Head and Neck Round Cell Sarcomas: A Comparative Clinicopathologic Analysis of 2 Molecular Subsets: Ewing and *CIC*-Rearranged Sarcomas

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Abstract *CIC*-rearranged sarcoma (CRS) is a relatively new entity defined by its pathognomonic genetic signature and undifferentiated round cell phenotype, initially grouped together with the ‘Ewing sarcoma-like tumors’. However, increasing data suggest that these tumors should be regarded as a stand-alone pathologic entity. We conducted a clinicopathologic analysis on molecularly conformed Ewing sarcoma (ES) and CRS arising in the head and neck (HN) and compared to a well characterized cohort of ES and CRS from other locations. A total of 41 HN round cell sarcoma patients were selected from our institutional and consultation files, including 25 ES (median 20 years) and 16 CRS (median 29 years). Clinical follow-up information was available for all ES patients, ranging from 4 to 436 months (median 70 months), while for CRS, follow-up information was available in 11 patients (69%), ranging from 1 to 269 months (median 27 months). The most common location for ES was the facial and jaw bones (56%), while CRS occurred exclusively in the soft tissue, commonly in the neck. CRS showed variable CD99 staining in 75% of cases and diffuse WT1 (6/6) reactivity, while all ES expressed diffuse membranous staining for CD99 but none for WT1 (0/6). The 2-year overall survival (OS) rate for HN-CRS patients was 78%, while for HN-ES it was 100%. The OS of ES and CRS showed a trend toward a favorable

outcome for HN-round cell sarcomas compared to other sites. Our findings suggest that HN-CRS have different clinical presentation and pathologic features compared to ES and should be classified as a stand-alone pathologic entity.

Keywords *CIC* · *DUX4* · *CIC-DUX4* · *EWSR1* · *SBRCT* · Sarcoma

Introduction

Undifferentiated round cell sarcomas are characterized by monomorphic small round to ovoid cytomorphology with a high nuclear to cytoplasmic ratio, lacking a definite line of differentiation. Ewing sarcoma (ES) is the prototypical round cell sarcoma characterized by pathognomonic gene fusions between the *EWSR1* gene and member of the *ETS* transcription factor family (*FLII*, *ERG*, *ETV1*, *ETV4*, or *FEV*) [1–6] and less commonly to a non-ETS family gene, such as *NFATc2* [7]. In rare examples of ES, *FUS* gene can substitute for *EWSR1* in the fusion, retaining a similar phenotype [8–10]. Other round cell sarcomas with specific translocations include alveolar rhabdomyosarcoma, myxoid/round cell liposarcoma, desmoplastic small round cell tumor and poorly differentiated monophasic synovial sarcomas. In addition, there is a subset of tumors morphologically resembling ES with either non-*EWSR1-ETS* fusions or lacking known genetic abnormalities, which have been termed as ‘Ewing sarcoma-like tumors’. The recent application of genomic methods, including whole transcriptome sequencing, has led to the discovery of new recurrent translocations in this pathologic spectrum of undifferentiated round cell sarcomas, including *CIC-DUX4* fusion, resulting from either a t(4;19) or t(10;19) translocation, *CIC-FOXO4*

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and *BCOR*-related fusions involving *CCNB3*, *MAML3*, and *ZC3H7B* gene partners [11–18]. As most of the initial series have included a limited number of cases of each molecular subset, definitive classification of these tumors within or outside the more common ES spectrum has been difficult to establish. Despite the emerging data that sarcomas with alternative fusions have somewhat distinct morphology and immunoprofile, large series of molecularly confirmed groups are lacking. Furthermore, no reliable survival analysis has been done comparing the outcome of patients with Ewing sarcoma-like versus classic ES, matched for age, anatomic site and stage. In this study we took advantage of our large database of pathologically and molecularly well-defined round cell sarcomas, and investigated a cohort of HN Ewing sarcoma family of tumors, either with the canonical *EWSR1-ETS* fusion or with alternative gene fusions. As *CIC*-related fusions account for 2/3rds of *EWSR1*-negative round cell sarcomas, we have focused on this particular molecular subset compared to classic ES in the HN location. These 2 cohorts were then further compared to similar tumors from other anatomic sites.

Materials and Methods

The Pathology files of Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY as well as the consultation files of the senior authors (CRA and CDF) were searched for the diagnosis of small blue round cell tumors, Ewing sarcoma, Ewing-like sarcoma, atypical Ewing sarcoma and undifferentiated round cell sarcoma involving the head and neck (HN). Only cases that had either previous molecular confirmation or had available material for further molecular work-up were included in this study. The molecular confirmation of ES was based either on RT-PCR evidence of an *EWSR1-FLI1/ERG* fusion transcript or an *EWSR1/FUS* gene rearrangement by FISH, as previously described [10]. For *CIC*-rearranged sarcomas (CRS), the diagnosis was confirmed by *CIC* gene rearrangement by FISH, followed by further FISH-fusion assay investigation to determine a *CIC-DUX4* fusion [13]. Cases without a positive molecular result or without available material for further FISH testing were excluded. The following clinical data were retrieved: age, gender, anatomic site, tumor size, history of metastasis at diagnosis, modality of therapy, recurrence, vital status and survival time. The overall survival of each of the HN molecular groups was compared to similar cohorts of all anatomic sites.

The existing treatment guideline for ES at our Institution includes standard, multiagent induction chemotherapy (vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide), followed by surgical resection if feasible.

Adjuvant radiotherapy is employed in tumors >5 cm and for anatomic sites where local control may be difficult by surgery. A similar chemotherapy regimen and management has been offered for Ewing sarcoma-like patients, including the CRS, as alternative options are not available at this time. Patients with high risk of recurrence are often placed on irinotecan and temozolomide medications.

Statistical Analysis

Statistical analysis was performed on an SPSS platform (version 24.0; IBM Corp., Armonk, NY). The overall survival (OS) time was measured in months from diagnosis to the date of death. Kaplan–Meier estimate was used to calculate the OS, and the statistical significance of different clinicopathologic variables [age group (adult/pediatric), tumor size (>5 cm/≤5 cm), and recurrence (no recurrence/local recurrence/distant recurrence)] in relation to survival of ES patients was assessed by log-rank analysis. The OS between HN ES and CRS patient cohorts, as well as the site-specific comparisons, such as HN ES vs. all ES and HN CRS vs. all sites, were compared by log-rank analysis. A $p < 0.05$ was considered as significant for all statistical analysis.

Results

Clinicopathologic Features

Ewing Sarcoma (ES)

There were 25 HN ES patients treated at our Institution (MSKCC) with confirmed molecular diagnosis, including: 17 (68%) males and 8 (32%) females, with a median age of 20 years (age range 1–67 years). Among them, 10 (40%) were of pediatric age (<18 years). The anatomic locations included: facial bones ($n=8$, 32%), jaw bones (mandible $n=5$, maxilla $n=1$), skull base bone ($n=3$) and soft tissue (parotid $n=2$, neck $n=2$, scalp $n=2$, submandibular region $n=1$, buccal masticator space $n=1$). The tumor size available in 21 patients ranged from 1.6 to 6.7 cm, mean of 4.3 cm. A large tumor size >5 cm was seen in 7 (28%) patients. Only one patient presented with metastasis at presentation to other bones.

Morphologically, all except one case showed classic microscopic features with solid sheets of primitive small blue round cells, lacking any signs of differentiation or any significant stromal component. One case showed features in keeping with the so-called adamantinoma-like ES [19], being composed of nests and trabecular growth, with cohesive rounded to ovoid cells, arranged in a somewhat basaloid pattern (Fig. 1). In fact this case, due to its atypical

Fig. 1 Adamantinoma-like Ewing sarcoma—diagnostic pitfalls. Morphologic appearance shows cohesive round to ovoid cells with a distinctive basaloid nested appearance (a), with focal areas of squa-

moid pearls (b) and glandular differentiation (c). Immunohistochemical stains showed positivity for cytokeratin AE1:AE3 (d), and P40 (e), as well as strong, membranous staining for CD99 (f)

histologic features and expression for epithelial markers, such as cytokeratin and P40 (Fig. 1) was initially misdiagnosed as a multiply recurrent mandibular ameloblastic carcinoma.

Immunophenotypically, all cases evaluated were positive for CD99 (n=24) with a strong and diffuse membranous pattern. All cases (n=6) evaluated for WT1 were negative. All cases except one were molecularly confirmed as having an *EWSR1*-related fusion, including an *EWSR1* gene rearrangement by FISH (n=17), or an *EWSR-FLI* (n=5) and *EWSR-ERG* (n=2) fusion transcript by RT-PCR. One case had instead a *FUS* gene rearrangement by FISH.

CIC-Rearranged Sarcoma (CRS)

The clinical features of the 16 CRS selected are summarized in Table 1; 4 patients being treated at MSKCC and 12 patients included from the personal consults of the senior authors (CRA, CDF). Fifteen patients were previously included in a larger series of CRS spanning all anatomic locations [20]. There were 9 (56%) males and 7 (44%) females, with an age range of 10–66 years (median of 28.5 years). Three (19%) patients were of pediatric age (<18 years). All tumors occurred at extraskeletal soft tissue locations, with the neck being the most common, accounting for over two-thirds of all HN sites. Other anatomic sites included scalp (n=2), submandibular region

(n=2) and tonsil (n=1). The tumor size, available in 10 patients, ranged from 3.2 to 7 cm (mean, 4.8 cm), with 4 (36%) patients having tumor sizes larger >5 cm. Based on the MRI images reviewed, the lesions of large size were associated with a mass-effect on nearby structures (Fig. 2). All patients presented with localized disease at diagnosis.

The morphologic appearance showed tumors with a compact solid growth, often displaying a nodular pattern, separated by fibrous septa (Fig. 3). Most tumors were composed predominantly of primitive small to medium-sized round to ovoid cells, with mild irregularity of nuclear contour and heterogeneity in size and shape (Fig. 3). However, in half of the cases there were intermixed focal areas of spindle and more epithelioid cells with pale eosinophilic cytoplasm (Fig. 3). All tumors showed high mitotic activity of >10 MF/10 HPFs (Fig. 3) and most showed geographic necrosis.

Immunohistochemical analysis showed reactivity for CD99 in 15/16 cases: 3 with diffuse positivity, 5 multifocal staining and 7 only focal. Six cases evaluated for WT1 showed diffuse nuclear expression (Fig. 3). Desmin, myogenin and S100 immunohistochemical stains were negative in all cases tested.

All cases showed a *CIC* break-apart signal by FISH. Of these, a *CIC-DUX4* fusion was confirmed in 9 cases by 3-color FISH fusion assay, with 7 (44%) cases involving

Table 1 Clinical features and follow-up information of *CIC*-rearranged sarcoma arising in the head and neck

Case #	Age/sex	Site	Size (cm)	Therapy ^a	Recurrence (site)	Follow-up duration	Vital status
1 ^{a,b,c}	30/F	Tonsil	5.0	Surgery , CT and RT	DR (Lung)	17 mos	DOD
2 ^{b,c}	25/M	Neck	5.0	Surgery	NR	56 mos	Alive (NED)
3 ^c	21/M	Parapharyngeal (Neck)	5.1	CT , Surgery and RT	LR, DR (Lung)	27 mos	DOD
4 ^c	50/M	Scalp	4.0	Surgery and CT	NR	34 mos	Alive (NED)
5 ^c	19/F	Neck	6.2	CT and RT	LR	33 mos	Alive (NED)
6 ^c	44/F	Neck	3.5			1 mo	Alive (AWD)
7 ^c	13/M	Submandibular area	5.0	Surgery and CT		1 mo	Alive (AWD)
8 ^c	27/F	Supraclavicular area (Neck)	3.2		NR	36 mos	Alive (NED)
9 ^c	21/M	Neck	NA	CT , Surgery and RT	NR	269 mos	Alive (NED)
10 ^{b,c}	47/M	Supraclavicular area (Neck)	7.0	Surgery , CT and RT	DR (Lung)	20 mos	Alive (NED)
11	66/M	Neck	6.1	Surgery and CT	DR (Lung)	13 mos	DOD
12 ^c	10/F	Neck	NA	NA	NA	NA	NA
13 ^c	30/F	Neck	3.8	NA	NA	NA	NA
14 ^c	10/M	Scalp	NA	NA	NA	NA	NA
15 ^c	41/F	Neck	NA	NA	NA	NA	NA
16 ^c	46/M	Submandibular area	NA	NA	NA	NA	NA

^aTherapy in bold was the first modality administered; *CT* chemotherapy, *RT* radiotherapy, *LR* local recurrence, *DR* distant recurrence, *NR* no recurrence, *DOD* died of disease, *NED* no evidence of disease, *AWD* alive with disease; *NA* not available

Previously reported in: ^aItaliano et al. [13]; ^bSpecht et al. [23]; ^cAntonescu et al. [20]

Fig. 2 MRI findings of HN *CIC*-rearranged sarcoma. Images illustrate large soft tissue mass associated with mass effect on nearby structures. **a** Neck *CIC*-rearranged sarcoma occurring in a 21-year-old male, developed LR and DR to the lung, deceased at 27 months. **b** Tonsil *CIC*-rearranged sarcoma in a 30-year-old female, developed DR to the lung, deceased at 17 months

the *DUX4* gene on 4q35 and 2 (13%) the *DUX4* on 10q26 (Fig. 4).

Follow-Up Data

Ewing Sarcoma

Clinical follow-up information was available for all patients, ranging from 4 to 436 months, median 70 months. One patient presented with distant metastasis at initial diagnosis. Among the remaining patients presenting with localized disease, 11 developed recurrence following treatment:

3 local recurrence (LR) alone, 4 distant recurrence (DR) and 4 patients developed both LR and DR. Overall, the metastatic rate was 36% (9/25) and 33% (8/24) in the localized group. The lungs were the most common site for distant recurrence (4/8, 50%); other sites including chest wall, spine, mediastinum and pelvic bone. Four (16%) patients had died of disease (DOD) at last follow up. Of the patients who died, 1 presented with distant metastasis at initial diagnosis and the remaining 3 after subsequently developing DR.

From the 22 ES patients with available therapy information, 12 patients received initial surgery with curative

Fig. 3 Histologic spectrum of CIC-rearranged sarcoma (CRS). Tumors were composed of solid sheets of round to ovoid cells with vesicular chromatin and conspicuous nucleoli and a high mitotic

activity (**a**); focal areas of spindle (**b**) or epithelioid cells with scattered enlarged, pleomorphic nuclei (**c**). Immunohistochemically the tumor showed strong and diffuse WT1 nuclear expression (**d**)

intent, followed by adjuvant chemo-radiation in 8 patients, adjuvant radiotherapy in 3 and 1 with no adjuvant therapy. Ten patients received neoadjuvant chemotherapy (9 with localized disease and 1 with distant metastasis at initial presentation), followed by surgical resection (n=10) and radiotherapy in 5 patients. All patients received an ES-type chemotherapy regimen. All patients with localized disease who received neoadjuvant chemotherapy are alive and only one patient who received ES neoadjuvant chemotherapy developed DR. Six patients who had surgical excision first developed DR, including 4 patients who received adjuvant chemoradiotherapy, 1 patient with adjuvant radiation alone and 1 patient who received no adjuvant therapy. Within the cohort of patients treated with initial surgery, 3 DOD, including 2 patients who received adjuvant chemoradiotherapy and 1 without any other adjuvant modalities.

CIC-Rearranged Sarcoma

Clinical follow-up information was available in 11 patients (69%), ranging from 1 to 269 months, median 27 months.

Five (45%) developed recurrence following treatment: 1 patient had LR alone, 3 DR and 1 patient developed both LR and DR. Overall the metastatic rate was 36% (4/11), with lungs being the dominant site for DR. Three (27%) patients DOD at last follow up; all after developing DR. Nine patients had therapy information, with 6 patients receiving initial surgery with curative intent, followed by adjuvant chemoradiation in 2 patients, chemotherapy alone in 3 and 1 without adjuvant modalities. Three patients received neoadjuvant chemotherapy, followed by surgical resection in 2 patients and radiotherapy in one. Five patients received an ES-type chemotherapy regimen. One patient who received neoadjuvant chemotherapy developed DR and DOD. Of the 6 patients who received initial surgery, 3 developed DR (2 adjuvant chemoradiotherapy, 1 adjuvant chemotherapy) and 2 DOD.

Survival Analysis

On survival analysis, the 2, 5 and 10-year overall survival (OS) rates for HN-ES patients were 100, 90 and

Fig. 4 FISH studies as reliable molecular methods in differential diagnosis of HN round cell sarcomas. Adamantinoma-like Ewing sarcoma case showing *EWSR1* break-apart (arrows, red, centromeric; green, telomeric) (a) and *FLII* split signals (arrows, red, centromeric; green, telomeric) (b). CRS showing an unbalanced *CIC* rearrange-

ment (arrow, red, centromeric) with deletion of the telomeric part (green) (c). FISH fusion assay in a different CRS case showing *CIC* (green, telomeric) signal fused together with *DUX4* on 4q35 (red, centromeric) signals (arrows) (d)

74%. Only a 2-year OS rate could be calculated for the HN-CRS patients which was 78%, due to a limited number of patients with follow-up. There was no significant effect on OS in HN-ES patients when comparing age group (adult vs. pediatric; $p=0.426$), tumor size (≤ 5 cm vs. >5 cm; $p=0.102$) and recurrence (NR vs. LR vs. DR; $p=0.109$).

There was no significant difference between the OS of HN ES and CRS patients ($p=0.117$) (Fig. 5a), even when comparing the extraskelatal HN-ES ($n=8$) to HN-CRS ($p=0.104$) (Fig. 5b). Furthermore, only a trend toward favorable outcome ($p=0.082$) was noted in the localized HN-ES cohort ($n=24$), compared to the localized ES of other sites ($n=82$) (Fig. 5c). Similarly, there was no

difference in OS between the localized CRS of HN or other locations ($p=0.297$) (Fig. 5d).

Discussion

This study investigates a cohort of molecularly defined round cell sarcomas arising in the head and neck. The analysis also evaluates for the first time the clinicopathologic features of *CIC*-rearranged sarcomas (CRS) in this anatomic location and provides the incidence of CRS among tumors previously diagnosed as Ewing sarcoma-like tumors. *CIC-DUX4* gene fusion, resulting from either a $t(4;19)$ or $t(10;19)$ translocation, is the most common

Fig. 5 Kaplan–Meier curves of OS of Ewing sarcomas and *CIC*-rearranged sarcoma. No difference in OS was noted between localized ES and CRS of HN ($p=0.117$) (a), or between extraskelletal ES and *CIC*-rearranged sarcomas of HN ($p=0.104$) (b); however, a

trend for better OS was seen in localized HN ES compared to other sites (localized) ($p=0.082$) (c) and for HN *CIC*-rearranged sarcomas compared to other sites (localized) there was no difference in OS ($p=0.297$) (d)

genetic abnormality detected in *EWSR1*-negative Ewing sarcoma-like tumors. Following their discovery it was debated if these tumors should be classified as variants of ES or as a stand-alone pathologic entity. As such, the WHO classification of soft tissue tumors in 2013 [21] grouped the CRS under undifferentiated sarcomas with round cell phenotype, at least temporarily until more clinical evidence became available. However, most studies reported so far include small series with limited follow-up information inadequate for a definitive assessment. The present work undertakes a comparative clinical and pathologic investigation of a large cohort of HN ES and CRS, in order to define

their potential differences in clinical presentation, morphologic spectrum, and outcome.

Among the 31 HN round cell sarcomas with a known gene fusion treated at MSKCC there were: 24 (77%) with *EWSR1*-related fusion, 1 (3%) with *FUS* rearrangement, 4 (13%) with *CIC* gene break-part and 2 (6%) with *BCOR* gene abnormalities. The analysis was then focused only on the 2 more prevalent groups: ES with canonical *EWSR1/FUS-ETS* fusions and CRS. First, the anatomic distribution of CRS was exclusively in the neck soft tissues, whereas more than half of the ES occurred in the bone, mostly in the facial bones. This finding is similar to CRS

studies from other primary sites, highlighting the predilection for deep soft tissues, with only rare presentation in the viscera or bone [22, 23]. Second, the age at presentation for HN CRS was a decade older than ES patients, occurring in young adults in their 3rd decade of life, while a significant proportion of ES patients are children. This older age distribution is in keeping with what has been reported in CRS of other anatomic locations [22–24]. Our results also showed a male gender preponderance in HN ES (M:F ratio > 2), compared to HN CRS which showed an even gender distribution.

At the morphologic level, CRS showed subtle but distinct microscopic features from the highly monomorphic round cell proliferation typical of ES. Although most CRS tumors showed a predominant round to ovoid cell phenotype, of small to intermediate sized cells, there was in addition a focal component of other cell types, such as spindle or more often epithelioid, with a moderate amount of pale eosinophilic cytoplasm, present in more than half of the cases. Furthermore, the cells lacked the uniformity of ES and showed more heterogeneous nuclear features, such as variability in size, shape, chromatin pattern and nucleoli, as previously described in other CRS presentations outside HN [13, 22]. Immunophenotypically, although similar to ES, 94% of CRS showed CD99 positivity, the pattern of expression was diffuse only in 19%, while being focal or patchy in 75%. Nuclear reactivity for WT1, as previously described at other sites, was positive only in CRS but not in the ES tested [23].

One additional level of complexity when dealing with HN round cell malignancies is the wide differential diagnosis encountered in this anatomic location, with a wide spectrum of round cell/undifferentiated epithelial tumors being more common than sarcomas at this site. Even in the setting of a round cell lesion in a young patient, where diagnostic considerations typically include ES, the occasional nested appearance and cytokeratin expression may suggest instead a carcinoma diagnosis. Indeed, 2 of our ES cases were initially misdiagnosed as epithelial tumors. The first case, a 22-year-old male with a maxillary bone and ethmoid sinus tumor was misdiagnosed as a poorly differentiated squamous cell carcinoma, due to positivity for epithelial markers (AE1:AE3 and 34BE12). The patient was initially treated with neoadjuvant cisplatin and 5-fluorouracil, followed by surgery and adjuvant radiation. He subsequently developed osseous and spinal metastases 3 years later, from which material was analyzed by RT-PCR and found to have an *EWSR1-FLII* fusion. The patient was subsequently treated with ES-like chemotherapy regimen and radiation to C7-T4 and is alive with disease in the lung at 171 months follow-up. The 2nd case, a 28-year-old male with a mandibular lesion was misdiagnosed as an ameloblastic carcinoma due to its distinctive basaloid appearance

with cohesive nests and trabeculae of round to ovoid cells showing focal squamous and glandular differentiation. Its immunoprofile was further suggestive of epithelial differentiation, being positive for AE1/3, CK5/6, P40 and p16. The patient developed 2 local recurrences and one distant recurrence to the dura, being treated with surgical resection and radiotherapy. Subsequent targeted sequencing from one of the recurrences identified a *EWSR1-FLII* fusion in keeping with a diagnosis of adamantinoma-like ES. The diagnostic challenges of adamantinoma-like ES arising in the HN have been recently highlighted in a study of 7 cases, spanning a wide variety of anatomic sites (paranasal sinuses, orbit, parotid and thyroid glands) and age range (7–56 years) [19]. Diagnostic pitfalls stem from the epithelial features identified in these tumors, such as squamoid pearls, intra-epithelial growth, as well as immunoreactivity for epithelial markers. As carcinomas typically outnumber round cell sarcomas in the H&N, the more common entities that adamantinoma-like ES were confused with were: basaloid squamous cell carcinoma, basal cell adenocarcinoma [25], and sinonasal undifferentiated/poorly differentiated carcinoma [19]. The young age at diagnosis and the diffuse/strong membranous pattern of CD99 reactivity are important clues for a diagnosis of adamantinoma-like ES, which should trigger additional molecular testing.

A further diagnostic challenge is the HN *EWSR1*-positive myoepithelial carcinoma, which can mimic a HN ES due to similar *EWSR1* gene rearrangement and clinical presentation in children and young adults. Myoepithelial tumors occasionally show a predominantly round to ovoid phenotype with clear cytoplasm [26], however, in contrast to ES, they often display an abundant myxoid or hyalinized stromal component and at least focally epithelioid cells arranged in cords and nests. Furthermore, myoepithelial tumors often show diffuse expression for EMA and S100 protein, while lacking CD99 reactivity and *FLII* gene rearrangements by FISH.

The diagnosis of CRS in the HN is extremely rare, only 11 cases being described to date [12, 13, 17, 18, 22, 23, 27]. As CRS is a relatively new pathologic entity, without a specific immunoprofile, it remains under-recognized outside specialist sarcoma centers. Some of its pathologic features, such as variable CD99 staining, the increased cytologic variability and the lack of *EWSR1* break-apart by FISH, have historically discouraged its classification into the Ewing sarcoma family of tumors.

At the molecular level, ES is characterized by recurrent fusions between the *EWSR1* gene and a member of the *ETS* transcription factor family. In contrast, the *CIC-DUX4* fusions in CRS are functionally unrelated to *EWSR1-ETS*. *CIC*, the human homologue of *Drosophila capicua*, encodes a transcriptional repressor with a high-mobility group (HMG)-box containing DNA binding

domain [28]. *CIC* gene abnormalities have been implicated in other tumors, either as loss of function mutations in oligodendrogliomas or as gene fusions in central nervous system primitive neuroectodermal tumors (PNET) and angiosarcomas [29–32]. *DUX4* is normally expressed in germ cells and is epigenetically silenced in somatic differentiated tissues through CpG methylation [33]. Aberrant expression of *DUX4* has been implicated in the development of facioscapulohumeral muscular dystrophy [34].

Our survival analysis focusing on the HN location did not identify a significant difference between CRS and ES overall survival (OS). In contrast, Yoshida et al. showed that CRS (of all anatomic sites) are resistant to ES-type chemotherapeutic regimens and that the OS was significantly worse than ES patients [22]. Other studies further emphasized that CRS is a highly aggressive tumor with high metastatic rate in comparison to ES [35, 36]. In this study, the 2-year OS rate of patients with HN-ES (100%) was better than that of patients with HN-CRS (78%), but not statistically significant ($p=0.117$). This may be related to the small sample size of HN-CRS patients with follow-up information ($n=11$) and the short follow-up interval (median of 27 months). Interestingly, the comparison of OS of HN-ES to ES of other sites showed a trend toward a favorable outcome for HN-ES ($p=0.082$). A similar observation was noted when comparing the OS of HN-CRS to CRS of other sites. A recent study using the Surveillance, Epidemiology, and End Results (SEER) database, showed that patients with HN-ES have a better OS compared to ES of other sites ($p=0.015$), possibly related to the lower disease burden [smaller tumor size and lower initial metastasis at diagnosis ($p<0.001$)] at presentation compared to ES of other sites [37]. Moreover, in our prior study of primary jaw ES, all 6 patients were alive with no evidence of disease at last follow-up and none presented with initial metastasis at diagnosis [38].

In conclusion, this is the largest analysis of molecularly confirmed HN round cell sarcomas, with particular emphasis on the 2 most common molecular subsets, the classic Ewing sarcoma with *EWSR1*-canonical fusions and the *CIC*-rearranged sarcoma. Similar to other anatomic sites, HN-CRS shows significant clinical, pathologic and molecular differences compared to the more common ES, and should be classified as a stand-alone pathologic entity.

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Compliance with Ethical Standards

Conflict of interest All authors have contributed to this work and we declare that there are no financial conflicts associated with this study

and that the funding source has no role in conceiving and performing the study.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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