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## *Review*

# Ewing sarcoma and the new emerging Ewing-like sarcomas: (*CIC* and *BCOR*-rearranged-sarcomas). A systematic review

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**Summary.** Ewing-like sarcomas (ELS) are a heterogenous group of tumors that frequently affect pediatric and young adult patients. Accurate classification and distinction from the Ewing sarcoma family of tumor (ESFT) is decisive in patient management. ELS share a significant morphologic, immunohistochemical and clinical overlap with ESFT, thus the differential diagnosis is challenging, especially with atypical ESFT and tumors with unusual immunoprofiles or uncommon clinicoradiological findings. A subset of ELS harboring the *CIC-DUX4* or *BCOR-CCNB3* fusions has been described recently. The spectrum of ELS is now expanding, and additional gene fusion partners besides *DUX4* or *CCNB3* have been detected, and the terms *CIC* or *BCOR*-rearranged sarcomas have recently been proposed. We review the clinical, histological, phenotypic and molecular findings of ESFT and these new emerging ELS.

**Key words:** Ewing sarcoma, Ewing-like sarcomas, *CIC*-rearranged sarcomas, *BCOR*-rearranged sarcomas

### Introduction

Ewing sarcoma (ES) and Ewing-like sarcoma (ELS) are aggressive bone/soft tissue tumors arising mainly in pediatric, adolescents and young adult patients (Italiano et al., 2012; Machado et al., 2013; Antonescu, 2014; Fletcher, 2014; Mariño-Enríquez and Fletcher, 2014). The diagnosis of both tumors depends on the integration of clinicoradiological, histological, immunohistochemical (IHC) and specific molecular findings (Italiano et al., 2012; Machado et al., 2013; Antonescu, 2014; Puls et al., 2014; Smith et al., 2015). Three histological subtypes of ES have been recognized, comprising conventional/classic ES, PNET and the atypical variant. The first two can be easily recognized by microscopical examination and a specific IHC profile, however the diagnosis of the atypical histological variant is more challenging and requires molecular studies for a convincing diagnosis (Llombart-Bosch et al., 2009). Likewise, the atypical variant of ES shares some histological and IHC similarities with ELS, making it difficult to distinguish between these two tumors without specific genetic testing (Llombart-Bosch et al., 2009; Italiano et al., 2012; Antonescu, 2014; Specht et al., 2014).

As shown in Table 1, any small round cell sarcoma (SRCS) that harbors a reciprocal translocation of *EWSR1* gene with any member of the *ETS* gene family represents a true molecular variant of ES, and should be classified as a member of the Ewing sarcoma family of tumor (ESFT). Other SRCS with Ewing morphology and *EWSR1* rearrangement have non-*ETS* genes as partners, and although the designation of "ELS" or "atypical ES" for these tumors has been proposed, we believe that they also represent molecular variants of ESFT. In addition, uncommon SRCS with Ewing morphology reveal a *FUS* rearrangement instead of *EWSR1*. These tumors have *ETS*-genes as partners, for instance *ERG* or *FEV* and should also be included in the ESFT (Llombart-Bosch et al., 2009; Machado et al., 2009; Fletcher et al., 2013;

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Antonescu, 2014).

A new emerging group of round/ovoid cell sarcomas histologically mimicking ESFT has been characterized with the implementation of novel molecular biology studies in the work-up of unclassified/undifferentiated SRCS (Kawamura-Saito et al., 2006; Graham et al., 2012; Italiano et al., 2012; Pierron et al., 2012; Choi et al., 2013; Machado et al., 2013; Antonescu, 2014; Cohen-Gogo et al., 2014; Kajtar et al., 2014; Haidar et al., 2015; Gambarotti et al., 2016; Le Guellec et al., 2016; Liao et al., 2016; Yoshida et al., 2016). These molecular assays have facilitated the identification of specific genetically defined tumors, the so-called ELS (*CIC* or *BCOR* sarcomas), but it is uncertain and still under assessment as to whether or not these emerging entities may represent distinct biologic tumors (Graham et al., 2012; Italiano et al., 2012; Pierron et al., 2012; Choi et al., 2013; Machado et al., 2013; Cohen-Gogo et al., 2014; Sugita et al., 2014; Tardio et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). Although conventional treatments with Ewing regimens are effective against ESFT, the therapeutic options available for ELS are under discussion.

The aims of this review are to describe the recent advances in histological and phenotypical characterization of ESFT and ELS. To define whether or not the morphological findings might distinguish between ESFT and ELS. To update current knowledge on *CIC/DUX4* and *BCOR/CCNB3* sarcomas as well as other SRCS with the *CIC* or *BCOR* rearrangement; and finally to define whether or not specific gene fusions match the histological subtypes in ESFT and ELS.

## Ewing sarcoma family of tumor with EWSR1 or FUS rearrangement

ESFT is an aggressive tumor arising frequently in the long bones or pelvic bones of pediatric and young patients; however extraosseous tumors have also been recognized (de Alava et al., 1998; Llombart-Bosch et al., 2009; Fletcher et al., 2013; Antonescu, 2014; Doyle, 2014). Three histological variants sharing IHC and molecular characteristics were described in a large series of ESFT published by Llombart-Bosch et al. (2009). Both conventional and PNET subtypes are characterized by a monotonous small round cell proliferation, with fine chromatin, inconspicuous nucleoli and usually scant cytoplasm; additionally with pseudorosette formation in PNET (Llombart-Bosch et al., 2009). The atypical variant (Fig. 1A-D) can display areas of spindle cell formation, nuclear pleomorphism, large cells, clear cells, hemangioendothelial pattern and adamantinoma-like/sclerotic/desmoplastic areas (Llombart-Bosch et al., 2009). The IHC study usually reveals strong membranous CD99 (Fig. 1E,F) and FLi1 nuclear positivity in most of the tumors (Folpe et al., 2000; Llombart-Bosch et al., 2009; Machado et al., 2009, 2013). SRCS with a presumptive diagnosis of ESFT but displaying atypical morphology associated with poor or negative CD99 expression should prompt the exclusion of other tumors, especially those that belong to the ELS group (*CIC* or *BCOR* sarcomas). ERG can be expressed in some ESFT and their expression is not necessarily related with the presence or absence of the *EWSR1/ERG* gene fusion. Neuro-endocrine markers are sometimes positive, and epithelial differentiation can be observed in around 20-30% of ESFT, however, myogenic differentiation is exceptional in this family of tumors (Folpe et al., 2000; Llombart-Bosch et al., 2009; Antonescu, 2014).

ESFT are almost always characterized by reciprocal translocations between *EWSR1* and a gene of the *ETS* family of transcription factors. The most frequent partners are *Fli1* and *ERG* (de Alava et al., 1998; Llombart-Bosch et al., 2009; Machado et al., 2009; Le Deley et al., 2010; Fletcher et al., 2013; Antonescu, 2014; Mariño-Enríquez and Fletcher, 2014). Less commonly, the gene partner includes *ETV1, ETV4, FEV* and *E1A–F. EWSR1* exon 7 to *Fli1* exon 6 (type 1) and fusion of *EWSR1* exon 7 to *Fli1* exon 5 (type 2) are the most frequent *EWSR1/Fli1* fusion subtypes (Llombart-Bosch et al., 2009; Le Deley et al., 2010; Fletcher et al., 2013; Antonescu, 2014; Mariño-Enríquez and Fletcher 2014). ESFT may occasionally reveal a fusion between *EWSR1* and *non-ETS* gene family members (*PATZ1, SP3; NFATc2, SMARCA5*). Finally, a small group of ESFT shows the *FUS* instead of the *EWSR1* rearrangement with an *ERG* or *FEV* gene partner (de Alava et al., 1998; Llombart-Bosch et al., 2009; Machado et al., 2009; Le Deley et al., 2010; Fletcher et al., 2013; Antonescu, 2014; Mariño-Enríquez and Fletcher, 2014; Chen et al., 2016). The incidence of these gene fusions is too low in ESFT to make a

**Table 1.** Round cell sarcomas of bone and soft tissue.

| 1-Ewing sarcoma family of tumor (ESFT) with *EWSR1/FUS* rearrangement | | |
|---|---|---|
| t(11;22)(q24;q12) | *EWSR1-FLI1* | ESFT |
| t(21;22)(q22;q12) | *EWSR1-ERG* | ESFT |
| t(7;22)(p22;q12) | *EWSR1-ETV1* | ESFT |
| t(17;22)(q21;q12) | *EWSR1-ETV4* | ESFT |
| t(2;22)(q35;q12) | *EWSR1-FEV* | ESFT |
| t(20;22)(q13;q12) | *EWSR1-NFATC2* | ESFT |
| t(2;22)(q31;q12) | *EWSR1-SP3* | ESFT |
| inv (22) | *EWSR1-PATZ1* | ESFT |
| t(4;22)(q31;q12) | *EWSR1-SMARCA5* | ESFT |
| t(16;21)(p11;q22) | *FUS-ERG* | ESFT |
| t(2;16)(q35;p11) | *FUS-FEV* | ESFT |
| 2-Ewing-like sarcoma/ round cell sarcoma (*CIC-DUX4*, *CIC-FOX04*, *BCOR-CCNB3*, *CIC* or *BCOR* rearrangement). | | |
| 3-Round cell sarcoma/no-Ewing sarcoma with *EWSR1* rearrangement (myoepithelial carcinoma, desmoplastic small round cell tumor, myxoid liposarcoma, extraskeletal myxoid condrosarcoma, clear cell sarcoma). | | |
| 4-Undifferentiated/unclassified round cell sarcoma (URCS). | | |

definitive conclusion, but it seems that all these ESFT with a rare/alternative gene fusion show a noteworthy pathological overlap with conventional ESFT, thus they should remain in the category of molecular variant of ESFT to avoid any confusion with the term: "Ewing-like sarcomas". Treatment protocol and patient surveillance should follow the same line as Ewing protocols (Grier et al., 2003; Granowetter et al., 2009; Le Deley et al., 2010, 2014; Womer et al., 2012; Bedetti et al., 2015; van den Berg et al., 2015).

Genetic confirmation for a convincing diagnosis of ESFT can be performed by RT-PCR and/or FISH analysis. FISH *EWSR1* analysis does not provide a specific genetic partner and may not be enough to exclude other ESFT mimics with *EWSR1*-rearrangement for instance: myoepithelial carcinoma, round cell liposarcoma, myxoid chondrosarcoma or desmoplastic small round cell tumor (DSRCT). RT-PCR provides the specific gene fusion including cases with insertion instead of reciprocal translocation but the reliability of the results of this approach depends strongly on the condition, fixation, decalcification, and age of the tumor sample (Machado et al., 2009; Pierron et al., 2012; Antonescu, 2014).

Despite the utility of RT-PCR assays in ESFT diagnosis, Le Deley et al., in a large prospective study of *EWS-ETS* fusion type and disease progression found no prognostic benefit for any fusion type (Le Deley et al., 2010). In addition, the fusion types did not match any specific histological subtypes in ESFT and do not predispose to any particular clinical evolution or specific tumor immunoprofile as demonstrated by Llombart-Bosch et al. (2009) in a large series of genetically confirmed ESFT.

The diagnosis of classic ESFT or PNET with a common clinical and immunophenotypic context do not frequently require molecular confirmation except for inclusion in clinical trials. However the atypical histological variants of ESFT, even more so with unexpected immunoprofile (CD99 negativity, desmin, EMA or WT1 positivity) require a genetic confirmation for a convincing diagnosis (Llombart-Bosch et al., 2009; Machado et al., 2009; Antonescu, 2014). In the same regard, conventional ESFT but with unusual clinicopathological presentation (atypical primary tumor sites or age) also require genetic confirmation, for instance: cutaneous ESFT, ESFT in patients over 45 years, intra-abdominal desmoplastic ESFT, primary visceral ESFT, ESFT of bone with absent or poor response to conventional chemotherapy Ewing protocols

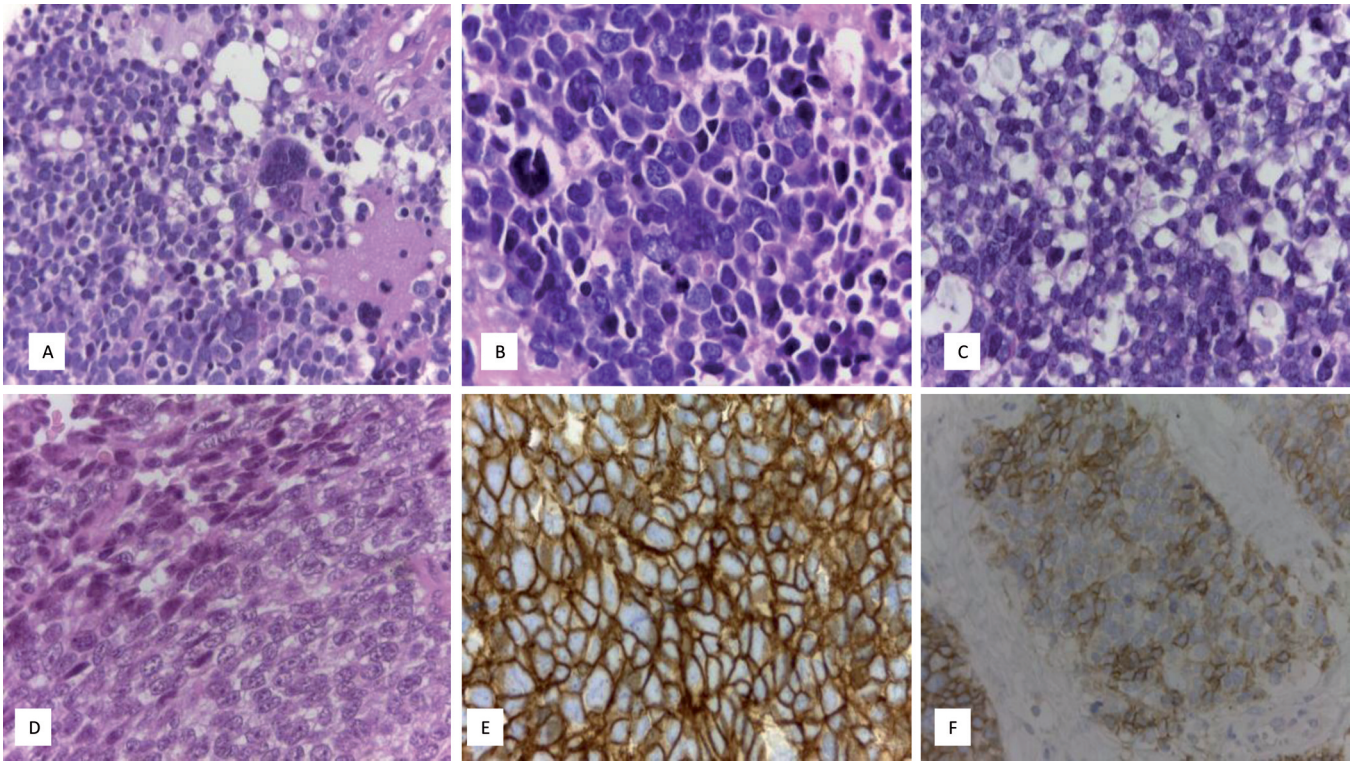


**Fig. 1.** (Ewing sarcomas with genetic confirmation by FISH and RT-PCR) **A, B**. Atypical large cell Ewing sarcoma resembling a *CIC*-rearranged sarcoma, hematoxylin/eosin staining, H&E. **C.** Atypical cell Ewing sarcoma showing clear cells mimicking a *CIC-DUX4* sarcoma, H&E. **D.** Atypical spindle cell Ewing sarcoma similar to *BCOR*-rearranged sarcoma, H&E. **E.** Strong membranous CD99 expression in Ewing sarcoma. **F.** Moderate and patchy membranous CD99 expression in atypical Ewing sarcoma with desmoplasia. A, C, F, x 20; B, D, E, x 40;

and conventional ESFT with strong epithelial and neuroendocrine differentiation (Llombart-Bosch et al., 2009; Machado et al., 2009; Mariño-Enríquez et al., 2014).

## Ewing-like sarcomas with *CIC* or *BCOR*-rearrangement

### *Ewing-like sarcomas with CIC/DUX4 gene fusion or CIC-rearrangement*

Recently, emerging molecular alterations have been identified in *EWSR1/FUS*-negative unclassified/undifferentiated SRCS. The most frequent alteration at present is a recurrent translocation, involving the *CIC* gene on chromosome 19, fused with either *DUX4* on chromosome 4 or *DUX4L* on chromosome 10, resulting in either t(4;19)(q35;q13) or t(10;19)(q26;q13) with *CIC/DUX4* and *CIC/DUX4L* gene fusion, respectively (Roberts et al., 1992; Richkind et al., 1996; Kawamura-Saito et al., 2006; Rakheja et al., 2008; Sirvent et al., 2009; Yoshimoto et al., 2009; Riccardi et al., 2010; Graham et al., 2012; Italiano et al., 2012; Machado et al., 2013; Antonescu, 2014; Kajtar et al., 2014; Panagopoulos et al., 2014; Specht et al., 2014; Smith et al., 2015; Chebib and Jo, 2016; Gambarotti et al., 2016; Le Guellec et al., 2016; Yoshida et al., 2016). As reported in a small series of cases, there are no significant differences between tumors harboring a t(4;19) and t(10;19) in terms of histological features, clinical presentation, or outcome (Graham et al., 2012; Italiano et al., 2012; Bielle et al., 2014; Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). Additionally, some tumors with undifferentiated SRCS morphology may harbor an alternative gene fusion partner (*CIC/FOX04*) or reveal *CIC* rearrangements with unknown partner genes (Solomon et al., 2014; Sugita et al., 2014; Yoshida et al., 2016). Up to now, the spectrum of "*CIC-rearranged family of tumors*" seems to be less wide than "*EWSR1-rearranged family of tumors*". In contrast to "*EWSR1-rearranged family of tumors*", the members of the "*CIC-rearrangement family of tumor*" share many morphological and phenotypic findings (Graham et al., 2012; Italiano et al., 2012; Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016).

*CIC-DUX4* fusion is now the most frequent genetic alteration in *EWSR1/FUS*-negative undifferentiated SRCS. Italiano and Yoshida et al. have reported the two largest series of *CIC-rearranged sarcomas* ("*CRS*") so

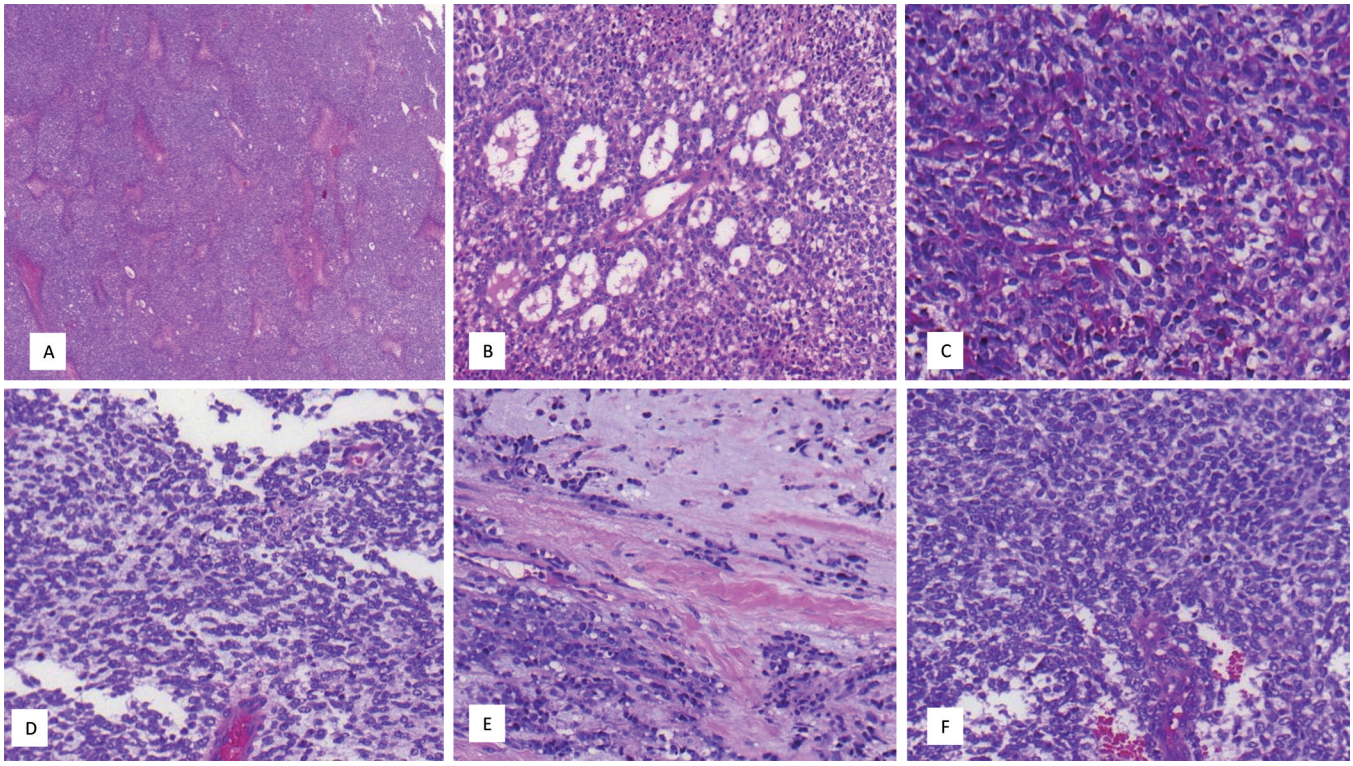


**Fig. 2.** *(CIC*-rearranged sarcomas with genetic confirmation by RT-PCR). **A**. Geographic necrosis in *CIC*-rearranged sarcoma, H&E. **B.** Pseudoacinar appearance in *CIC*-rearranged sarcoma, H&E. **C.** Spindle cell formation in *CIC-DUX4* sarcoma resembling an atypical Ewing sarcoma with spindle cells or *BCOR*-rearranged sarcoma. **D and E**. Myxoid stromal change in *CIC*-rearranged sarcoma resembling an extraskeletal myxoid chondrosarcoma. **F.** *CIC-DUX4* sarcoma with round and ovoid cells mimicking a poorly differentiated synovial sarcoma, H&E. A, x 10; B, C, E, F, x 20; D, x 40

far (Italiano et al., 2012; Yoshida et al., 2016) and the analyses of such reports as well as additional studies have identified this rearrangement in approximately 65-70% of *EWSR1*/*FUS*-negative undifferentiated SRCS. Given the small number of cases categorized so far, the clinical features of this emerging group of sarcoma are still somewhat unclear, a meta-analysis of the 85 Ewing-like tumors with *CIC* or *DUX4* rearrangement described to date is summarized in Table 2.

Distribution of "*CRS*" by gender is very similar to ESFT, whereas distribution by age is different, as shown in Table 2. "*CRS*" occurs more frequently in young adults, particularly in the 21 to 40-year age group, although more than 15 cases with a diagnosis of "*CRS*" have been reported in patients over 40 years of age. Indeed, ESFT arises more frequently in children and teenagers but is very infrequent in patients over 40, thus the histological diagnosis should be genetically confirmed.

Almost all "*CRS*" arise in soft tissue with very

**Table 2.** *CIC*-rearranged sarcoma meta-analysis.

| Cases with *CIC* or *DUX* rearrangement (Total of cases: 85 ) | N / (%) | Not reported |
|---|---|---|
| 1-Sex | | |
| Male | 45 (52.9%) | |
| Female | 40 (47.1%) | |
| 2-Age group (age range) | | |
| <10 years | 4 (4.7%) | |
| 10-20 years | 22 (25.8%) | |
| 21-40 years | 42 (49.4%) | |
| >40 years | 17 (20%) | |
| 3-Primary tumor localization(predominant) | | |
| soft tissue | 76 (89.4%) | |
| bone | 1 (1.1%) | |
| visceral | 8/9.4% | |
| 4-Primary tumor localization (region) | | |
| Head/neck | 8 (9.4%) | |
| Trunk and abdominal region | 31 (36.4%) | |
| Extremities and pelvis: | 46/54.1% | |
| 5-Morphological findings (predominant pattern) | | |
| USRCS Ewing-like | 75 (88.2%) | |
| USRCS with focal spindle pattern | 10 (11.8%) | |
| US with predominant spindle pattern | 0 | |
| Stromal myxoid changes (focal or extensive) | 23 (27%) | |
| 6-CD99 immunohistochemical result | | |
| negative | 5 (5.8%) | |
| poor and focal positivity (+) | 37 (43.5%) | |
| moderate positivity cytoplasmatic and/or membranous (++) | 14 (16.4%) | |
| strong positivity cytoplasmatic and/or membranous (+++) | 9 (10.6%) | |
| positive, not datailed intensity | 20 (23.5%) | |
| 7-Gene fusion or gene rearrangement | | |
| *CIC/DUX4*/ t(4;19) | 45 (52.9%) | |
| *CIC/DUX4L*/ t(10;19) | 11 (12.9%) | |
| *CIC/FOX01* | 2 (2.3%) | |
| *CIC* rearrangement (partner unknow or not reported) | 25 (29.4%) | |
| *DUX4* rearrangement | 2 (2.3%) | |
| 8-Metastasis at diagnosis or during the follow-up | | |
| Yes | 40 (71.4%) | 29 |
| No | 16 (28,6%) | |
| 9-Metastasis localization | | |
| Lung | 24 (63.2%) | 2 |
| Extra-pulmonary | 6 (15.8%) | |
| Mixed | 8 (21%) | |
| 10-Outcome | | |
| Dead of disease (DOD) | 39 (60%) | 20 |
| No evidence of disease | 17 (26.1%) | |
| Alive with disease | 9 (13.9%) | |
| 11-DOD, months | | |
| <12 months | 22 (59.4%) | 2 |
| >12 months | 15 (40.6%) | |

occasional visceral location and exceptionally in bone, as reported recently by Gambarotti et al. (2016). Extraskeletal ESFT have been well documented but the majority of this family of tumors arise in bone. Thus, in a work-up of a soft tissue undifferentiated *EWSR1/FUS* negative-rearranged SRCS in a young adult the possibility of "*CIC-rearranged sarcoma*" should be excluded.

The histology of "*CRS*" reveals a lobular growth pattern or diffuse neoplastic proliferation of small-to medium-sized round to oval cells with Ewing-like morphology and variable stromal tissue with desmoplastic, sclerotic, edematous or myxoid areas generating a microcystic, reticular or pseudo-acinar appearance (Fig. 2B,C). Myxoid areas (Fig. 2D,E) resulted in resemblance to extraskeletal myxoid chondrosarcoma or myoepithelioma and should be included in the differential diagnosis as well as ESFT. Geographic necrosis as observed in ESFT is a frequent feature and many of these tumors have focal areas morphologically indistinguishable from conventional ESFT (Fig. 2A). The neoplastic cells show vesicular nuclei with conspicuous enlarged nucleoli, moderate nuclear pleomorphism and variable amounts of amphophilic, clear or eosinophilic cytoplasm (Fig. 2F). The tumor cells can display a spindle, plasmacytoid, rhabdoid (Fig. 3A-C) or epithelioid shape but not usually as a predominant pattern (Graham et al., 2012; Italiano et al., 2012; Machado et al., 2013; Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). The tumor usually shows a more heterogenous nuclear shape and size (Fig. 3A,B) as well as usually more abundant cytoplasm when compared with ESFT. The mitotic index in "*CRS*" is normally higher than in ESFT.

Gambarotti et al., have reported that some distinctive morphologic features of ESFT, such as Homer-Wright rosettes, have never been reported in "*CRS*", while the morphologically distinctive features commonly observed in "*CRS*" (myxoid change of the stroma, nuclear spindling and overt multifocal cytologic atypia) are very rare in ESFT (Gambarotti et al., 2016). We agree with such histological differences but only in the case of conventional ESFT or PNET, because either atypical ESFT or post-treatment ESFT can also reveal myxoid stromal changes, spindle cell formation or nuclear

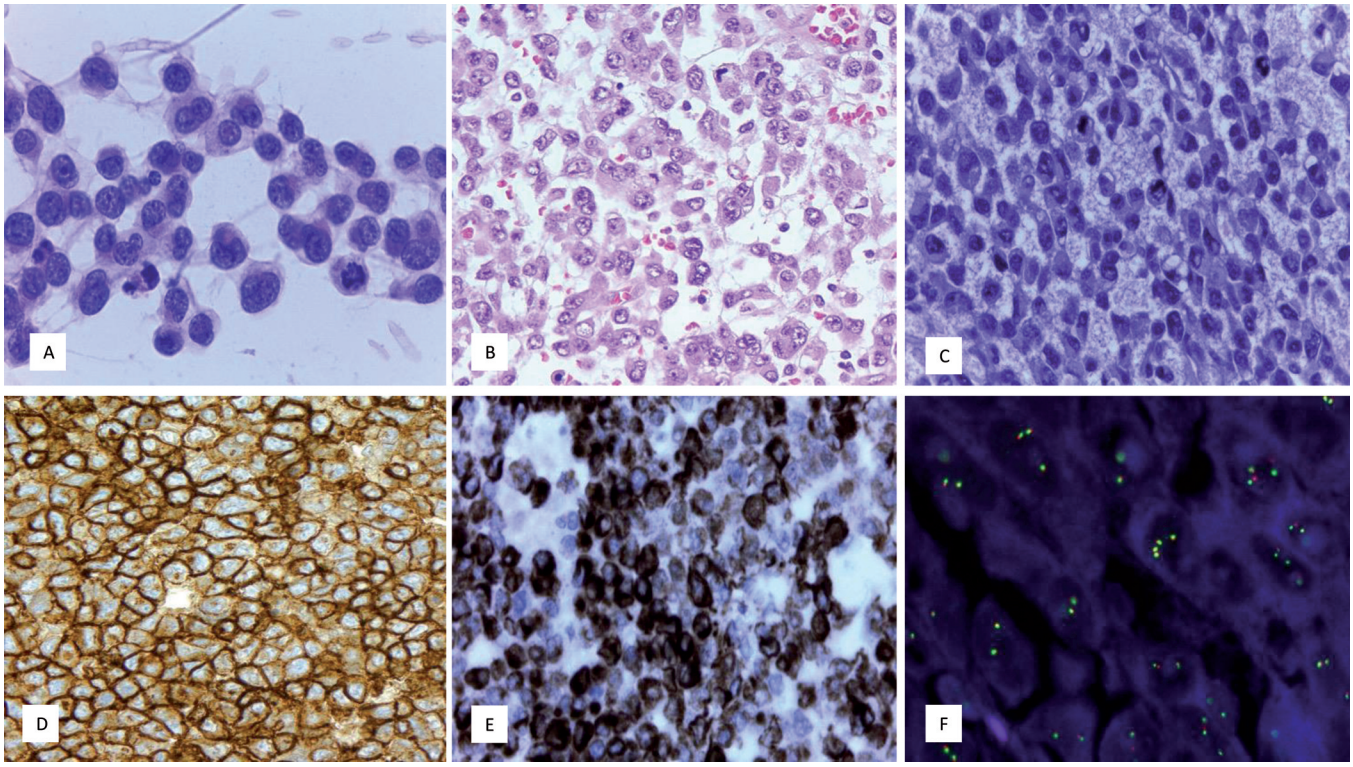


**Fig. 3.** *(CIC*-rearranged sarcomas with genetic confirmation by RT-PCR). **A.** Cytology smear of *CIC-DUX4* sarcomas show discohesive tumor cells with abundant cytoplasm, focal rhabdoid morphology, high degree of nuclear shape and mitosis, H&E. **B.** *CIC-DUX4* sarcoma with tumor cells displaying vesicular nuclei with conspicuous enlarged nucleoli, moderate nuclear pleomorphism and variable amounts of amphophilic, clear or eosinophilic cytoplasm, H&E. **C.** *CIC*-rearranged sarcoma showing myxoid stromal appearance and tumor cells with plasmocytoid and rhabdoid morphology, H&E. **D.** Strong membranous and dot-like CD99 expression in *CIC-DUX4* resembling the staining pattern observed in Ewing sarcoma. **E.** Cytoplasmic and focal nuclear WT1 expression in *CIC*-rearranged sarcoma. **F**. FISH in *CIC*-rearranged sarcoma lacking *EWSR1* rearrangement. A-C, E, x 40; D, F, x 60

pleomorphism (Llombart-Bosch et al., 2009).

In summary, as shown in Table 2, many of the "*CRS*" reported showed a Ewing-like morphology with varied myxoid stromal changes. A small subset of this tumor reveals focal spindle cell formation but always with a predominance of round cells. Overall, it can be a challenge for pathologists to distinguish *CIC-DUX4* fusion sarcoma from other Ewing-like sarcomas with *CIC* rearrangement without specific partners and from ESFT exclusively on the basis of their morphologic and IHC features. *CIC/FOX1* sarcomas are even uncommon and only the presence of a wide desmoplastic stroma was relevant in one of the two reported cases (Solomon et al., 2014; Sugita et al., 2014).

At present, it seems there is no specific gene fusion that matches the histological subtypes in Ewing-like sarcomas that harbor "*CIC*" rearrangement since no substantial morphological differences exist between Ewing-like sarcomas with *CIC/DUX4* or *CIC/FOX01* (Italiano et al., 2012; Specht et al., 2014; Antonescu, 2014; Solomon et al., 2014; Sugita et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). In addition, in the large series of Yoshida et al., the histological characteristics of tumors with *CIC* rearrangement were similar to those histological findings described in *CIC/DUX4* sarcoma series or *CIC/FOXO1* sarcoma case reports (Italiano et al., 2012; Solomon et al., 2014; Specht et al., 2014; Sugita et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016).

Parallel to these morphological similarities are the IHC findings of those tumors with *CIC* rearrangement. CD99 expression is variable with focal or diffuse expression (Fig. 3D) but almost always not the typical strong membranous staining as observed in ESFT (Llombart-Bosch et al., 2009; Machado et al., 2013; Gambarotti et al., 2016; Yoshida et al., 2016). Fli-1 and ERG expression is also variable and the result with such antibodies does not offer additional information in the differential diagnosis with ESFT. "*CRS*" are typically negative for neuroendocrine, melanic and lymphoid markers, however epithelial differentiation can be observed in this family of tumors with focal cytokeratin and/or EMA expression (Italiano et al., 2012; Antonescu, 2014; Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). Myogenic differentiation is unusual., but focal desmin expression has been described (Yoshida et al., 2016). Nuclear or cytoplasmic WT1 expression (Fig. 3E) as well as MYC and ETV4 IHC positivity have emerged as new interesting IHC findings in "*CRS*" (Specht et al., 2014; Smith et al., 2015; Le Guellec et al., 2016; Gambarotti et al., 2016; Yoshida et al., 2016). In addition, calretinin, MUC-4 and D2-40 can be IHC expressed in this family of tumors as reported in the large series of Yoshida et al. (2016). Due to the virtual absence of WT1 and ETV4 expression in many of the ESFT, staining with both these markers can help in the differential diagnosis between "CRS" and ESFT, in the applicable clinical and morphological context (Le Guellec et al., 2016). Thus, positive WT1 and ETV4 IHC results in *EWSR1/FUS*-negative URCS may facilitate a better selection of cases for FISH analysis allowing the definitive diagnosis of "*CRS*".

The clinical features and outcome of this new sarcoma are still somewhat uncertain, given the small number of cases described so far. As documented in Table 2, "*CRS*" have an aggressive clinical course with frequent distant metastatic disease and predominant lung localization. The outcome of patients with "*CRS*" diagnosis has been described in 63 out of 85 patients, finding more than half of the patients died of disease mainly before 12 months of follow-up. These data support the aggressive evolution of this type of sarcoma and strongly suggest the need to establish a more close surveillance and specific oncologic treatment protocol for such a patient.

In fact, "*CRS*" seems to be less sensitive than ESFT to standard Ewing chemotherapy agents (le Deley et al., 2010, 2014; Applebaum et al., 2011; Mariño-Enríquez and Fletcher 2014; Bedetti et al., 2015; van den Berg et al., 2015) but the predominant incidence of "*CRS*" in

**Table 3.** List of cases with *CIC* and/or *DUX* rearrangement (85 cases).

| Authors | Year | N | Journal |
|---|---|---|---|
| Roberts et al. | 1992 | 1 | Cancer Genet Cytogenet |
| Richkind et al. | 1996 | 1 | Cancer Genet Cytogenet |
| Somers et al. | 2004 | 1 | Pediatr Dev Pathol |
| Kawamura-Saito et al. | 2006 | 2 | Hum Mol Genet |
| Rakheja et al. | 2008 | 1 | Pediatr Dev Pathol |
| Alaggio et al. | 2009 | 1 | Hum Pathol |
| Yoshimoto et al. | 2009 | 2 | Cancer Genet Cytogenet |
| Sirvent et al. | 2009 | 1 | Cancer Genet Cytogenet |
| Riccardi et al. | 2010 | 1 | Cancer Genet Cytogenet |
| Italiano et al. | 2012 | 15 | Genes, Chromosomes & Cancer |
| Graham et al. | 2012 | 3 | Hum Pathol |
| Machado et al. | 2013 | 1 | Virchow Arch |
| Choi et al. | 2013 | 4 | Am J Surg Pathol |
| Kajtar et al. | 2014 | 1 | Cytopathology |
| Specht et al. | 2014 | 9 | Genes Chromosomes Cancer |
| Bielle et al. | 2014 | 1 | Acta Neuropathol |
| Sugita et al. | 2014 | 1 | Am J Surg Pathol |
| Solomon et al. | 2014 | 1 | Am J Surg Pathol |
| Panagopoulos et al. | 2014 | 1 | PloS One |
| Haidar et al. | 2015 | 1 | Am J Case Rep |
| Tardio et al. | 2015 | 1 | Pathology - Research and Practice |
| Smith et al. | 2015 | 6 | Modern Pathology |
| Chebib and Jo | 2016 | 2 | Cancer Cytopathol |
| Yoshida et al. | 2016 | 20 | Am J Surg Pathol |
| Gambarotti et al. | 2016 | 7 | Histopathology |
| List of cases with BCOR-rearrangement (68 cases). | | | |
| Cohen-Gogo et al., | 2014 | 26 | Pediatr Blood Cancer |
| Puls et al., | 2014 | 10 | Am J Surg Pathol |
| Peters et al., | 2015 | 6 | Mod Pathol |
| Shibayama et al. | 2015 | 3 | Pathol Int |
| Specht et al., | 2016 | 19 | Am J Surg Pathol |
| Huang et al., | 2016 | 4 | USCAP meeting 2016 |

young adult patients may suggest that such patients should be treated according to ESFT protocols instead of conventional adult soft-tissue sarcoma treatment protocols (Italiano et al., 2012; Machado et al., 2013; Antonescu, 2014; Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). Although additional studies are needed to elucidate the role of chemotherapy in "*CRS*", the fact that such tumors commonly have an aggressive clinical course with a high prevalence of distant metastasis suggests that neoadjuvant Ewing chemotherapy regimens can benefit these patients (Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). Clinical trials to search for a targeted therapy could offer additional benefits in this group of tumors.

In summary, despite the fact that the *CIC-DUX4* fusion oncoprotein leads to upregulation of ETS family genes such as *ETV1, ETV4* and *ETV5*, similar to the genes upregulated in ESFT suggesting a close biologic relationship between "*CRS*" and ESFT, the fact is that "*CRS*" have a distinctive clinical evolution (predominant in young adult patient, soft tissue tumor, frequent highly aggressive clinical course) and a distinct transcriptional profile that warrants separation from ESFT, as a distinct entity (Italiano et al., 2012; Antonescu, 2014; Specht et al., 2014; Smith et al., 2015; Gambarotti et al., 2016; Yoshida et al., 2016). "*CRS*" and atypical ESFT can share morphologically and phenotypic characteristics. Although the molecular confirmation by either FISH (Fig. 3F) and/or RT-PCR represents at present the most appropriate method to establish a definitive diagnosis of "*CRS*", these assessments are not yet extensively available in all cancer institutions.

### *Ewing-like sarcomas with BCOR-CCNB3 gene fusion or BCOR-rearranged sarcomas*

A new subset of primitive undifferentiated oval-round cell sarcoma characterized by a *BCOR-CCNB3* gene fusion, resulting from a paracentric inversion of chromosome X was first described by (Pierron et al., 2012; Cohen-Gogo et al., 2014). This tumor represents around 5% of all *EWSR1/FUS*-negative Ewing-like sarcomas and the initial clinical and morphologic analyses on a small series revealed that such neoplasms appear to share clinical and histopathological similarities with ESFT (Pierron et al., 2012; Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Shibayama et al., 2015; Liao et al., 2016; Specht et al., 2016). As detailed in Table 3, the number of publications describing *BCOR-CCNB3* sarcomas is clearly less than those for "*CRS*". The similarities between ESFT and *BCOR-CCNB3* sarcomas include the common occurrence in long and pelvic bones of teenagers and

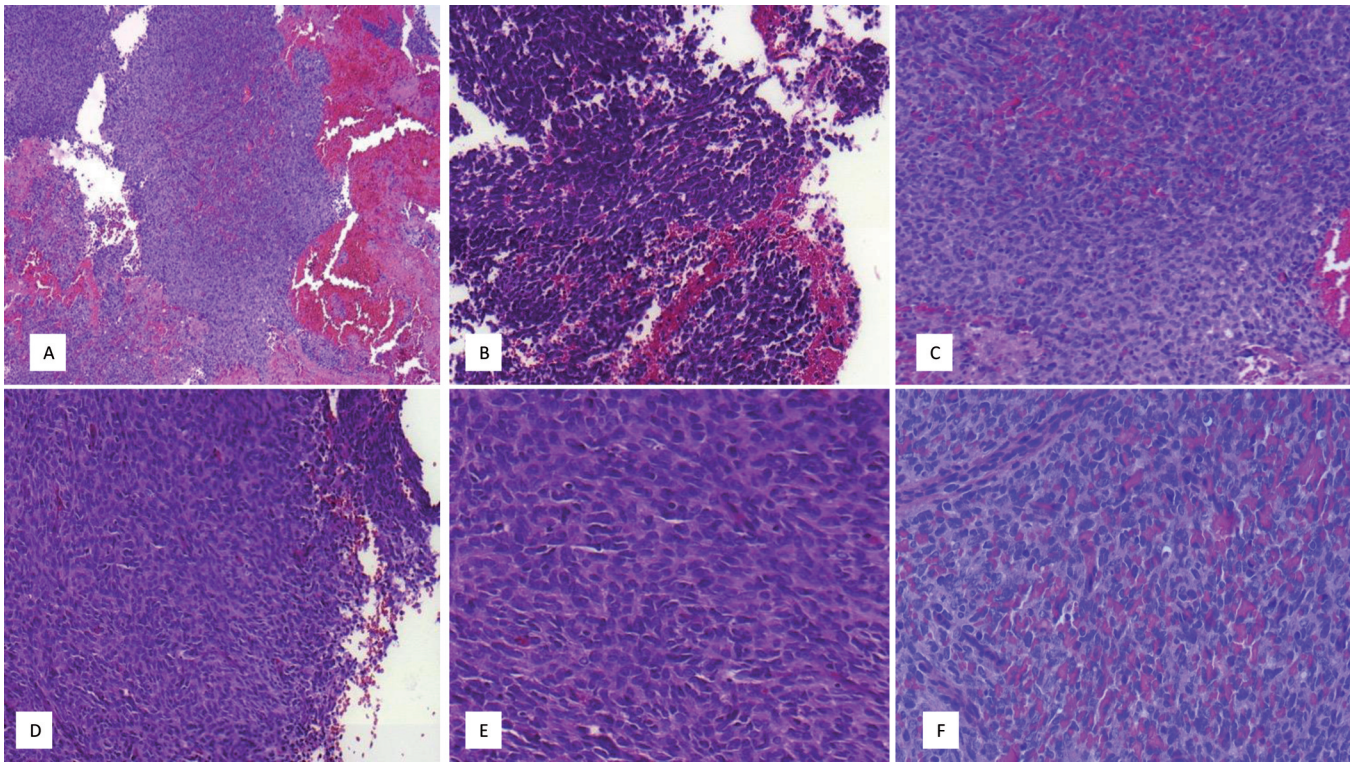


**Fig. 4.** *(BCOR*-rearranged sarcoma). **A-C.** Spindle cell pattern in *BCOR*-rearranged sarcomas resembling fibrosarcoma or poorly differentiated synovial sarcoma. **D-F.** Spindle, ovoid and focal round cell in *BCOR-CCNB3* sarcoma. A, B, x 10; C, D, x 20; E, F, x 40

young adults; nevertheless there are also differences, such as a strong male predilection and a potentially less aggressive clinical course in *BCOR-CCNB3* sarcomas. In addition, *BCOR-CCNB3* sarcomas have a distinct gene expression profiling without an *EWSR1-ETS* expression signature, in light of these findings, *BCOR-CCNB3* sarcomas are considered as a new subtype of bone sarcomas distinct from ESFT (Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Specht et al., 2016).

Some clinical features of *BCOR-CCNB3* sarcomas also appear to some degree distinctive when compared with *CIC/DUX4* sarcomas. *BCOR-CCNB3* sarcomas occur frequently in a younger age group (adolescents) in contrast to *CIC/DUX4* sarcomas that appear predominantly in young adults. *BCOR-CCNB3* sarcomas have a higher prevalence in male and primary bone locations in comparison with *CIC-DUX4* sarcomas where almost all cases arise in soft tissue (Pierron et al., 2012; Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Shibayama et al., 2015; Specht et al., 2016; Liao et al., 2016).

**Table 4.** *BCOR*-rearranged sarcomas meta-analysis.

| Cases with *BCOR* rearrangement (Total of cases: 68) | N/% | Not reported |
|---|---|---|
| 1-Sex | | |
| Male | 52 (81.2%) | 4 |
| Female | 12 (18.8%) | |
| 2-Age group (age range) | | |
| <10 years | 10 (17.5%) | 11 |
| 10-17 years | 32 (56.1%) | |
| >17 years | 15 (26.4%) | |
| 3-Primary tumor localization(predominant) | | |
| soft tissue | 24 (36.9%) | 3 |
| bone | 40 (61.5%) | |
| visceral | 1 (1.6%) | |
| 4-Primary tumor localization (region) | | |
| Head/neck | 1 (2.3%) | 24 |
| Trunk and abdominal region | 5 (11.4%) | |
| Pelvis and extremities: | 36 (81.8%) | |
| Axial skeletum: | 2 (4.5%) | |
| 5-Morphological findings (predominant pattern) | | |
| USRCS/Ewing-like morphology | 19 (33.3%) | 11 |
| Undifferentiated sarcoma (US) with predominant spindle cell formation | 8 (14%) | |
| US with small round cell Ewing-like and spindle cell formation (mixed) | 30 (52.7%) | |
| Stromal myxoid changes (focal or extensive) | 16 (28%) | |
| 6-CD99 immunohistochemical result | | |
| negative | 9 (17.3%) | 16 |
| positive | 43 (82.7%) | |
| 7-CCNB3 immunohistochemical results (nuclear staining) | | |
| negative | 2 (4%) | 19 |
| positive | 47 (96%) | |
| 8-Gene fusion or gene rearrangement | | |
| *BCOR/CCNB3* | 59 (88%) | 1 |
| *BCOR/MAML3* | 2 (1.5%) | |
| *BCOR/ZC3H7B* | 2 (1.5%) | |
| *BCOR* with unknow partner | 4 (6%) | |
| 9-Metastasis at diagnosis or during the follow-up | | |
| Yes | 16 (30.7%) | 16 |
| No | 36/69.3% | |
| 10-Metastasis localization | | |
| Lung | 6 (37.5%) | |
| Extra-pulmonary | 8 (50%) | |
| Mixed | 2 (12.5%) | |
| 11-Outcome | | |
| Dead of disease (DOD) | 18 (36.7%) | 19 |
| No evidence of disease | 11 (22.4%) | |
| Alive with disease | 20 (40.9%) | |
| 12-DOD, months | | |
| <12 months | 0 | 12 |
| >12 months | 6 (100%) | |

*CCNB3* is not the only partner fused with *BCOR* gene, as other new emerging gene partners have recently been described, such as *MAML3* and *ZC3H7B*. In addition, *BCOR* sarcomas with unknown partners have also been reported (Specht et al., 2016). Thus, we prefer to use the term *BCOR*-rearranged sarcomas ("*BRS*") that comprise all the molecular variants.

Gene fusions involving *BCOR*, with either *CCNB3* or *ZC3H7B* partner genes, have been reported in several translocation-associated mesenchymal tumors (Specht et al., 2016) and this finding of identical chromosomal translocations in various pathologic and clinical entities also suggests that, as in ESFT, there is no specific gene fusion that matches any of these particular histological subtypes.

As shown in Table 4, 68 "*BRS*" have been described so far, at least in the English literature (Pierron et al., 2012; Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Shibayama et al., 2015; Liao et al., 2016; Specht et al., 2016). The strong male occurrence associated with a high prevalence of neoplasms arising in extremities or pelvis in the 10-17 year-old age group are characteristics of such tumors. Although bone is the most frequent primary tumor location, soft tissue tumors have also been described, in contrast to the very uncommon primary visceral localization.

The histopathology of "*BRS*" usually reveals a neoplasm with a primitive appearance resulting in uniform small round/ovoid tumor cell proliferation intermixed with spindle cell formation (Fig. 4A-F). In some cases, the spindle cell arrangement is the predominant pattern resembling fibrosarcoma (Fig. 4A-C). The neoplastic cells show vesicular nuclei, fine chromatin, scant to moderate amounts of eosinophilic cytoplasm and usually inconspicuous nucleoli (Fig. 5 A,B). Nuclear pleomorphism is rare. The stromal tissue can reveal fibrosis, increased collagenous, hemorrhagic or myxoid changes similar to "*CRS*". As described in Table 3, myxoid stromal changes have been detected in several "*BRS*". The mitotic count is variable and necrosis can also be detected. "*BRS*" can resemble atypical ESFT or spindle cell sarcomas such as (synovial sarcoma, malignant peripheral nerve sheath tumor, fibrosarcoma, low-grade fibromyxoid sarcoma etc) depending on the predominant pattern (Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Specht et al., 2016). The differential diagnosis between "*BRS*" and these other entities should be performed through IHC (Fig. 5C) and/or molecular testing for the corresponding fusion transcripts with strong correlation with the clinical context.

Almost all *BCOR-CCNB3*-sarcomas show strong

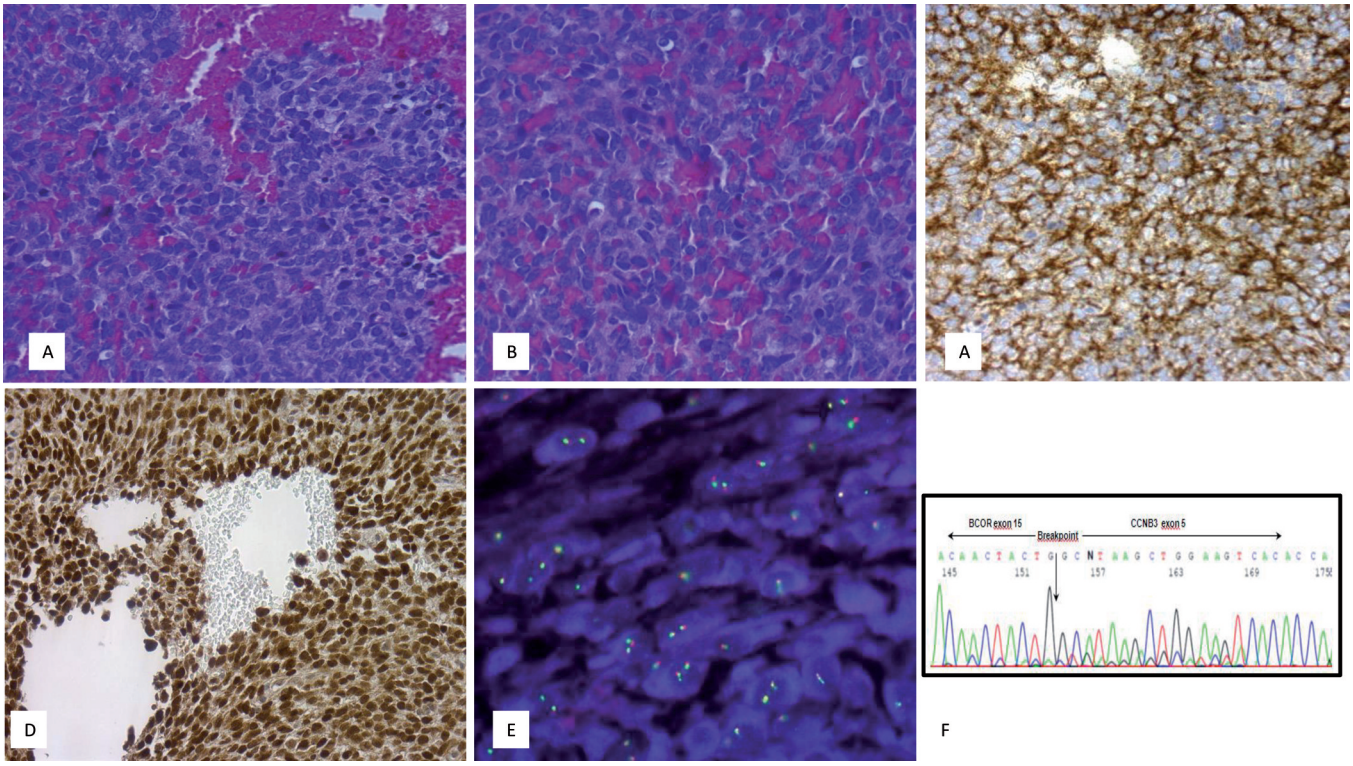


**Fig. 5.** (*BCOR*-rearranged sarcoma). **A, B.** *BCOR*-rearranged sarcoma with round, ovoid and focal spindle cells with hemorrhagic tissue, H&E. **C.** Moderate cytoplasmic and focal membranous CD99 expression in *BCOR-CCNB3* sarcoma. **D.** Strong nuclear CCNB3 expression in *BCOR-CCNB3* sarcoma. **E.** *EWSR1* FISH analysis lacking *EWSR1* rearrangement in *BCOR-CCNB3* sarcoma. **F.** *BCOR-CCNB3* gene fusion, RT-PCR analysis. x 40

nuclear CCNB3 immunoreactivity (Fig. 5D), however loss of expression or poor cytoplasmic staining can be observed in post-treatment samples or in "*BRS*" with an alternative gene partner. In fact CNNB3 staining is highly specific and sensitive for detecting *BCOR/CCNB3* sarcomas as reported by Pierron et al., (Pierron et al., 2012), thus a CCNB3 IHC study is strongly recommended for the first screening of *EWSR1/FUS*-negative undifferentiated sarcomas of bone or soft tissue. CD99 is also usually positive in "*BRS*" but the staining is patchy, cytoplasmic and focal, similar to many "*CRS*", although some "*BRS*" can reveal strong membranous CD99 stained mimicking an ESFT. CD56 can be positive in "*BRS*" but epithelial., myogenic or melanic differentiation is not observed in immunohistochemical analyses (Puls et al., 2014; Peter et al., 2015; Shibayama et al., 2015; Liao et al., 2016; Specht et al., 2016).

The clinical outcomes for patients with "*BRS*" are presently imprecise, given the small number of cases reported to date with all the clinical information and the heterogeneity of treatment (Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Specht et al., 2016). Early publications describe that survival is not significantly different than in ESFT (Cohen-Gogo et al., 2014). As observed in Tables 1 and 3, patients with "*BRS*" seem to have a better outcome in comparison with "*CRS*". In fact, further clinical studies including additional cases are necessary to confirm this statement. Induction chemotherapy has a positive impact in survival and is associated with long-term remissions (Cohen-Gogo et al., 2014; Puls et al., 2014; Peter et al., 2015; Specht et al., 2016).

In summary, bear in mind "*BRS*" as a possible diagnosis in male adolescents with a primitive/unclassified high-grade bone or soft tissue tumor with spindle or Ewing-like morphology lacking the *EWSR1/FUS*-rearrangement. A combination of CCNB3 IHC study with a confirmatory RT-PCR analysis (Fig. 5F) is the most appropriate diagnostic approach for this emerging group of sarcomas (Puls et al., 2014; Peter et al., 2015; Shibayama et al., 2015; Specht et al., 2016; Liao et al., 2016).

Despite thorough IHC and/or molecular studies of undifferentiated/unclassified sarcomas, some tumors still remain in this 'catch-all' WHO category (Alaggio et al., 2009; Fletcher et al., 2013, 2014; Antonescu, 2014; Mariño-Enríquez and Fletcher, 2014). Next-generation genomic sequencing technologies as well as new emerging molecular assays will probably help to decrease the list of cases with diagnosis of undifferentiated/unclassified sarcoma. The morphological., IHC and molecular characteristics of undifferentiated/unclassified sarcoma are beyond the scope of the present review.

In conclusion, regardless of overlapping clinical, histological and IHC features, the distinction between "*BRS*", "*CRS*" and ESFT appears to be of prognostic significance and would allow prospective therapeutic management with selection of appropriate chemotherapy regimens or target-specific therapy. Considering the morphological overlapping in those tumors, a molecular study is needed to achieve an accurate diagnosis, while taking into account that fusion subtypes do not match any specific histological subtypes, a definitive diagnosis will still depends on a strong correlation between clinical., morphological and phenotypic characteristics.

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