



DICER1-associated metastatic abdominopelvic primitive neuroectodermal tumor with an *EWSR1* rearrangement in a 16-yr-old female

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Abstract We report a case of a *DICER1*-associated *EWSR1*-rearranged malignant primitive neuroectodermal tumor (PNET) arising in a patient with *DICER1* tumor predisposition syndrome. A 16-yr-old female with a history of multinodular goiter presented with a widely metastatic abdominal small round blue cell tumor with neuroectodermal differentiation. *EWSR1* gene rearrangement was identified in the tumor by fluorescence in situ hybridization (FISH). Genetic analysis revealed biallelic pathogenic *DICER1* variation. The patient was treated with an aggressive course of chemotherapy, surgery, and radiation with complete pathologic response. We believe this case to represent a new expression of the *DICER1* tumor predisposition syndrome, an entity caused by deleterious germline mutations in the *DICER1* gene, encoding a ribonuclease active in the processing of miRNA. Patients with germline mutations in *DICER1* develop a diverse group of benign and malignant tumors. Some of these tumors have been noted to have immature neuroepithelium as a component, including the ciliary body medulloepithelioma and the recently described *DICER1*-associated presacral malignant teratoid neoplasm. To our knowledge, abdominal sarcomas that resemble PNET histology with an *EWSR1* rearrangement have not previously been described as a classical expression of the *DICER1* syndrome phenotype.

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INTRODUCTION

DICER1 syndrome is an autosomal dominant hereditary tumor predisposition syndrome caused by deleterious germline mutations of the *DICER1* gene (Warren et al. 2020). The *DICER1* gene is located on Chromosome 14 and encodes a RNase III endoribonuclease that processes precursor micro RNAs (miRNAs) into functional mature miRNAs, which serve

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to negatively regulate gene expression by messenger RNA (mRNA) silencing or repressing translation (Foulkes et al. 2014; Wormald et al. 2018). The predicted effects of mutations in *DICER1* include reduction of DICER1 protein level, consequently reduction of miRNA levels, and thereby reduction in tumor-suppression activity. *DICER1* function in cancer, however, may vary, as its inactivation has been associated with tumorigenesis in some tumor types, whereas increased DICER1 protein expression has been associated with invasion and metastasis in others (Kumar et al. 2007; Hata and Kashima 2016). The expression of DICER1 can be increased or decreased in various types of cancers, leading to expression changes in the large number of cellular miRNAs. Instances of global down-regulation of miRNAs in tumors have been reported, especially in poorly differentiated ones, and one explanation proposed is that the function of miRNAs is to define lineage-specific properties so that a low abundance of miRNAs could promote the undifferentiated state of tumor cells (Lu et al. 2005; Hata and Kashima 2016). On the other hand, elevated levels of DICER1 have been found in tumor cells, even if global up-regulation of miRNA is uncommon (Hata and Kashima 2016).

DICER1-associated tumors appear to arise as a result of the second hit hypothesis, whereby, in addition to a heterozygous germline mutation, typically a truncating loss of function mutation, a second, tumor-specific missense mutation occurs (Schultz 2018; Wormald et al. 2018). These somatic missense mutations predominantly occur within exons 24 and 25 in the RNaselllb domain at one of five hotspot codons, E105, D1709, G1809, D1810, or E1813 (Pugh et al. 2014).

Initially described in familial pleuropulmonary blastoma (Hill et al. 2009; Slade et al. 2011), in which 70% of cases harbor a germline heterozygous *DICER1* loss-of-function mutation, *DICER1* syndrome has since been associated with a variety of additional benign and malignant conditions including lung cysts, cystic nephroma (Bahubeshi et al. 2010; Faure et al. 2016), Wilms tumor (Foulkes et al. 2014), multinodular goiter (Rio Frio et al. 2011; Schultz 2018), thyroid adenoma, juvenile-type intestinal polyps, ciliary body medulloepithelioma, nasal chondromesenchymal hamartoma, pituitary blastoma, pinealoblastoma (Schultz 2018), cervical embryonal rhabdomyosarcoma (Doros et al. 2012), Sertoli–Leydig tumors (Fremerey et al. 2017), and malignant sacrococcygeal tumors (Nakano et al. 2019; Warren et al. 2020). *DICER1* syndrome exhibits incomplete penetrance, and up to 95% of *DICER1* carriers do not develop any significant clinical features by the age of 10 (Doros et al. 1993; Wormald et al. 2018; de Kock et al. 2019; Stewart et al. 2019).

Although embryonal rhabdomyosarcomas and renal sarcomas are recognized in association with *DICER1* syndrome, and recent reports have identified a small cohort of patients with intracranial sarcomas, other sarcoma types are not known to occur in these patients. Here, we report the unique case of a teenage girl who presented with a large abdominopelvic malignancy that was identified as a *DICER1*-associated, *EWSR1*-rearranged primitive neuroectodermal tumor (PNET), and thereby adds to the spectrum of malignant tumors that occur in patients with this cancer predisposition syndrome.

RESULTS

Clinical Presentation

A 16-yr-old female presented with chest pain and several months of worsening abdominal distention. Her past medical history was notable for iron deficiency anemia and multinodular goiter with a prior biopsy demonstrating a benign adenomatoid nodule. Computed tomography (CT) imaging of the chest, abdomen, and pelvis revealed multiple abdominal and pelvic masses with an enlarged uterus, omental caking, and ascites (Fig. 1). Both ovaries were normal and uninvolved by tumor. Fluorodeoxyglucose (FDG)-positron emission tomography (PET)/CT confirmed the presence of large FDG-avid masses within the pelvis and abdomen

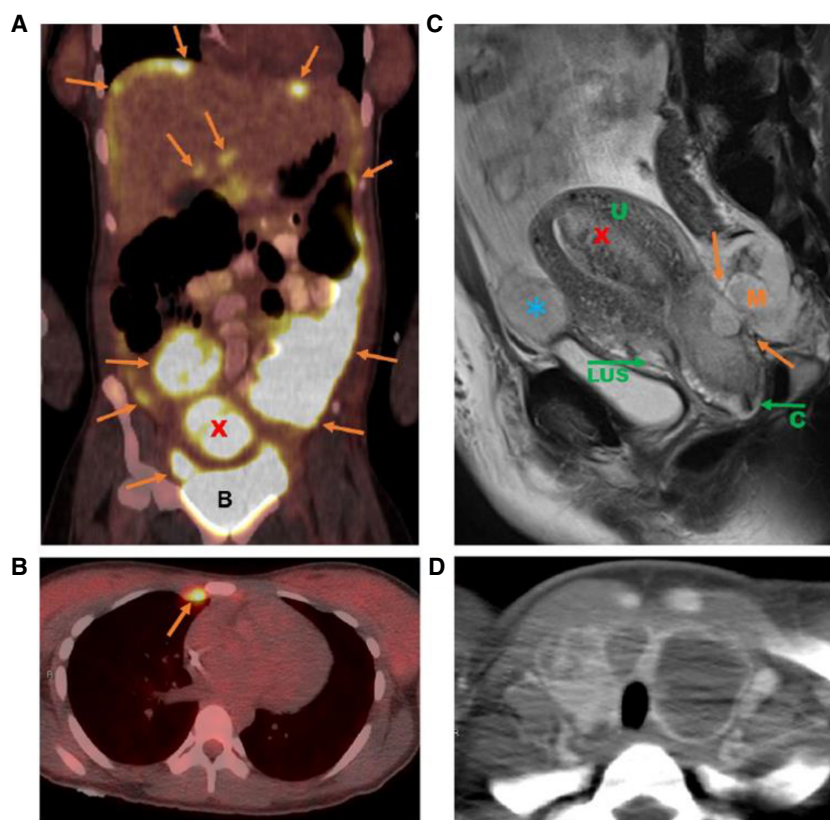


Figure 1. Imaging findings at diagnosis. (A) Abdominal fluorodeoxyglucose (FDG)-positron emission tomography (PET) scan image demonstrating diffuse hypermetabolic omental, peritoneal, and perihepatic tumor implants (orange arrows) as well as hypermetabolic intrauterine mass (red X). (B) Bladder. (B) Chest FDG-PET scan image demonstrating hypermetabolic internal mammary chain node (orange arrow). (C) Sagittal pelvic magnetic resonance imaging (MRI) image demonstrating pelvic mass invading cervix and lower uterine segment (orange arrows), intrauterine mass (red X), and peritoneal implant anterior to uterus (blue *). (U) Uterus, (LUS) lower uterine segment, (C) cervix, (M) mass. (D) Axial CT image showing thyroid gland with multifocal cystic lesions consistent with multinodular goiter.

with uterine involvement (including both intrauterine mass and invasion of lower uterine segment), and FDG-avid bilateral mammary chain lymph nodes. FDG-avid thyroid enlargement, consistent with multinodular goiter, was also noted (Fig. 1). Serologic studies revealed a mildly elevated CA-125 (77U/mL), normal AFP, and normal CEA.

Diagnostic laparoscopy was performed with biopsy of peritoneal and omental implants. Histologic examination showed a high-grade undifferentiated malignancy consisting of primitive or blastemal-like small blue cells with scattered pseudo-rosette formation and gland-like structures. The tumor cells were variably round to spindled with a high nuclear to cytoplasmic ratio. Densely cellular areas alternated with less cellular areas with intervening loose fibroblastic stroma. The intervening fibroblastic stroma was negative for desmin. Chondroid foci and foci of rhabdomyosarcoma were absent. There was no evidence of teratoma. Areas of desmin positivity were noted, however, some of these foci also stained with calretinin, raising the possibility of entrapped mesothelium. Immunohistochemistry showed focal positive staining for NKX2.2, synaptophysin, and PAX8; dot-like positive staining for CD99 with areas of faint incomplete membranous staining; and positive WT-1 (Table 1; Fig. 2A–E). The staining patterns were not that of a classic Ewing sarcoma, which shows

Table 1. Diagnostic immunohistochemical stains

Marker	Staining pattern
AE1/AE3	Rare positive cells
AFP	Negative
BCL-2	Patchy, weak positivity
β -catenin	Diffuse cytoplasmic and focal nuclear positivity
BRG-1	Intact
Calretinin	Negative (highlights entrapped mesothelial cells)
CAM5.2	Rare positive cells
CD99	Focal weak cytoplasmic and rare membranous positivity
Chromogranin	Negative
CK7, CK20	Negative
Desmin	Rare positive cells
ER	Negative
GFAP	Negative
Glypican-3	Positive
Inhibin	Patchy positivity
INI-1	Intact
NKX2.2	Focal positivity
OCT3/4	Negative
PAX-8	Focal positivity
S-100	Rare positive cells
SF-1	Negative
Thyroglobulin, TTF-1	Negative

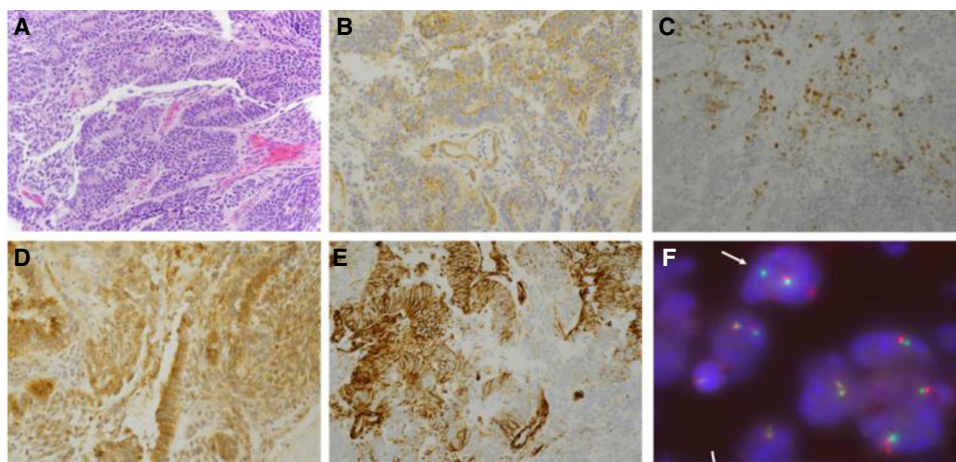


Figure 2. (A) Hematoxylin and eosin (H&E)-stained sections demonstrating a proliferation of small round blue cells with immature neuroepithelial components, areas of pseudo-rosette formation, and gland-like structures in a background of loose fibroblastic stroma with scattered spindle cells. H&E, 20 \times . (B) CD99 immunostain with dot-like positivity and faint cytoplasmic staining. Note lack of diffuse complete membranous staining typical of Ewing sarcoma, 40 \times . (C) NKX2.2 stain with rare positive cells, 20 \times . (D) Glypican-3 highlighting the neuroepithelial elements, 40 \times (Desai and Jambhekar 2010). (E) WT1 immunostain with cytoplasmic staining and absence of nuclear staining, 40 \times . (F) EWSR1 fluorescence in situ hybridization (FISH) break-apart probe demonstrating split signal (arrow) indicating gene rearrangement in a subset of cells.

diffuse membranous staining for CD99. To further characterize the tumor, additional immunohistochemical stains were performed and were notable for negative cytokeratins (AE1/AE3, CAM5.2, CK7, CK20) and intact INI-1. Additional negative stains included thyroid markers (thyroglobulin, TTF-1), inhibin, α -feto protein (AFP), chromogranin, GFAP, SF-1, S-100, and OCT3/4. Yolk sac tumor was considered given glypican-3 positivity but thought to be unlikely given the minimal cytokeratin staining and negative AFP (Euscher 2019). In addition, positive glypican-3 staining can be seen in immature neural elements (Zynger et al. 2008). Taken together, the morphology and immunohistochemical profile of this difficult-to-classify tumor was felt to be most compatible with tumors within the primitive round-cell sarcoma family, including Ewing sarcoma/primitive neuroectodermal tumor (Desai and Jambhekar 2010).

The patient's worsening clinical status, with increasing ascites and lower extremity edema secondary to venous compression by pelvic masses, prompted initiation of chemotherapy. Ewing sarcoma/PNET type chemotherapy was chosen, using the schema of the Children's Oncology Group (COG) protocol AEWS0031 Regimen B1 (interval compression) consisting of cycles of vincristine, doxorubicin, and cyclophosphamide (VDC) alternating with cycles of ifosfamide and etoposide (IE), administered every 2 wk (Womer et al. 2012), with the addition of a second weekly vincristine dose in VDC cycles, similar to that given in COG AEWS1031 (Mascarenhas et al. 2016). Significant clinical improvement was noted following one cycle of VDC with resolution of abdominal ascites and lower extremity edema.

Molecular Studies

Molecular testing for the *EWSR1* gene rearrangement was performed by fluorescence in situ hybridization (FISH) and was positive for the *EWSR1* rearrangement in ~16% of nuclei (Fig. 2F). Polymerase chain reaction (PCR) confirmation for *FLI1*, *ERG*, and *WT1* as partner genes, however, resulted negative. Additional molecular profiling of the tumor was performed through a commercial platform (Personal Genome Diagnostics; Jones et al. 2015) and revealed a somatic missense G1809R mutation in *DICER1* in 44% of transcripts, a previously described hotspot mutation affecting the RNaseIIIb domain. No other sequence mutations, amplifications, or translocations were identified within a panel of 203 cancer-associated genes that included those previously shown to be more commonly altered in sarcomas such as *TP53*, *PTEN*, *MDM2*, *KRAS*, *NRAS*, *NF1*, *ATRX*, *CDK4*, and *RB1*. The assay failed to detect a rearrangement in *EWSR1*, with coverage for breakpoints within introns 7–13 to a sequencing depth of 1500 \times (Table 2; Supplemental Table S2).

Given the patient's history of multinodular goiter in conjunction with the somatic *DICER1* mutation identified in the tumor, there was strong suspicion for inherited *DICER1* syndrome. Germline testing was performed and detected a heterozygous mutation in exon 3 of the *DICER1* gene, with insertion of two nucleotides (NM_177438.2: c.282.283dupAA) causing a frameshift mutation leading to a premature translational stop signal (p.Arg95Lysfs*34). This was a variant not previously reported but predicted to be a pathogenic loss of function mutation (Table 2; Supplemental Table S2). Thus, this *DICER1*-associated tumor appeared to arise from a typical tumor-specific *DICER1* second hit in the setting of a deleterious germline mutation.

Treatment Outcome

The patient continued treatment with VDC/IE chemotherapy, which was tolerated well. Restaging imaging after six cycles of chemotherapy showed a striking response to treatment with resolution of omental and peritoneal disease and multiple pelvic masses, a residual right pelvic mass that was decreased in size and FDG avidity (Fig. 3). At this time, the decision was made to proceed with aggressive surgical resection of all gross residual disease and

Table 2. Sequence variants identified by next-generation sequencing (Personal Genome Diagnostics, Cancer Select-203)

Gene	Origin	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/dbVar ID	Genotype	ClinVar ID
DICER1	Tumor	14:95557642	NM_177438.2:c.5425G > A	G1809R	Substitution (missense)	Inactivation	rs1595314951	Heterozygous	SCV000959315.1 and SCV001372111.1
DICER1	Tumor	14:95598875	NM_1774382.2:c.282_283dupAA	p.Arg95Lysfs*34	Insertion (frameshift)	Inactivation	rs1555376368	Heterozygous	SCV000658204.1
DICER1	Normal	14:95598875	NM_1774382.2:c.282_283dupAA	p.Arg95Lysfs*34	Insertion (frameshift)	Inactivation	rs1555376368	Heterozygous	SCV000658204.1

previously involved areas. Surgery was performed, and included total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, resection of the right lower quadrant mass, and removal of all grossly visible peritoneal implants. Pathological examination of the resected tumor specimens showed extensive chemotherapy treatment effect with no evidence of remaining viable tumor. The postoperative course was uncomplicated, and chemotherapy with VDC/IE was resumed until completion of 14 total cycles. Given the extent of disease at presentation, consolidative radiation therapy was recommended upon completion of chemotherapy. Whole-abdomen–pelvis radiation therapy (WAPRT) and whole-lung irradiation (WLI) was administered to a total of 1500 cGy to the chest and 2400 cGy to the abdomen/pelvis. The patient tolerated the intensive therapy well with no undue side effects.

Total thyroidectomy for multinodular goiter was performed at ~1.5 yr post-treatment completion. The patient is now more than 2 years postcompletion of planned treatment and is doing well. Regular surveillance imaging continues, with no concerns for recurrent disease to date.

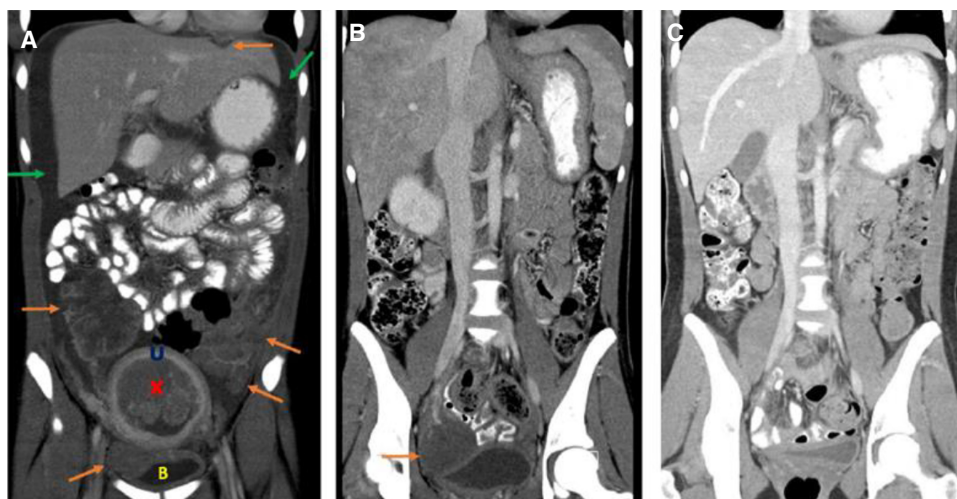


Figure 3. Coronal contrast-enhanced computed tomographic (CT) images of the abdomen and pelvis at presentation and after treatment. (A) At presentation demonstrating presence of multiple abdominal/pelvic tumors (orange arrows), intrauterine mass (red X), and malignant ascites (green arrows). (U) Uterus, (B) bladder. (B) After six cycles of chemotherapy demonstrating treatment response with resolution of malignant ascites and significant decrease in tumor burden with residual right pelvic tumor (orange arrow). (C) After surgical resection and completion of chemotherapy demonstrating complete treatment response with no detectable tumor.

DISCUSSION

Since the initial description of *DICER1* as the causative gene in the pleuropulmonary blastoma inherited cancer syndrome (Hill et al. 2009), *DICER1* mutations have been associated with an increasing number of childhood and adult cancers. Several unique cancer entities have been reported in patients with pathogenic *DICER1* mutations and include cystic nephroma, anaplastic renal sarcoma (Wu et al. 2018), Wilms tumor, Sertoli–Leydig cell tumor (SLCT) (Schultz et al. 2011), embryonal rhabdomyosarcoma, and, more recently, intracranial sarcomas (Koelsche et al. 2018b). Sarcomas comprise a minority of *DICER1*-associated cancers, but of the 86 *DICER1*-associated sarcomas reported in the literature to date (Warren et al. 2020), embryonal rhabdomyosarcoma of the uterus or uterine cervix ($n = 28$), intracranial sarcoma ($n = 26$), and anaplastic sarcoma of the kidney ($n = 10$) are the most common (Stewart et al. 2019; Warren et al. 2020). To our knowledge, only one case of PNET of the abdomen/pelvis, which was reported to arise from the uterine cervix, in a patient with *DICER1* mutation has been described (Foulkes et al. 2011). Regardless of their location, *DICER1*-associated sarcomas reported in the literature demonstrate a heterogeneous histologic appearance including undifferentiated small round blue cells, spindle cells, and/or large anaplastic cells with or without rhabdomyoblastic, chondroid, myxoid differentiation, and bone/osteoid formation (Warren et al. 2020).

Here we describe the case of a 16-yr-old female with a metastatic high-grade malignancy that was difficult to characterize histologically and appeared to have rearrangement of the *EWSR1* gene by FISH. The partner gene was not successfully identified via next-generation sequencing and the FISH positivity was low (16%). The focal and incomplete membranous staining pattern of CD99 and rare focal staining pattern of NKX2.2 were not classic for Ewing sarcoma. Also noted by immunostaining was focal positive PAX8, a transcription factor most strongly expressed by thyroid tumors and tumors of Mullerian origin. This tumor was morphologically more similar to primary *DICER1*-associated central nervous system sarcomas and embryonal tumors with multilayered primitive epithelium. Features of rhabdomyoblastic or chondroid differentiation often seen in *DICER1*-associated sarcomas were not identified; however, the biopsy specimen was small and the possibility of sampling error cannot be excluded (Warren et al. 2020). Overall, this tumor is best considered a *DICER1*-associated sarcoma with neuroectodermal differentiation, a morphology that has been described in other *DICER1*-associated tumors, specifically ciliary body medulloepithelioma and the recently described *DICER1*-associated presacral malignant teratoid neoplasm (Nakano et al. 2019). The finding of a subset of cells showing *EWSR1* rearrangement raises the possibility that this additional mutation was the driver for malignant or more aggressive transformation.

To our knowledge, *EWSR1*-rearranged sarcomas have not been identified as members of the clinical spectrum of tumors that occur in *DICER1* syndrome. *EWSR1* is a member of the TET (*TLS*, *EWSR1*, *TAFII68*) family (Thway and Fisher 2019) and a gene commonly involved in translocation-associated sarcomas (Fisher 2014). *EWSR1* rearrangements lead to formation of chimeric genes in which the *EWSR1* amino-terminal transcriptional activation domain is fused to the carboxy-terminal DNA-binding domain of the partner gene, usually a transcription factor. Although most commonly rearranged in Ewing sarcoma, with the ETS transcription factors *FLI1* or *ERG*, the spectrum of *EWSR1*-rearranged neoplasms now includes many soft-tissue sarcomas including desmoplastic small round cell tumor (*EWSR1*-WT1), myxoid liposarcoma (*EWSR1*-DDIT3), clear cell sarcoma (*EWSR1*-ATF1 or CREB1), extraskeletal myxoid chondrosarcoma (*EWSR1*-NR4A3), and other *EWSR1*-associated tumors (Fisher 2014; Krystel-Whittemore et al. 2019). Of note, germline *DICER1* mutations were not identified in a whole-genome sequencing study of Ewing sarcoma patients, which identified germline mutations most frequently affecting genes involved in DNA repair pathways (Brohl et al. 2017).

The striking finding of multinodular goiter in our patient at presentation raised the possibility of DICER1 syndrome, despite the absence of family history to suggest an inherited cancer predisposition. Germline testing for *DICER1*, in fact, detected a mutation in exon 3 of the *DICER1* gene with insertion of two nucleotides (c.282.283dupAA) causing a frameshift mutation leading to a premature translational stop signal (p.Arg95Lysfs*34). This is a variant not previously reported but predicted to be a pathogenic loss of function mutation. Consistent with a two-hit model, next-generation sequencing detected the G1809R missense mutation (variant allele frequency [VAF] 44%), confirming that this malignancy, although not a reported type in DICER1 syndrome, was potentially a novel cancer type in a syndrome increasingly being identified with predisposition to other sarcomas.

Despite an extremely high tumor burden and the extensive metastatic disease at presentation, our patient's sarcoma proved extremely chemotherapy-sensitive. Little is known overall about the optimal multimodality treatment for patients with *DICER1*-associated sarcomas, and treatment paradigms often rely on those of the tumor type it most closely resembles. In many cases, treatment choices were not those used for sarcomas at all and reflect an emerging knowledge regarding the spectrum of tumors that occur in DICER1 syndrome (Koelsche et al. 2018a). We elected to treat our patient following the U.S. Ewing sarcoma-like paradigm, and that approach proved successful. No viable tumor remained after neo-adjuvant chemotherapy, and the surgical approach was similar to the aggressive type of resection usually reserved for desmoplastic small round cell tumor (DSRCT), in which the extent of resection has proven to be prognostic (La Quaglia 2014; Hayes-Jordan et al. 2016). Whole-abdomen–pelvis radiation was given, also following the approach used for DSRCT (Casey et al. 2014), which seemed logical in our patient's case, given widespread omental and peritoneal studding. It is unknown which, or perhaps all, of these therapeutic choices resulted in the positive outcome for our patient.

Based on available data from similar patients with extensively metastatic extra-osseous Ewing sarcoma, the chances of survival were low (Miser et al. 2004), which suggests that the biology of the DICER1-PNET is perhaps of a different nature. This hypothesis may also be supported by a similar report of a woman with a malignant thyroid teratoma with neuroectodermal differentiation and a somatic *DICER1* mutation. She also received chemotherapy like that given for Ewing sarcoma and had a complete response (Rabinowits et al. 2017). Regardless, this is the first case to our knowledge of a confirmed *EWSR1*-rearranged sarcoma in a patient with DICER1 syndrome, who was treated successfully and remains alive and well.

METHODS

Next-generation sequencing was conducted using the Cancer Select-203 panel commercially available from Personal Genome Diagnostics (Jones et al. 2015), which evaluates a total of 203 cancer-associated genes for mutations, copy-number variation, and/or translocations. Sequencing was performed using both tumor (abdominal peritoneal implant) and normal (blood) specimens to identify somatic mutations. Sequences were aligned to Human Reference genome hg19. A complete list of genes included in the assay can be found in Supplemental Table S1 and sequencing depth data can be found in Supplemental Table S2. Additional information on the bioinformatics analysis pipeline is available at <https://www.personalgenome.com/>.

Targeted sequencing of *DICER1* for germline mutation testing was performed by Invitae (www.invitae.com) with reads aligned to Human Reference genome hg19.

ADDITIONAL INFORMATION

Data Deposition and Access

Coding variants identified in the Personal Genome Diagnostics Cancer Select-203 analysis are reported in the body of the manuscript. The patient did not provide consent for public deposition of all raw sequencing data for all genes included in the assay. The somatic *DICER1* variant was submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and can be found under accession number SCV001429657.1. The germline *DICER1* mutation was previously submitted to ClinVar by Invitae under accession number SCV000658204.1.

Ethics Statement

The patient has provided written consent to publication of relevant clinical information presented in the manuscript and was given the opportunity to read the manuscript prior to publication. The patient understands that material will be freely available on the web and can be freely redistributed and that complete anonymity cannot be guaranteed.

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Author Contributions

L.P., D.S.R., J.A.F., and C.A.P. were responsible for patient care and clinical decision making. A.P., L.P., and C.A.P. were responsible for the concept and design. E.D. provided radiographic interpretation and figures. D.B. and J.A.F. provided pathologic diagnosis and figures. A.P., L.P., and C.A.P. wrote the manuscript. All authors performed a final review of the manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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