

Evaluation of ETV4 and WT1 expression in *CIC*-rearranged sarcomas and histologic mimics

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A distinct subset of round cell sarcomas harbors capicua transcriptional repressor (*CIC*) rearrangement. Diagnosing these sarcomas can be difficult owing to their resemblance to Ewing sarcoma and other 'small round blue cell tumors'; molecular techniques are generally required. Recent gene expression studies of *CIC*-rearranged sarcomas identified the upregulation of ETV4. We assessed the sensitivity and specificity of ETV4 and WT1 immunohistochemistry for *CIC*-rearranged sarcoma. We evaluated whole-tissue sections from 40 *CIC*-rearranged sarcomas, 40 Ewing sarcomas, 4 *BCOR-CCNB3* sarcomas, 6 unclassified round cell sarcomas, and 150 histologic mimics. Moderate-to-strong nuclear immunoreactivity for ETV4 in at least 50% of cells was observed in 36 (90%) *CIC*-rearranged sarcomas and 10 (5%) other tumors, including 5 unclassified round cell sarcomas, 2 Wilms tumors, and 1 each desmoplastic small round cell tumor, melanoma, and small cell carcinoma. Thirty-eight (95%) *CIC*-rearranged sarcomas showed nuclear staining for WT1, and 34 (85%) were positive for both ETV4 and WT1. Of 182 other tumors evaluated, 34 (19%) showed nuclear WT1 positivity, including all Wilms tumors and desmoplastic small round cell tumors, 5 unclassified round cell sarcomas, and a subset of lymphoblastic lymphomas, rhabdomyosarcomas, mesenchymal chondrosarcomas, carcinomas, and melanomas. In summary, diffuse moderate-to-strong ETV4 expression is present in most *CIC*-rearranged sarcomas and unclassified round cell sarcomas. More limited expression is seen in small subsets of various other round cell neoplasms. Nuclear WT1 expression is also present in most *CIC*-rearranged sarcomas and unclassified round cell sarcomas, along with Wilms tumors and desmoplastic small round cell tumors, and subsets of various histologic mimics. The sensitivity and specificity of diffuse ETV4 expression for *CIC*-rearranged sarcomas are 90% and 95%, respectively, whereas the sensitivity and specificity of WT1 are 95% and 81%, respectively. Diffuse ETV4 along with at least focal WT1 expression is helpful to distinguish *CIC*-rearranged sarcoma from Ewing sarcoma and other histologic mimics.

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A distinct subset of round cell sarcomas that resemble Ewing sarcoma is negative for Ewing sarcoma breakpoint region 1 (*EWSR1*) rearrangement and instead harbors recurrent capicua transcriptional repressor (*CIC*) rearrangement,¹ predominantly characterized by gene fusion between *CIC* on 19q13 and one of the two *DUX4* retrogenes on 4q35 or 10q26.^{2,3} *CIC*-rearranged sarcomas often present at extraskeletal sites and are highly aggressive with poor prognosis.^{4,5} Diagnosing *CIC*-rearranged sarcoma can be difficult because of its histologic overlap with other 'small round blue cell tumors,' including various sarcomas (Ewing sarcoma, *BCOR-CCNB3* round cell sarcoma, synovial

sarcoma, myxoid liposarcoma, rhabdomyosarcomas, and desmoplastic small round cell tumor), carcinomas (small cell, NUT midline, and Merkel cell), lymphoblastic lymphomas, melanomas, and pediatric tumors (neuroblastomas and Wilms tumor). The diagnosis of *CIC*-rearranged sarcomas relies on demonstrating the presence of characteristic karyotype such as t(4;19)(q35;q13.1) or t(10;19)(q26;q13.1), reverse-transcription PCR (RT-PCR), or fluorescence *in situ* hybridization (FISH). By immunohistochemistry, *CIC*-rearranged sarcomas show nuclear WT1, ERG, and FLI1 expression, along with variable CD99 immunoreactivity.^{4,6,7} These markers are not specific, however, and can be seen in various other round cell neoplasms.^{8–13}

ETV4 (ETS variant 4; also known as PEA3 or E1AF), a member of the PEA3 subgroup in the ETS transcription factor family, is involved in regulating early embryogenesis¹⁴ and branching morphogenesis of epithelial structures including ureteric buds and limb buds.^{15,16} Somatic mutations in *ETV4* have

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been implicated in the progression of breast, colonic, gastric, endometrial, and squamous cell carcinomas.¹⁵ Translocations involving *ETV4* are present in <1% of Ewing sarcomas (*EWSR1-ETV4*) and 1–2% of prostatic adenocarcinomas (*TMPRSS2-ETV4*).^{15,17,18} Furthermore, *ETV4* expression is upregulated in *CIC*-mutant 1p/19q-codeleted oligodendrogliomas.¹⁹ Recent gene expression studies in *CIC*-rearranged sarcomas identified upregulation of *WT1* and *ETV4*,^{3,7} along with *ETV5* (*ERM*) and *ETV1*,^{1,3,7} which appear to distinguish *CIC*-rearranged sarcomas from Ewing sarcomas. A recent study suggested that immunohistochemistry for *ETV4* is sensitive and specific for *CIC-DUX4* sarcomas.²⁰ This study aimed to validate the diagnostic utility of *ETV4* as an immunohistochemical marker for *CIC*-rearranged sarcomas versus other round cell histologic mimics, in comparison with *WT1*.

Materials and methods

Cases were retrieved after approval of the Institution Review Board from the surgical pathology and consultation files of Brigham and Women's Hospital, Boston, MA, USA, and from the consultation files of two of the authors (CDMF and JLH). Representative hematoxylin and eosin-stained slides were reviewed. Whole-tissue sections were evaluated for *ETV4* expression in 240 cases: 40 *CIC*-rearranged sarcomas, 40 Ewing sarcomas, 4 *BCOR-CCNB3* round cell sarcomas, 6 unclassified round cell sarcomas, 10 poorly differentiated synovial sarcomas, 10 high-grade (round cell) myxoid liposarcomas, 10 lymphoblastic lymphomas, 10 alveolar rhabdomyosarcomas, 10 embryonal rhabdomyosarcomas, 10 mesenchymal chondrosarcomas, 10 Merkel cell carcinomas, 10 Wilms tumors, 10 neuroblastomas, 10 olfactory neuroblastomas, 5 *NUT* midline carcinomas, 5 desmoplastic small round cell tumors, 20 small cell carcinomas, and 20 melanomas. Immunohistochemistry for *WT1* was performed on all 40 *CIC*-rearranged sarcomas and 182 other tumors (additional materials from 18 of the cases listed above were not available). Immunohistochemistry for *CD99* was previously performed on 37 *CIC*-rearranged sarcomas, 39 Ewing sarcomas, 4 *BCOR-CCNB3* sarcomas, and 6 unclassified round cell sarcomas. Immunohistochemistry for *NKX2-2* was previously performed on 17 *CIC*-rearranged sarcomas, 38 Ewing sarcomas, 4 *BCOR-CCNB3* sarcomas, and 5 unclassified round cell sarcomas.²¹

Genetic confirmation by FISH and/or RT-PCR was previously obtained in all *CIC*-rearranged sarcomas, Ewing sarcomas, poorly differentiated synovial sarcomas, high-grade myxoid liposarcomas, as well as 4 of 5 desmoplastic small round cell tumors and 2 of 4 *BCOR-CCNB3* sarcomas (see below). Of the 40 *CIC*-rearranged sarcomas, 38 were confirmed by break-apart FISH for *CIC* rearrangement, with the remaining two cases confirmed by RT-PCR for *CIC*-

DUX4 fusion. Of the 40 Ewing sarcomas, 36 were confirmed by FISH for *EWSR1* rearrangement, with the remaining 4 cases confirmed by RT-PCR; in addition, 4 cases were confirmed to be negative for *CIC* rearrangement by FISH, and 4 cases underwent targeted next generation sequencing by OncoPanel (a massively parallel sequencing platform using Agilent SureSelect hybrid capture kit and Illumina HiSeq 2500 sequencer).²² *EWSR1-FLI1* fusion was confirmed in seven cases: three by cytogenetics with t(11;22)(q24;q12), two by both RT-PCR and OncoPanel, one by RT-PCR alone, and one by OncoPanel alone. *EWSR1-ERG* fusion was confirmed in three cases: one by cytogenetics with t(21;22)(q22;q12), one by RT-PCR, and one by OncoPanel. Of the four *BCOR-CCNB3* sarcomas, two were confirmed by RT-PCR (one additionally confirmed by FISH), and two were confirmed by immunohistochemistry for *CCNB3*.²³ All 10 poorly differentiated synovial sarcomas were confirmed by FISH for *SS18* rearrangement. All 10 high-grade myxoid liposarcomas were confirmed by FISH for *DDIT3* (*CHOP*) rearrangement, with one case showing concurrent *EWSR1* rearrangement and no *FUS* rearrangement consistent with *EWSR1-DDIT3* fusion. Of five desmoplastic small round cell tumors, four were confirmed by FISH for *EWSR1* gene rearrangement.

Immunohistochemistry for *ETV4* was performed on 4 µm-thick formalin-fixed paraffin-embedded whole-tissue sections following pressure cooker antigen retrieval (Target Retrieval Solution, pH 6.1; Dako, Carpinteria, CA, USA) using a mouse anti-PEA3 (*ETV4*) monoclonal antibody (1:50 dilution; 40 min incubation; clone 16/sc-113; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the Novolink Polymer Detection System (Leica, Buffalo Grove, IL, USA). The latter detection system was used after preliminary testing with the EnVision+ kit (Dako) showed at most weak staining, even at low primary antibody dilution. Immunohistochemistry for *WT1* was performed using a mouse anti-*WT1* monoclonal antibody (1:50 dilution; 40 min incubation; clone 6F-H2; Dako), *CD99* using a mouse anti-*CD99* antibody (1:150 dilution; 40 min incubation; clone O13; BioLegend, Dedham, MA, USA), and *NKX2-2* using a mouse anti-*NKX2-2* monoclonal antibody (1:25 dilution; 40 min incubation; clone 74.5A5; Developmental Studies Hybridoma Bank, Iowa City, IA, USA), as previously described.²¹ Appropriate positive and negative controls were used throughout the study. The extent of immunoreactivity was graded according to the percentage of cells with nuclear staining (0, <5%; 1+, 5–25%; 2+, 25–50%; 3+, 50–75%; or 4+, 75–100%) and intensity (weak, moderate, or strong). *ETV4* positivity was defined as moderate-to-strong nuclear immunoreactivity in at least 50% of cells. *WT1* positivity was defined as moderate-to-strong nuclear immunoreactivity in at least 5% of cells.

Results

Clinicopathologic Characteristics of the *CIC*-Rearranged Sarcoma and Other Round Cell Sarcoma Study Groups

The *CIC*-rearranged sarcomas affected 21 males and 19 females, with a median age of 32 years (age range, 0.5–81 years). The anatomic distribution of the primary *CIC*-rearranged sarcomas included lower limb ($n=13$), abdominopelvic cavity ($n=12$), thoracic cavity ($n=5$), upper limb ($n=3$), retroperitoneum ($n=3$), paravertebral region ($n=2$), and head and neck ($n=2$). Thirty-eight primary tumors were included, along with one locally recurrent and one metastatic case (in the lung); the study group typically involved soft tissue ($n=37$), rarely bone ($n=1$), lung ($n=1$), or colon ($n=1$). *CIC*-rearranged sarcomas displayed a wide histologic spectrum. The tumors predominantly showed a diffuse or vaguely lobular architecture, with small to intermediate-sized, round-to ovoid and occasionally spindled cells with vesicular chromatin and prominent nucleoli; the cytoplasm could be moderate or scant, eosinophilic and occasionally clear or amphophilic (Figure 1a and c). Geographic necrosis, hemorrhage, and focal sclerotic stroma were commonly seen, along with frequent mitoses and single-cell apoptosis. Two *CIC*-rearranged sarcomas showed a reticular or pseudoalveolar architecture within a myxoid stroma (Figure 1e), mimicking extraskeletal myxoid chondrosarcoma or myoepithelial carcinoma.^{4,6} Immunohistochemical expression of CD99 was present in 32 of 37 cases (87%), with staining pattern ranging from focal ($n=5$) or multifocal ($n=24$), mixed membranous and cytoplasmic, to diffuse and predominantly membranous ($n=3$). All 17 cases tested were negative for NKX2-2 expression.

The Ewing sarcomas affected 22 males and 18 females, with a median age of 29 years (age range, 10–76 years). The anatomic distribution of primary Ewing sarcomas included lower limb ($n=9$), abdominopelvic cavity ($n=9$), head and neck ($n=7$), upper limb ($n=6$), thoracic cavity ($n=6$), retroperitoneum ($n=2$), and paravertebral region ($n=1$); this study group typically involved soft tissue ($n=19$) or bone ($n=10$), rarely lung ($n=4$), brain ($n=2$), submandibular gland ($n=2$), skin ($n=1$), oral cavity ($n=1$), or uterus ($n=1$), with 35 primary, 2 locally recurrent, and 3 metastatic cases (in the lung or liver). Ewing sarcoma showed classic histomorphology with uniform round cells in a solid or lobular arrangement (Figure 2a) in 36 cases, with the remainder 4 characterized by atypical cytomorphology with small nucleoli and small amounts of cytoplasm. CD99 expression was observed in all 39 cases tested, predominantly with diffuse ($n=36$) and rarely focal or weak ($n=3$) membranous staining patterns. NKX2-2 expression was present in 32 of 38 cases (84%).

The *BCOR-CCNB3* sarcomas affected four males, with a median age of 12 years (age range, 2–44 years) and included two osseous (femur and sacrum) and two extraosseous primary sites (neck and thigh). *BCOR-CCNB3* sarcomas showed ovoid to focally spindled tumor cells in a myxoid or collagenous stroma (Figure 2c). CD99 and NKX2-2 expression were absent in all four cases tested.

The six unclassified round cell sarcomas affected three males and three females, with a median age of 20 years (age range, 13–34 years), all extraosseous and involving lower limb in two cases, chest wall, abdominopelvic cavity, tonsil, and the paravertebral region in one case each. All six cases showed diffuse sheets or vague lobules of round-to-ovoid and focally spindled tumor cells with eosinophilic or focally clear cytoplasm (Figure 3a and c). Of the six cases, all showed diffuse to multifocal membranous CD99 expression; none of the five tested cases were positive for NKX2-2. All six unclassified round cell sarcomas were genetically confirmed by FISH to lack *EWSR1* and *CIC* gene rearrangement; *BCOR*, *FUS*, and *ERG* gene rearrangements were confirmed by FISH to be absent in five, four, and three cases, respectively.

ETV4 Expression in *CIC*-Rearranged Sarcomas and Other Round Cell Neoplasms

The results of ETV4 immunohistochemistry in *CIC*-rearranged sarcomas and other round cell tumors are summarized in Table 1. Diffuse moderate-to-strong nuclear ETV4 staining was present in 36 of 40 (90%) *CIC*-rearranged sarcomas (Figure 1b, d, and f), with at least 1+ staining of any intensity in 38 of 40 (95%) cases. The intensity of ETV4 staining in the 40 *CIC*-rearranged sarcomas was as follows: strong in 29, moderate in 10, and weak in 1. In contrast, Ewing sarcomas showed at most focal weak-to-moderate ETV4 expression (1+ in 12, including moderate in 6 and weak in 6; 0 in 28). There was no ETV4 immunoreactivity in *BCOR-CCNB3* sarcomas (Figure 2d), olfactory neuroblastomas, Merkel cell carcinomas, and NUT midline carcinomas (all 0). Of the 200 non-*CIC*-rearranged tumors, ETV4 staining was present in 30 (15%). Focal (5–50%) moderate nuclear ETV4 expression was present in 20 (10%) cases, including 6 (15%) Ewing sarcomas (Figure 2b), 6 melanomas, 3 Wilms tumors, and 1 each poorly differentiated synovial sarcoma, lymphoblastic lymphoma, alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, and neuroblastoma. Diffuse moderate-to-strong nuclear ETV4 expression was present in 10 (5%) cases overall, including 5 unclassified round cell sarcomas (strong in three and moderate in two) (Figure 3), 2 Wilms tumors (1 moderate and 1 strong) (Figure 4f), 1 desmoplastic small round cell tumor (moderate) (Figure 5f), 1 melanoma (strong) (Figure 4b), and 1 small cell carcinoma (strong) (Figure 4d).

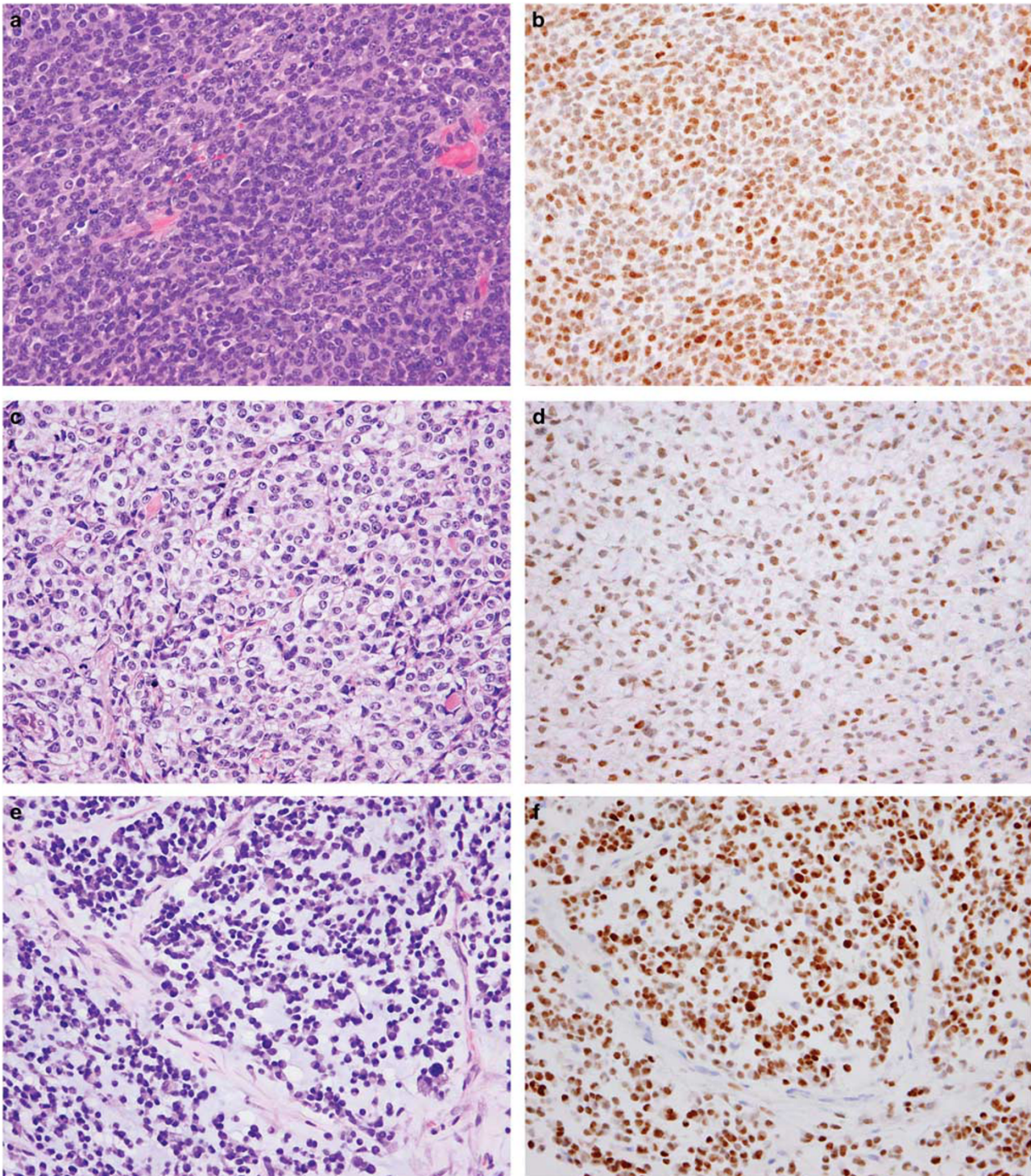


Figure 1 *CIC*-rearranged sarcoma in the gluteal region, composed of ovoid cells with amphophilic cytoplasm and prominent nucleoli (a), showing diffuse ETV4 staining (b). *CIC*-rearranged sarcoma in the soft tissue of the posterior neck, composed of sheets and vague nests of epithelioid cells with clear cytoplasm and small nucleoli (c), showing diffuse nuclear immunoreactivity for ETV4 (d). *CIC*-rearranged sarcoma in the buttock, characterized by round-to-epithelioid cells with hyperchromatic nuclei in a reticular architecture within a myxoid stroma (e), mimicking extraskeletal myxoid chondrosarcoma or a myoepithelial tumor; diffuse strong nuclear ETV4 expression is present (f).

Although most *CIC*-rearranged sarcomas showed diffuse strong nuclear ETV4 expression, focal weak-to-moderate ETV4 staining was present in a minor subset of various other round cell tumors.

As antibodies directed against transcription factors occasionally show weak nuclear staining in clinical practice, which in most instances we interpret as nonspecific, ETV4 positivity was defined

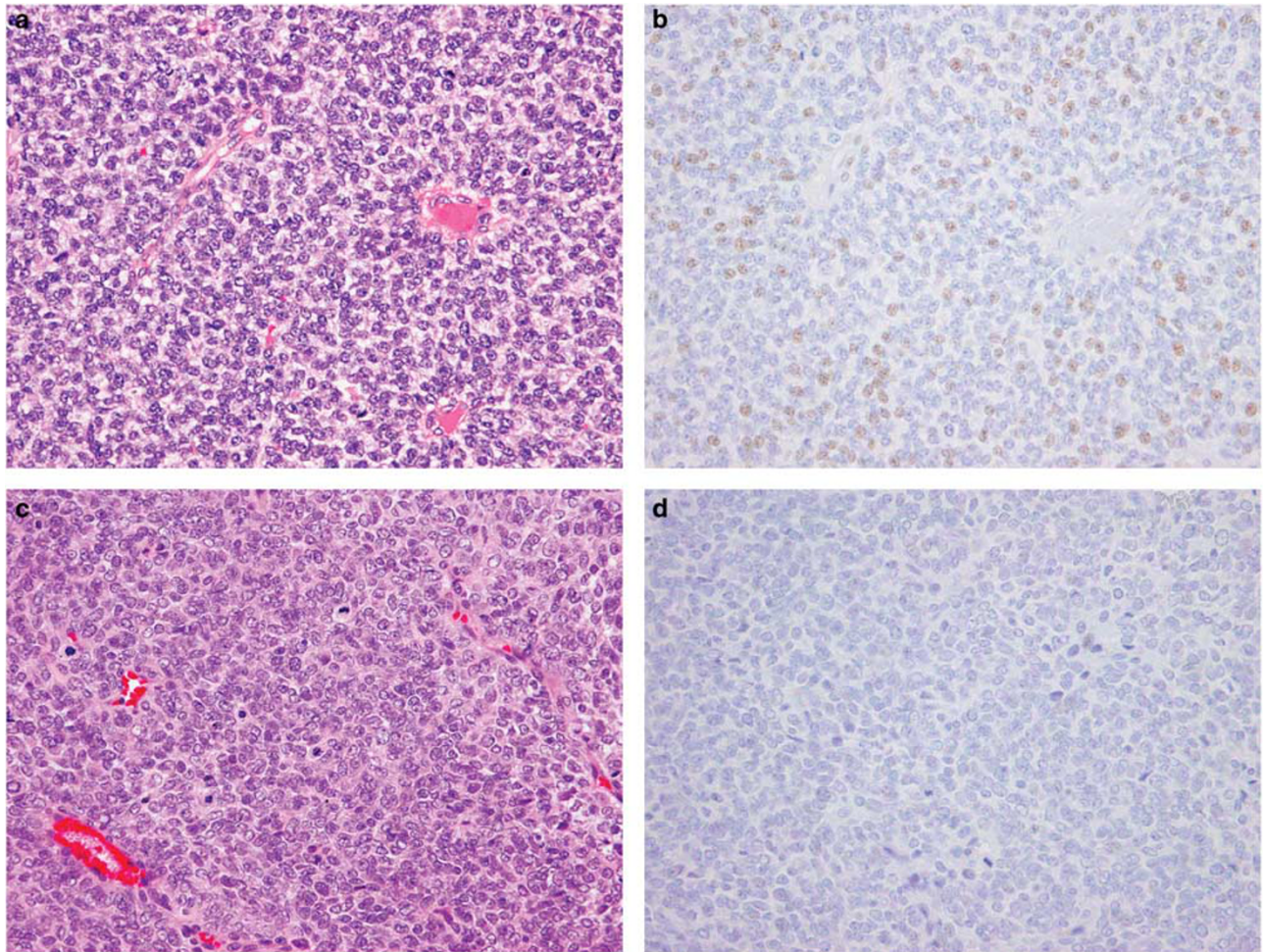


Figure 2 Ewing sarcoma showing uniform round cells (a) and focal weak nuclear immunoreactivity for ETV4 (b). *BCOR-CCNB3* sarcoma (c) showing an absence of ETV4 staining (d).

as moderate-to-strong nuclear staining in at least 50% of cells. With this stringent threshold, ETV4 immunohistochemistry was positive in 90% of *CIC*-rearranged sarcomas and negative in all Ewing sarcomas, *BCOR-CCNB3* sarcomas, poorly differentiated synovial sarcomas, high-grade myxoid liposarcomas, lymphoblastic lymphomas, alveolar rhabdomyosarcomas, embryonal rhabdomyosarcomas, mesenchymal chondrosarcomas, Merkel cell carcinomas, neuroblastomas, olfactory neuroblastomas, and NUT midline carcinomas. Overall, ETV4 positivity was present in 5% of non-*CIC*-rearranged tumors, including 5 of 6 unclassified round cell sarcomas, 2 of 10 Wilms tumors, 1 of 5 desmoplastic small round cell tumors, and 1 of 20 small cell carcinomas and melanomas.

WT1 Expression in *CIC*-Rearranged Sarcomas and Other Round Cell Neoplasms

The results of WT1 immunohistochemistry in *CIC*-rearranged sarcomas and other round cell

tumors are summarized in Table 2. Of 40 *CIC*-rearranged sarcomas, 38 (95%) showed nuclear staining for WT1, including 31 (78%) with diffuse (>50%) moderate-to-strong reactivity; 34 tumors (85%) were positive for both ETV4 and WT1. Nuclear WT1 expression was absent in Ewing sarcomas, *BCOR-CCNB3* sarcomas, poorly differentiated synovial sarcomas, high-grade myxoid liposarcomas, neuroblastomas, and olfactory neuroblastomas (Table 2). Of 182 non-*CIC*-rearranged tumors tested, 34 (19%) showed nuclear WT1 positivity, including all Wilms tumors and desmoplastic small round cell tumors, 5 of 6 unclassified round cell sarcomas, and a subset of lymphoblastic lymphomas, rhabdomyosarcomas, mesenchymal chondrosarcomas, carcinomas, and melanomas.

The sensitivity and specificity of diffuse ETV4 expression for *CIC*-rearranged sarcomas were 90% and 95%, respectively, whereas the sensitivity and specificity of nuclear WT1 were 95% and 81%, respectively. The specificity of expression of both markers was 96%.

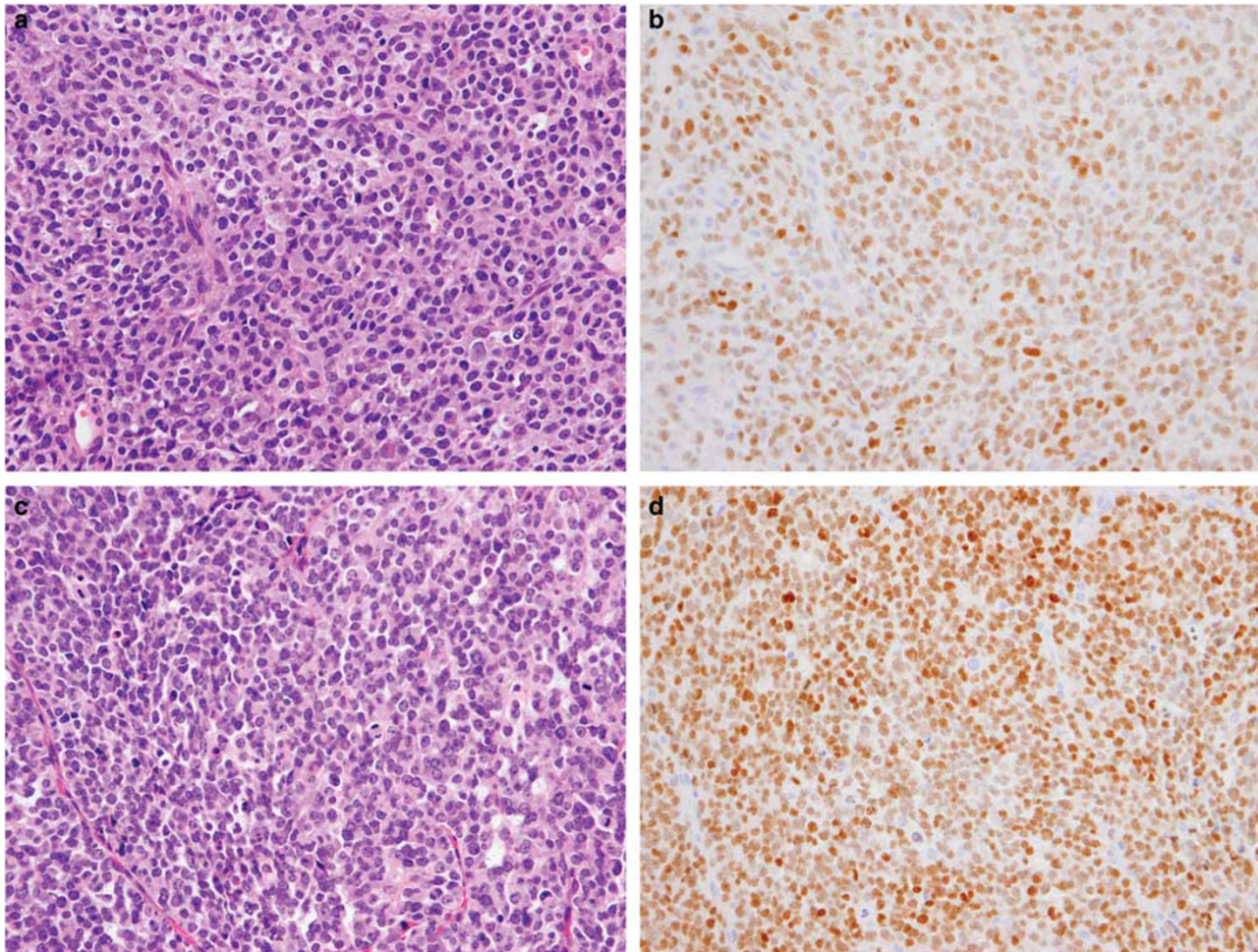


Figure 3 An unclassified round cell sarcoma in the abdominal cavity of a 17-year-old woman (a), without *CIC*, *EWSR1*, or *BCOR* rearrangements, showing diffuse strong ETV4 expression (b). An unclassified round cell sarcoma in the supraclavicular soft tissue of a 34-year-old man (c), showing diffuse strong nuclear ETV4 expression (d).

Table 1 Summary of immunohistochemical staining for ETV4

Tumor type	Total cases	ETV4 positive (%) ^a	0	1+	2+	3+	4+	Both ETV4 and WT1 positive (%) ^b
<i>CIC</i> -rearranged sarcoma	40	36 (90)	2	1	1	4	32	34 (85)
Non- <i>CIC</i> -rearranged tumors	200	10 (5)	145	33	11	4	7	8 (4) ^c
Ewing sarcoma	40	0 (0)	28	12	0	0	0	0 (0)
<i>BCOR-CCNB3</i> sarcoma	4	0 (0)	4	0	0	0	0	0 (0)
Unclassified round cell sarcoma	6	5 (83)	1	0	0	0	5	5 (83)
Synovial sarcoma, poorly differentiated	10	0 (0)	6	3	1	0	0	0 (0)
Myxoid liposarcoma, high grade (round cell)	10	0 (0)	8	2	0	0	0	0 (0)
Lymphoblastic lymphoma	10	0 (0)	9	1	0	0	0	0 (0)
Alveolar rhabdomyosarcoma	10	0 (0)	8	2	0	0	0	0 (0)
Embryonal rhabdomyosarcoma	10	0 (0)	8	1	1	0	0	0 (0)
Mesenchymal chondrosarcoma	10	0 (0)	10	0	0	0	0	0 (0)
Merkel cell carcinoma	10	0 (0)	10	0	0	0	0	0 (0)
Neuroblastoma	10	0 (0)	9	0	1	0	0	0 (0)
Olfactory neuroblastoma	10	0 (0)	9	1	0	0	0	0 (0)
NUT midline carcinoma	5	0 (0)	5	0	0	0	0	0 (0)
Desmoplastic small round cell tumor	5	1 (20)	3	1	0	1	0	0 (0)
Wilms tumor	10	2 (20)	3	2	3	1	1	2 (22)
Small cell carcinoma	20	1 (5)	16	3	0	1	0	0 (0)
Melanoma	20	1 (5)	8	5	5	1 ^d	1	1 (5)

0, < 5%; 1+, 5–25%; 2+, 25–50%; 3+, 50–75%; 4+, 75–100%.

^aETV4 positivity was defined as moderate-to-strong nuclear staining in at least 50% of cells.

^bWT1 positivity was defined as moderate-to-strong nuclear staining in at least 5% of cells.

^cImmunohistochemistry for both ETV4 and WT1 was performed in 182 of 200 non-*CIC*-rearranged cases.

^dOne melanoma case showed 3+ weak nuclear ETV4 staining.

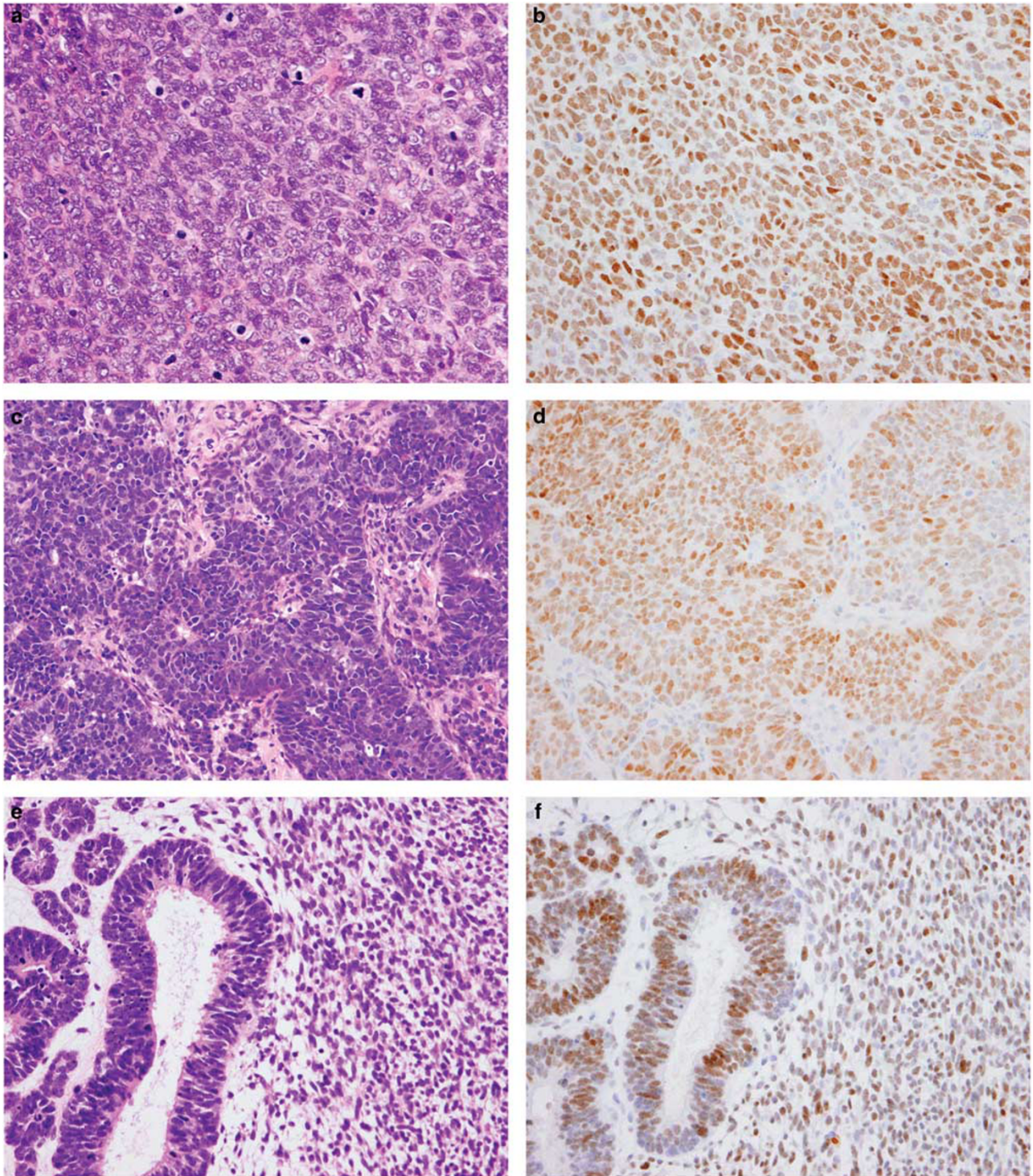


Figure 4 Metastatic melanoma in the inguinal soft tissue (a), showing diffuse strong nuclear ETV4 expression (b). Small cell carcinoma of the bladder (c) with diffuse ETV4 staining (d). Metastatic Wilms tumor (e) showing focal moderate-to-strong nuclear ETV4 expression (f).

Discussion

A primitive round cell sarcoma characterized by a recurrent chromosomal translocation $t(4;19)(q35;q13.1)$ was first described in 1996 in the ankle of a 12-year-old boy, who developed bilateral pulmonary

metastasis and died within 10 months.²⁴ In 2006, two adult extraosseous round cell sarcomas with Ewing sarcoma-like morphology and $t(4;19)(q35;q13.1)$ translocation were shown to harbor a fusion between *CIC* on 19q13 and *DUX4* on 4q35, creating an oncogenic transcription factor *CIC-DUX4*.¹

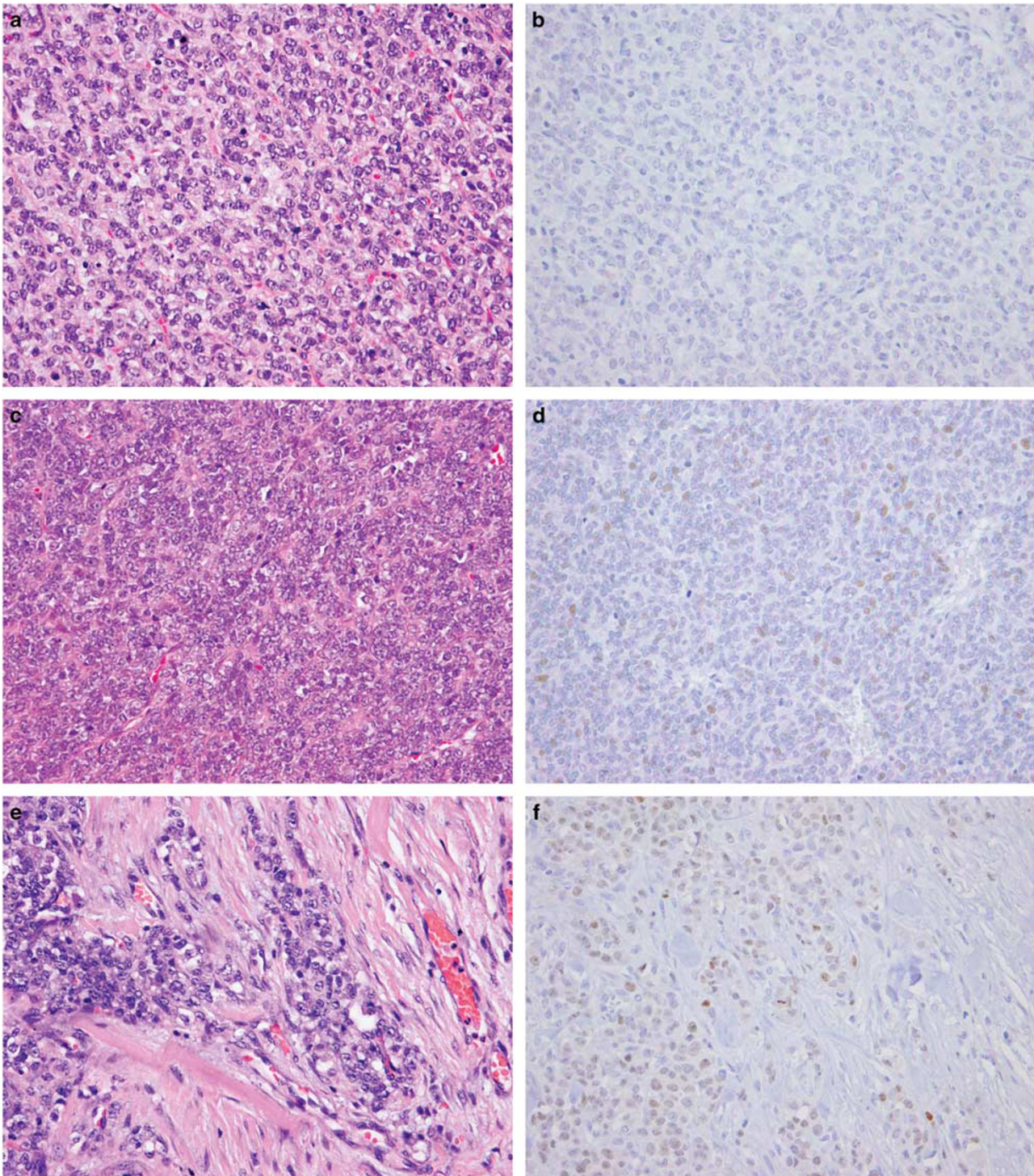


Figure 5 High-grade myxoid (round cell) liposarcoma (a) showing an absence of ETV4 staining (b). Poorly differentiated synovial sarcoma (c) showing focal weak ETV4 staining (d). Desmoplastic small round cell tumor (e) showing nests of round-to ovoid cells and multifocal weak-to-moderate ETV4 immunoreactivity (f).

CIC, a human homolog of *Drosophila capicua*, encodes a high mobility group box transcription repressor in a receptor tyrosine kinase pathway.²⁵ *CIC* is normally expressed in cerebellar granular neurons; its dysregulation is implicated in

spinocerebellar ataxia type 1 neurodegeneration, and inactivating *CIC* mutations have been identified in oligodendrogliomas¹⁹ and breast cancer.²⁶ *DUX4* and its paralog, located in the D4Z4 macrosatellite repeat at 4q35 and 10q26, respectively,²⁷ encode a

Table 2 Summary of immunohistochemical staining for WT1

Tumor type	Total cases	WT1 positive (%) ^a	0	1+	2+	3+	4+
CIC-rearranged sarcoma	40	38 (95)	2	5	2	7	24
Non-CIC-rearranged tumors	182	34 (19)	143	15	5	3	16
Ewing sarcoma	37	0 (0)	37	0	0	0	0
BCOR-CCNB3 sarcoma	4	0 (0)	4	0	0	0	0
Unclassified round cell sarcoma	6	5 (83)	1	0	0	0	5
Synovial sarcoma, poorly differentiated	5	0 (0)	5	0	0	0	0
Myxoid liposarcoma, high grade (round cell)	10	0 (0)	10	0	0	0	0
Lymphoblastic lymphoma	8	2 (25)	5	0	1 ^b	2	0
Alveolar rhabdomyosarcoma	10	4 (40)	3	4	2 ^b	1 ^b	0
Embryonal rhabdomyosarcoma	10	4 (40)	5	5 ^b	0	0	0
Mesenchymal chondrosarcoma	10	2 (20)	8	1	1	0	0
Merkel cell carcinoma	10	1 (10)	9	1	0	0	0
Neuroblastoma	10	0 (0)	10	0	0	0	0
Olfactory neuroblastoma	9	0 (0)	9	0	0	0	0
NUT midline carcinoma	4	1 (25)	3	1	0	0	0
Desmoplastic small round cell tumor	2	2 (100)	0	1	0	0	1
Wilms tumor	9	9 (100)	0	0	1	0	8
Small cell carcinoma	19	1 (5)	18	0	0	0	1
Melanoma	19	3 (16)	8	2	0	0	1

0, < 5%; 1+, 5–25%; 2+, 25–50%; 3+, 50–75%; 4+, 75–100%.

^aWT1 positivity was defined as moderate-to-strong nuclear staining in at least 5% of cells.

^bWeak nuclear WT1 staining was seen in one lymphoblastic lymphoma (2+), three alveolar rhabdomyosarcomas (3+, 2+, and 2+), and one embryonal rhabdomyosarcoma (1+); these cases were categorized as negative.

double homeodomain transcription factor that is normally expressed in testis and is epigenetically suppressed in differentiated cells.²⁸ Aberrant DUX4 expression due to D4Z4 contraction at 4q35 underlies facioscapulohumeral muscular dystrophy.²⁹ As compared with wild-type CIC, the chimeric CIC-DUX4 enhances transcriptional activities of selected genes by >100 fold¹ with activation of WT1 along with PEA3 subgroup of ETS transcription factors including ETV4, ETV1, and ETV5.^{1,7} Since its initial description, CIC rearrangement has been documented in over 50 cases of pediatric and adult Ewing sarcoma-like round cell sarcomas^{1–7,30–34} and accounts for up to 68% of *EWSR1*-negative round cell sarcomas.³

Although referred to as ‘Ewing-like sarcoma’¹ and morphologically overlapping with Ewing sarcoma,^{35,36} CIC-rearranged sarcoma shows distinct clinical, histologic, immunophenotypic, and molecular features: clinically, whereas CIC-rearranged sarcoma and Ewing sarcoma present with a wide age distribution in both sexes,⁴ osseous presentation is rare in CIC-rearranged sarcomas but common in Ewing sarcoma;^{4,21} CIC-rearranged sarcoma is associated with chemoresistance and worse prognosis as compared to Ewing sarcoma.^{4–6} Histologically, unlike typical Ewing sarcoma with small uniform round cells, CIC-rearranged sarcoma displays variable cytomorphology and intratumoral heterogeneity, with ovoid to spindle and occasionally clear cells, vesicular chromatin, prominent nucleoli, and amphophilic cytoplasm. The architecture is usually diffuse or lobular, reminiscent of Ewing sarcomas; however, rare cases can show a reticular or pseudoalveolar patterns (Figure 1e), reminiscent of extraskeletal myxoid chondrosarcoma or

myoepithelial carcinoma.^{4,6} Immunophenotypically, CIC-rearranged sarcoma shows variable CD99 but strong nuclear WT1 expression and is negative for NKX2-2,^{4,6,7,21} whereas Ewing sarcoma is diffusely and strongly positive for CD99 and NKX2-2 but negative for nuclear WT1 in most cases.^{4,11,13,21,37} FLI1 and ERG are positive in both CIC-rearranged and Ewing sarcomas and thus are not discriminatory.^{6,7,9,10,12} CIC-rearranged sarcoma rarely expresses desmin, keratin,^{3–5,33} or S-100 protein,^{2,3,34} but often shows calretinin⁴ and diffuse MYC expression,⁶ which may be secondary to trisomy 8 with MYC amplification in subsets of cases.⁵ Genetically, Ewing sarcoma is characterized by fusion of *EWSR1* on 22q12 (or rarely *FUS*) to *FLI1* on 11q24 (or less frequently *ERG* or other ETS family members including *FEV*, *ETV1*, or *E1AF/ETV4*),³⁸ whereas 70–80% of CIC-rearranged sarcomas published to date involves fusion between *CIC* and one of the two *DUX4* retrogenes on 4q35 or 10q26.^{3,7}

This study describes ETV4 as a diagnostic immunohistochemical marker for CIC-rearranged sarcoma and compares its diagnostic performance with WT1. ETV4 immunohistochemistry is sensitive for CIC-rearranged sarcomas, with at least 1+ staining in 95% and diffuse moderate-to-strong expression in 90% of cases. As most tumors not associated with CIC rearrangement showed no to only focal immunoreactivity, diffuse ETV4 expression appears relatively specific for CIC-rearranged sarcomas among most histologic mimics including Ewing and *BCOR-CCNB3* sarcomas. Nevertheless, diffuse moderate-to-strong ETV4 staining was noted in 5% of small cell carcinomas and melanomas, 2 of 10 Wilms tumors, 1 of 5 desmoplastic small round cell

tumors, and 5 of 6 unclassified round cell sarcomas. Despite similar sensitivities of ETV4 and WT1 for CIC-rearranged sarcomas (90% and 95%, respectively), diffuse ETV4 expression is more specific than WT1 (95% versus 81%). We observed nuclear WT1 positivity in 19% of round cell malignant neoplasms other than CIC-rearranged sarcomas, including 100% of Wilms tumors and desmoplastic small round cell tumors, five of six unclassified round cell sarcomas, and a subset of lymphoblastic lymphomas, rhabdomyosarcomas, mesenchymal chondrosarcomas, carcinomas, and melanomas. The specificity of expression of both markers was 96%. Of note, as the five unclassified round cell sarcomas were histologically and immunophenotypically similar to CIC-rearranged sarcomas (that is, with expression of both ETV4 and WT1), we speculate that these cases may harbor cryptic CIC rearrangements that were not detected by conventional break-apart FISH.

In summary, ETV4 is a sensitive but not entirely specific immunohistochemical marker for CIC-rearranged sarcomas. Although weak ETV4 expression is present focally in small subsets of various other round cell tumors, diffuse moderate-to-strong ETV4 expression is 90% sensitive and 95% specific for CIC-rearranged sarcomas, with exceptional positive cases including occasional Wilms tumors and desmoplastic small round cell tumors, and rare small cell carcinomas and melanomas. ETV4 may thus be helpful to distinguish CIC-rearranged sarcomas from histologic mimics, such as Ewing sarcomas and those with *BCOR-CCNB3* fusion, although we would expect the rare Ewing sarcomas with *EWSR1-ETV4* fusion (<1% of all Ewing sarcomas) to express ETV4 as well. Most tumors not associated with CIC rearrangement are negative for ETV4. Although nuclear WT1 positivity is 95% sensitive for CIC-rearranged sarcoma, its specificity is only 81%. The combination of diffuse ETV4 and at least focal nuclear WT1 expression is helpful to distinguish CIC-rearranged sarcoma from histologic mimics.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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