Published in final edited form as:

Am J Surg Pathol. 2017 July ; 41(7): 941–949. doi:10.1097/PAS.0000000000000846.

Sarcomas with *CIC*-rearrangements are a distinct pathologic entity with aggressive outcome: A clinicopathologic and molecular study of 115 cases

Cristina R. Antonescu¹, Adeptan A. Owosho², Lei Zhang¹, Sonja Chen¹, Kemal Deniz³, Joseph M. Hurn², Yu-Chien Kao^{1,4}, Shih-Chiang Huang^{1,5}, Samuel Singer², William Tap⁶, Inga-Marie Schaefer⁷, and Christopher D Fletcher⁷

¹Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

²Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

³Erciyes University Department of Pathology; Kayseri, Turkey

⁴Department of Pathology, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan

⁵Department of Anatomical Pathology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan

⁶Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

⁷Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Abstract

CIC-DUX4 gene fusion, resulting from either a t(4;19) or t(10;19) translocation, is the most common genetic abnormality detected in *EWSR1*-negative small blue round cell tumors (SBRCTs). Following their discovery it was debated if these tumors should be classified as variants of Ewing sarcoma (i.e. atypical Ewing sarcoma) or as a stand-alone pathologic entity. As such the WHO classification temporarily grouped the *CIC*-rearranged tumors under undifferentiated sarcomas with round cell phenotype, until further clinical evidence was available. However, most studies reported so far include small series with limited follow-up information which preclude a more definitive assessment. The present work investigates the clinicopathologic features of a large cohort of sarcomas with *CIC* gene rearrangement, in order to define their clinical presentation, morphologic spectrum, and outcome. Our study further examines the overall survival of the *CIC*-positive cohort compared to a control group of *EWSR1*-rearranged Ewing sarcoma matched for age and stage. The study cohort included 115 patients, with a mean age of 32 years and a slight male predominance. Most tumors occurred in the soft tissue (86%), predominantly deep-seated and equally divided among trunk and extremity, followed by visceral locations (12%) and rarely in the bone (3%). Microscopically, most tumors showed round to ovoid cytomorphology but half of the cases showed also focal areas of spindling and epithelioid/

*Corresponding Author: Cristina R Antonescu, MD, Memorial Sloan Kettering Cancer Center, Department of Pathology, 1275 York Ave, New York, NY, Phone: (212) 639-5905; antonesc@mskcc.org.

Conflicts of interest: none

rhabdoid phenotype, with frequent myxoid stromal changes. Variable CD99 reactivity was seen in 84% cases, with a diffuse pattern only in 23% of cases, while nuclear WT1 was seen in 92%. A *CIC-DUX4* fusion was detected in 57% of cases, with either *DUX4* on 4q35 (35%) or on 10q26 in 25 (22%) cases. No *FOXO4* gene rearrangements were present in 39 cases tested. Clinical follow-up was available in 57 patients, with a 5-year survival of 43%, which was significantly lower than the 77% 5-year survival in the control Ewing sarcoma group ($p=0.002$). Our findings show that *CIC-DUX4* sarcomas occur most commonly in young adults within the somatic soft tissues, having a wide spectrum of morphology including round, epithelioid and spindle cells, and associated with an aggressive clinical course, with an inferior overall survival compared to Ewing sarcoma. The results support the classification of *CIC*-rearranged tumors as an independent molecular and clinical subset of SBRCTs distinct from Ewing sarcoma.

Keywords

CIC; *DUX4*; SBRCT; round cell sarcoma

INTRODUCTION

According to the current WHO classification¹, undifferentiated round cell sarcomas are characterized by relatively monotonous round to ovoid cytomorphology, with a high nuclear to cytoplasmic ratio, and no distinct line of differentiation, lacking consistent genetic abnormalities. However, as they most often resemble Ewing sarcoma, for practical and treatment purposes, round cell undifferentiated sarcomas have been regarded as ‘Ewing sarcoma-like’ and are often managed similarly to the Ewing sarcoma family of tumors. In contrast to classic Ewing sarcoma, round cell undifferentiated sarcomas lack the pathognomonic translocations involving the *EWSR1* gene on chromosome 22 fused to a member of the *ETS* transcription factor family, either *FLI1*,² *ERG*,^{3,4} or other less common variants. Within this undifferentiated subgroup, the present WHO classification included round cell sarcomas with *CIC-DUX4* fusions, as a temporary subset, until further evidence, both at the clinical and molecular level, could determine if they represent an independent pathologic entity.

CIC-rearranged sarcomas have been relatively recently described as aggressive tumors arising in soft tissues of children and young adults. Although they share partial morphologic overlap with Ewing sarcoma and variable CD99 expression, emerging molecular data suggest that *CIC-DUX4* tumors have a distinct pathogenesis. The *CIC-DUX4* fusion results from either a t(4;19)(q35;q13) or a t(10;19)(q26;q13) translocation^{5,6,7,8,9}. The genes involved in the fusion are *CIC*, a transcriptional repressor on chromosome 19q13.1, and *DUX4*, a double homeobox transcription factor, located on either 4q35 or chromosome 10q26.3. Whether the group of *CIC-DUX4*-positive round cell sarcomas represents a stand-alone category of tumors or a subgroup of the Ewing sarcoma family has been a matter of debate. In the present study we attempt to elucidate this controversy by investigating a large cohort of 115 molecularly confirmed cases to provide a more definitive classification based on detailed clinical presentation, histologic spectrum, immunoprofile and outcome.

MATERIAL AND METHODS

Patients and Tumor Characteristics

The MSKCC files and personal consultation files of the senior authors (CRA, CDF) were searched for the diagnosis of small blue round cell tumors (SBRCTs) and Ewing sarcoma-like tumors with available tissue for molecular analysis. All cases included were positive for *CIC* gene rearrangements by FISH, being selected from SBRCTs lacking *EWSR1* and *FUS* gene rearrangements (or other common sarcoma-associated translocations, e.g. *SS18*). A total of 115 SBRCTs with *CIC*-related fusions were identified during a two-decade period (1995–2016). Hematoxylin and eosin-stained slides and previously performed immunohistochemical stains were reviewed in all cases. All cases were handled in accordance with the ethical rules of the respective institutions.

For each case, the location of the tumor was recorded, along with the anatomic structures involved. The tumors were assessed for growth pattern, cytomorphology (round, oval, spindle, epithelioid, plasmacytoid/rhabdoid phenotype), cellular pleomorphism, nuclear features including nuclear contour, chromatin pattern and presence of nucleoli, mitotic activity, necrosis, type of stroma and myxoid change. The immunohistochemical stains were re-reviewed and a minimum panel was available for review in most cases, including CD99, WT1, cytokeratin and/or EMA, and desmin. However, most cases in fact had a much wider panel of stains performed, including neural/neuroendocrine markers, lymphoid markers which were negative and which excluded other diagnostic considerations.

As most of the cases tested were received in consultation, complete follow-up data was available in only 57 (50%) patients. The following clinical data were retrieved: tumor size, stage at diagnosis (primary versus distant metastasis at diagnosis), modality of initial therapy, recurrence, vital status and survival time. In addition, from a large cohort of 127 Ewing sarcoma patients with molecular confirmation treated at MSKCC, we selected a group of 57 Ewing sarcomas equivalent to the number of the *CIC*-positive cohort, closely matched for age and stage, for an accurate comparison for overall survival (OS). A further statistical analysis was performed focusing only on patients with localized disease at diagnosis, thus comparing 45 *CIC*-rearranged SBRCTs with 45 primary Ewing sarcoma patients for OS. The diagnosis of Ewing sarcoma was confirmed by either RT-PCR amplification of an *EWSR1-FLI1/ERG* transcript or an *EWSR1* gene rearrangement by FISH.

Fluorescence in situ Hybridization (FISH)

FISH analysis was performed on interphase nuclei from paraffin-embedded 4 µm sections using bacterial artificial chromosomes (BAC clones), flanking *CIC* in 19q13 and *DUX4* on 4q35 and 10q26.3 as previously described⁷. Two hundred tumor nuclei were evaluated using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems). Cases that were negative for *DUX4* gene abnormalities were also investigated for the *FOXO4* gene rearrangements, as previously described^{10,11} (Supplementary Table 1). A cut-off of >20% nuclei showing a break-apart signal was

considered to be positive for rearrangement. Nuclei with incomplete set of signals were omitted from the score.

Statistical analysis

Statistical analysis was performed on an SPSS platform (version 24.0; IBM Corp., Armonk, NY). The associations between the clinical variables and matched groups were evaluated by Fisher's exact test. The OS time was measured in months from the date of diagnosis to the date of death. Kaplan-Meier estimate was used to calculate the OS. The statistical significance of different clinicopathologic variables (gender, age group [adult/pediatric], tumor size [≤5cm/>5cm], presence of metastasis at diagnosis, and recurrence status) in relation to survival was assessed by log-rank analysis. The OS between *CIC* fusion positive SBRCT and Ewing sarcoma patients were compared by log-rank analysis. A $p<0.05$ was considered as significant for all statistical analyses.

RESULTS

Patients Demographics and Clinical Presentation

Among the 115 patients included, there was a slight male dominance, with 63 (55%) males and 52 (45%) females. The age at diagnosis ranged from 6–81 years, with a mean of 32 years. Twenty-five patients (22%) were in the pediatric age group (<18 years of age). The anatomic location of the primary tumor was known in 111 cases (Table 1). Two additional patients presented with metastases to brain and lung (unknown primary tumors). Ninety-five cases (86%) arose within soft-tissue, 3 (3%) cases in bone (all involving pelvic bones) and 13 (12%) cases were visceral (mostly involving GI and GU organs, see Table 1). Among the soft tissue tumors, most were located deep within the muscle, with only 7 (7%) cases being superficial. Soft tissue tumors were equally distributed between trunk ($n=39$) and extremity ($n=38$), with less common presentation in the head and neck ($n=12$) and retroperitoneum/intra-abdominal/pelvic location ($n=6$). Within the limbs, 31 tumors occurred in the lower and 7 in the upper extremity.

Microscopic Findings

All cases showed solid proliferation of small to medium-sized neoplastic cells. One-third of the cases showed a nodular or vaguely nodular appearance with thick collagenous septa separating the tumor into compartments (Fig. 1A). Less common growth patterns included intersecting short fascicles in 2 cases (Fig. 1B) and a reticular architecture pattern (Fig. 1C) in one case. Thirty-five cases showed variably prominent myxoid stromal change, which was moderate to diffuse in 12 cases (Fig. 1D). No prominent collagenous stroma was identified in these tumors.

Most of the cases displayed round to ovoid cytomorphology, with 57 (50%) cases being composed purely of round/ovoid cells (Fig 1E). Twelve cases (10%) showed a mixture of round/ovoid and spindle cells, with one of the cases displaying a predominant (>50%) spindle cell morphology (Fig 1B). Twenty-seven (23%) cases had a mixture of round/ovoid and plasmacytoid/rhabdoid cells, with 12 (11%) showing predominantly (>50%) a plasmacytoid/rhabdoid phenotype (Fig. 1F). Nineteen (17%) cases showed a mixture of

round/ovoid, spindle and plasmacytoid/rhabdoid, however, the predominant (>50%) component was composed of round/ovoid cells.

Most tumors showed cells with scant lightly eosinophilic to clear cytoplasm, which was more abundant in the epithelioid/rhabdoid component. The nuclear features displayed various chromatin patterns: including 33 cases with fine (Fig. 1G), 22 cases with hyperchromatic (Fig. 1H), 60 with a vesicular chromatin pattern (Fig. 1I). Multifocal prominent nucleoli were observed in 38 (33%) of the tumors (Fig. 1G, I). Most tumors had relatively monomorphic cytomorphology, with only mild nuclear pleomorphism. However, in 15 (13%) cases there was some degree of moderate nuclear pleomorphism (Fig. 1H), in 6 being more diffuse and in 9 cases only focal. The mitotic count varied from 6–99 per 10 HPFs, with a mean of 32 MF/10 HPFs. Most (84%) cases showed geographic necrosis.

Immunohistochemical Findings

Immunohistochemical stains including CD99, WT1, AE1/AE3, and desmin were evaluated. One hundred and ten cases were evaluated for CD99: 25 (23%) showed diffuse positivity (Fig. 2A), 67 (36 multifocal, 31 focal)(61%) cases multifocal/focal positivity (Fig. 2B), while 18 (16%) were negative. Forty-eight (74%) of 65 cases evaluated showed diffuse WT1 nuclear expression (Fig. 2C), while 12 (18%) cases were multifocally/focally positive. No staining for WT1 was noted in 4 cases. Focal staining for AE1:AE3 and desmin was present in 16 and 4 cases, respectively, of the 110 cases evaluated.

Fluorescence in situ Hybridization (FISH)

All cases showed a *CIC* gene break-apart signal. Cases with other *CIC* gene abnormalities, such as small, constant gaps suspicious for inversion, were not considered as a positive result and were not included in the study cohort. As the *DUX4* gene is located in the subtelomeric region of 4q35 or 10q26, a FISH split assay could not be designed due its proximity to the telomeric area. Thus, the alternative method was to interrogate the *CIC* gene partner by using a 3-color FISH assay for telomeric *CIC* and centromeric *DUX4*, as previously described.⁷ The results showed that 40 (35%) cases were positive for fusion to *DUX4* gene on 4q35 (Fig. 2D), while 25 (22%) were fused to *DUX4* on 10q26 (Fig. 2E). In the remaining 43% of the cases a fusion partner could not be demonstrated (Fig. 2 F); however, in about one-third of these cases (n=14) the fusion assay was not informative due to a *CIC* telomeric deletion, which precluded evaluation of its fusion partner. From the 50 cases lacking a *DUX4* gene partner, 39 cases had available material for further FISH *FOXO4* break-apart assay, as previously described in 2 cases,^{10,11} however, no *FOXO4* gene abnormalities were identified.

Follow-up data

Clinical follow-up information was available in 57 patients (50%), ranging from 1 to 269 months, median of 14 months. This cohort was representative of the entire group, with 33 (58%) males and 24 (42%) females, with an age range of 10–81 years (median of 31 years). Forty-eight (84%) patients were of adult age (>18 years). The tumor size ranged from 0.7–23 cm, with 32 (64%) patients presenting with large sized tumors (>5 cm). Nine (16%) patients presented with distant metastasis at initial diagnosis to the lung (n=9), liver, brain or

supraclavicular lymph nodes. Among the patients who presented with primary disease, 25 developed recurrence following treatment: 4 patients had local recurrence (LR) alone, 17 patients developed distant recurrence (DR) and 4 patients developed both LR and DR (lungs and pelvis). Overall the metastatic rate was 53% (30/57) and 44% (21/48) in the localized group. The lungs were the most common site for distant recurrence (16/21, 76%). Twenty-three (40%) patients died of disease (DOD) at last follow up. Twenty-two of 23 (96%) patients who remained free of disease are alive, while only 5/21 (24%) patients who recurred distantly are still alive.

***CIC*-positive tumors treated with neoadjuvant chemotherapy showed an inferior pathologic response and poor outcome**

52 patients had detailed therapy information, with 22 receiving neoadjuvant chemotherapy after the diagnosis was established on a core biopsy, while 29 patients had an initial surgical resection with curative intent, followed by adjuvant chemotherapy in 22 patients and radiation in 2. The overwhelming number of patients (>90%) were treated following a Ewing sarcoma chemotherapy regimen. Five patients were treated with surgery alone. One patient received only radiotherapy, due to advanced age and anatomic location of the tumor (80/M, paraspinal). Ten patients presenting with localized disease and treated with neoadjuvant chemotherapy could be evaluated for treatment response in the resection specimen showing that only 3 had >90% therapy-related changes (fibrosis), consistent with a grade III chemotherapy response. The remaining 7 (70%) patients had an inferior degree of pathologic response, either <50% necrosis (n=4, grade I response) or >50% but <90% (n=3, grade II), using the 4-tier grading system applied for Ewing sarcoma. However there was no correlation between the degree of pathologic response and survival in these 10 patients analyzed.

Interestingly, patients treated with neoadjuvant chemotherapy (n=22) showed an inferior survival compared to patients managed by surgery first (n=29) (p=0.025), although the latter group was heterogeneous and included 22 patients with adjuvant chemotherapy, 2 with adjuvant radiation, and 5 with surgery alone. This survival advantage was retained when comparing only patients with localized disease who received neoadjuvant chemotherapy (n=17, of whom 13 underwent surgical resection) versus those managed by surgery first (n=25, of whom 18 were treated with adjuvant chemo)(p=0.041). However, patients selected for neoadjuvant therapy had a larger tumor size (p<0.0001) compared to patients who were managed by surgery first, with no difference in age or anatomic location. Patients who were treated for localized disease with surgery and adjuvant chemotherapy (n=18) had only a trend for improved survival compared to patients treated with neoadjuvant chemotherapy (n=17)(p=0.084). Among the 5 patients who had surgery alone (tumor size range 3.5–5, mean 4.1 cm), none developed distant recurrences or died of disease. The 2 patients who were treated with surgery followed by radiation alone are both alive without distant recurrence.

Survival analysis of *CIC*-rearranged sarcoma patients

On survival analysis, the *CIC*-rearranged sarcoma patients showed a 53% 2-year OS and a 43% 5-year OS rates (Fig. 3A). When limited to the 45 *CIC*-rearranged sarcoma patients

presenting with localized disease, the 2-year and 5-year OS rates were 59% and 49%. There was no significant effect on OS related to gender (male vs. female; $p=0.178$), age (adult vs. pediatric; $p=0.269$) and tumor size (≤ 5 cm vs. >5 cm; $p=0.378$). Patients who presented with metastatic disease at diagnosis had a poor prognosis compared to patients with primary disease ($p<0.0001$)(Fig. 3B). Also, presence of recurrence (NR vs. LR vs. DR) was statistically significant on OS ($p<0.0001$)(Fig. 3C). The survival analysis of *CIC*-rearranged sarcoma patients based on clinical variables is presented in Supplementary Table 2.

***CIC*-fusion positive SBRCTs have a significantly unfavorable outcome compared to Ewing sarcoma**

The survival comparison between *CIC*-rearranged SBRCTs and Ewing sarcoma patients is presented in Supplementary Table 3. The 57 Ewing sarcoma cohort (matched for stage and age) had a significantly longer OS compared to *CIC*-rearranged sarcoma patients ($p=0.002$), with a 2-year and 5-year survival of 87% and 77%, respectively (Fig. 3D). This survival difference was retained when comparing only patients with localized disease for both cohorts ($n=45$), with the 5-year survival for *CIC*-positive cases at 49% compared to 76% for Ewing sarcoma patients ($p=0.035$). In fact, the 5 year-survival for the whole cohort of localized Ewing sarcoma patients, not skewed by age ($n=106$, out of 127 cases total available from our database), was even more favorable at 82% ($p=0.005$).

DISCUSSION

This study investigates a large cohort of 115 *CIC*-rearranged sarcomas providing further and more definitive evidence on its distinctive clinical and pathologic characteristics, in favor of a stand-alone molecularly defined entity. Initially classified under the umbrella of the Ewing sarcoma family, our findings reinforce important differences between these two round cell sarcomas. First, the anatomic distribution is different, with the overwhelming majority of *CIC-DUX4*-sarcomas, 86% in this series, occurring in the somatic soft tissues, equally distributed between extremities and trunk, followed by visceral location and infrequently in the bone. In contrast, most Ewing sarcomas occur in skeletal locations, either in the flat bones of the pelvis or chest wall or the diaphysis of long bones.¹ Second, *CIC-DUX4*-positive tumors preferentially affect young adults with a peak incidence in the fourth decade, with slight male predominance. In fact only 22% of the patients included in this study were of pediatric age group. In contrast, patients with Ewing sarcoma have a mean age at diagnosis of 15 years,¹² also with a slight male predominance. Although both tumor entities share an undifferentiated and mostly monotonous proliferation of round to ovoid cells, there are certain distinctive features associated with *CIC-DUX4*-positive tumors, most importantly a wider spectrum of cytomorphologies, with mixture of round, spindle and epithelioid cells. Although half of the cases showed mainly a round to ovoid phenotype, the remaining cases revealed, in addition, focal areas of spindle or epithelioid cells. Furthermore, the tumor cells showed increased nuclear size and shape variability, vesicular chromatin with focally prominent nucleoli, in addition to more abundant, typically light eosinophilic cytoplasm. Stromal myxoid change was a common finding in *CIC*-positive tumors, which is typically absent in Ewing sarcoma.^{13,14}

Immunophenotypically, as previously reported, most *CIC*-rearranged tumors (84%) showed variable expression of CD99, but only 23% with a diffuse pattern and 16% were completely negative. This is in contrast with classic Ewing sarcomas which typically show diffuse and strong membranous reactivity with CD99 in most if not all cases. Furthermore, nuclear WT1 reactivity was also a consistent finding in *CIC*-positive sarcomas, in contrast with Ewing sarcoma, as previously noted, most likely related to its transcriptional up-regulation.¹⁵

A *CIC-DUX4* fusion was detected in 57% of cases with either *DUX4* on 4q35 (35%) or *DUX4* on 10q26 in 25 (22%) cases. *CIC-DUX4* fusion appears functionally unrelated to *EWSR1-ETS*, with the transcriptional profile of *CIC-DUX4*-positive sarcomas being distinct from that of Ewing sarcoma or other sarcoma subtypes, in keeping with separate tumor entities.¹⁵ Prior evidence has demonstrated that *CIC-DUX4* sarcomas overexpress the PEA3 (polyoma enhancer activator 3) subfamily of transcription factors, including *ETV1*, *ETV4*, and *ETV5*, both at mRNA and protein levels.^{5,15–17} *CIC* is the human homologue of *Drosophila capicua*, a gene identified in a screen for mutations affecting the anterior-posterior pattern of *Drosophila* embryos.¹⁸ *CIC* gene abnormalities have been implicated in various neoplastic conditions, such as *CIC* loss of function mutations being identified in 83% of oligodendrogliomas.^{19,20} *CIC* encodes a transcriptional repressor with a high-mobility group (HMG)-box containing DNA binding domain that normally inhibits *ETV1/4/5* expression and regulates receptor tyrosine kinase signaling pathways.^{21,22} Using an experimental cell line model system, Kawamura-Saito et al. demonstrated binding of this *CIC* HMG box to the promoter of *PEA* genes *ETV1* and *ETV5*. Their results further revealed that fusion of *DUX4* to *CIC* sequence provides strong transcriptional activity, resulting in mostly upregulated gene expression, with minimal down-regulated genes.⁵ Similar *ETV1/4/5* upregulation is also present in other *CIC* related fusions, such as *CIC-LEUTX* positive angiosarcoma²³ *CIC-NUTM1*-positive central nervous system primitive neuroectodermal tumor (PNET).^{23,24}

In contrast, *DUX4* gene is normally expressed in germ cells and is epigenetically silenced in somatic differentiated tissues through CpG methylation.²⁵ Aberrant expression of *DUX4* has been implicated in the development of facioscapulohumeral muscular dystrophy.²⁶ The exact role in tumorigenesis of *DUX4* dysregulation in the setting of *CIC-DUX4* fusion remains poorly defined. Recently, *CIC-DUX4* sarcoma has been shown to exhibit strong *DUX4* immunoexpression, while other translocation-positive round cell sarcomas (i.e. Ewing sarcoma, alveolar rhabdomyosarcoma, synovial sarcoma, desmoplastic small round cell tumor) did not.²⁷ *DUX4* gene is located within the D4Z4 macrosatellite repeat region (11–100 copies of the repeat units) of the chromosomes 4 and 10 subtelomeric regions.²⁸

Two SBRCT cases were reported quite recently to have *CIC* alternative fusions involving *FOXO4* gene on Xq13.^{10,11} Of interest both cases occurred in the head and neck soft tissue (neck and scalp) in a 65 year-old male and 13 year-old male, respectively. No *FOXO4* gene abnormalities were detected in any of the 39 cases tested lacking *DUX4* abnormalities in the present series, including head and neck lesions, suggesting that *CIC-FOXO4* fusion is a rare genetic event in the pathogenesis of SBRCTs. Also of interest, a small subset of high grade angiosarcomas occurring in the soft tissue of young adults have been recently shown to

harbor *CIC*-related fusions, including one case fused to the *LEUTX* gene, which, similar to *DUX4*, belongs to the paired (PRD) homeobox genes.^{29,30}

Finally patients with *CIC*-rearranged tumors followed an aggressive clinical course with a high metastatic rate, mainly to the lung. The 5-year overall survival was 43% for the entire group and 49% for the patients who presented with localized disease at diagnosis. The overall survival was significantly lower compared to the localized Ewing sarcoma cohort, matched for stage and age, which showed a 76% 5 year-survival. These findings confirm the results of Yoshida et al who found a statistically significant inferior overall survival in a smaller cohort of 20 *CIC*-rearranged sarcomas compared to a group of 53 Ewing sarcoma patients (unmatched for age or stage).¹⁴

In summary, this is the largest cohort to date of molecularly confirmed *CIC*-fusion positive sarcomas showing a relatively uniform clinical presentation, distinct from Ewing sarcoma. Most study group patients were young adults in their 4th decade (mean 32 years of age), presenting with a deep soft tissue tumor, either in the extremity or trunk. Microscopically, tumors had a more heterogeneous cytology, with a mixture of round, spindle and epithelioid cell types and often myxoid stroma. Immunohistochemically, the variable CD99 reactivity and strong WT1 are helpful ancillary techniques, diagnosis can be further confirmed by FISH for *CIC* gene rearrangements. A *CIC-DUX4* fusion was documented in only half of the cases, while no *FOXO4* gene abnormality was identified. Most importantly, the follow-up information revealed highly aggressive behavior, with a high metastatic rate of 53%, mainly to the lung and a 5 year-survival of 44%, which was significantly inferior to comparable Ewing sarcoma patients. Furthermore the patients who were treated with neoadjuvant chemotherapy using a similar regimen as for Ewing sarcoma showed a poor pathologic response in 70% of cases. All these findings reinforce the prior data combined from smaller series that *CIC*-rearranged sarcoma has distinct clinicopathologic features, which clearly warrant a stand-alone entity, separate from Ewing sarcoma. The challenging aspect for the future remains to design novel therapies, independent from Ewing sarcoma, possibly targeting the highly up-regulated downstream signature, such as the PEA3 family of transcription factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported in part by: P50 CA140146-01 (CRA, SS); P30-CA008748 (CRA, SS); Kristen Ann Carr Foundation (CRA); Cycle for Survival (CRA)

References

1. Fletcher, C., Bridge, JA., Hogendoorn, PC., et al. WHO Classification of Tumours of Soft Tissue and Bone. IARC; Lyon: 2013.
2. Delattre O, Zucman J, Plougastel B, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature. 1992; 359:162–165. [PubMed: 1522903]

3. Zucman J, Melot T, Desmaze C, et al. Combinatorial generation of variable fusion proteins in the Ewing family of tumours. *Embo J*. 1993; 12:4481–4487. [PubMed: 8223458]
4. Sorensen PH, Lessnick SL, Lopez-Terrada D, et al. A second Ewing's sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. *Nat Genet*. 1994; 6:146–151. [PubMed: 8162068]
5. 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. [PubMed: 16717057]
6. Yoshimoto M, Graham C, Chilton-MacNeill S, et al. Detailed cytogenetic and array analysis of pediatric primitive sarcomas reveals a recurrent CIC-DUX4 fusion gene event. *Cancer Genet Cytogenet*. 2009; 195:1–11. [PubMed: 19837261]
7. Italiano A, Sung YS, Zhang L, et al. High prevalence of CIC fusion with double-homeobox (DUX4) transcription factors in EWSR1-negative undifferentiated small blue round cell sarcomas. *Genes Chromosomes Cancer*. 2012; 51:207–218. [PubMed: 22072439]
8. 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. [PubMed: 21813156]
9. 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 Surg Pathol*. 2013; 37:1379–1386. [PubMed: 23887164]
10. 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. [PubMed: 25007147]
11. Solomon DA, Brohl AS, Khan J, et al. Clinicopathologic features of a second patient with Ewing-like sarcoma harboring CIC-FOXO4 gene fusion. *Am J Surg Pathol*. 2014; 38:1724–1725. [PubMed: 25321332]
12. Sankar S, Lessnick SL. Promiscuous partnerships in Ewing's sarcoma. *Cancer Genet*. 2011; 204:351–365. [PubMed: 21872822]
13. Gambarotti M, Benini S, Gamberi G, et al. CIC-DUX4 fusion-positive round-cell sarcomas of soft tissue and bone: a single-institution morphological and molecular analysis of seven cases. *Histopathology*. 2016; 69:624–634. [PubMed: 27079694]
14. Yoshida A, Goto K, Kodaira M, et al. CIC-rearranged Sarcomas: A Study of 20 Cases and Comparisons With Ewing Sarcomas. *Am J Surg Pathol*. 2016; 40:313–323. [PubMed: 26685084]
15. 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. [PubMed: 24723486]
16. 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. [PubMed: 27443513]
17. 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; 29:1523–1531. [PubMed: 27562494]
18. Jimenez G, Guichet A, Ephrussi A, et al. Relief of gene repression by torso RTK signaling: role of capicua in Drosophila terminal and dorsoventral patterning. *Genes Dev*. 2000; 14:224–231. [PubMed: 10652276]
19. Bettegowda C, Agrawal N, Jiao Y, et al. Mutations in CIC and FUBP1 contribute to human oligodendroglioma. *Science*. 2011; 333:1453–1455. [PubMed: 21817013]
20. Sahm F, Koelsche C, Meyer J, et al. CIC and FUBP1 mutations in oligodendrogliomas, oligoastrocytomas and astrocytomas. *Acta Neuropathol*. 2012; 123:853–860. [PubMed: 22588899]
21. Dissanayake K, Toth R, Blakey J, et al. ERK/p90(RSK)/14-3-3 signalling has an impact on expression of PEA3 Ets transcription factors via the transcriptional repressor capicua. *Biochem J*. 2011; 433:515–525. [PubMed: 21087211]
22. Jimenez G, Shvartsman SY, Paroush Z. The Capicua repressor—a general sensor of RTK signaling in development and disease. *J Cell Sci*. 2012; 125:1383–1391. [PubMed: 22526417]

23. Huang SC, Zhang L, Sung YS, et al. Recurrent CIC Gene Abnormalities in Angiosarcomas: A Molecular Study of 120 Cases With Concurrent Investigation of PLCG1, KDR, MYC, and FLT4 Gene Alterations. *Am J Surg Pathol*. 2016; 40:645–655. [PubMed: 26735859]
24. Sturm D, Orr BA, Toprak UH, et al. New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. *Cell*. 2016; 164:1060–1072. [PubMed: 26919435]
25. Krom YD, Thijssen PE, Young JM, et al. Intrinsic epigenetic regulation of the D4Z4 macrosatellite repeat in a transgenic mouse model for FSHD. *PLoS Genet*. 2013; 9:e1003415. [PubMed: 23593020]
26. van der Maarel SM, Tawil R, Tapscott SJ. Facioscapulohumeral muscular dystrophy and DUX4: breaking the silence. *Trends Mol Med*. 2011; 17:252–258. [PubMed: 21288772]
27. Siegele B, Roberts J, Black JO, et al. DUX4 Immunohistochemistry is a Highly Sensitive and Specific Marker for CIC-DUX4 Fusion-positive Round Cell Tumor. *Am J Surg Pathol*. 2016 [In Press].
28. van der Maarel SM, Frants RR. The D4Z4 repeat-mediated pathogenesis of facioscapulohumeral muscular dystrophy. *Am J Hum Genet*. 2005; 76:375–386. [PubMed: 15674778]
29. Huang SC, Zhang L, Sung YS, et al. Recurrent CIC Gene Abnormalities in Angiosarcomas: A Molecular Study of 120 Cases With Concurrent Investigation of PLCG1, KDR, MYC, and FLT4 Gene Alterations. *Am J Surg Pathol*. 2016
30. Holland PW, Booth HA, Bruford EA. Classification and nomenclature of all human homeobox genes. *BMC Biol*. 2007; 5:47. [PubMed: 17963489]

Figure 1. Morphologic spectrum of CIC-rearranged sarcomas

Tumors showed a solid and often nodular growth pattern (A). Less common architectural patterns included intersecting short fascicles in tumors with a spindle cell component (B), a rare reticular growth (C). Myxoid stromal component was seen in a third of cases (D). The predominant phenotype was that of solid sheets of round to ovoid cells (E), however, focal areas of more epithelioid or plasmacytoid appearance was also noted with light eosinophilic cytoplasm and eccentric nuclei (F). The nuclear features showed variable chromatin patterns, with either fine (G), dark, hyperchromatic (H) or vesicular (I). Most cases showed minor

variability in nuclear size and shape, except for a small subset revealing moderate degree of nuclear pleomorphism (H). Small to medium sized nucleoli were a common finding (G,I).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Immunohistochemical and FISH ancillary methods in diagnosis of *CIC* fusion positive sarcomas

CD99 reactivity a hallmark for Ewing sarcoma was present with a diffuse membranous pattern in a minority of cases (A), while most showed patchy, focal staining (B). WT1 nuclear staining was a consistent finding in overwhelming majority of cases (C). 3-color fusion FISH assay showing *CIC* (green, telomeric) fused to *DUX4* (red, centromeric) gene located on 4q35 (D) or 10q26 (E). FISH break-apart showing split red centromeric and green telomeric *CIC* signals, in an SBRCT lacking *DUX4* or *FOXO4* gene abnormalities.

Figure 3. Survival analysis of *CIC*-rearranged sarcoma patients

Overall survival of the 57 patients with follow-up information showed a 2-year OS: 53% and a 5-year OS: 43% (A). Patients who presented with metastatic disease at diagnosis followed an unfavorable clinical course compared to patients with localized disease ($p < 0.0001$) (B). Distant recurrence was associated with an inferior survival ($p < 0.0001$) (C). Patients with *CIC*-rearranged sarcoma have a significantly worse outcome compared to patients with Ewing sarcoma (matched for age/stage) ($p = 0.002$) (D).

Table 1

Anatomic location of CIC-rearranged sarcomas

Location of the tumor	Number of cases (n=111)
Soft tissue	95 (86%)
Trunk	39
Lower extremity	31
Upper extremity	7
Head/neck	12
Retroperitoneum/perineum/pelvis	6
Viscera	13 (12%)
Stomach	1
Small/large intestine	5
Kidney/prostate	4
Tonsils/parapharyngeal	3
Bone	3 (3%)
Pelvic bones	3