehner LP, Pfeifer JD, Perry A (2006) Molecular diagnosis of Ewing sarcoma/primitive neuroectodermal tumor in routinely processed tissue: a comparison of two FISH strategies and RT-PCR in malignant round cell tumors. Mod Pathol 19:1–8
14. Kleinman GM, Young RH, Scully RE (1993) Primary neuroectodermal tumors of the ovary. A report of 25 cases. Am J Surg Pathol 17:764–778
15. Anfinan NM, Sait KH, Al-Maghrabi JA (2008) Primitive neuroectodermal tumor of the ovary. Saudi Med J 29:444–446
16. Ambros IM, Ambros PF, Strehl S, Kovar H, Gardner H, Salzer-Kuntschik M (1991) MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors. Cancer 67:1886–1893
17. Gordon MD, Corless C, Renshaw AA, Beckstead J (1998) CD99, keratin, and vimentin staining of sex cord-stromal tumors, normal ovary, and testis. Mod Pathol 11:769–773
18. Lin PP, Brody RI, Hamelin AC, Bradner JE, Healey JH, Ladanyi M (1999) Differential transactivation by alternative EWS-FLI1 fusion proteins correlates with clinical heterogeneity in Ewing's sarcoma. Cancer Res 59:1428–1432
19. Giovannini M, Biegel JA, Serra M, Wang JY, Wei YH, Nycum L, Emanuel BS, Evans GA (1994) EWS-erg and EWS-Fli1 fusion transcripts in Ewing's sarcoma and primitive neuroectodermal tumors with variant translocations. J Clin Invest 94:489–496
20. Kovar H, Aryee DN, Jug G, Henockl C, Schemper M, Delattre O, Thomas G, Gadner H (1996) EWS/FLI-1 antagonists induce growth inhibition of Ewing tumor cells in vitro. Cell Growth Differ 7:429–437
21. Toretsky JA, Connell Y, Neckers L, Bhat NK (1997) Inhibition of EWS-FLI-1 fusion protein with antisense oligodeoxynucleotides. J Neurooncol 31:9–16
22. Tanaka K, Iwakuma T, Harimaya K, Sato H, Iwamoto Y (1997) EWS-Fli1 antisense oligodeoxynucleotide inhibits proliferation of human Ewing's sarcoma and primitive neuroectodermal tumor cells. J Clin Invest 99:239–247