DUX4 Immunohistochemistry Is a Highly Sensitive and Specific Marker for CIC-DUX4 Fusion-positive Round Cell Tumor

Bradford Siegele, MD, JD,†‡ Jon Roberts, BA,† Jennifer O. Black, MD,*† Erin Rudzinski, MD,§ Sara O. Vargas, MD,‡ and Csaba Galambos, MD, PhD*†*

Abstract: The histologic differential diagnosis of pediatric and adult round cell tumors is vast and includes the recently recognized entity *CIC-DUX4* fusion-positive round cell tumor. The diagnosis of *CIC-DUX4* tumor can be suggested by light microscopic and immunohistochemical features, but currently, definitive diagnosis requires ancillary genetic testing such as conventional karyotyping, fluorescence in situ hybridization, or molecular methods. We sought to determine whether DUX4 expression would serve as a fusion-specific immunohistochemical marker distinguishing *CIC-DUX4* tumor from potential histologic mimics. A cohort of *CIC-DUX4* fusion-positive round cell tumors harboring t(4;19)(q35;q13) and t(10;19)(q26;q13) translocations was designed, with additional inclusion of a case with a translocation confirmed to involve the *CIC* gene without delineation of the partner. Round cell tumors with potentially overlapping histologic features were also collected. Staining with a monoclonal antibody raised against the C-terminus of the DUX4 protein was applied to all cases. DUX4 immunohistochemistry exhibited diffuse, crisp, strong nuclear staining in all *CIC-DUX4* fusion-positive round cell tumors (5/5, 100% sensitivity), and exhibited negative staining in nuclei of all of the other tested round cell tumors, including 20 Ewing sarcomas, 1 Ewing-like sarcoma, 11 alveolar rhabdomyosarcomas, 9 embryonal rhabdomyosarcomas, 12 synovial sarcomas, 7 desmoplastic small round cell tumors, 3 malignant rhabdoid tumors, 9 neuroblastomas, and 4 clear cell sarcomas (0/76, 100% specificity). Thus, in our experience, DUX4 immunostaining distinguishes *CIC-DUX4* tumors from other round cell mimics. We recommend its use when *CIC-DUX4* fusion-positive round cell tumor enters the histologic differential diagnosis.

Key Words: CIC-DUX4 fusion-positive round cell tumor, undifferentiated sarcoma, Ewing sarcoma, monoclonal antibody, immunohistochemistry

(*Am J Surg Pathol* 2017;41:423–429)

An emerging class of tumors is that of undifferentiated sarcomas resembling Ewing sarcoma but not harboring characteristic Ewing sarcoma gene fusions.[1–3] Among the most prevalent subset of this class of tumors is an entity with the t(4;19)(q35;q13) translocation between the *CIC* and *DUX4* genes or, less frequently, the t(10;19)(q26;q13) translocation between *CIC* and the *DUX4* paralog *DUX4L*. This subset of tumors is variously referred to as "round cell sarcoma with *CIC* translocation," "*CIC-DUX* sarcoma," and "*CIC-DUX4* fusion-positive round cell tumor." Tumors harboring these fusions express a distinct transcriptional signature and have been associated with very poor clinical outcomes, suggesting a pathobiology distinct from classic Ewing sarcoma, and emphasizing the importance of their diagnostic distinction.[4–6]

CIC is a human homolog of *Drosophilia capuica* that encodes a high mobility group box transcription factor. The CIC protein functions to transduce receptor tyrosine kinase signaling through mechanisms involving transcriptional repression. CIC is overexpressed in the cerebellum and in subsets of medulloblastoma,[7] whereas genetic alterations including mutations, deletions, and amplifications have been detected in cohorts of a wide assortment of tumors, including oligodendrogliomas, gastric adenocarcinomas, pancreatic cancers, and malignancies of the gynecologic tract.[8]

DUX4, also known as double-homeobox 4, is a retrogene contained within the D4Z4 polymorphic macrosatellite repeat. The gene encodes the double-homeobox transcription factor. The DUX4 protein is expressed during normal development in fetal skeletal muscle as well as within mature human testes.[9] Expression of DUX4 in differentiated tissues is normally suppressed through epigenetic mechanisms, with residual DUX4

From the *Department of Pathology, University of Colorado School of Medicine; †Department of Pathology and Laboratory Medicine, Children's Hospital Colorado, Aurora, CO; ‡Department of Pathology, Boston Children's Hospital, Boston, MA; and §Department of Pathology, Seattle Children's Hospital, Seattle, WA.

Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Bradford Siegele, MD, JD, Department of Pathology, Boston Children's Hospital, 300 Longwood Ave. Boston, MA 02115 (e-mail: bsiegele@post.harvard.edu).

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website, www.ajsp.com.

transcripts spliced to remove cytotoxic carboxyterminal domains.

The *CIC-DUX4* fusion gene has been shown to induce oncogenic transformation in transfected human NIH 3T3 fibroblasts by a mechanism hypothesized to involve upregulation of *ERM* and other *PEA3* family members by enhanced binding of the *ERM* promoter.[10] In tumors harboring *CIC-DUX4* fusions, chromosomal gains and losses have additionally been reported, with trisomy 8, and associated *MYC* amplification, constituting the most common numerical abnormality reported to date.[4,11] The recent discoveries of the overexpression of *ETS*-family transcription factor proteins FLI1, ERG, ETV1, ETV4, and ETV5 as well as the frequent expression of WT1[6,11–13] have increasingly brought the biological and molecular mechanisms involved in *CIC-DUX4* tumors to bear on the workup and pathologic diagnosis of these tumors.

CIC-DUX4 fusion-positive tumors often demonstrate characteristic morphologic features, including nuclear pleomorphism, nuclear vacuolation, prominent nucleoli, and geographic necrosis. Cells are typically round to ovoid but may occasionally be spindled (see Fig. 1 for a representative case S1). Multiple ancillary diagnostic modalities, including but not limited to conventional karyotyping, fluorescence in situ hybridization (FISH), broad-based immunohistochemical profiling, and molecular methods such as reverse-transcription polymerase chain reaction (RT-PCR), are also commonly utilized tools for narrowing the broad differential and arriving at a final diagnosis of this tumor entity; however, there remain limitations inherent to each of these modalities. Conventional karyotyping showing a translocation at 19q13.2 can provide strong support for a *CIC* rearrangement, but by necessity this modality requires the submission of fresh tissue for cell culture in addition to the time required for tumor cell colony growth and evaluation. RT-PCR assays generally require either the immediate processing of fresh tissue or snap freezing of tissue at the time of specimen processing for the preservation of evaluable mRNA. Break-apart FISH studies can confirm the presence of a *CIC* translocation in paraffin-embedded tissue, but at this time regular testing is confined to a handful of specialized research laboratories and large reference laboratories.

To date no series of *CIC-DUX4* fusion-positive round cell tumors has been systematically evaluated and reported for immunohistochemical staining by a monoclonal antibody raised either against the N-terminal CIC or against the C-terminal DUX4 or DUX4L components of the characteristic chimeric oncoproteins. Using previously unreported cases of *CIC-DUX4* fusion-positive tumors along with a set of potential histologic mimics, we aimed to determine whether routine immunohistochemistry using a DUX4 monoclonal antibody serves as a fusion-specific immunohistochemical stain of practical use in the diagnostic workup of round cell tumors and undifferentiated sarcomas.

MATERIALS AND METHODS

Representative formalin-fixed, paraffin-embedded tissue samples from pediatric and adult *CIC-DUX4* tumors and potential histologic mimics were examined

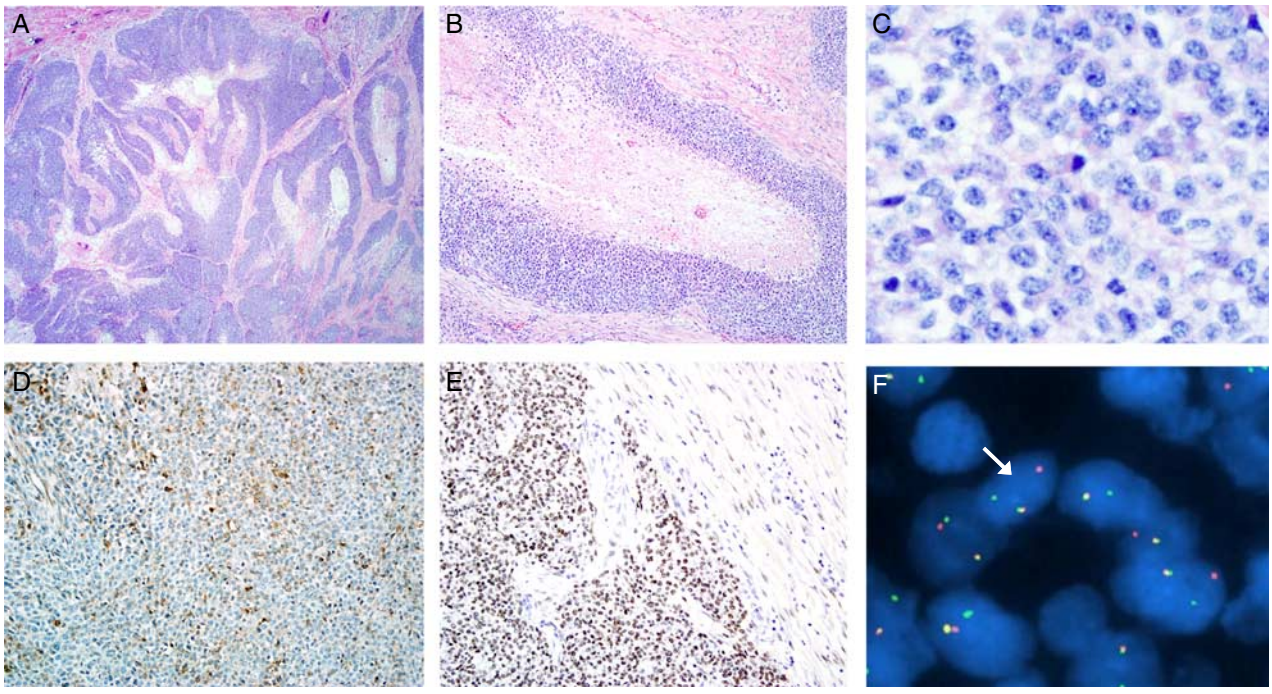

FIGURE 1. Posterior midline neck mass (patient S1) at low-power magnification (H&E) (A), at intermediate-power magnification (H&E) (B), and at high-power magnification (H&E) (C). D, CD99 immunostaining demonstrating patchy cytoplasmic and membranous positivity (CD99). E, WT1 showing uniform strong nuclear staining (WT1). F, Sample *CIC* rearrangement (arrow) demonstrated by break-apart FISH (courtesy of Dr Julia Bridge).

TABLE 1. Clinical, Molecular, and Immunohistochemical Findings of *CIC-DUX4* Sarcoma

Case	Age/ Sex	Location	Translocation/Genetic Confirmation	CD99	WT1	CK	Desmin	Myogenin	Clinical Course and Outcome
S1	10/F	Neck	*CIC* translocation FISH	+ (mem/ cyt)	+	–	–	–	Surgical resection Chemotherapy and radiation Alive at 12 mo, lung nodules
S2	19/F	Pelvis	t(4;19) Conventional karyotype	+ (mem)	–	+ (foc)	–	–	Neoadjuvant chemotherapy Surgical resection Alive, NED at 24 mo
S3	13/F	Neck	t(4;19) RT-PCR	+	+	+ (foc)	–	–	Neoadjuvant chemotherapy and local proton-beam radiation Surgical resection with negative margins DFS 16 mo (lung metastases) Alive at 22 mo, lung nodules
S4	15/M	Pelvis	t(4;19) FISH	+ (foc mem)	+	ND	–	–	Neoadjuvant chemotherapy and radiation Hemipelvectomy Alive, at 7.5 mo
S5	14/M	Paraspinal	t(10;19) Conventional karyotype	+ (mem)	+	+ (foc)	–	–	Neoadjuvant chemotherapy and radiation Surgical resection DFS 6 mo (myocardial metastasis) Died, OS 7 mo

Cyt indicates cytoplasmic; DFS, disease-free survival; F, female; Foc, focal; M, male; Mem, membranous; N, nuclear; ND, not done; NED, no evidence of disease; Neg, negative; OS, overall survival.

(n = 81, from 81 unique patients). The samples included surgically resected *CIC-DUX4* fusion-positive tumors (n = 5, including 3 harboring the t(4;19) translocation, 1 harboring the t(10;19) translocation, and 1 harboring a translocation involving the *CIC* gene locus without separate confirmation of the translocation partner) obtained either before or following the initiation of chemotherapy and radiotherapy regimens (3 and 2 cases, respectively). Genetic confirmation of the tumor-defining t(4;19)(q35;q13) or t(10;19)(q26;q13) translocation involving the *CIC* gene and either the *DUX4* gene or its paralog *DUX4L*, respectively, was achieved during the course of the clinical workup in 4 of 5 cases (S2 to S5) by conventional karyotyping performed on fresh tumor tissue, by RT-PCR or by break-apart FISH assay. One case (S1) was diagnosed by a positive FISH assay using break-apart probes solely to the *CIC* gene (Table 1; Supplemental Data 1, Supplemental Digital Content 1, http://links.lww.com/PAS/A443).

Other round cell tumors not characteristically harboring a *CIC-DUX4* fusion were collected. Pediatric and adult round cell tumors evaluated included Ewing sarcoma (20), Ewing-like sarcoma (*BCOR-CCNB3*) (1), alveolar rhabdomyosarcoma (11), embryonal rhabdomyosarcoma (9), synovial sarcoma (12), desmoplastic small round cell tumor (7), malignant rhabdoid tumor (3), neuroblastoma (9), and clear cell sarcoma (4) (Supplemental Data 1, Supplemental Digital Content 1, http://links.lww.com/PAS/A443).

To evaluate the likelihood for cross-reactivity of the DUX4 antibody to chimeric oncoproteins derived from both the t(4;19)(q35;q13) and the t(10;19)(q26;q13) translocations in a preexperimental, in silico setting, we conducted a comparative BLAST analysis of nucleotide sequences from *DUX4* gene and its *DUX4L* paralog (NCBI Blast Align, Bethesda, MD).

Representative formalin-fixed, paraffin-embedded sections from each sample were immunohistochemically stained for DUX4 using a mouse IgG1 monoclonal antibody (clone P4H2) raised against a synthetic peptide corresponding to the C-terminus of the human DUX4 protein (catalog no MA5-16147; Thermo Fisher Scientific, Waltham, MA). An automated Ventana Ultra staining platform was used to perform all immunohistochemical procedures, with the aid of an Optiview detection kit. Antibody staining was performed at a 1:200 dilution, and staining on tissue samples was conducted in parallel with appropriate positive (human adult testis) and negative (human skeletal muscle) controls, as per manufacturer recommendations.

DUX4 immunohistochemical staining was considered positive when definitive diffuse strong, crisp nuclear immunoreactivity was observed in tumor cells (Figs. 2A–C). Samples were scored as negative if tumor cells demonstrated no nuclear staining (Figs. 2D–F).

We additionally collected data from those immunohistochemical stains performed during the course of the clinical workup, including WT1, CD99, CK, desmin, and myogenin, and where not available, performed supplementary immunoassays using routine automated staining methods as described above (Table 1). Immunohistochemical stains for other markers recently reported to bear high levels of sensitivity and specificity in the distinction of *CIC-DUX4* fusion-positive round cell tumors, including ETV4,[6,12,13] were not performed in this study.

The above study was approved by the institutional review boards of all participating institutions.

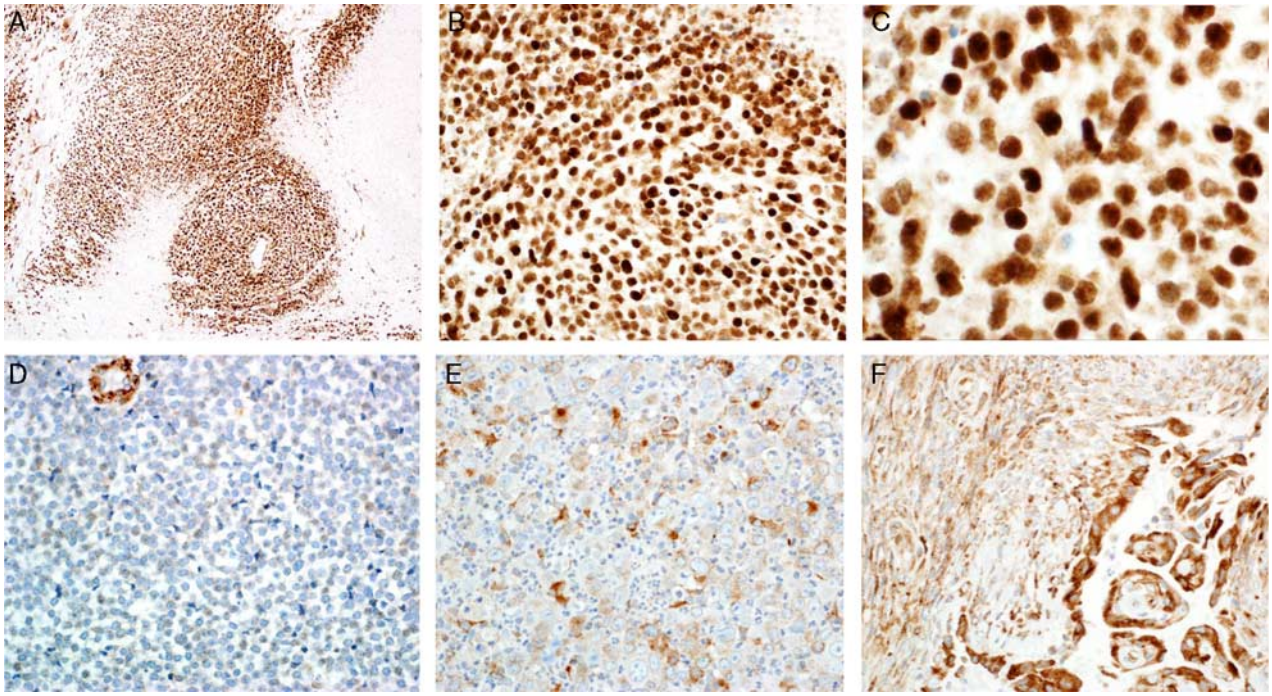

FIGURE 2. Positive DUX4 monoclonal antibody staining demonstrating tumor cell-specific strong, crisp nuclear staining in representative sections of a *CIC-DUX4* fusion-positive round cell tumor at low-power magnification (DUX4) (A) and at high-power magnifications (DUX4) (B and C). D, Negative DUX4 staining demonstrating complete lack of tumor cell staining in a Ewing sarcoma (DUX4). E, Negative DUX4 staining demonstrating absent nuclear staining and patchy mild cytoplasmic staining in a malignant rhabdoid tumor (DUX4). F, Negative DUX4 staining demonstrating absent nuclear staining and diffuse moderate, with focal strong, cytoplasmic staining in a synovial sarcoma, biphasic type (DUX4).

RESULTS

Clinical characteristics from the cohort of patients with *CIC-DUX4* fusion-positive round cell tumors are summarized in Table 1. Age at biopsy ranged from 10 to 19 years (mean, 14.2 y), and 3 of 5 patients were female. All tumors were extraosseous. Primary tumors arose in axial soft tissue, including the neck, paraspinal region, and pelvis.

Immunohistochemical profiling with broad panels of stains was the norm in the diagnostic workup in all cases, with CD99 showing positive membranous staining in a majority of cases, though membranous staining frequently proved patchy and admixed with cytoplasmic staining (Table 1). WT1 staining showed positive nuclear staining in 4 of 5 cases. Cytokeratin showed focal staining in the majority of cases. Muscle lineage–specific markers were uniformly negative.

A frequent presence of advanced disease at initial diagnosis and a short disease-free survival interval was seen among those with recurrent disease after definitive therapy (mean 11 mo; range, 6 to 16 mo), consistent with the well-established aggressive nature of *CIC-DUX4* fusion-positive round cell tumors.[6]

A BLAST analysis of nucleotide sequences from the *DUX4* gene and its *DUX4L* paralog demonstrated high levels of homology of nucleotide sequences, with Identities of 1277/1285 (99%) and Gaps of 6/1285 (< 1%) (Supplemental Data 2, Supplemental Digital Content 2, http://links.lww.com/PAS/A444), predicting that a monoclonal antibody raised against a part of or the whole DUX4 protein product would demonstrate sufficient cross-reactivity with the DUX4L component of the CIC-DUX4L chimeric oncoprotein that the immunoassay studied would be highly sensitive for the diagnosis of both the t(4;19)(q35;q13) and the t(10;19)(q26;q13) variants of *CIC-DUX4* fusion-positive round cell tumor.

Results of immunohistochemical staining on *CIC-DUX4* fusion-positive round cell tumors are illustrated in Figure 3. DUX4 immunohistochemical staining showed perfect correlation with translocation status in *CIC-DUX4* tumors, demonstrating uniform, strong nuclear staining in tumor cells of all 5 fusion-positive cases (5/5, sensitivity 100%) (Fig. 3). In all other round cell tumors studied, DUX4 staining was negative in tumor cell nuclei (0/76, specificity 100%), with cytoplasmic staining in tumor cells ranging from negative staining to diffuse moderate, with focally strong, positivity (Fig. 2). The positively staining *CIC-DUX4* fusion-positive round cell tumors notably included pretreatment and posttreatment cases in addition to cases harboring the t(4;19)(q35;q13) as well as the less common t(10;19)(q26;q13) variant translocation (S5).

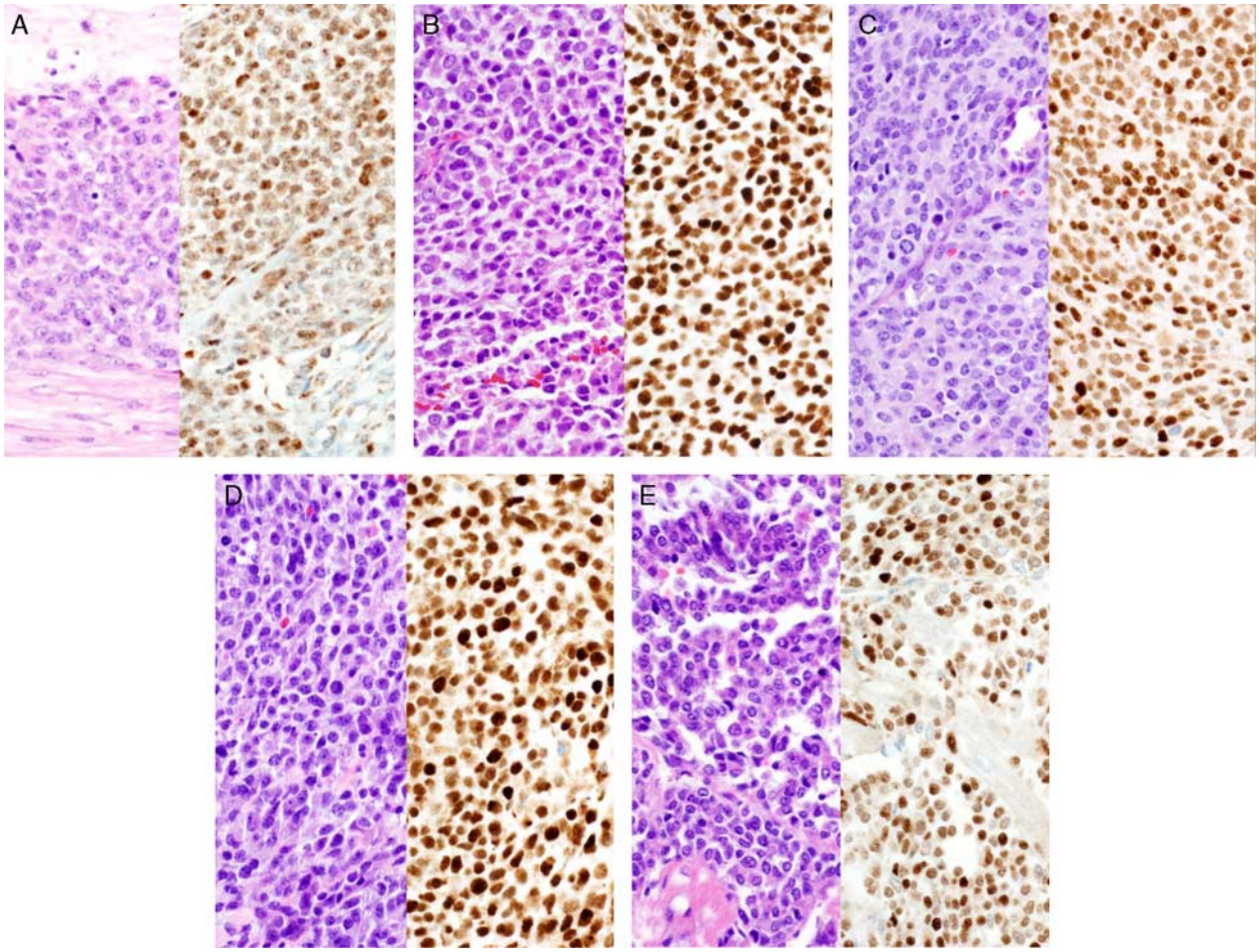

FIGURE 3. A–E, S1-S5, respectively. Positive DUX4 monoclonal antibody staining in 5 separate *CIC-DUX4* fusion-positive round cell tumors (H&E, DUX4).

DISCUSSION

CIC-DUX4 fusion-positive round cell tumors harboring the t(4;19)(q35;q13) or t(10;19)(q26;q13) translocation are a recently described class of round cell tumors resembling Ewing sarcoma but lacking characteristic Ewing sarcoma gene fusions and bearing a significantly worse clinical prognosis. Recent studies have provided evidence of a characteristic immunohistochemical profile and distinct transcriptional signature for these tumors, supporting the assertion that *CIC-DUX4* fusion-positive round cell tumors represent a distinct pathologic entity and laying the groundwork for their immunohistochemical characterization and workup.[6,12,13]

There is currently a small but growing cohort of translocation-associated tumors, including alveolar soft part sarcoma (*ASPSCR1-TFE3*), Xp11.2 translocation renal cell carcinoma (*ASPSCR1-TFE3*), NUT-midline carcinoma (*BRD3/4-NUT*), solitary fibrous tumor (*NAB2-STAT6*), and *ALK+* anaplastic large cell lymphoma (*NPM-ALK*), for which staining with a monoclonal antibody raised against a single protein constituent of the chimeric oncoprotein is clinically utilized as a surrogate marker for the presence of the chimeric oncoprotein and for diagnosis of the tumor entity.[14–17] These assays are used, variously, to establish a diagnosis and/or to direct decisions about ancillary cytogenetic or genetic testing.

In our study, the use of a monoclonal antibody raised against the C-terminus of the DUX4 protein under standard immunohistochemical conditions showed perfect correlation with *CIC-DUX4* fusion status in a cohort of *CIC-DUX4* fusion-positive round cell tumors and other round cell tumor controls. Five cases with a previously confirmed translocation all demonstrated diffuse and strong nuclear staining with the DUX4 antibody (5/5, 100% sensitivity, Fig. 3). Other round cell tumors, including a wide range of pediatric and adult tumors, were uniformly negative for nuclear staining by the DUX4 antibody (0/76, 100% specificity). Our finding that staining against the CIC-DUX4 protein is localized to the nucleus is consistent with previous observations of the localization of the chimeric protein not being significantly different from the localization of wild-type CIC.[10,18]

The crisp nuclear staining pattern of the DUX4 antibody offered easy-to-interpret results for the ready, unequivocal distinction of positive and negative *CIC-DUX4* fusion status. In our study the uniform DUX4 nuclear staining was observed in all samples originating from multiple hospital systems, suggesting that the DUX4 antibody with standard immunohistochemistry protocol will perform across a broad spectrum of fixation and processing platforms in a wide range of pathology laboratories.

Turnaround time for the protocol was rapid, requiring $\leq$1 day from the initiation of the protocol on unstained slides to the availability of a microscopically evaluable immunohistochemically stained slide. By comparison, currently available cytogenetic and molecular methods to identify gene fusions in undifferentiated round cell sarcomas can be time consuming and costly and may engender trade-offs between rapidity of diagnosis and optimal resource utilization. In the setting of the workup of an undifferentiated round cell sarcoma, for instance, *EWSR1* rearrangement may be the first test ordered, and, if followed by ancillary testing conducted in a stepwise manner, can set in place a time frame of days or weeks before specific genetic testing for the *CIC-DUX4* translocation is pursued. The availability and utilization of a reliable, molecularly targeted, easy-to-interpret immunoassay for the rapid evaluation of *CIC-DUX4* translocation status may avoid the diagnostic delay and added costs of a substantial contingent of such cases.

Our immunohistochemistry results supported the prediction, based upon in silico modeling from BLAST data, that a monoclonal antibody raised against the DUX4 protein would demonstrate cross-reactivity and correspondingly equivalent immunohistochemical staining of *CIC-DUX4* fusion-positive round cell tumors harboring t(4;19)(q35;q13) and t(10;19)(q26;q13) translocations. These results collectively reinforce the potentially broad-based utility of the DUX4 antibody as a diagnostic marker for the *CIC-DUX4* fusion-positive round cell tumor class as a whole.

Recent studies have proposed that immunohistochemical staining for WT1 and ETV4 may constitute fairly sensitive diagnostic markers for *CIC-DUX4* translocation round cell tumors.[6,12,13] Data for WT1 staining in our series (4/5, sensitivity 80%) support the previously reported findings that WT1 has high but not perfect sensitivity for diagnosis of this entity. The molecularly targeted immunohistochemical stain using a monoclonal antibody raised against DUX4 was shown in our cohort of cases to have a comparable, if not superior, sensitivity and specificity profile, and, as such, might be utilized as a critical adjunctive marker in the immunohistochemical workup of these tumors.

Although the sample size of our study is small, *CIC-DUX4* fusion-positive round cell tumor is recognized as an exceedingly rare entity. Three major hospital centers were included in the study to maximize the quantity of cases assessed. Several additional medium-sized to-large-sized case cohorts have been amassed in recent years,[6,11–13,19] any of which may constitute an opportune resource for further validation of these findings.

A potential limitation of this immunohistochemical protocol as proposed is that the subset of recently reported but exceedingly rare tumors harboring translocations involving the *CIC* gene but without either the *DUX4* or the *DUX4L* partner, for example, *CIC-FOXO4*,[20] would not be detected by the DUX4 antibody as described. If these so-far exceptionally rare tumors are eventually grouped with tumors containing either the *CIC-DUX4* or *CIC-DUX4L* translocation to comprise a single clinicopathologic entity, an alternative diagnostic modality would be required to produce a complete and accurate diagnosis. Additional efforts are currently underway to identify and evaluate rare and unique variants for future analysis by this immunohistochemical protocol.

An additional limitation of the protocol elaborated in this study is that raised from the recent description of a subtype of adolescent and young adult (AYA) B-cell precursor acute lymphoblastic leukemia cases characterized by *IGH-DUX4* and *ERG-DUX4* fusion proteins.[21] In light of the demonstration of this leukemia subtype, which would likewise be anticipated to stain positively for an immunohistochemical stain directed against the C-terminal region of the DUX4 protein, it is expected that DUX4 staining will not be wholly exclusive to solid *CIC-DUX4* fusion-positive round cell tumors, representing a real-world specificity of $<100\%$. Case reports of rhabdomyosarcomas harboring translocations involving the *DUX4* gene suggest further isolated limitations to the overall specificity of the immunohistochemical stain.[22]

These latter limitations, beyond marginally reducing the potential real-world specificity of the immunohistochemical protocol with the DUX4 antibody, also suggest a broader utility for this immunohistochemical marker in the diagnostic workup and molecular characterization of a range of pediatric and adult malignancies. We anticipate the refinement and expansion of this utility in ongoing and future studies.

In conclusion, we found a monoclonal antibody raised against DUX4 to be a highly specific and sensitive test for the differentiation of *CIC-DUX4* fusion-positive round cell tumor from its histologic mimics. We recommend including this tool in the diagnostic workup of round cell tumors and undifferentiated sarcomas.

ACKNOWLEDGMENT

The authors thank Dr Julia Bridge who kindly contributed the FISH image in Figure 1F.

REFERENCES

1. Antonescu C. Round cell sarcomas beyond Ewing: emerging entities. *Histopathology*. 2014;64:26–37.
2. Fletcher CD, Bridge JA, Hogendoorn P, et al. *WHO Classification of Tumors of Soft Tissue and Bone*, 4th ed. Lyon, France: IARC Press; 2013.
3. Marino-Enriquez A, Fletcher CD. Round cell sarcomas—biologically important refinements in subclassification. *Intl J Biochem Cell Biol*. 2014;53:493–504.
4. Choi EY, Thomas DG, McHugh JB, et al. Undifferentiated small round cell sarcoma with t(4;19)(q35;q13.1) CIC-DUX4 fusion: a

novel highly aggressive soft tissue tumor with distinctive histopathology. *Am J Pathol*. 2013;37:1379–1386.
5. Graham C, Chilton-MacNeill S, Zielenska M, et al. The CIC-DUX4 fusion transcript is present in a subgroup of pediatric primitive round cell sarcomas. *Hum Pathol*. 2012;43:180–189.
6. Specht K, Sung YS, Zhang L, et al. Distinct transcriptional signature and immunoprofile of CIC-DUX4 fusion-positive round cell tumors compared to EWSR1-rearranged Ewing sarcomas: further evidence toward distinct pathologic entities. *Genes Chromosomes Cancer*. 2014;53:622–633.
7. Lee CJ, Chan WI, Scotting PJ. CIC, a gene involved in cerebellar development and ErbB signaling, is significantly expressed in medulloblastomas. *J Neurooncol*. 2005;73:101–108.
8. Cerami E, Gao J, Dogrusoz U, et al. The cBio Cancer Genomics Portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401–404.
9. Van Geel M, Dickson MC, Beck AF, et al. Genomic analysis of human chromosome 10q and 4q telomeres suggests a common origin. *Genomics*. 2002;79:210–217.
10. Kawamura-Saito M, Yamazaki Y, Kaneko K, et al. Fusion between CIC and DUX4 up-regulates PEA3 family genes in Ewing-like sarcomas with t(4; 19)(q35; q13) translocation. *Hum Mol Genet*. 2006;15:2125–2137.
11. Smith SC, Buehler D, Choi EY, et al. CIC-DUX sarcomas demonstrate frequent MYC amplification and ETS-family transcription factor expression. *Mod Pathol*. 2015;28:57–68.
12. Hung YP, Fletcher CD, Hornick JL. Evaluation of ETV4 and WT1 expression in CIC-rearranged sarcomas and histologic mimics. *Mod Pathol*. 2016;29:1324–1334.
13. Le Guellec S, Velasco V, Perot G, et al. ETV4 is a useful marker for the diagnosis of CIC-rearranged undifferentiated round-cell sarcomas: a study of 127 cases including mimicking lesions. *Mod Pathol*. 2016. doi:10.1038/modpathol.2016.155. [Epub ahead of print].
14. Doyle LA, Vivero M, Fletcher CD, et al. Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics. *Mod Pathol*. 2014;27:390–395.
15. Armah HB, Parwani AV. Xp11.2 translocation renal cell carcinoma. *Arch Pathol Lab Med*. 2010;134:124–129.
16. Haack H, Johnson LA, Fry CJ, et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. *Am J Surg Pathol*. 2009;33:984–991.
17. Sheffield BS. Immunohistochemistry as a practical tool in molecular pathology. *Am J Surg Pathol*. 2016;140:766–769.
18. The Human Protein Atlas. CIC. 2016. Available at: http://www.proteinatlas.org/ENSG00000079432-CIC/antibody. Accessed June 13, 2016.
19. Gambarotti M, Benini S, Gamberi G, et al. CIC-DUX4 fusion-positive round cell sarcomas of soft tissue and bone: a single institution morphologic and molecular analysis of 7 cases. *Histopathology*. 2016;69:624–634.
20. Sugita S, Arai Y, Tonooka A, et al. A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. *Am J Surg Pathol*. 2014;38:1571–1576.
21. Lilljebjorn H, Henningsson R, Hyrenius-Wittsten A, et al. Identification of ETV6-RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblastic leukemia. *Nat Commun*. 2016;7:11790.
22. Sirvent N, Trassard M, Ebran N, et al. Fusion of EWSR1 with the DUX4 facioscapulohumeral muscular dystrophy region resulting from t(4;22)(q35;q12) in a case of embryonal rhabdomyosarcoma. *Cancer Genet Cytogenet*. 2009;195:12–18.