

## Case Report

# Beware of immunohistochemistry - report of a cytokeratin-, desmin- and INI-1-negative pelvic desmoplastic small round cell tumor in a 51 year old woman

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**Abstract:** We present a 51 year old female patient with a pelvic desmoplastic small round cell tumor with an unusual immunohistochemical profile, including absence of significant cytokeratin expression, complete negativity for desmin and widespread loss of nuclear INI-1 expression (>90% of tumor cells). The neoplastic cells were positive for epithelial membrane antigen (EMA), vimentin, and WT-1 (antibody against the C-terminus). The tumor showed classic histopathological features with no rhabdoid cells. Fluorescent in situ hybridization revealed *EWSR1* gene rearrangement and absent *SYT* gene rearrangement. Reverse transcriptase polymerase chain reaction showed presence of *EWSR1-WT1* transcript.

**Keywords:** Desmoplastic small round cell tumor, immunohistochemistry, molecular genetics

## Introduction

Desmoplastic small round cell tumor (DSRCT) was first described by Gerald and Rosai in 1989 [1] and subsequently characterized in a series comprising 19 cases (16 males, 3 females and age range 8 to 38 years, median 18.6 years) [2]. Histologically, DSRCT is typified by variably sized, well-defined invasive tumor islands separated by a desmoplastic stroma. The malignant cells have scant cytoplasm and small to medium round/oval hyperchromatic nuclei. DSRCT characteristically shows a complex "polyphenotypic" immunohistochemical expression profile, with most cases being immunoreactive for cytokeratins, epithelial membrane antigen (EMA), vimentin, desmin and neuron specific enolase (NSE), as well as exhibiting nuclear expression of WT-1 [2-9]. DSRCT was initially believed to occur nearly exclusively in an intra-/peri-abdominal location. The clinicopathological spectrum of DSRCT is, however, much wider than initially thought. DSRCT has been described to occur in a multitude of sites/organs other than the abdomen, such as in the thoracic cavity, lung, posterior

cranial fossa, ethmoid sinuses, soft tissue, paratesticular region and parotid gland [10-17]. Similarly, both light microscopically and immunohistochemically, several variants of DSRCT are on record, including areas with an epithelial architecture of tubules and glands [10, 15, 16], extensive pleomorphism [11], rhabdoid features [2], spindle cell or signet-ring morphology [16], lack of significant stromal desmoplasia [16, 18, 19], and cytokeratin- and desmin-negative tumors [11, 15]. On a molecular genetic level, DSRCT is characterized by a t(11;22)(p13;q12) translocation, resulting in the fusion of *EWSR1* and *WT1* which may be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) [19-26]. However, even on the genetic level, variants have been described [11, 20, 27, 28]. Herein, we present yet another case of a DSRCT with atypical/unusual immunohistochemical features, notably with absent significant expression of cytokeratins, no expression of desmin and loss of nuclear INI-1 expression in >90% of the neoplastic cells. The latter finding has, to the best of our knowledge, not previously been documented in DSRCT.



**Figure 1.** CT findings. CT image (coronal plane) of large pelvic mass (single arrow), and liver lesions (double arrow).

### Case presentation

A 51 year old female presented with abdominal bloating for the past 3 weeks and loss of weight (5 kg over 6 months). She had well-controlled diabetes mellitus and hypertension, as well as a vaginal hysterectomy for uterine prolapse 2 years prior to presentation. On physical examination, there was a palpable firm and immobile suprapubic mass. A serum tumor marker panel showed raised CA-125 (81.9 U/ml), and unremarkable alpha-fetoprotein, beta-hcg and carcinoembryonic antigen levels. Imaging studies included a computed tomography (CT) scan, revealing a 10 cm lobulated heterogeneous solid cystic mass in the pelvis (**Figure 1**), which was suspicious for a primary ovarian malignancy, as well as peritoneal nodules and liver lesions suspicious for metastases. An ultrasound-guided biopsy performed on the liver and pelvic masses showed a malignant small round cell tumor. The patient underwent a diagnostic laparoscopy 2 weeks later, with the following findings: enlarged 4 cm left ovary with surface tumor nodules, unremarkable right ovary, multiple small (<1 cm) nodules on the mesentery, pelvic peritoneum and omentum, as well as a 2 cm nodule on the sigmoid colon serosa. Biopsies of the nodules on the sigmoid colon, serosa, omentum and pelvic peritoneum were performed. The patient was initially plan-

ned for 6 cycles of chemotherapy, but developed severe septicemia during admission for the first cycle secondary to tumor invasion and small bowel perforation. She recovered from the septicemia but subsequently opted for palliative management.

### Materials and methods

The tissue was fixed in formalin and embedded in paraffin. 4  $\mu$ m thin sections were cut and stained with Hematoxylin and Eosin (H&E). An immunohistochemical study using the primary antibodies listed in **Table 1** was performed, using the Roche Ventana Benchmark XT auto-stainer, with appropriate positive controls.

Fluorescent in-situ hybridization (FISH) to detect *SYT* (18q11.2) gene rearrangement using Dual Color, Break Apart Rearrangement Probe was performed. FISH for detection of *EWSR1* (22q12) gene rearrangements using Vysis LSI *EWSR1* Dual Color, Break Apart Rearrangement Probe was also performed. Five-micron-thick sections of the received formalin-fixed, paraffin-embedded tissue were pre-treated and subjected to protease digestion. The cells and probes were co-denatured at 86 degrees Celsius (90 degrees Celsius for detection of *EWSR1* (22q12) gene rearrangement) for 2 minutes and incubated at 37 degrees Celsius overnight using the ThermoBrite denaturation/hybridization system (Vysis, Downer's Grove, IL, USA). At least 100 non-overlapped nuclei with distinct signals were scored and the interpretation of intact and split signals based on generally accepted guidelines recommended by Vysis. A positive result was defined as 15% or more of cells with split signals of more than 1 signal diameter apart.

RT-PCR was performed for detection of *EWSR1-WT1* fusion transcript t(11;22). RNA was isolated from prepared sections of the received formalin-fixed, paraffin-embedded tissue with the High Pure RNA Paraffin Kit method by Roche, and reverse transcription performed using the High Capacity cDNA Reverse Transcription Kit by Applied Biosystems. The PCR amplification was carried out according to published protocols [15]. The PCR product was run in a conventional agarose gel. A positive product was defined as unequivocal bands in the expected range, and confirmed by routine sequencing.

## INI-1 negative desmoplastic small round cell tumor

**Table 1.** Panel of antibodies used in this study

Antibody	Clone	Dilution	Antigen retrieval	Source
Cam5.2	Cam5.2	1:100	pH9 buffer, 30' @ 100 °C	Becton Dickinson
MNF116	MNF116	1:250	pH9 buffer, 60' @ 100 °C	DAKO
AE1/3	AE1/AE3	1:100	Protease – 8' @ 37 °C	DAKO
EMA	E29	1:200	pH9 buffer, 36' @ 95 °C	DAKO
Vimentin	V9	1:3000	pH9 buffer, 30' @ 100 °C	DAKO
Desmin	D33	1:100	pH9 buffer, 30' @ 100 °C	DAKO
Myogenin	F5D	Ready to use (prediluted)	pH9 buffer, 60' @ 100 °C	Cell Marque
Synaptophysin	MRQ-40	Ready to use (prediluted)	pH9 buffer, 30' @ 100 °C	Cell Marque
Chromogranin	Rabbit polyclonal	1:800	pH9 buffer, 8' @ 100 °C	DAKO
NSE	BBS/NC/VI-H14	Ready to use (prediluted)	pH9 buffer, 8' @ 100 °C	DAKO
S-100	Rabbit polyclonal	1:2000	No retrieval	DAKO
CD99	MIC2	1:100	pH9 buffer, 8' @ 100 °C	DAKO
CD34	QBEnd-10	1:100	pH9 buffer, 30' @ 100 °C	DAKO
INI-1	25/BAF47	1:250	pH9 buffer, 20' @ 100 °C	BD BioSciences
WT-1	WT49	1:100	ER2 for 20' using bond Leica autostainer	Novocastra
WT-1	Rabbit polyclonal	1:50	Acidic buffer, 68' @ 91 °C	Santa Cruz
Alpha-inhibin	R1	Ready to use (prediluted)	pH9 buffer, 30' @ 100 °C	Cell Marque, USA
CD117	c-kit	1:200	pH9 buffer, 30' @ 100 °C	DAKO
LCA	2B11+PD7126	1:750	pH9 buffer, 60' @ 100 °C	DAKO
CD3	Rabbit polyclonal	1:100	pH9 buffer, 30' @ 100 °C	DAKO
CD20	L26	1:1500	pH9 buffer, 30' @ 100 °C	DAKO
Ki-67	MIB-1	1:100	pH9 buffer, 30' @ 100 °C	DAKO

### Results

#### *Routine morphology*

The tumor was composed of sheets and large highly cellular, sharply demarcated nests surrounded by a variably prominent desmoplastic stroma (**Figure 2A**). The stroma was composed of spindle-shaped reactive fibroblasts, featuring no nuclear atypia, embedded in a matrix of loose myxoid material and collagen (**Figure 2A, 2B**). The tumor comprised sheets and islands of densely packed cells exhibiting high nuclear to cytoplasmic ratio, round to oval nuclei with coarse chromatin and limited amount of amphophilic cytoplasm (**Figure 2C**). There was mild nuclear pleomorphism and high mitotic activity (>35 per 10 high-power fields). No rhabdoid tumor cells were identified.

#### *Immunohistochemistry*

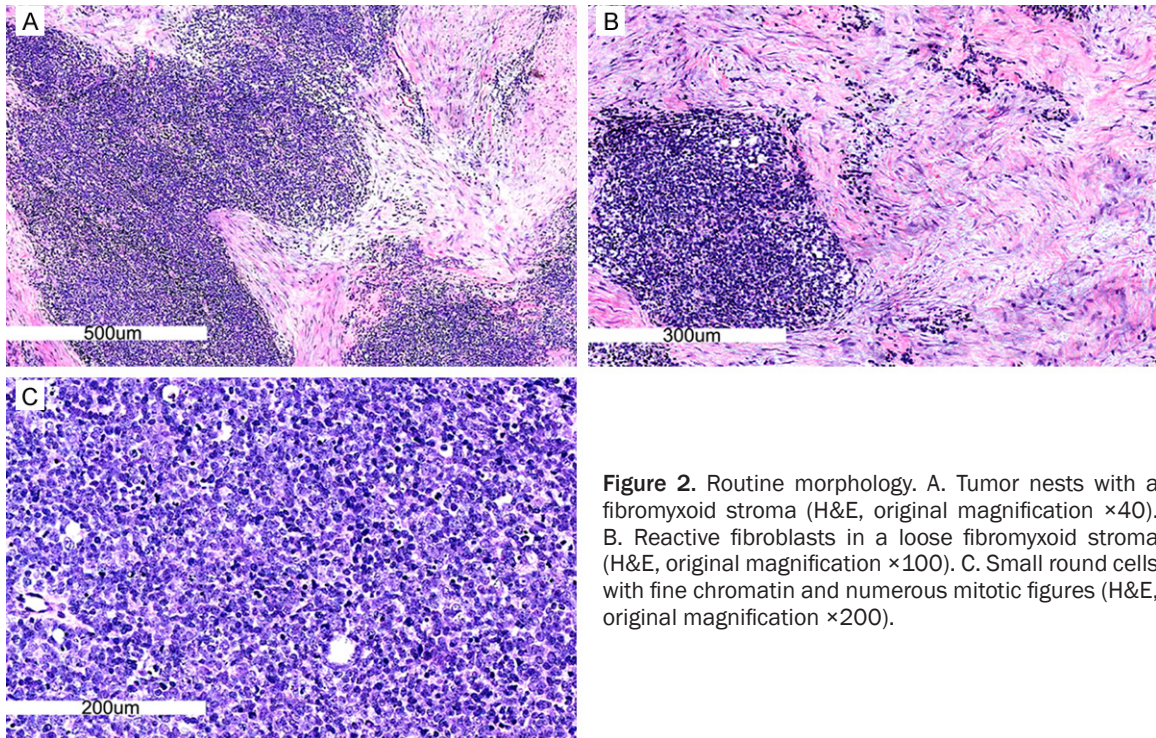
The tumour cells were diffusely positive for vimentin, EMA and WT-1 (antibody directed against the C-terminus). There was weak to moderate membranous staining with CD99, and weak cytoplasmic staining with NSE. The

tumor cells in the initial biopsy did not express cytokeratins (Cam5.2 and MNF116). Focally, very few scattered neoplastic cells in the second biopsy done at laparoscopy showed expression of cytokeratins, detected by AE1/3. There was loss of INI-1 expression in more than 90% of the neoplastic cells (100% of the neoplastic cells in the first biopsy), with weak nuclear expression in the remaining cells (**Figure 3A-D**). There was no expression of desmin, myogenin, synaptophysin, chromogranin, S-100 protein, CD34, CD117, leucocyte common antigen (LCA), CD3, CD20, alpha-inhibin and WT-1 (antibody directed against the N-terminus). The proliferative index (Mib-1/Ki-67) was approximately 30%.

#### *Molecular genetic study*

FISH (dual colour break apart probe) for SYT gene rearrangement did not reveal any disruption of the gene. FISH for *EWSR1* gene rearrangement showed break-apart/rearrangement in 92% of neoplastic cells (**Figure 4A**). RT-PCR showed presence of an *EWSR1-WT1* transcript (**Figure 4B, 4C**).

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**Figure 2.** Routine morphology. A. Tumor nests with a fibromyxoid stroma (H&E, original magnification  $\times 40$ ). B. Reactive fibroblasts in a loose fibromyxoid stroma (H&E, original magnification  $\times 100$ ). C. Small round cells with fine chromatin and numerous mitotic figures (H&E, original magnification  $\times 200$ ).

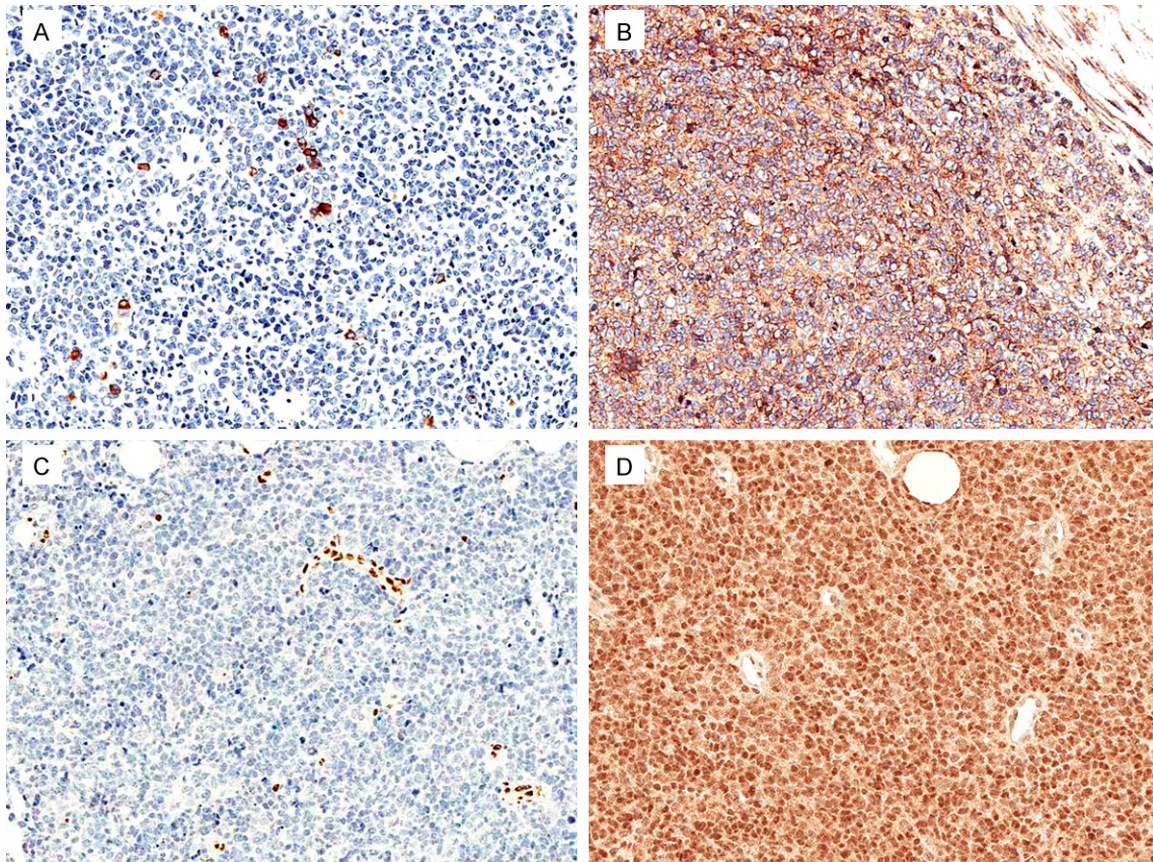
### Discussion

Desmoplastic small round cell tumor has been recognized as a neoplastic entity since 1989 [1]. DSRCT is characteristically composed of variably sized nests of malignant cells separated by a desmoplastic stroma. The cell morphology is, as the name implies, that of malignant small round blue cells, and the neoplastic cells typically exhibit a “polyphenotypic” immunohistochemical expression profile, with most cases being immunoreactive for cytokeratins, EMA, vimentin, desmin (frequently perinuclear dot-like) and NSE, as well as exhibiting nuclear expression of WT1 (detected with antibodies to the carboxy terminal end of the protein) [2-9]. DSRCT was initially believed to occur nearly exclusively in an intra-/peri-abdominal location. The clinicopathological spectrum has, however, turned out to be much wider than initially thought with reported cases of DSRCT occurring in a multitude of sites/organs [10-17]. Similarly, both light microscopically and immunohistochemically, several “variants” of DSRCT are on record, including tumors containing areas with an epithelial-like architecture of tubules and glands [10, 15, 16], extensive nuclear pleomorphism [11], rhabdoid features [2], spindle cell or signet-ring morphology [16],

lack of significant stromal desmoplasia [16, 18, 19], and absence of expression of cytokeratins and desmin [11, 15]. Highly characteristic of DSRCT is the presence of a  $t(11;22)(p13;q12)$  chromosomal translocation, resulting in the fusion of the *EWSR1*- and *WT1*-genes which can be detected by RT-PCR [19-26]. To add yet another layer of variability of DSRCT, variants of the molecular genetic change are on record [11, 20, 27, 28].

Our case had several unusual features that confounded a straightforward diagnosis of DSRCT. In the clinical presentation of an ovarian mass in a woman with a raised CA-125 level, a primary ovarian carcinoma was the first diagnostic consideration [29, 30]. The range of differential diagnoses for an ovarian neoplasm composed of small round cells is wide [31]. Ovarian small cell carcinoma of hypercalcemic type (OSCCHT) is a relatively rare but well characterized primary ovarian neoplasm frequently displaying a diffuse growth pattern of small round tumor cells. Expression of EMA and cytokeratins is often focal in OSCCHT, and the tumor cells may show diffuse nuclear staining with antibodies directed against the N-terminus of WT-1 [32]. Recent studies have demonstrated inactivating biallelic *SMARCA4* mutations

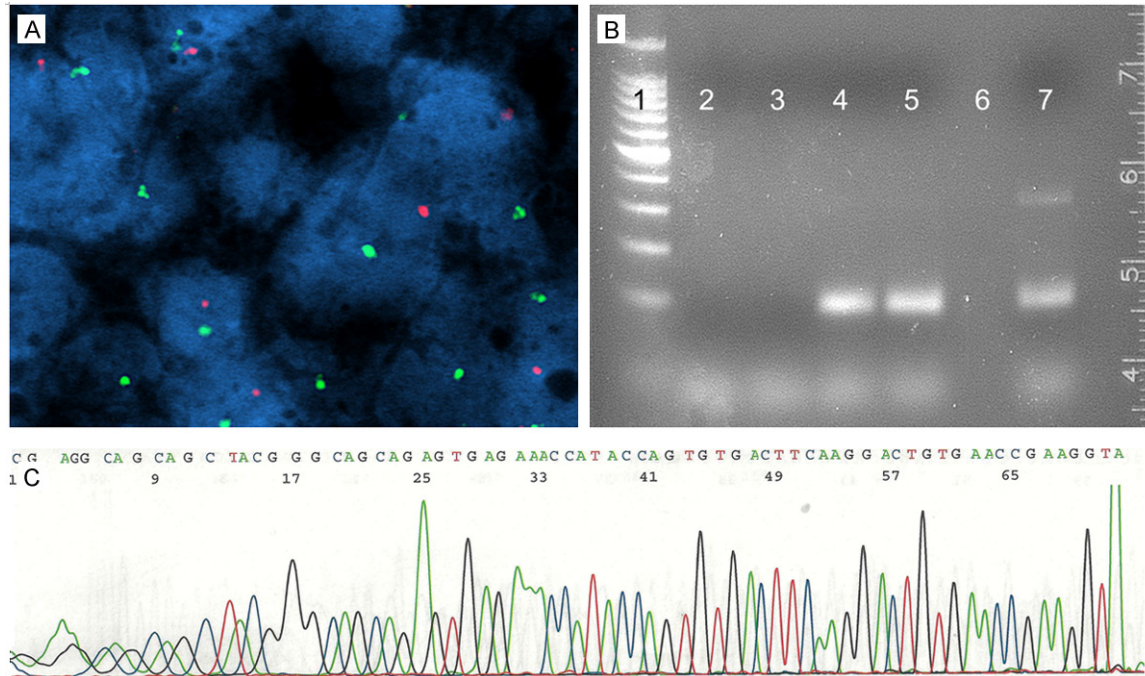
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**Figure 3.** Results of the immunohistochemical study (All figures original magnification x200). A. Only very focally, few tumor cells expressed cytokeratins (detected exclusively with AE1/3). B. Tumor cells expressed CD99. C. The majority of cells displayed loss of nuclear INI-1 expression. D. There was diffuse nuclear expression of WT-1 (antibody to the C-terminus end).

and loss of SMARCA4 nuclear immunostaining [33-35] but retained INI-1 nuclear expression in OSCCHT [36]. In our case, the absence of follicle-like structures, which is a characteristic histomorphological feature of OSCCHT, and the immunohistochemical findings of diffuse EMA expression and strong nuclear staining with antibodies directed against the C-terminus of WT-1 but not the N-terminus, argued against this differential diagnosis. A malignant sex-cord stromal tumor with a diffuse or insular pattern such as a granulosa cell tumor may also impart a 'small blue cell tumor' appearance; the presence of follicular structures and/or positive alpha-inhibin immunostaining is helpful in establishing such a diagnosis (absent in our case) [37]. Other tumors that should be included in the differential diagnosis are primary or metastatic small cell carcinoma of pulmonary type (neuroendocrine-type small cell carcinoma), although the near complete absence of

cytokeratin expression (which was investigated with several broad spectrum cytokeratin antibodies in our case), strongly argue against this possibility. In addition, primitive neuroectodermal tumor (PNET) can also occur in the ovary, sometimes arising from the neural tissue of dermoid cyst/mature cystic teratoma [38]. PNETs are characterized by strong and diffuse membranous expression of CD99, nuclear expression of FLI-1 and variable immunoreactivity for synaptophysin, NSE and S-100 protein. Membranous CD99-expression is, however, a non-specific feature and may be encountered in a whole range of other malignant small round blue cell tumors, such as lymphoblastic lymphoma, rhabdomyosarcoma, synovial sarcoma, mesenchymal chondrosarcoma, the blastemal component of Wilms tumor, and rarely in DSRCT [39]. As stated above, all these neoplasms with typically nondescript small cell morphology should be reflected on as differential diagnos-



**Figure 4.** Results of the molecular genetic study. A. Neoplastic cells demonstrating break-apart/rearrangement (FISH for *EWSR1* gene rearrangement). B. Detection of gene rearrangement by PCR. Lane 1: DNA ladder. Lane 2: No template control. Lane 3: Negative control. Lane 4: Band corresponding to *EWSR1*-*WT1* rearrangement in our patient. Lane 5: Positive control. Lane 7: Re-confirmation of *EWSR1*-*WT1* rearrangement in our patient. C. Sequencing data for *EWSR1*-*WT1* fusion transcript.

tic possibilities. Adequate sampling and performing a broad enough immunohistochemical study would resolve most of these differential diagnostic problems. However, as emphasized by the case presented herein, molecular genetic studies are invaluable in cases with unusual morphological and/or immunohistochemical findings. There have been only few reported cases of primary ovarian DSRCT [40-46]. However, keeping this differential diagnosis in mind has clinical relevance, given that maximal tumor debulking in ovarian involvement by DSRCT may not be as efficacious when compared to ovarian epithelial neoplasms [47] and different chemotherapeutic protocols are employed [42].

The immunohistochemical findings in our tumor posed diagnostic difficulties. In the original case series describing and characterizing DSRCT, which has been confirmed by others [3, 4, 10], nearly all cases exhibited immunohistochemical positivity for cytokeratins, EMA, NSE, vimentin and desmin, the latter frequently with a dot-like cytoplasmic appearance. In one study, cytokeratins (AE1/3, Cam5.2) and des-

min had a high sensitivity (85.9% and 89.7% respectively) [10], and the authors felt that extensive co-expression of both markers was a unique feature of this entity. Subsequent studies performed on molecularly confirmed tumors have supported the utility of Cam5.2 and desmin [4]. In our case, however, very few neoplastic cells displayed cytokeratin expression (by AE1/3); the vast majority (>99%) of tumor cells exhibited no cytokeratin expression whatsoever. In addition, no immunorexpression of cytokeratins was detected in any of the tumor cells with Cam5.2 and MNF116. Moreover, the neoplastic cells lacked expression of desmin. This led us to consider the possibilities of a poorly differentiated synovial sarcoma and proximal-type epithelioid sarcoma (although no rhabdoid cells were identified). A significant morphologic overlap between DSRCT and synovial sarcoma has been noted by other investigators [48]. EMA has been demonstrated to have an equal or higher sensitivity (~96%) than cytokeratins in DSRCT [3, 40, 49]. The utility of EMA in cases with equivocal or negative cytokeratin expression is also supported by our case, although molecularly confirmed cases lacking both cyto-

keratin or EMA staining have also been described [50].

In addition to epithelial markers, there have been several unusual cases of DSRCT which demonstrated a lack of expression of not only of epithelial markers, but also desmin and neural markers. A case published by Rekhi et al. [40] (located in the maxilla) was negative for cytokeratin, EMA, desmin, myogenin, Myo-D1, WT-1 (N-terminus), synaptophysin, chromogranin and NSE, and was only diffusely positive for vimentin. In addition the tumor cells expressed MIC2/CD99, FLI1 and focally CD56. This case was initially thought to be a PNET, but the tumor was demonstrated to have the characteristic molecular transcript of DSRCT. The higher sensitivity (97%) of molecular techniques as compared to immunohistochemical methods has been demonstrated in several studies [15, 20, 27]. We were also puzzled by the significant loss (>90%) of nuclear INI-1 expression. Synovial sarcoma has been shown to lack nuclear INI-1 expression [51, 52]. We therefore undertook a FISH study to investigate the presence of disruption of SYT (18q11.2) gene rearrangements, which was negative.

Although characteristic of malignant rhabdoid tumors, loss of INI-1 expression has also been documented in many other neoplasms, including epithelioid sarcoma, epithelioid malignant peripheral nerve sheath tumors, myoepithelial carcinoma, and extraskeletal myxoid chondrosarcoma [51-55]. Few carcinomas such as sinonasal basaloid carcinoma, collecting duct carcinomas of the kidney and rhabdoid carcinomas of the gastrointestinal tract are adding to the growing list of INI-negative tumors [56-58]. The absence of INI-1 nuclear staining is usually associated with at least some degree of rhabdoid morphology [59], which was completely absent in our case. Thus far, testing performed on limited number of DSRCT cases have shown retained INI-1 expression [51, 60], regardless of the absence or presence of rhabdoid morphology [40, 55]. Hence, to the best of our knowledge, this is the first case of DSRCT featuring loss of INI-1 expression. *SMARCB1/INI-1* has been postulated to be a tumor suppressor gene which may be disrupted in genetically unstable tumors, thus explaining the broad and increasing spectrum of tumors with loss of or decreased INI-expression [53].

### Conclusion

In summary, we present a case of desmoplastic small round cell tumor with unusual clinical and immunohistochemical features. Despite the absence of the characteristic polyphenotypic immunohistochemical profile of DSRCT, the histopathologic features as well as the detection of the *EWSR1-WT1* transcript in conjunction with the high rate of EWS rearranged cells in the FISH study established the diagnosis. Our case underscores that a high degree of diagnostic flexibility should be employed when evaluating immunohistochemical studies of malignant small round cell tumors and that the value of molecular genetic studies cannot be overemphasized in difficult cases.

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### Disclosure of conflict of interest

None.

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