

# Beyond Immunohistochemistry: Molecular Insights Into Round Cell Soft Tissue Tumors

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## ABSTRACT

Round cell soft tissue tumors (RCSTTs) are a diverse group of aggressive malignancies that share overlapping clinical, morphological, and immunohistochemical features, posing significant diagnostic challenges. Recent advances in molecular pathology have revolutionized the understanding and classification of these tumors by identifying tumor-specific genetic alterations. This review highlights the role of molecular insights in complementing histopathology and immunohistochemistry to achieve precise diagnosis, risk stratification, and therapeutic decision-making. Key subtypes, including Ewing sarcoma, CIC- and BCOR-rearranged sarcomas, alveolar rhabdomyosarcoma, desmoplastic small round cell tumor, and extraskeletal myxoid chondrosarcoma, are discussed with emphasis on their molecular signatures and clinical significance. The advent of techniques such as fluorescence in situ hybridization (FISH), reverse transcriptase-polymerase chain reaction (RT-PCR), and next-generation sequencing (NGS) has enabled detection of genetic fusions and aberrations critical for tumor identification and targeted therapies. Integration of molecular pathology with traditional diagnostic approaches is essential for improving diagnostic accuracy, prognostication, and therapeutic strategies, paving the way for personalized medicine in the management of RCSTTs. Full form of ABBREVIATIONS are provided at the end.

**KEYWORDS:** Chromosomal Aberrations, Molecular Pathology, Round Cell Tumors, Therapeutic Targets

## INTRODUCTION

Small round cell tumors of soft tissue are a diverse group of neoplasms that share many clinical, radiological, his-

tomorphological similarities and immunophenotypes but diverse clinical outcomes. The WHO criteria serve as the cornerstone for their diagnosis and classification based on histogenesis and molecular aspects.<sup>[1]</sup> These tumors are characterized as highly aggressive malignant tumors composed of relatively small, round, hyperchromatic and monotonous undifferentiated cells with an increased nuclear to cytoplasmic ratio.<sup>[1]</sup> The differential diagnosis of these tumors is particularly difficult because of their undifferentiated or primitive character. Immunohistochemistry plays a vital role in rendering a specific diagnosis or narrowing the differential diagnosis (See Table 1). Molecular genetic studies are often needed, especially for those lesions with unusual histologic features, an uncommon immunoprofile, and/or unusual clinical presentation. Recent molecular genetic advances have identified a growing list of round cell sarcomas, thus having revolutionized the diagnosis of sarcomas and provided insight into potential therapeutic targets as well as prognostic biomarkers.<sup>[2]</sup> These tumors include Ewing sarcoma (ES), characterized by specific EWSR1-ETS family gene fusions; CIC-rearranged and BCOR-rearranged Ewing-like sarcomas, which share histologic features with ES but lack EWSR1 aberrations<sup>[3]</sup>; alveolar rhabdomyosarcoma (ARMS), recognized by distinct PAX-FOXO1 fusions; desmoplastic small round cell tumor (DSRCT), defined by EWSR1-WT1 translocations; and extraskeletal myxoid chondrosarcoma (EMC), which harbors NR4A3 gene rearrangements. Understanding these distinct subtypes is essential for accurate classification, risk stratification, and management.

Chromosomal aberrations have been found in virtually all tumor types, some of which are primary and clearly central to the pathogenesis of a given tumor. In contrast, others are secondary, probably occurring later in the tumor development and progression. Approximately 20% of soft

tissue sarcomas are characterized by a specific balanced translocation resulting in the creation of the fusion gene. The ability to detect these translocations by molecular methods such as FISH, RT-PCR and NGS is increasingly becoming critical to the diagnosis and management of these diseases.<sup>[4]</sup>

### EWINGS SARCOMA

Histologically, classic ES shows a solidly packed lobular architecture of strikingly uniform round cells with inapparent or small nucleoli. Intracellular glycogen is present in most cases. Approximately 75% of the cases show these classic morphological features while a minority display moderate nuclear enlargement, irregular nuclear contours and prominent nucleoli corresponding to atypical or large cell variant of ES.<sup>[1]</sup> The morphological spectrum of ES is now known to include Adamantinoma-like cases, Keratin positive cases and rare desmin positive cases.<sup>[5,6]</sup> Numerous IHC markers such as CD99<sup>[7-9]</sup>, FLI-1<sup>[10]</sup>, NKX-2.2<sup>[11]</sup>, Keratin and Desmin<sup>[6]</sup> are considered (See Table 1). Some of them are sensitive but their aberrant expressions and lack of specificity are limiting their potential as diagnostic tools. Molecular pathology including cytogenetics, FISH, RT-PCR and NGS is providing finer data aiding in the specific diagnosis of Ewings Sarcoma and Ewings like tumors. (See Table 2). A minority of ES cases also express secondary cytogenetic abnormalities including Trisomy 8, trisomy 12, unbalanced t(1;16) leading to gain of 1q and loss of 16q.<sup>[12]</sup> TP53 and p16/p14ARF are detected in chemotherapy refractory tumors and associated with aggressive clinical course.<sup>[13]</sup>

### CIC- REARRANGED EWING-LIKE SARCOMAS

Histological features in CIC-rearranged sarcomas include small round cells with vesicular nuclei (greater nuclear pleomorphism than in ES), prominent nucleoli and amphophilic to slightly eosinophilic cytoplasm. Distinct lobular architecture, extensive areas of necrosis and mitosis (including atypical forms) is common. Other uncommon features include spindle cell areas, myxoid stroma, large epithelioid or rhabdoid cells.<sup>[17]</sup>

This subset of SBRCT closely resembling ES but lacking aberrations of EWSR1 have been delineated over the past few years, many of which show rearrangement of the CIC gene on 19q13.<sup>[17,18]</sup> Some of these tumors show t(4;19)(q35;q13.1) involving the DUX4 and CIC genes on chromosomes 4 and 19, respectively, whereas others show a t(10;19) involving a gene on chromosome 10q26, which is highly homologous to the DUX-4 gene on 4q35.<sup>[17-19]</sup> Although referred to in the WHO classification as "Ewings like sarcoma", it is clear that CIC-rearranged sarcomas account for the majority (60-70%) of these Ewings like tumors.<sup>[17]</sup>

While CD99<sup>[20]</sup> and WT-1<sup>[14,15]</sup> are sensitive markers in IHC, they lack specificity. In contrast, DUX-4 is gaining

prominence for its specific diagnostic utility<sup>[14,15]</sup> (See Tables 1 and 3). Molecular pathology detection of fusion genes is crucial for identifying CIC-rearranged sarcomas, providing a definitive diagnostic advantage<sup>[17,18,21]</sup> (Refer to Tables 2 and 3).

### BCOR- REARRANGED EWING-LIKE SARCOMAS

Among the "Ewing-like" sarcomas, those with BCOR-rearrangements are much less common than CIC-rearranged sarcomas accounting for approximately 10% of Ewing-like sarcomas.<sup>[20]</sup>

Histologically tumors may show an exclusive round cell population or a spindle cell population or an admixture of both round and spindle cells. Other features seen include whorling fascicles or fascicles with herring-bone pattern. Mitosis, necrosis, and hemorrhage are quite common. By IHC, apart from BCOR and CCNB3, other markers such as CD99, SATB2, TLE1, Keratin, S100, SOX10, melanocytic, and myoid markers have proven less helpful due to aberrant or absent expression<sup>[20,26]</sup> (see Table 1). As most of these tumors harbor BCOR- fusion genes, molecular pathology detection of BCOR associated fusion genes is crucial<sup>[26]</sup> (Refer to Tables 2 and 3).

### ALVEOLAR RHABDOMYOSARCOMA

ARMS is prevalent in adolescents and young adults and characterized by proliferation of tumor cells with the characteristic alveolar growth pattern. The solid subtype shows proliferation of tumor cells in a sheet-like pattern without fibrovascular septa. Some tumors show a morphological admixture mimicking embryonal component<sup>[1]</sup>. Although rhabdomyoblastic differentiation is confirmed by immunohistochemistry with desmin, myogenin and MyoD1<sup>[1]</sup> molecular pathology studies allow efficient distinction of ARMS from other rhabdomyosarcoma subtypes (Embryonal RMS lacks defined chromosomal abnormalities).<sup>[28]</sup> Expression of myogenin, AP-2beta, NOS-1 and HMGA2, by IHC, varies with fusion positive / negative status.<sup>[1,4]</sup> Gene fusion status, as detected by Cytogenetics, RT-PCR, FISH<sup>[29]</sup>, is playing a critical role in risk stratification and prognostication of ARMS cases<sup>[30-33]</sup> (Refer to Tables 2 and 4).

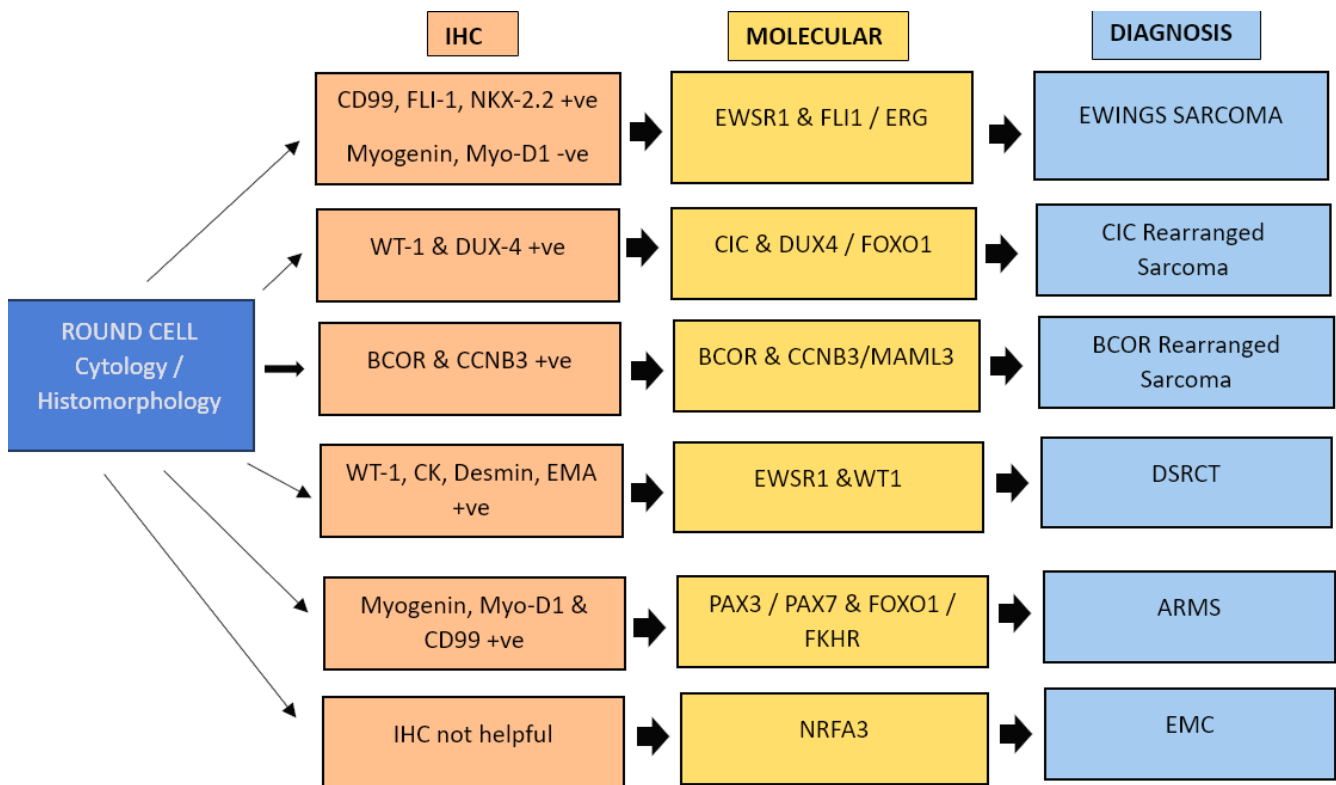
### DESMOPLASTIC SMALL ROUND CELL TUMOR

DSRCT primarily affects children and young adults and must be differentiated from other small round cell tumors, including Ewing sarcoma, alveolar rhabdomyosarcoma, neuroblastoma, lymphoma, poorly differentiated carcinoma, small cell carcinoma, merkel cell carcinoma and malignant mesothelioma. DSRCT typically presents with widespread abdominal serosal involvement and exhibits polyphenotypic differentiation. With IHC, lesional cells show positivity for Cytokeratins, EMA, Desmin, Vimentin and WT-1.<sup>[1,4]</sup>

In DSRCTs, molecular pathology consistently shows the t(11;22)(p13;q12) translocation, leading to EWSR1-WT1

Marker	Expression Pattern / Characteristics	Relevance / Significance
CD99	Product of MIC-2 gene; patchy or diffuse expression	Highly sensitive; seen in Ewing sarcoma, CIC sarcomas [7-9]
Keratin	Dot-like or aberrant pattern	Seen in 25% of Ewing sarcoma cases; diagnostic pitfall. [5]
Desmin	Distinctive dot-like positivity	Aberrant expression in a small subset of tumors. [5]
Desmin, Myo-D1, Myogenin	Positive	Rhabdomyoblastic differentiation [1]
FLI-1	Nuclear immunoreactivity (70-94%)	Non-specific; also seen in lymphomas and vascular tumors. [10]
NKX2.2	Recent marker	Specific for Ewing sarcoma; also seen in mesenchymal tumors. [11]
WT-1	Nuclear positivity	Specific for DSRCT and CIC-rearranged sarcomas [14, 15]
DUX-4	Positive	Marker for CIC-DUX4 fusion sarcomas. [14, 15]
CD117	Variable expression (20-71%)	Indicates therapeutic targeting opportunities. [16]
Vimentin	Positive	Common mesenchymal marker; also expressed in DSRCT. [1, 4]
Myogenin	Negative	Helps distinguish fusion-negative alveolar RMS. [1, 4]

**Table 1: Immunohistochemical Markers in Round Cell Tumors**



**Figure 1: Algorithm for testing RCTs.**

Tumor Type	Fusion Genes / Translocations	Detection Methods*	Significance
Ewing Sarcoma	EWSR1-FLI1 (t(11;22)), EWSR1-ERG (t(21;22))	FISH, RT-PCR, NGS	Pathognomonic molecular features for diagnosis [12, 13, 22–25]
CIC-Rearranged Sarcoma	CIC-DUX4, CIC-FOXO4, CIC-NUTMTA	RT-PCR, FISH, RNA ISH	Identifies CIC-specific genetic abnormalities [17–19, 21]
BCOR-Rearranged Sarcoma	BCOR-CCNB3, BCOR-MAML3, BCOR-ZC3H7B	RT-PCR, FISH	Highly specific for BCOR sarcomas [26]
Alveolar Rhabdomyosarcoma (ARMS)	PAX3 / PAX7 with -FOXO1 / FKHR	Cytogenetics, FISH, RT-PCR	Associated with poor prognosis in pediatric cases [27–34]
Desmoplastic Small Round Cell Tumor (DSRCT)	EWSR1-WT1 (t(11;22))	FISH, RT-PCR	Diagnostic marker unique to DSRCT [4, 21]
Extraskeletal Myxoid Chondrosarcoma (EMC)	NR4A3 gene rearrangements	FISH for NR4A3	Molecular hallmark of EMC [35–37]

\*Detection methods using frozen tissue (preferred), cytology smears or formalin fixed paraffin embedded tissue.

**Table 2: Molecular Features and Detection Methods in Round Cell Tumors**

Tumor Type	Marker	Expression / Pattern	Significance
CIC-Rearranged Sarcomas	CD99	Patchy positivity	Identifies CIC-rearranged sarcomas [20]
	WT-1	Nuclear positivity	Marker for CIC-DUX4 sarcomas [14, 15]
	DUX-4	Positive	CIC-DUX4 fusion specific [14, 15]
BCOR-Rearranged Sarcomas	BCOR	Nuclear immunoreactivity	Highly sensitive and specific for BCOR sarcomas [20, 26]
	CCNB3	Nuclear positivity	Specific marker for BCOR-CCNB3 fusions [20, 26]
	CD99	Variable expression	Less diagnostic relevance compared to CIC [20, 26]

**Table 3: CIC- and BCOR-Rearranged Sarcomas: Immunoprofile and Molecular Markers**

fusion. Because other sarcomas can also express EWSR1, identifying the EWSR1-WT1 fusion gene via RT-PCR or FISH is crucial for specific diagnosis (see Tables 2 and 4). Chromosome banding analysis shows that about 60% of DSRCTs are near-diploid with this translocation, while RT-PCR reveals the EWSR1-WT1 fusion transcript [4, 21] in nearly 100% of cases, underscoring the importance of molecular studies.

#### EXTRASKELETAL MYXOID CHONDROSARCOMA

EMC observed predominantly in adults and rarely in children, lacks convincing evidence of cartilaginous differentiation. EMC shares histological and IHC features with mixed tumors of soft tissue and myoepitheliomas, presenting a diagnostic challenge with crucial therapeutic and prognostic implications. IHC is unhelpful necessitating molecular

testing. [35] NR4A3 gene rearrangements detection is therefore pivotal and is unique to EMC [35–37] (Refer Tables 2 and 4).

Other round cell tumors of soft tissue including Extramedullary leukemia, undifferentiated sarcoma, and poorly differentiated synovial sarcoma lesions, despite sharing a round cell histomorphology, can be effectively distinguished through comprehensive analysis involving clinical evaluation, immunohistochemical profiling, and ultrastructural studies and molecular studies are usually not required. [4]

#### DISCUSSION

In ES, until the introduction of modern therapy, the outlook for patients was bleak. The prognosis for ES has

Tumor Type	Chromosomal Abnormalities / Features	Genetic or Molecular Findings
Ewing Sarcoma	Balanced translocations: t(11;22), t(21;22)	EWSR1-ETS family gene fusions [12, 13, 22-25]
CIC-Rearranged Sarcoma	Translocations: t(4;19), t(10;19)	CIC-DUX4, CIC-FOXO4 fusion genes [17-19, 21]
BCOR-Rearranged Sarcoma	Rearrangements involving BCOR	BCOR-CCNB3 and related fusions [26]
Alveolar Rhabdomyosarcoma (ARMS)	t(2;13)(q35;q14) and t(1;13)(q36;q14)	PAX3/ PAX7 with FOXO1 / FKHR fusions [27-34]
Desmoplastic Small Round Cell Tumor (DSRCT)	t(11;22)(p13;q12)	EWSR1-WT1 fusion [4, 21]
Extraskeletal Myxoid Chondrosarcoma (EMC)	t(9;22)(q22;q12), t(9;17)(q22;q11)	NR4A3 rearrangements [35-37]

**Table 4: Key Genetic Features in Round Cell Tumors**

steadily improved. About 75% of cases present with localized disease and a combination of surgery +/- radiotherapy and systemic chemotherapy, are associated with a cure rate of almost 75%. [38] However, little progress has been made for patients who present with metastatic disease. The role of megatherapy (Myeloablative high dose chemotherapy with or without total body irradiation followed by stem cell infusion) in the treatment of metastatic disease remains unclear. [39] With increasing understanding of the molecular pathways, opportunities for targeted therapy have emerged. Potential targets can be broadly classified into those related to EWSR1-ETS fusion (eg- Trabectedin, lurbinectedin) [40, 41], receptor tyrosine kinases and associated signalling pathways [41], TP53 and retinoblastoma pathways, angiogenesis and apoptosis. [42] Key prognostic factors that adversely influence the outcome of the disease are the presence of metastatic disease, large tumor size, extensive necrosis (filigree pattern), central axis tumors and poor response to initial chemotherapy. [43] Gene expression profiles associated with the presence of metastases, prognosis, and response to therapy have been identified. [44]

CIC-rearranged sarcomas have now been clearly delineated as the most common type of "Ewing-like" sarcoma, with histologic, immunohistochemical and molecular genetic features that distinguish it from ES and all other RCTs. Its recognition is important because these tumors usually follow an aggressive clinical course with high rates of metastases and a lower sensitivity to ES-Chemotherapeutic protocols. [45] CIC-rearranged sarcomas also more often present with disseminated disease showing greater chemoresistance and a poorer overall survival than conventional ES. [46]

BCOR-rearranged sarcomas seem to have a clinical course not too dissimilar from ES but less aggressive than CIC-rearranged sarcoma. In a study by Kao et al, BCOR-

rearranged sarcomas showed an overall 5year survival of 72%, similar to patients with ES (79%), but significantly better than CIC-rearranged sarcomas(43%). [20]

In ARMS, molecular tests identify characteristic fusions, differentiating ARMS from embryonal rhabdomyosarcoma and other small round cell tumors. Fusion-positive ARMS (especially PAX3::FOXO1) is associated with worse prognosis, guiding treatment intensity. Fusion-negative cases generally have a better prognosis. [47] Anderson et al. recently reported the translocation, t(2;13)/PAX3-FKHR, to be an adverse prognostic factor for alveolar rhabdomyosarcoma. In contrast, t(1;13)/PAX7-FKHR was associated with a favorable prognosis and was more frequently observed in younger patients with relatively localized disease. [34] Genetic testing may reveal actionable mutations (e.g., ALK alterations) that can be targeted with therapies like ALK inhibitors. [48] Molecular markers guide inclusion in clinical trials for novel agents or immunotherapies.

In DSRCT, molecular detection of the EWSR1::WT1 fusion helps to differentiate it from other small round blue cell tumors such as Ewing sarcoma or rhabdomyosarcoma. The presence of the fusion confirms the aggressive nature of DSRCT. Additional molecular markers are under investigation for better risk stratification. [49] WT1 is being explored as a target for immunotherapy (e.g., WT1 vaccines or WT1-targeted CAR-T cells). Molecular profiling may guide participation in clinical trials exploring novel targeted approaches. [50]

In EMC, detection of NR4A3 rearrangements is pathognomonic. This distinguishes EMC from other chondrosarcomas and small round cell tumors. Molecular subtype analysis can provide insights into tumor behavior, though most EMC cases follow an indolent but persistent course. No standard targeted therapies are yet established for EMC, but molecular diagnostics may identify candidates for clinical trials tar-

getting NR4A3-related pathways. Investigational therapies are focused on disrupting the oncogenic fusion protein or associated signaling pathways. <sup>[51]</sup>

## CONCLUSION

Navigating the intricacies of small round blue cell tumors poses a diagnostic challenge due to their primitive appearances. Histopathology alone struggles to differentiate these neoplasms, which may originate from epithelial, mesenchymal, hematopoietic, or melanocytic sources. Immunohistochemistry serves as a valuable tool in narrowing down the differential. Despite its effectiveness, IHC often falls short in distinguishing between different round cell sarcomas. Molecular genetic features, including fusion status, will undoubtedly play a much larger role in diagnosing these lesions along with risk stratification and designing appropriate therapeutic strategies.

## ABBREVIATIONS:

- ARMS – Alveolar Rhabdomyosarcoma
- BCOR – BCL6 transcriptional corepressor
- CD – Cluster of differentiation
- CIC - Capicua Transcriptional Repressor
- DSRCT - Desmoplastic Small Round Cell Tumor
- ES – Ewings Sarcoma
- ETS - E26 transformation-specific
- EWSR1 - Ewings Sarcoma Breakpoint Region-1
- EMC - Extra skeletal Myxoid Chondrosarcoma
- FISH - Fluorescent Insitu Hybridization
- FLI-1 – Friend Leukemia Integration 1
- HMW - High molecular weight
- IHC – Immunohistochemistry
- NGS – Next generation sequencing
- RC – Round Cell
- RT-PCR – Reverse Transcriptase Polymerase chain reaction
- SBRCT – Small blue round cell tumors
- WHO – World health organization

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