Scientific Research Publishing

Journal of Biosciences and Medicines, 2025, 13(8), 158-187
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Ewing Sarcoma: A Comprehensive Review of Its Classification, Diagnosis, and Emerging Treatments

Hugo Uriel Suaste-Vera[1], Rafael Villalobos-Molina[2]

[1]Carrera de Medicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, México
[2]Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, México
Email: villalobos@unam.mx

How to cite this paper: Suaste-Vera, H.U. and Villalobos-Molina, R. (2025) Ewing Sarcoma: A Comprehensive Review of Its Classification, Diagnosis, and Emerging Treatments. *Journal of Biosciences and Medicines*, **13**, 158-187.
https://doi.org/10.4236/jbm.2025.138014

Received: July 1, 2025
Accepted: August 12, 2025
Published: August 15, 2025

Abstract

Ewing sarcoma comprises a group of highly aggressive malignant neoplasms that predominantly affect children, adolescents, and young adults. It is classified among small round cell tumors and is cytogenetically characterized by specific chromosomal translocations, the most common being t(11;22) (q24;q12), which results in the fusion of the EWSR1 gene with members of the ETS transcription factor family, primarily FLI1. This genetic alteration plays a pivotal role not only in malignant transformation but also serves as a key diagnostic biomarker. Epidemiologically, Ewing sarcoma represents the second most frequent primary bone tumor in the pediatric population, with a clear predominance in males and individuals of Caucasian descent. Clinically, it typically presents as localized bone pain, often accompanied by swelling or a palpable mass, and in some cases, systemic symptoms. The most common locations include the long bones of the lower limbs and the pelvis. Diagnosis requires a multidisciplinary approach encompassing advanced radiological studies (magnetic resonance imaging, computed tomography), bone scintigraphy, and histological confirmation through biopsy, supported by immunohistochemical techniques (such as CD99 expression), and molecular analyses for the detection of gene fusions. The standard treatment is based on a combination of intensive systemic chemotherapy, radical surgery, and, in some cases, radiotherapy. Multicenter clinical trials, such as the Euro-Ewing 99 protocol, have optimized therapeutic strategies, improving survival rates in localized disease (up to 70% - 75%), and reducing the long-term adverse effects. Nevertheless, the prognosis remains poor in patients with metastatic or recurrent disease, prompting the development of targeted therapies, immunotherapies (such as IGF-1R inhibitors or T cell-based strategies), and personalized approaches guided by

molecular profiles. This article presents an up-to-date and comprehensive review of Ewing sarcoma, focusing on its biological classification, clinical manifestations, diagnostic methods, current therapeutic options, and emerging advances in translational research, with an emphasis on the need to individualize clinical management, and explore new therapeutic targets to improve outcomes in high-risk subgroups.

Keywords

Ewing Sarcoma, Cancer, Diagnosis, Classification, Therapy

1. Introduction

Ewing sarcoma (EwS) is a neoplasm of considerable clinical importance, as it may originate in either bone or soft tissues, and exhibits a high prevalence among adolescents and young adults (AYA). Its therapeutic management requires a multidisciplinary approach combining intensive chemotherapy with local treatment, which may consist of surgical resection, radiotherapy, or a combination of both. With an incidence estimated at approximately 1.5 cases per million in the AYA population, progress in the treatment of this rare malignancy has largely been driven by collaborative clinical trials. These studies have addressed key issues, such as the optimization of chemotherapeutic protocols and the development of more precise risk stratification strategies, thereby enabling the formulation of more individualized therapeutic approaches based on patient-specific characteristics, including the type of genetic fusion present [1]. In this context, patients with localized disease have achieved event-free survival (EFS) rates approaching 75%. However, the treatment regimens employed are not devoid of significant adverse effects, both immediate and long-term, and patients with metastatic or recurrent disease continue to face a markedly unfavorable prognosis. Consequently, there remains a critical need to refine therapeutic interventions and to explore new alternatives that may improve clinical outcomes while simultaneously minimizing the toxicities associated with current treatments [1] [2].

2. History

In 1921, James Ewing first described what is now known as Ewing sarcoma, distinguishing it as a malignant bone tumor separate from osteogenic sarcoma. He noted that the tumor was composed of small round cells, unlike osteosarcoma, which is characterized by large, spindle-shaped cells. Ewing observed that this tumor tended to originate diffusely in the mid-diaphysis of long bones while sparing the epiphyses, leading him to hypothesize an origin in the endothelium of blood vessels within the medullary cavity. Consequently, he named it "diffuse endothelioma of bone" [3]. Aware that osteosarcoma was resistant to radiation, Ewing proposed that this distinct tumor type might be radiosensitive. In 1923, clinical and radiologic similarities between Ewing sarcoma and osteomyelitis were noted,

and Ewing observed that many of his patients presented with a febrile syndrome [3]. Subsequently, Coley proposed a possible infectious etiology; however, this hypothesis was later refuted by Pitchard and colleagues, who found no alterations in the host's immune defense mechanisms. In 1965, Phillips and Higinbotham demonstrated that irradiating the entire diaphysis of the affected bone with doses exceeding 4000 rads reduced local recurrence, a common indicator of metastatic dissemination [4]. Although some surgeons advocated for amputation, patients often developed metastases within one year. The combination of radiotherapy and chemotherapy began to yield promising results, and in 1969, the use of cyclophosphamide led to prolonged disease-free survival [4] [5]. In 1979, S. Weintroub reported a case of avascular necrosis with histological features suggestive of Ewing sarcoma; although only one similar case had been recorded at the time, subsequent studies confirmed that the tumor described by Weintroub was indeed Ewing sarcoma [6].

3. Epidemiology

Ewing sarcoma is the second most common malignant bone tumor in children, with an incidence rate of approximately three cases per million individuals in the United States. The majority of diagnoses occur during adolescence, with over 50% of cases presenting between the ages of 10 and 20. Fewer than 23% of patients are under the age of 10, and the incidence decreases significantly in adults over 20 years old. This tumor affects males more frequently, accounting for approximately 61% of cases, and occurs almost exclusively in individuals of Caucasian descent, who represent 92% of all cases [2] [7].

4. Etiology

Ewing sarcoma is characterized by a specific genetic translocation that results in the fusion of the EWSR1 gene, located on chromosome 22, with members of the ETS and FET transcription factor families (**Figure 1**). The FET family (also known as TET) comprises a group of genes encoding proteins with key roles in transcriptional regulation and RNA processing. The acronym FET derives from its three main members:

- FUS (also known as TLS)
- EWSR1 (EWS)
- TAF15

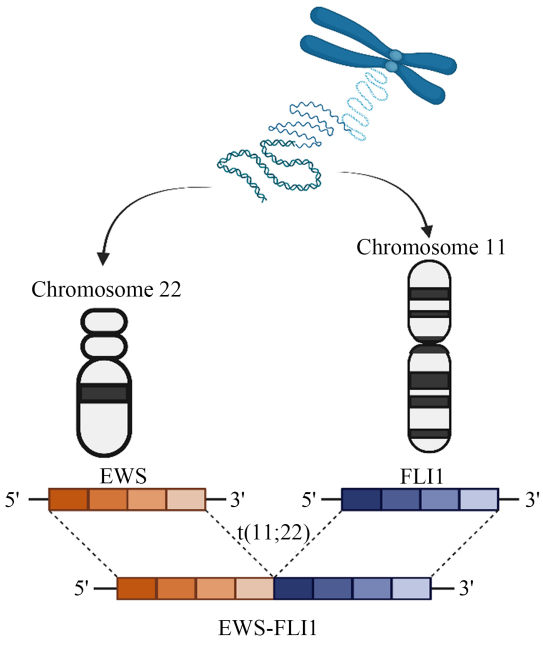
These proteins share RNA-binding domains and participate in the regulation of gene expression. Among them, EWSR1 is the most frequently involved in gene fusions in Ewing sarcoma. The ETS family (Erythroblast Transformation Specific) consists of over 20 transcription factors that share a conserved DNA-binding domain known as the ETS domain. This domain recognizes specific DNA sequences and regulates the expression of numerous genes involved in:

- Cell growth and differentiation
- Embryonic development

- Apoptosis

In Ewing sarcoma, the most commonly involved ETS genes in fusions with EWSR1 include:

- FLI1
- ERG
- ETV1
- ETV4
- FEV



FUS-ERG	1%	t(16;21) (p11;q22)
FUS-FEV	1%	t(2;16) (q35;p11)
EWS-FLI1	80%	t(11;22) (q24;q12)
EWS-ERG	10%	t(16;21) (p11;q22)
EWS-ETV1	2%	t(7;22) (p22;q12)
EWS-E1AF	2%	-
EWS-FEV	1%	t(2;22) (q33;q12)
EWS-ETV4	1%	t(17;22) (q21;q12)
EWS-ETV5	1%	-

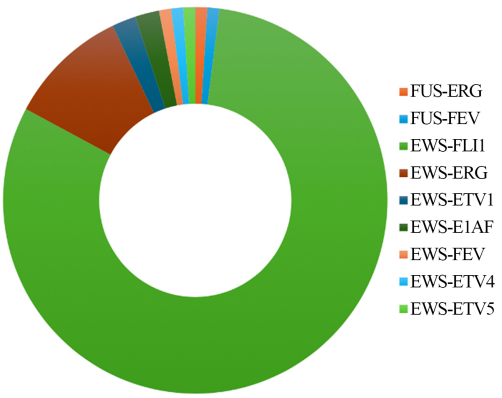


Figure 1. Various translocations, fusion genes, and their respective frequencies in Ewing sarcomas. Source: own work.

These gene fusions (e.g., EWSR1-FLI1 or EWSR1-ERG) result in aberrant fusion proteins that drive tumor proliferation by disrupting normal gene transcription [8] [9]. The most frequent translocation, present in approximately 85% of cases, involves the FLI1 gene on chromosome 11 and produces the t(11;22)(q24;q12) rearrangement. The EWSR1 gene encodes a protein involved in multiple cellu-

lar processes, including gene expression regulation and RNA processing. The EWSR1::FLI1 fusion results in a chimeric oncoprotein that functions as an abnormal transcription factor essential for maintaining tumor cell malignancy. Less common fusions include EWSR1::ERG and FUS::ERG. These rearrangements are typically detected using fluorescence in situ hybridization (FISH) (**Figure 2**) and reverse transcription polymerase chain reaction (RT-PCR). Notably, detection of the EWSR1::ERG fusion by FISH has a higher false-negative rate compared to EWSR1::FLI1 [10].

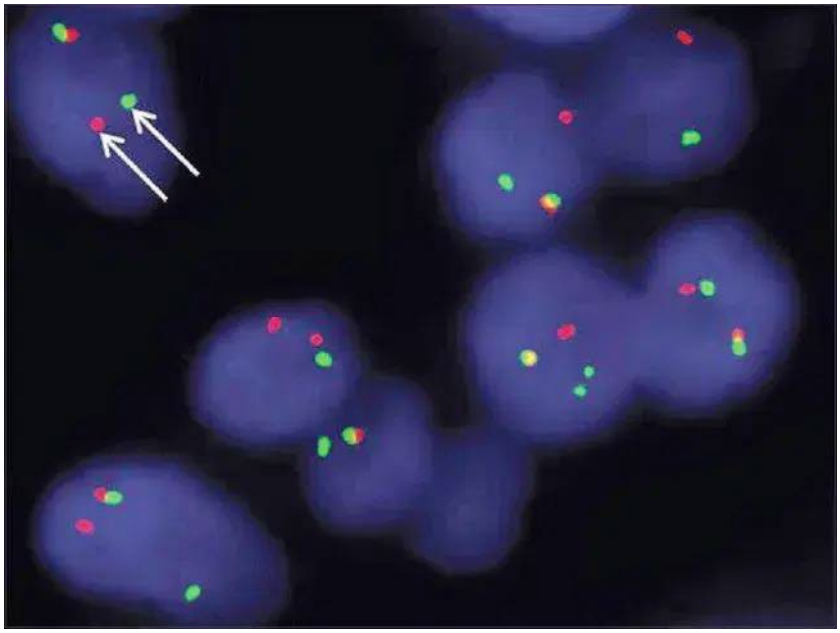


Figure 2. This image displays a break-apart fluorescence *in situ* hybridization (FISH) assay for detecting EWSR1 gene rearrangement. The separation of green and red signals, as indicated by arrows, confirms the presence of EWSR1 rearrangement. In negative cases, red and green signals appear fused. Image source: Mardekian *et al.*, 2014 [11]. Licensed under CC BY 3.0.

5. Classification

Specific chromosomal translocations are frequently identified in human lymphomas and leukemias. The molecular study of these rearrangements has enabled the discovery of novel mechanisms of malignant transformation. Although such translocations are less common in solid tumors, a recurrent t(11;22)(q24;q12) translocation has been described in a group of closely related neoplasms, including osseous Ewing sarcoma, extraskeletal Ewing sarcoma, peripheral neuroepithelioma (PNET), and Askin tumor. Increasing evidence suggests that these tumors share a common origin from the neural crest [6] [9], albeit with variable degrees of neural differentiation and diverse tissue locations. Collectively, these neoplasms are now referred to as the Ewing sarcoma family of tumors (ESFT). The ESFT includes four major subtypes: extraskeletal Ewing sarcoma, osseous Ewing sarcoma, Askin tumor (localized in the thoracic wall), and peripheral primitive neu-

roectodermal tumor (PNET). It is important to specify "peripheral PNET" to distinguish it from unrelated tumors originating in the central nervous system. Extraskeletal Ewing sarcoma most commonly occurs in the paravertebral region and lower limbs [6], while its appearance in the upper extremities is relatively rare (Table 1).

Table 1. Ewing sarcoma classification.

Tumor type	Frecuent location	Commom fusion gene	Age of incidence	Clínical characteristics	Differential diagnosis	Relative prognosis
Bone Ewing sarcoma	Diaphysis of large bones	EWSR1-FLI1 (85-90%)	10 to 20 years (75% < 20 years)	Bone pain, palpable mass, pathologic fractures	Osteosarcoma, bone lymphoma	Best located prognosis
Extraosseus Ewing Sarcoma	Soft tissues (muscle-skeletal, thoracic wall, retroperitoneal)	EWSR1-ERG (10%)	15 to 30 years	Fast growing mass, localized pain	Rhabdomyosarcoma, synovial sarcoma	Best located prognosis, size dependent
Askin tumor (PNET of thoracic wall)	Thoracic wall (intercostal spaces)	EWSR1-FLI1	10 to 25 years	Thoracic pain, pleural effusion, superior vena cava syndrome	Neuroblastoma, lymphoma	Very aggressive due to its high metastasis rate
Peripheral PNET	Soft tissues (limbs and pelvis)	EWSR1-ETS (FLI1, ERG)	5 to 25 years	Painful mass with neural differentiation (Homer-Wright rossetes)	Neuroblastoma, desmoplastic tumor	Best located prognosis, resectability dependent
Early neuroectodermal tumor (PNET) abdominal	Abdomen (retroperitoneal and pelvis)	EWSR1-FLI1	20 to 40 years	Abdominal mass, obstructive symptoms	GIST, liposarcoma	Cautios prognosis (high recurrence rate)
Head and neck Ewing sarcoma	Mandible, craneal bones, cervical soft tissues	EWSR1-FLI1	5 to15 years	Neurological disorders	Granular cells tumor, carcinoma	Surgical challenge (best treatment with chemotherapy and radiotherapy)
Adamantioma ES like	Large bones, head and neck	EWSR1-FLI1 + keratin expression	10 to 30 years	Pseudoadamantine histological pattern	Adamantinoma, carcinoma	Similar to classic ES
"Ewing-like" tumors	Soft tissues	CIC-DUX4 (no EWSR1)	15 to 50 years	High aggressivity, poor response to standard chemotherapy	Undifferentiated sarcoma	Worst prognosis (survival rate < 30% at 5 years)

6. Genetic Predisposition

Tumors of the Ewing sarcoma family (ESFT) are characterized by a recurrent cytogenetic alteration involving a translocation between chromosomes 11 and 22. This genetic anomaly is the most common and is detected in approximately 90% of these tumors. The translocation involves the EWS gene, located on the long arm of chromosome 22 (22q12), and the FLI1 gene, situated on chromosome 11q24. The resulting fusion gene, known as EWS-FLI1, is highly specific to the

tumor and is present in nearly all cases of ESFT, making it a valuable diagnostic marker. Additional translocations involving EWS have also been identified, although their clinical significance remains unclear. In recent years, several hereditary cancer syndromes have been studied for their potential association with an increased risk of Ewing sarcoma:

- **Li-Fraumeni Syndrome (LFS)**

The Li-Fraumeni syndrome phenotype can be influenced by various genetic and epigenetic factors. Notably, certain polymorphic variants in the TP53 and MDM2 genes modulate the LFS phenotype. Studies have examined how the TP53 Arg72Pro and MDM2 T309G single nucleotide polymorphisms (SNPs) affect the risk of developing Ewing sarcoma, independent of a clinical diagnosis of LFS. The G allele of the MDM2 T309G SNP was significantly associated with an increased risk of Ewing sarcoma, whereas no relevant association was found with the TP53 Arg72Pro variant. The T309G polymorphism in MDM2 should be prospectively evaluated in Ewing sarcoma patients, regardless of the mutational status of TP53, CHEK2, or POT1 [12]. TP53 analysis remains crucial when exploring germline contributions in Ewing sarcoma. Although current scientific evidence does not establish a clear link between TP53 mutations and Ewing sarcoma risk, assessing deleterious TP53 germline variants in patients and their families may provide the basis for personalized genetic counseling based on familial risk [12]. Current evidence does not establish a direct relationship between pathogenic germline mutations in TP53 and Ewing sarcoma. However, its role remains under investigation, particularly in the context of Li-Fraumeni syndrome. TP53 testing may be considered in selected cases, especially when there is a family history suggestive of hereditary cancer. MDM2: A significant association has been identified between the MDM2 T309G polymorphism and increased susceptibility to Ewing sarcoma, independently of TP53 mutation status. Although these findings are promising, prospective validation is still required before routine clinical implementation can be recommended [12] [13].

- **Retinoblastoma Predisposition Syndrome**

Hereditary retinoblastoma is caused by a germline mutation in the RB1 gene, which not only predisposes to ocular tumors but also significantly increases the risk of developing second primary malignancies. Previous studies have identified recurrent RB1 mutations in Ewing sarcoma cases, prompting investigations into a potential association. Large cohort analyses have shown that individuals carrying RB1 mutations have a higher risk of developing soft tissue sarcomas, even in the absence of prior radiotherapy. Among secondary sarcomas in these patients, leiomyosarcoma is the most frequently observed subtype [12]. While somatic mutations in RB1 have been documented in some cases of Ewing sarcoma, there is no strong evidence supporting a germline predisposition. Therefore, RB1 analysis is not considered part of the standard genetic assessment for this malignancy.

- **Bloom Syndrome**

Bloom syndrome is a rare autosomal recessive disorder caused by mutations in the BLM gene (Bloom Syndrome RecQ Like Helicase), which plays a crucial role

in genomic stability. The most frequently associated malignancies in Bloom syndrome include leukemias and lymphomas. While the cancer types observed in these patients generally mirror those found in the general population, they tend to occur at significantly younger ages. Although cases of sarcomas have been reported, no significant increase in sarcoma risk has been identified in Bloom syndrome patients [12]. Brohl and colleagues, through sequencing analyses, identified pathogenic or likely pathogenic BLM variants in patients with Ewing sarcoma. While intriguing, these findings warrant further investigation to determine their clinical significance. Given this preliminary evidence, screening for BLM pathogenic variants may be considered in the germline evaluation of patients with Ewing sarcoma, particularly in cases suggestive of genetic predisposition [12] [14] [15]. Pathogenic or likely pathogenic germline variants in BLM have been reported in certain patients with Ewing sarcoma, suggesting a potential predisposing role [14] [15]. Nevertheless, current data are limited, and further studies are necessary to determine its clinical relevance and to justify inclusion in diagnostic protocols.

7. Pathogenesis of Ewing Sarcoma

Step 1. Cell of origin and characteristics

For decades, the cell of origin of Ewing sarcoma (EwS has been a subject of debate. Initially, a neuroectodermal lineage was proposed based on the expression of neuronal markers in tumor cells. However, more recent studies indicate that these neuroectodermal phenotypes are largely induced by the activity of the EWS-FLI1 fusion protein, rather than reflecting a true neural origin [16]. The currently prevailing hypothesis posits that EwS arises from a mesenchymal stem cell (MSC), likely derived from the bone marrow. MSCs exhibit self-renewal capacity and multipotency, allowing differentiation into osteogenic, chondrogenic, and adipogenic lineages. Experimental models have demonstrated that the introduction of EWS-FLI1 into murine or human MSCs can generate cells with a morphology, transcriptomic profile, and epigenetic behavior highly similar to EwS, supporting their role as the cell of origin [17] [18].

Step 2. Initiating genetic event—t(11;22) translocation

The primary genomic event in EwS pathogenesis is a balanced chromosomal translocation, specifically t(11;22)(q24;q12), resulting in fusion of the EWSR1 and FLI1 genes. This rearrangement occurs in approximately 85% of cases and produces the chimeric oncogenic protein EWS-FLI1, which combines the transcriptional activation domain of EWSR1 with the DNA-binding domain of FLI1, a member of the ETS transcription factor family [19]. In a smaller subset of cases (~10%), similar translocations such as EWS-ERG are observed. These fusions are highly specific and represent a defining molecular hallmark of EwS [16].

Step 3. Epigenetic reprogramming and differentiation blockade

The oncogenic function of EWS-FLI1 is primarily exerted through epigenetic reprogramming of the host cell genome. EWS-FLI1 binds to GGAA microsatellite repeats and recruits epigenetic co-activators such as p300/CBP, thereby promot-

ing histone acetylation (H3K27ac) and the formation of de novo enhancers [16] [18]. This process activates a network of genes associated with proliferation and maintenance of an undifferentiated state, including IGF1R, MYC, NKX2-2, and EZH2. Simultaneously, EWS-FLI1 represses differentiation genes characteristic of mesenchymal lineages, blocking normal osteogenic and adipogenic pathways. For example, it has been shown to suppress the expression of the cell cycle inhibitor CDKN1A/p21, thereby interfering with physiological cell cycle regulation [20]. This dual mechanism—oncogene activation and differentiation blockade—establishes a highly proliferative, immature, and senescence-resistant phenotype typical of Ewing sarcoma.

Step 4. Cooperating alterations

Although EWS-FLI1 is the central initiating event in EwS tumorigenesis, it is not sufficient to induce full malignant transformation on its own. Multiple studies have demonstrated that additional genetic alterations act cooperatively. Common secondary events include deletion of the CDKN2A gene (~10%), TP53 mutations (~5% - 10%), and functional loss of STAG2 (~15% - 20%), a cohesin complex component essential for chromosomal architecture and segregation [21]. These alterations enhance genetic plasticity and tolerance to replicative stress. Notably, STAG2 loss is associated with more aggressive clinical behavior and increased metastatic potential [16]. These cooperating mutations enable tumor cells to better withstand the transcriptional dysregulation imposed by EWS-FLI1 and to progress toward advanced malignancy.

Step 5. Impact on cell cycle and proliferation

The mitogenic effect of EWS-FLI1 leads to a marked acceleration of the cell cycle. This chimeric protein upregulates genes involved in G1/S and G2/M transitions, including CCND1 (cyclin D1), MYC, and IGF1R, all of which promote cellular proliferation and survival [16]. Concurrently, it represses transcription of negative cell cycle regulators such as CDKN1A/p21 [20]. This dysregulation promotes unchecked proliferation, enabling rapid tumor expansion.

Step 6. Tumor migration and invasion: EMT, CXCR4/CXCL12 signaling, and the role of M2 macrophages

Ewing sarcoma's ability to invade tissues and metastasize is mediated by a complex interplay of autocrine and paracrine signals within the tumor microenvironment. A key pathway involves the CXCR4 receptor and its ligand CXCL12 (also known as SDF-1). EwS cells express CXCR4, and its activation by CXCL12—highly expressed in tissues such as lung and bone marrow—facilitates directed migration and metastatic seeding [20]. Tumor-associated macrophages (TAMs), predominantly of the M2 phenotype, also play a major pro-metastatic role. These immunosuppressive cells secrete factors such as TGF-β and IL-10 that promote an epithelial-mesenchymal transition (EMT-like program), characterized by loss of intercellular adhesion, cytoskeletal reorganization, and acquisition of motility [22]. In animal models, pharmacologic inhibition of M2 macrophage activity significantly reduces metastatic burden [23].

Step 7. Immune evasion

Ewing sarcoma exhibits a marked ability to evade host immune surveillance. One of the primary mechanisms is the downregulation or absence of major histocompatibility complex class I (HLA-I) expression, which impairs antigen presentation to cytotoxic CD8+ T cells [20]. Additionally, the tumor expresses immune checkpoint proteins such as PD-L1, HLA-G, and HLA-E, which bind to inhibitory receptors on T cells (e.g., PD-1, NKG2A), inducing T cell anergy and impairing cytotoxic responses. This immunosuppressive environment is further reinforced by high levels of TGF-β secreted by M2 TAMs and tumor cells, which promote the differentiation of regulatory T cells (Tregs) via FOXP3 induction. These Tregs suppress dendritic cell activation and limit the expansion of effector T cells. Collectively, this immunotolerant niche protects the tumor from immune-mediated destruction [20] [22].

Step 8. Metastatic progression

Metastatic progression remains the most challenging therapeutic hurdle in Ewing sarcoma. Clinically, metastases most frequently involve the lungs, bone, and bone marrow—sites where CXCL12 expression is abundant (**Figure 3**). Signaling through the CXCR4/CXCL12 axis guides tumor cell homing to these permissive environments [20]. At the molecular level, in addition to chemokine signaling, EWS-FLI1-regulated genes such as CAV1, EZH2, and TNC contribute to extracellular matrix remodeling and increased motility [23]. Loss of STAG2 also plays a dual role, disrupting chromatin organization and promoting a more aggressive metastatic phenotype [21]. These interconnected pathways promote early systemic dissemination and account for the high relapse rates observed in EwS patients.

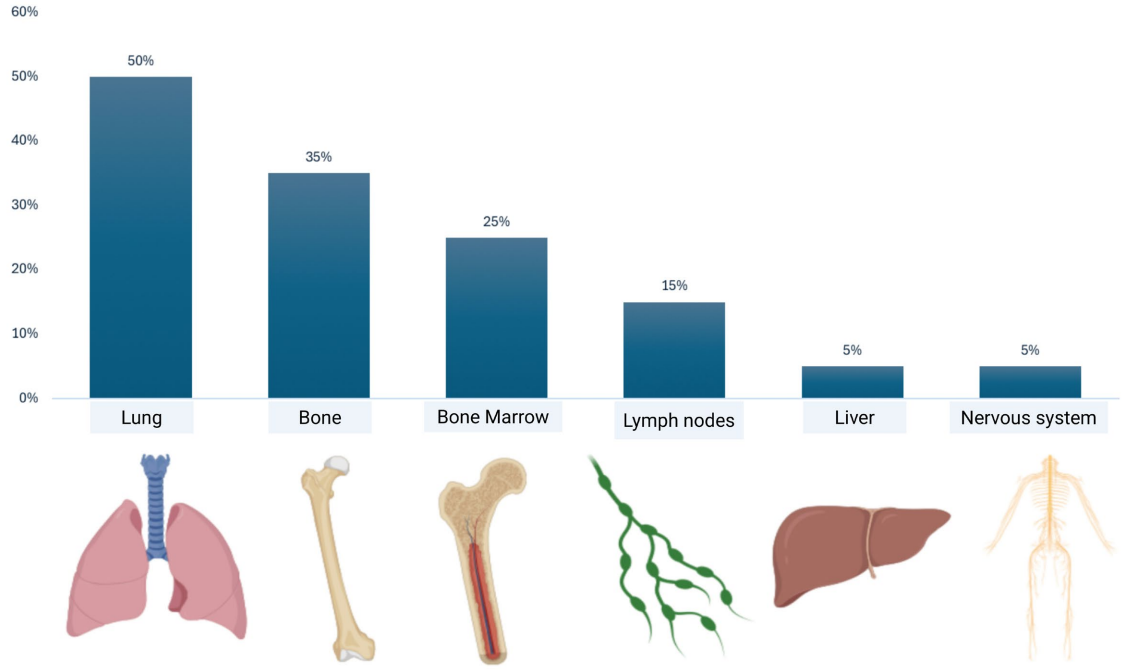


Figure 3. Organs and systems with their respective propensity for metastatic involvement. Source: own work.

8. Clinical Features

A population-based study in Sweden, conducted between 1983 and 1995 using data from the Swedish Cancer Registry managed by the National Board of Health in Stockholm, analyzed all cases of osteosarcoma and Ewing sarcoma in patients aged ≤30 years, excluding tumors located in the skull and ribs. The study identified 102 patients with osteosarcoma (61 males and 41 females) and 47 with Ewing sarcoma (28 males and 19 females), revealing a male predominance in both tumor types, with a male-to-female ratio of 1.5:1. The most frequent location was around the knee, where 74% of osteosarcomas (75 cases) and 23% of Ewing sarcomas (11 cases) were found [7]. The most common initial symptom prompting medical consultation was regional pain, either isolated or accompanied by a palpable mass. Specifically, 70% of osteosarcoma patients (71 individuals) and 72% of Ewing sarcoma patients (34 individuals) sought medical attention due to pain alone. An additional 25% of osteosarcoma patients (26 cases) and 15% of Ewing sarcoma patients (7 cases) presented with both pain and a palpable mass. Only 4% of osteosarcoma patients (4 individuals) and 11% of those with Ewing sarcoma (5 individuals) reported no pain at initial consultation, instead presenting with other clinical manifestations [7]. Cases in infants are exceedingly rare. A report from Turkey documented a case in a 6-month-old infant with a femoral tumor, highlighting the diagnostic and therapeutic challenges unique to this population [24].

Initial Diagnosis

The study by Widhe and Widhe (2000) also reported the frequency of misdiagnoses at initial clinical evaluation for both osteosarcoma and Ewing sarcoma.

Osteosarcoma

The most frequent initial misdiagnosis was tendinitis, accounting for 31% of cases (25 males [41%] and 7 females [17%]). In 12% of patients (12 cases), the physician was unable to provide a definitive diagnosis. Only two patients were initially misdiagnosed with a pathological fracture.

Ewing Sarcoma

Tendinitis was also the most common misdiagnosis in Ewing sarcoma, recorded in 21% of cases (10 patients). An additional 19% (9 cases) presented diagnostic uncertainty. Misdiagnoses were particularly common in tumors located in the pelvis or proximal femur. Among younger patients, the most frequent alternative diagnoses were transient coxitis and osteomyelitis, whereas in older adults, tendinitis and sciatica predominated as erroneous diagnoses [7].

Staging

Accurate risk stratification in patients with Ewing sarcoma (ES) is essential for therapeutic decision-making and prognostic assessment. Over recent decades, multiple staging systems have been developed, integrating clinical, histological, surgical, and molecular variables.

1.- American Joint Committee on Cancer (AJCC-TNM) Classification

The AJCC-TNM classification is commonly employed to stage EwS and other neoplasms. Its objective is to aid in prognostication by considering tumor size,

histologic confirmation, and topographic localization [25] (**Figure 4**).

PRIMARY TUMOR (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumnor
T1	Tumor <8 cm in greatest dimension
T2	Tumor >8 cm in greatest dimension.
T3	Discontinuous tumors in the pimary bone site
REGIONAL LYMPH NODES (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis present.
DISTANT METASTASIS (M)	
M0	No distance metastasis
M1	Distant metastasis
M1a	Pulmonary metastasis
M1b	Metastases at other distant sites

Figure 4. Summary of the relevant TNM parameters.

2.- European Society for Medical Oncology (ESMO) Classification

The ESMO system categorizes ES into two fundamental groups: localized disease and metastatic disease. This approach prioritizes the presence of metastasis as the most significant survival predictor and guides treatment intensity accordingly. For instance, metastatic patients often receive intensified regimens such as VDC/IE (vincristine, doxorubicin, cyclophosphamide/ifosfamide, etoposide) [16]. A major limitation of this system is its lack of integration of molecular data and histologic response to chemotherapy [26].

3.- Euro-E.W.I.N.G. 99 Risk Stratification

The Euro-E.W.I.N.G. 99 trial introduced a more nuanced model, dividing patients into standard, high, and very high-risk categories based on tumor volume, anatomical location, and percentage of necrosis after neoadjuvant chemotherapy [27] (Euro-E.W.I.N.G. 99 Trial Investigators, 2012). One strength of this model is its inclusion of tumor necrosis as a prognostic marker, although reliance on imaging for volume estimation may introduce variability.

4.- Rosen Histologic Necrosis Grading (Grades I - III)

This system evaluates histological response to preoperative chemotherapy by estimating the percentage of tumor necrosis:

- Grade I: <50% necrosis (5-year survival: 30% - 40%)
- Grade II: 50% - 90% necrosis (5-year survival: 50% - 60%)
- Grade III: >90% necrosis (5-year survival: 70% - 80%) [28]

This grading informs decisions about adjuvant therapy, although intratumoral heterogeneity may underestimate true necrosis, and the system is not applicable to unresectable tumors.

5.- Children's Oncology Group (COG) Classification

The COG system stratifies patients into low- and high-risk groups using readily assessable clinical factors such as age, tumor size, location, and metastatic status.

High-risk patients receive intensified interval-compressed chemotherapy [29].

- Adverse factors: age > 14 years, pelvic/spinal location, tumor size >8 cm, presence of metastases
- Patients with ≥2 adverse factors are classified as high risk

6.- Italian Intergroup Staging System

This approach classifies ES based on surgical resectability:

- Stage I: Wide surgical margins
- Stage II: Marginal or positive surgical margins
- Stage III: Unresectable or metastatic disease [30]

Although useful in surgical planning, this system has been largely superseded by protocols emphasizing neoadjuvant chemotherapy.

7.- Molecular Prognostic Scale

Still under development, this scale incorporates secondary genetic alterations:

- High risk: TP53 mutations, CDKN2A deletions, STAG2 loss
- Low risk: EWS-FLI1 fusion without additional mutations [31]
- These markers predict chemotherapy resistance and relapse risk. For example, STAG2 loss is associated with reduced sensitivity to alkylating agents like cyclophosphamide [28]. However, lack of standardized genomic testing limits widespread adoption.

8.- MSTS Functional Scale (Musculoskeletal Tumor Society)

This scale assesses postoperative function, including pain, mobility, emotional acceptance, and return to work. Scores > 25 indicate satisfactory recovery, while scores < 20 suggest severe functional limitation [32]. Though subjective, it remains valuable for evaluating quality of life after surgery.

9.- NIH/NCI Scale for Bone Metastasis

This system classifies skeletal metastatic burden into three grades based on extent. While its prognostic value has been validated, it has been partially replaced by metabolic imaging modalities such as PET/CT [33].

10.- Enneking System for Musculoskeletal Tumors

Traditionally used for musculoskeletal neoplasms, this system distinguishes between low- and high-grade tumors and metastatic status. In ES, all cases are considered high-grade, limiting the system to differentiating between localized (Stage II) and metastatic (Stage III) disease [32]. Its limitation lies in the exclusion of molecular subtypes and treatment response.

Integration of Staging Systems in Clinical Practice

The combination of clinical [27], histologic (Rosen), and molecular staging systems [31], allows for more accurate risk stratification and facilitates personalized therapy. For instance, a patient with pelvic metastatic ES, TP53 mutation, and Grade I necrosis would be classified as very high-risk with an adverse genomic profile, thus qualifying for experimental therapies such as PARP inhibitors or immunotherapy [34]. Nevertheless, the lack of international standardization and inequitable access to genomic sequencing technologies remain significant challenges in global ES management (**Table 2**).

Table 2. Comparative overview of staging and risk stratification systems for Ewing Sarcoma.

System	Classification criteria	Risk groups/ Stages	Streghs	Limitations	Main clinical use
AJCC-TNM	Tumor size, location, metastasis, histologic confirmation	Stages I - III (based on T/N/M)	International standard, allows inter-study comparison	Does not include treatment response or molecular data	General prognosis and initial treatment planning
ESMO	Presence of metastases	Localized vs. Metastatic	Emphasizes prognostic impact of metastasis	Lacks integration of histologic or molecular data	Determines treatment intensity
Euro-EWING 99	Tumor volume, anatomical site, necrosis after neoadjuvant chemotherapy	Standard, high, very high risk	Includes response to chemotherapy (necrosis), more precise stratification	Imaging-based volume estimation introduces variability; lacks molecular input	Personalized prognostic and therapeutic stratification
Rosen Histologic Necrosis Grading	Percentage of tumor necrosis after chemotherapy	Grade I (<50%), II (50% - 90%), III (>90%)	Strong correlation with survival; guides adjuvant therapy	Not applicable to unresectable tumors; risk of underestimating necrosis	Postoperative prognostic assessment
COG	Age, tumor size, location, metastasis	Low risk (0 - 1 factors), high risk (≥2 factors)	Simple and clinically practical	Limited sensitivity per patient; omits necrosis and molecular data	Treatment planning and pediatric clinical trial enrollment
Italian Intergroup Staging System	Surgical resectability and margin status	I: wide margins, II: marginal/positive margins, III: unresectable or metastatic	Useful for surgical decision-making	Largely replaced by protocols favoring neoadjuvant chemotherapy	Early-stage surgical planning
Molecular Prognostic Scale	Secondary genetic alterations	Low risk (EWS-FLI1 only), high risk (TP53, CDKN2A, STAG2)	Predicts resistance and relapse; supports precision medicine	Still investigational; lack of standard genomic testing	Molecular prognosis and future targeted therapies
MSTS	Postoperative function: pain, mobility, emotional acceptance, work reintegration	Score 0 - 30 (higher = better function)	Assesses postoperative quality of life	Subjective; does not reflect oncologic control	Postsurgical follow-up and rehabilitation
NIH/ NCI	Extent of skeletal metastatic burden (Grades 1 to 3)	Three grades based on disease extent	Validated; helps guide treatment	Being replaced by PET/CT; only assesses bone disease	Prognostic evaluation in skeletal metastasis
Enneking System	Histologic grade, compartmentalization, metastatic status	I: low grade, II: high-grade localized, III: metastatic	Traditional and broadly applicable to musculoskeletal tumors	All Ewing sarcomas are high-grade → limited discrimination	Basic surgical planning and oncologic staging

9. Diagnosis

Tumor Locations

Ewing sarcoma most frequently arises in the long bones of the extremities, including the femur, tibia, fibula, humerus, and pelvis. A study involving 976 patients, conducted by the European Intergroup Cooperative Ewing Sarcoma Studies (EI-CESS), reported the distribution of primary tumor sites as follows (Figure 5):

- Axial skeleton: 54%
  - Pelvis: 25%
  - Ribs: 12%
  - Spine: 8%
  - Scapula: 3.8%
  - Clavicle: 1.2%
- Appendicular skeleton: 42%
  - Femur: 16.4%
  - Fibula: 6.7%
  - Tibia: 7.6%
  - Humerus: 4.8%
  - Feet: 2.4%
  - Radius: 1.9%
  - Hands: 1.2%
- Other bones: 0.7% (Cotterill *et al.*, 2000)

Nevertheless, atypical primary sites have been documented, including the pancreas [35], uterine cervix [36], male external genitalia [37], cervical esophagus [38], and small intestine [39]. These unusual presentations underscore the importance of considering Ewing sarcoma in the differential diagnosis of extraosseous masses.

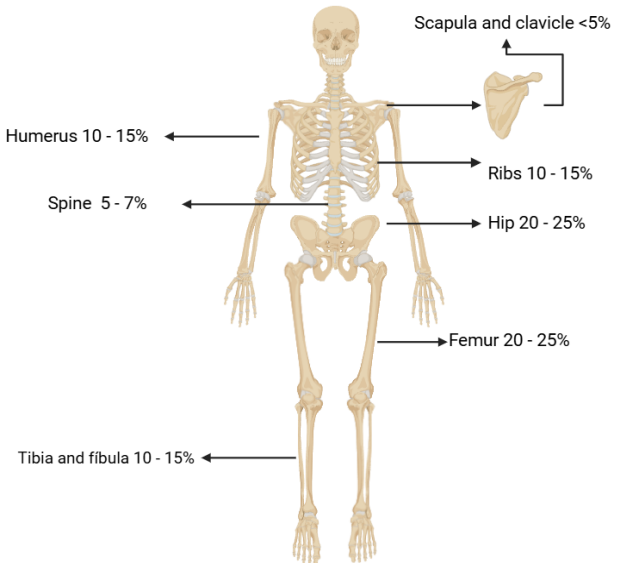


Figure 5. Distribution and frequency of primary neoplasms and Ewing sarcoma. Source: own work.

Initial Imaging

- **Radiography (First-Line Detection Tool)**

Conventional radiography remains the first-line imaging modality in the clinical suspicion of a malignant bone lesion. In Ewing sarcoma, plain radiographs frequently reveal a permeative osteolytic lesion in the diaphysis of long bones, accompanied by cortical destruction and characteristic periosteal reactions such as "onion-skin" layering, spiculated patterns, or a Codman triangle—hallmarks of aggressive tumor behavior [16] [40]. Additionally, in approximately 50% of cases, a soft tissue mass may be visualized, evidenced by displacement of fat planes or loss of soft tissue contours (**Figure 6**). However, radiography has significant limitations in assessing intramedullary tumor extent and involvement of neurovascular structures, thereby restricting its utility in advanced staging [41]. Despite these limitations, its accessibility and rapid execution make it an essential tool for initial evaluation and for guiding further imaging studies.

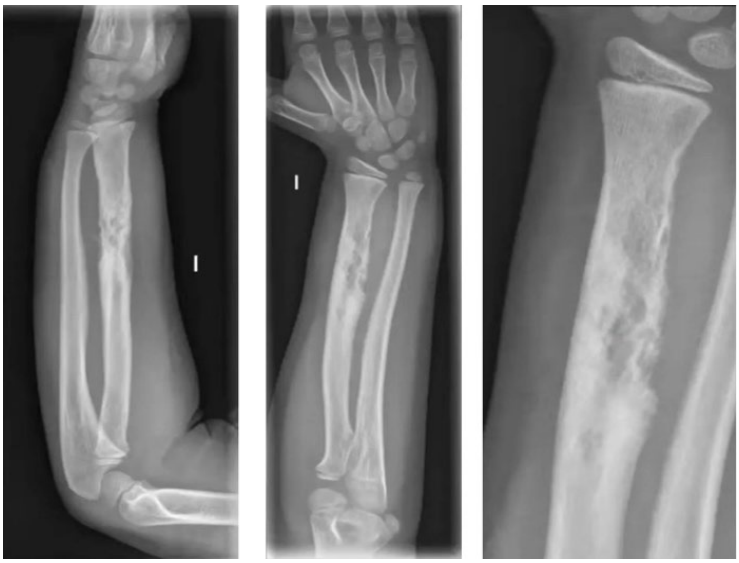
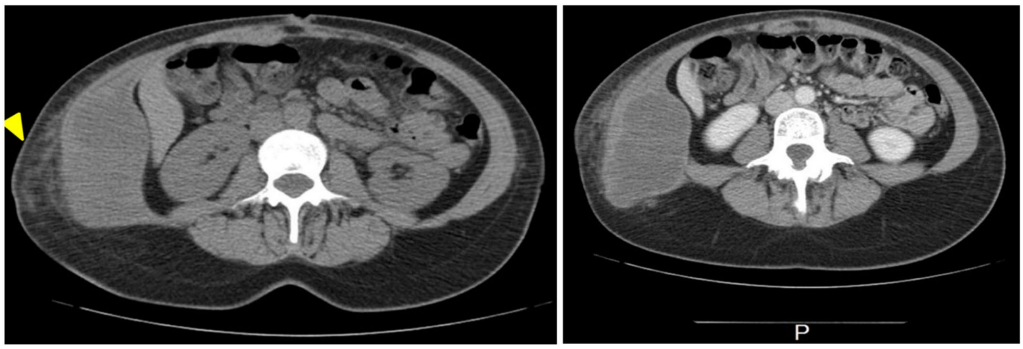


Figure 6. Lateral and anteroposterior radiographs of the radius and ulna in a 4-year-old female patient diagnosed with Ewing sarcoma. The diaphyseal region shows mottled areas with lytic and sclerotic changes, accompanied by cortical destruction.

- **Computed Tomography (CT)—"Anatomical Precision"**

CT provides high-resolution anatomical detail, particularly useful in assessing cortical destruction, involvement of trabecular bone, and detection of mineralized matrix components (**Figure 7(A)**). Although intralesional calcifications are uncommon in Ewing sarcoma, their identification may aid in differential diagnosis [40]. One of CT's most relevant applications is in the detection of pulmonary metastases, where its sensitivity surpasses that of radiography or MRI [42]. Additionally, CT is valuable for guiding interventional procedures such as percutaneous biopsies. However, its sensitivity for detecting bone marrow edema or soft tissue infiltration is limited, and ionizing radiation exposure is a concern—particularly

in pediatric populations (**Figure 7(B)**) [19] [43]. Consequently, CT is used selectively, mainly for thoracic staging.



(A) (B)

Figure 7. (A) Axial non-contrast CT reveals a 12 cm paravertebral mediastinal mass displacing the aorta, esophagus, and azygos vein. (B) Contrast-enhanced imaging shows heterogeneous enhancement and internal neovascularization. The lesion compresses the left atrium, though signs of pulmonary venous congestion are absent.

- **Magnetic Resonance Imaging (MRI)—"Defining Tumor Extent"**

MRI is the most accurate imaging modality for evaluating local extension of Ewing sarcoma. On T1-weighted sequences, the lesion typically appears hypointense, reflecting replacement of normal bone marrow. On T2-weighted sequences, it exhibits hyperintensity due to interstitial edema and high tumor cellularity. Following gadolinium contrast administration, heterogeneous enhancement helps differentiate viable tumor areas from necrosis [16] [40]. MRI is essential for assessing soft tissue invasion, neurovascular involvement, and intramedullary spread, playing a central role in surgical planning and response assessment. Despite its high sensitivity, MRI may overestimate tumor volume by including reactive edema or inflammation, a limitation to consider during post-treatment evaluation [41].

- **Bone Scintigraphy and PET Scan—"Searching for Metastases"**

Bone scintigraphy using technetium-99m has historically been used to detect osseous metastases, as it identifies areas of increased osteoblastic activity. However, its specificity is limited, as inflammatory or traumatic lesions can yield false positives [44]. In many centers, it has been supplanted by ^18F-FDG PET/CT, which detects areas of tumor hypermetabolism in both osseous and extrapulmonary sites. PET/CT not only offers superior sensitivity over bone scintigraphy but also provides metabolic data valuable for treatment monitoring and therapeutic decision-making [45]-[47]. Despite its advantages, PET/CT may produce false positives in inflammatory or infectious settings, and is limited by high cost and limited availability.

Clinical Integration of Imaging Techniques

The integration of various imaging modalities offers a comprehensive and stratified assessment of Ewing sarcoma. Radiography serves as the initial diagnostic

tool, providing orientation and justification for further studies. MRI is the most accurate modality for delineating anatomical extent and soft tissue involvement, whereas PET/CT stands out as an advanced tool for systemic staging and treatment monitoring [20] [47]. Recent studies have proposed the complementary use of liquid biomarkers—such as circulating tumor DNA (ctDNA)—as a means to detect early relapse and to enhance the monitoring of minimal residual disease [37]. However, factors such as tumor heterogeneity and limited access to high-complexity technologies in resource-limited countries continue to represent substantial challenges for the universal implementation of these strategies [19].

Biopsy

- **Histology**

From a morphological standpoint, Ewing sarcoma consists of sheets of small, round cells with a high nuclear-to-cytoplasmic ratio [48]. It is commonly categorized within the group of "small round blue cell tumors," which also includes neuroblastoma, alveolar rhabdomyosarcoma, and lymphoblastic lymphoma [49] (**Figure 8**).

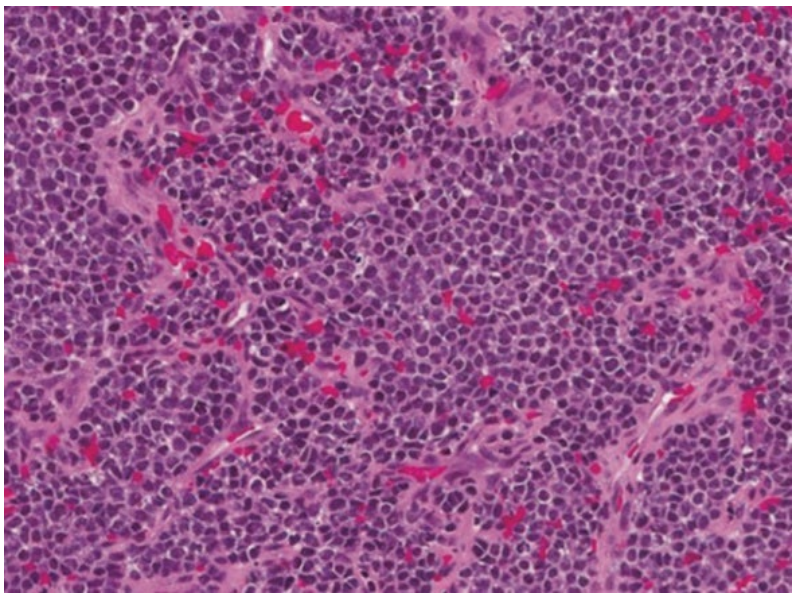


Figure 8. High-magnification image showing a characteristic pattern of small, round to ovoid cells, coarse chromatin, pleomorphic nuclei, and prominent nucleoli. Image source: Mardekian, *et al.* [11]. Licensed under CC BY 3.0.

- **Architecture**

The tumor cells typically present with scant, weakly eosinophilic cytoplasm, often containing glycogen granules that are positive for periodic acid-Schiff (PAS) staining and diastase-sensitive. Nuclei are round, with evenly distributed chromatin and low mitotic activity [15].

Immunohistochemistry

Immunohistochemical evaluation is essential for distinguishing Ewing sarcoma from other small round cell tumors. Markers such as CD99, NKX2.2, and FLI-1

are fundamental, though their interpretation must always be contextualized alongside molecular studies [50].

- **CD99: A Sensitive but Non-Specific Marker**

CD99 (MIC2) is the most commonly expressed immunohistochemical marker in Ewing sarcoma, exhibiting strong, diffuse membranous staining in over 95% of cases [10] [51]. Its high sensitivity makes it an excellent screening tool; however, its specificity is limited, as it is also expressed in other small round cell neoplasms, such as T-cell lymphomas, neuroblastomas, synovial sarcomas, and rhabdomyosarcomas [52] [53] (**Figure 9**). Therefore, it should be interpreted in conjunction with more specific markers and the histological context. In lymphomas, positivity for CD45 or TdT supports exclusion; in rhabdomyosarcomas, coexpression of myogenin and MyoD1 is diagnostically useful [54] [55].

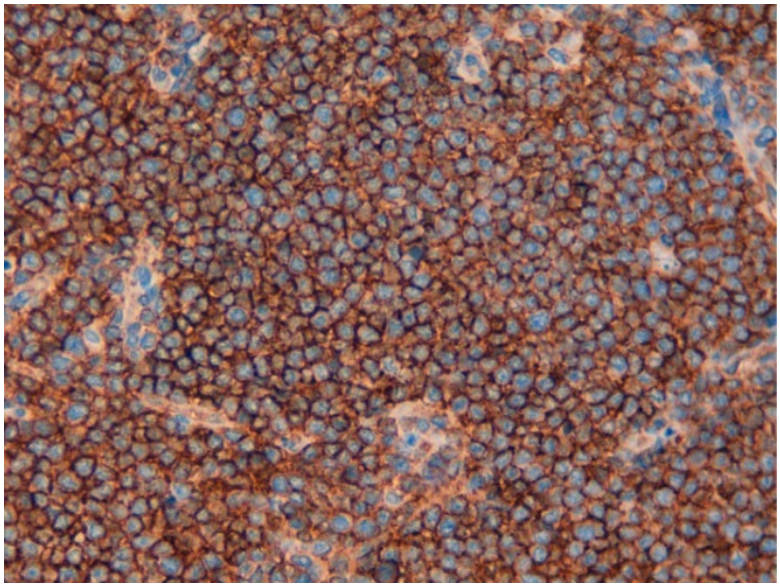


Figure 9. Ewing sarcoma consistently demonstrates membranous positivity for CD99, a surface glycoprotein encoded by the MIC2 gene located on the short arm of the X and Y chromosomes. Although CD99 is highly sensitive for this tumor, it lacks specificity, being expressed in other entities such as lymphoblastic lymphoma, small cell osteosarcoma, mesenchymal chondrosarcoma, and alveolar rhabdomyosarcoma. Nonetheless, absence of CD99 expression virtually rules out Ewing sarcoma. Source adapted from: Ventura-Martínez *et al.* (2017) [56]. Licensed under CC BY-NC-ND 4.0.

- **NKX2.2: Specificity for Neural Differentiation**

NKX2.2 is a transcription factor involved in neural differentiation and is directly induced by the oncogenic activity of the EWSR1-FLI1 fusion protein. It exhibits nuclear staining in approximately 90% - 93% of Ewing sarcomas, offering a sensitivity comparable to CD99 but with higher diagnostic specificity [10] [57]. It is typically negative in most other small round cell tumors, including small cell osteosarcoma and small cell lung carcinoma, although weak positivity may be seen in certain neuroendocrine neoplasms, such as desmoplastic small round cell tu-

mors. Therefore, NKX2.2 should be used cautiously and in conjunction with other markers [58].

- **FLI-1: A Reflection of the EWSR1-FLI1 Fusion**

FLI-1 is a nuclear marker from the ETS transcription factor family and is expressed as a result of the characteristic t(11;22)(q24;q12) translocation that produces the EWSR1-FLI1 fusion. Nuclear expression of FLI-1 is observed in 70% - 80% of Ewing sarcomas but also in lymphomas, angiosarcomas, and some melanomas, limiting its specificity [53] [55]. Thus, FLI-1 should be interpreted as part of a panel rather than in isolation. Furthermore, in tumors with alternative translocations such as EWSR1-ERG (~10% of cases), FLI-1 may be negative, emphasizing the need for complementary molecular testing [10] [59].

Negative Markers: Exclusion of Differential Diagnoses

Exclusion of morphologically similar entities is a critical step in the diagnostic algorithm for Ewing sarcoma. For instance:

- Cytokeratins (AE1/AE3) and EMA are positive in carcinomas and some epithelioid sarcomas but generally negative in Ewing sarcoma, except in rare cases with epithelial differentiation (Hameed & Mandahl, 2020) [60].
- S100 and SOX10, typical of nerve sheath tumors and melanomas, are negative in Ewing sarcoma.
- Desmin, myogenin, and MyoD1, which are key markers for rhabdomyosarcoma, are consistently negative in Ewing sarcoma, with rare exceptions in hybrid variants [61].

The absence of these markers, together with positivity for CD99 and NKX2.2, strongly supports the diagnosis of Ewing sarcoma when supported by appropriate histology.

Emerging Markers: PAX7 and EZH2

PAX7 has recently been proposed as a promising nuclear marker for Ewing sarcoma, with reported sensitivity ranging from 85% to 95% and high specificity relative to other small round cell tumors [57]. Its inclusion in immunohistochemical panels has improved diagnostic accuracy, particularly in small or poorly cellular biopsy specimens. Additionally, EZH2—a histone methyltransferase involved in transcriptional repression—has been found to be overexpressed in Ewing sarcoma, in part regulated by EWSR1-FLI1. While its diagnostic utility is still limited, EZH2 expression may hold future prognostic or therapeutic relevance [34].

Treatment

Historical Context: Euro-E.W.I.N.G. 99

The Euro-E.W.I.N.G. 99 was a landmark international clinical trial designed to improve the treatment and outcomes of patients with Ewing sarcoma, a malignant bone tumor that primarily affects children, adolescents, and young adults. Initiated in 1999, it became one of the largest and most comprehensive studies of its kind. Its primary objective was to evaluate different therapeutic strategies aimed at optimizing survival while minimizing long-term treatment-related sequelae (**Table 3**) [27].

Table 3. Current treatment for Ewing sarcoma.

Drug	Pharmacologic family	Mechanism of action	Typical dose	Adverse edfects	Contraindications	Pharmacokinetics
Vincristine	Periwinkle alcaloid	Inhibits tubuline polimerization	1.5 - 2 mg/m^2 i.v. weekly	Peripheral neuropathy, constipation, SIADH syndrome	Hypersensitivity, pre-existing neuromuscular disease	Half life: 19 - 155 hr, hepatic metabolism (CYP3A4)
Doxorubicin	Anthracycline	Inserts DNA and inhibits topoisomerase ll	60 - 75 mg/m^2 every 3 weeks	Cardiotoxicity, myelosuppression, alopecia	Heart failure	Half life: 12 - 18 hr, bile expression
Cyclophosphamide	Alkylating agent	Forms adducts of DNA	1200 mg/m^2 with Mesna	Myelosuppression, haemorrhagic cystitis, infertility	Chronic kidney disease	Half life: 3 - 12 hr, hepatic activation
Ifosfamide	Alkylating agent	Similar to cyclophosphamide	1800 - 3000 mg/m^2/day with Mesna	Encephalopaty, nefrotoxicity, myelosuppression	Chronic kidney disease	Half life: 7 - 15 hr, hepatic metabolism
Etoposide	Epipodophyllotoxin Inhibits topoisomerase ll	Induces DNA fragmentation	100 mg/m^2/day i.v. or 50 mg/m^2 oral	Myelosuppression, secondary leukemia, hypotension	Hipersensitivity to etoposide	Half life: 4 - 12 hrs, 95% bound to proteins
Busulphan	Alkylating agent	Forms DNA bridges	3.2 mg/kg/day i.v. 4 days	Pulmonary fibrosis, hyperpigmentation, venous obstruction syndrome	Known hypersensitivity	Half life: 2 - 3 hr
Melphalan	Alkylating agent	Forms DNA adducts	140 mg/m^2 i.v.	Severe myelosuppression, mucositis, nefrotoxicity	Hypersensitivity, chronic kidney disease	Half life: 1.5 hr
Irinotecan	Camptothecins Inhibits topoisomerase l	Blocks DNA replication	50 mg/m^2/day with temozolomide	Diarrhea, myelosuppression	Gilbert's syndrome, inflammatory bowel disease	Half life: 6 - 12 hr
Cabozantinib	Multikinase inhibitor	Blocks MET, VEGFR2, RET	60 mg/day oral	Hypertension, fatigue, diarrhea, hepatotoxicity	Recent hemorrage, gastrointestinal perforation	Half life: 55 - 99 hr
Trabectedin	Sea alkaloid	Binds the minor groove of DNA	1.3 mg/m^2 every 3 weeks	Rhabdomyolysis, hepatotoxicity, myelosuppression	Severe hepatic insufficiency	Half life: 40 - 60 hr

Objectives of the Euro-E.W.I.N.G. 99 Study

The trial focused on the following goals:

- Improve overall survival rates in patients with Ewing sarcoma
- Evaluate the efficacy of various chemotherapy regimens
- Investigate the role of radiotherapy and surgery in local tumor control [26]
- Reduce the long-term side effects associated with treatment

Study Design

Patients were stratified into risk groups based on tumor size, anatomical location, and the presence of metastases. The three main categories were:

1) Localized Disease (Standard Risk)

Patients with small, localized tumors without evidence of metastasis received standard therapy with chemotherapy, surgery, and/or radiotherapy [49].

2) Localized High-Risk Disease

Patients with large tumors or those located in anatomically challenging sites (e.g., pelvis) were considered high risk. This group received intensified treatment protocols, including high-dose chemotherapy and autologous stem cell transplantation [26].

3) Metastatic Disease

Patients with tumors that had spread to the lungs, bone, or other organs received more aggressive treatment approaches, including high-dose chemotherapy and targeted therapies [27].

Evaluated Treatments

The study investigated several treatment modalities, including:

- Chemotherapy: Regimens included vincristine, ifosfamide, doxorubicin, and etoposide. In some cases, high-dose chemotherapy followed by autologous stem cell rescue (ASCR) was evaluated.
- Surgery: Surgical resection of the primary tumor was performed when feasible, aiming for negative margins [27].
- Radiotherapy: Radiotherapy was used in cases where surgery was not feasible or as an adjunct to reduce local recurrence risk.

Key Findings

The Euro-E.W.I.N.G. 99 trial generated several pivotal outcomes:

- Survival: Five-year overall survival exceeded 70% in patients with localized disease. Although outcomes remained poorer in metastatic patients, the trial helped identify strategies to improve prognosis [27].
- High-Dose Chemotherapy: The study showed that high-dose chemotherapy followed by ASCR may benefit selected high-risk patients [26].
- Local Control: Combined use of surgery and radiotherapy proved effective in achieving local tumor control and reducing recurrence [27].
- Toxicity: The trial emphasized the importance of reducing long-term side effects, such as cardiotoxicity, infertility, and second malignancies, associated with intensive therapy.

Impact on Clinical Practice

The Euro-E.W.I.N.G. 99 trial significantly influenced the clinical management of Ewing sarcoma by:

- Establishing more effective and standardized chemotherapy regimens [29]
- Defining the role of high-dose chemotherapy and ASCR for high-risk patients [26]
- Providing robust evidence to guide decisions regarding surgery and radiotherapy [27]

Recent Therapeutic Advances

Since its first description by James Ewing in 1921, Ewing sarcoma (ES) has undergone a profound evolution in therapeutic management. During the early and mid-20th century, the only available treatments were surgery and radiotherapy, which provided limited local control and low cure rates—even in patients with localized disease [62]. It was not until the 1970s that the introduction of chemotherapy significantly improved survival outcomes. Pioneering studies demonstrated that systemic administration of vincristine, actinomycin D, cyclophosphamide, and doxorubicin markedly enhanced overall survival [63]. Subsequently, combination regimens such as VDC/IE (vincristine, doxorubicin, cyclophosphamide/ifosfamide, etoposide) became the standard of care, integrating systemic chemotherapy with local control via surgery or radiotherapy [29]. In the early 21st century, therapeutic efforts focused on intensifying these classical regimens. In the United States, the Children's Oncology Group (COG) AEWS0031 protocol demonstrated that reducing the interval between chemotherapy cycles from three to two weeks improved event-free survival [29]. In Europe, the Euro-E.W.I.N.G. 99 and Ewing 2008 trials confirmed that consolidation with busulfan and melphalan followed by autologous stem cell transplantation (ASCT) improved outcomes in high-risk patients [42] [63]. Concurrently, national and international clinical guidelines—such as those from the Spanish Sarcoma Research Group (GEIS)—highlighted the importance of a centralized and multidisciplinary approach to managing ES, particularly in patients with poor prognostic features such as inadequate histologic response or large tumor volume [64]. Predictive models for treatment response have refined criteria for therapy intensification. As the molecular biology of ES became better understood, research shifted toward the development of targeted therapies. The main oncogenic driver, the EWS-FLI1 fusion protein, is difficult to inhibit directly, but several associated proteins—such as BAF, CBP/p300, and MLL—have emerged as potential therapeutic targets, particularly through epigenetic modulation [65]. Tyrosine kinase inhibitors have also been explored. Cabozantinib, a dual inhibitor of MET and VEGFR2, demonstrated an objective response rate of 26% in a phase II trial involving patients with advanced ES, positioning it as a potential therapy for refractory disease [66]. The insulin-like growth factor 1 receptor (IGF-1R) pathway, known to promote ES cell proliferation and survival, has been targeted by agents such as ganitumab. However, a phase II trial failed to show improved event-free survival when ganitumab was added to standard chemotherapy [67]. Simultaneously, immunotherapy has gained attention. Immune checkpoint inhibitors such as nivolumab and ipilimumab have shown limited efficacy, though isolated cases have demonstrated prolonged responses [68]. Emerging strategies include CAR-T cells targeting antigens such as GD2, B7-H3, IGF1R, and VEGFR2, as well as bispecific antibodies and tumor vaccines—all in various stages of clinical development [69] [70].

Recent advancements in CAR-T cell research have introduced fourth-generation constructs with enhanced persistence and safety profiles. Notably, the C7R-

GD2.CAR-T cell therapy [71], and the use of anti-GD2 CAR-T cells in pediatric patients with relapsed/refractory neuroblastoma and other GD2-positive solid tumors [71], have shown promising preclinical and early clinical outcomes, some of which include patients with Ewing sarcoma. Furthermore, the EGFR806 CAR-T cell trial is evaluating its efficacy in recurrent/refractory solid tumors, including Ewing sarcoma, due to the overexpression of EGFR in a subset of cases [72]. Another relevant study is exploring safety-engineered CAR-T cells specifically targeting sarcomas, representing a significant step toward personalized immunotherapy in bone tumors [73] [74]. While most of these trials remain in early phases (I/II), their translational relevance is growing, especially for high-risk or refractory Ewing sarcoma, where conventional treatment options remain limited.

Ewing sarcoma exhibits a markedly immunosuppressive tumor microenvironment, characterized by a predominance of M2 macrophages, scarce T-cell infiltration, and elevated levels of inhibitory cytokines such as TGF-β and IL-10. The MIF/CD74 axis has recently been identified as a potential immunotherapeutic target to counteract this immunosuppression [75]. On the diagnostic front, detection of minimal residual disease using RT-PCR targeting the EWS-FLI1 fusion transcript has demonstrated greater sensitivity than conventional morphology [62]. Additionally, liquid biopsy techniques employing small extracellular vesicles (sEVs) containing tumor proteins (e.g., CD99, NGFR) and fusion transcripts offer a less invasive and dynamic method for disease monitoring [76].

In drug repurposing efforts, metformin combined with chemotherapy regimens such as VIT has shown disease control in patients with relapsed ES [77]. Other trials have evaluated the use of the PARP inhibitor talazoparib in combination with temozolomide, yielding partial responses in pediatric patients [78]. Thalidomide has also been reported as an antiangiogenic agent in refractory tumors [79]. At the experimental level, studies have shown that compounds like triamterene induce selective toxicity in cells with DNA repair defects, paving the way for more personalized therapeutic approaches [80]. Additionally, artificial intelligence tools are being applied to predict prognosis and survival by integrating clinical and molecular variables [81]. Given the increasing complexity of ES management, the National Ewing Sarcoma Tumor Board has issued consensus recommendations that include the use of compressed chemotherapy in adults, maintenance therapy, and autologous stem cell transplantation in high-risk settings. The need for international collaborative clinical trials that include patients of all ages is strongly emphasized [82].

10. Conclusion

In summary, the evolution of ES treatment reflects a transition from generalist approaches toward increasingly personalized and rational strategies. The integration of targeted therapies, immunomodulation, molecular diagnostics, and predictive computational models heralds a new era in the management of Ewing sarcoma, particularly for patients with advanced or relapsed disease [83].

Funding

The authors thank PAPIIT IN228825 DGAPA, UNAM for partial financial support.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Choong, P.F.M. (2021) Sarcoma. Springer. https://doi.org/10.1007/978-981-15-9414-4

[2] Moore, D.D. and Haydon, R.C. (2014) Ewing's Sarcoma of Bone. In: *Cancer Treatment and Research*, Springer, 93-115. https://doi.org/10.1007/978-3-319-07323-1_5

[3] Glazebrook, R.T. (1935) James Alfred Ewing, 1855-1935. *Obituary Notices of Fellows of the Royal Society*, **1**, 475-492.

[4] Phillips, R.F. and Higinbotham, N.L. (1967) The Curability of Ewing's Endothelioma of Bone in Children. *The Journal of Pediatrics*, **70**, 391-397. https://doi.org/10.1016/s0022-3476(67)80136-7

[5] Kilpatrick, S.E., Reith, J.D. and Rubin, B. (2018) Ewing Sarcoma and the History of Similar and Possibly Related Small Round Cell Tumors: From Whence Have We Come and Where Are We Going? *Advances in Anatomic Pathology*, **25**, 314-326. https://doi.org/10.1097/pap.0000000000000203

[6] Shashaa, M.N., Alkarrash, M.S., Kitaz, M.N., Hawash, S., Otaqy, M.B., Tarabishi, J., *et al.* (2022) Ewing's Sarcoma in Scapula, Epidemiology, Clinical Manifestation, Diagnosis and Treatment: A Literature Review. *Annals of Medicine & Surgery*, **77**, Article 103617. https://doi.org/10.1016/j.amsu.2022.103617

[7] Widhe, B. and Widhe, T. (2000) Initial Symptoms and Clinical Features in Osteosarcoma and Ewing Sarcoma. *The Journal of Bone and Joint Surgery*, **82**, 667-674. https://doi.org/10.2106/00004623-200005000-00007

[8] Riggi, N. and Stamenkovic, I. (2007) The Biology of Ewing Sarcoma. *Cancer Letters*, **254**, 1-10. https://doi.org/10.1016/j.canlet.2006.12.009

[9] Zucman, J., Melot, T., Desmaze, C., Ghysdael, J., Plougastel, B., Peter, M., *et al.* (1993) Combinatorial Generation of Variable Fusion Proteins in the Ewing Family of Tumours. *The EMBO Journal*, **12**, 4481-4487. https://doi.org/10.1002/j.1460-2075.1993.tb06137.x

[10] Yoshida, A. (2023) Ewing and Ewing-Like Sarcomas: A Morphological Guide through Genetically-Defined Entities. *Pathology International*, **73**, 12-26. https://doi.org/10.1111/pin.13293

[11] Mardekian, S.K., Gandhe, A., Miettinen, M., Pack, S., Curtis, M.T. and Abdullaev, Z. (2014) Two Cases of Spinal, Extraosseous, Intradural Ewing's Sarcoma/Peripheral Neuroectodermal Tumor: Radiologic, Pathologic, and Molecular Analysis. *Journal of Clinical Imaging Science*, **4**, Article 6. https://doi.org/10.4103/2156-7514.126050

[12] Gargallo, P., Yáñez, Y., Juan, A., Segura, V., Balaguer, J., Torres, B., *et al.* (2020) Review: Ewing Sarcoma Predisposition. *Pathology & Oncology Research*, **26**, 2057-2066. https://doi.org/10.1007/s12253-019-00765-3

[13] Gillani, R., Camp, S.Y., Han, S., Jones, J.K., Chu, H., O'Brien, S., *et al.* (2022) Germline Predisposition to Pediatric Ewing Sarcoma Is Characterized by Inherited Pathogenic

Variants in DNA Damage Repair Genes. *The American Journal of Human Genetics*, **109**, 1026-1037. https://doi.org/10.1016/j.ajhg.2022.04.007

[14] Carvalho, N.D.A., Santiago, K.M., Maia, J.M.L., *et al.* (2024) Prevalence and Clinical Implications of Germline Pathogenic Variants in Cancer Predisposing Genes in Young Patients across Sarcoma Subtypes. *Journal of Medical Genetics*, **61**, 61-68. https://doi.org/10.1136/jmg-2023-109269

[15] Shulman, D.S., Whittle, S.B., Surdez, D., Bailey, K.M., de Álava, E., Yustein, J.T., *et al.* (2022) An International Working Group Consensus Report for the Prioritization of Molecular Biomarkers for Ewing Sarcoma. *npj Precision Oncology*, **6**, Article No. 65. https://doi.org/10.1038/s41698-022-00307-2

[16] Grünewald, T.G.P., Cidre-Aranaz, F., Surdez, D., Tomazou, E.M., de Álava, E., Kovar, H., *et al.* (2018) Ewing Sarcoma. *Nature Reviews Disease Primers*, **4**, Article No. 5. https://doi.org/10.1038/s41572-018-0003-x

[17] Franzetti, G., Laud-Duval, K., van der Ent, W., Brisac, A., Irondelle, M., Aubert, S., *et al.* (2017) Cell-to-Cell Heterogeneity of EWSR1-FLI1 Activity Determines Proliferation/Migration Choices in Ewing Sarcoma Cells. *Oncogene*, **36**, 3505-3514. https://doi.org/10.1038/onc.2016.498

[18] Riggi, N., Suvà, M.L., Stamenkovic, I. and Ewing, P. (2020) EWS-FLI1, a Chimeric Transcription Factor with a Key Role in Ewing Sarcoma Pathogenesis. *Current Opinion in Genetics & Development*, **60**, 1-10.

[19] Crompton, B.D., Stewart, C., Taylor-Weiner, A., Alexe, G., Kurek, K.C., Calicchio, M.L., *et al.* (2014) The Genomic Landscape of Pediatric Ewing Sarcoma. *Cancer Discovery*, **4**, 1326-1341. https://doi.org/10.1158/2159-8290.cd-13-1037

[20] Zhang, H., Yang, X., Zhu, L., *et al.* (2020) Immunotherapy Strategies and Immune Microenvironment of Ewing Sarcoma. *Frontiers in Oncology*, **10**, Article 602279.

[21] Tirode, F., Surdez, D., Ma, X., Parker, M., Le Deley, M.C., Bahrami, A., *et al.* (2014) Genomic Landscape of Ewing Sarcoma Defines an Aggressive Subtype with Co-Association of STAG2 and TP53 Mutations. *Cancer Discovery*, **4**, 1342-1353. https://doi.org/10.1158/2159-8290.cd-14-0622

[22] Maharaj, R. (2021) Targeting Tumor-Associated Macrophages in Ewing Sarcoma: From Bench to Bedside. *Frontiers in Oncology*, **11**, Article 715556.

[23] Hahne, J.C., Okuducu, A.F., Fuchs, T., *et al.* (2020) The EWS-FLI1 Target Gene TNC Promotes Metastasis in Ewing Sarcoma. *Oncotarget*, **8**, 108103-108114.

[24] Bedük-Esen, Ç.S., Gültekin, M., Aydın, G.B., Akyüz, C., Oğuz, K.K., Orhan, D., *et al.* (2019) Ewing Sarcoma in an Infant and Review of the Literature. *The Turkish Journal of Pediatrics*, **61**, 760-764. https://doi.org/10.24953/turkjped.2019.05.016

[25] Escrig Sos, J., Gómez Quiles, L. and Maiocchi, K. (2019) The 8th Edition of the AJCC-TNM Classification: New Contributions to the Staging of Esophagogastric Junction Cancer. *Cirugía Española* (*English Edition*), **97**, 432-437. https://doi.org/10.1016/j.cireng.2019.09.004

[26] Gaspar, N., Hawkins, D.S., Dirksen, U., *et al.* (2018) Ewing Sarcoma: Current Management and Future Approaches through Collaboration. *Journal of Clinical Oncology*, **36**, 603-612.

[27] Euro-E.W.I.N.G. 99 Trial (2012) Improved Survival for Patients with Ewing Sarcoma: Results of the Euro-E.W.I.N.G. 99 Trial. *Journal of Clinical Oncology*, **30**, 1682-1689.

[28] Mondal, G., Stevers, M., Goode, B., Ashworth, A. and Solomon, D.A. (2019) A Requirement for STAG2 in Replication Fork Progression Creates a Targetable Synthetic Lethality in Cohesin-Mutant Cancers. *Nature Communications*, **10**, Article No. 1686.

https://doi.org/10.1038/s41467-019-09659-z

[29] Womer, R.B., West, D.C., Krailo, M.D., Dickman, P.S., Pawel, B.R., Grier, H.E., *et al.* (2012) Randomized Controlled Trial of Interval-Compressed Chemotherapy for the Treatment of Localized Ewing Sarcoma: A Report from the Children's Oncology Group. *Journal of Clinical Oncology*, **30**, 4148-4154. https://doi.org/10.1200/jco.2011.41.5703

[30] Wan, W., Lou, Y., Hu, Z., Wang, T., Li, J., Tang, Y., *et al.* (2016) Factors Affecting Survival Outcomes of Patients with Non-Metastatic Ewing's Sarcoma Family Tumors in the Spine: A Retrospective Analysis of 63 Patients in a Single Center. *Journal of Neuro-Oncology*, **131**, 313-320. https://doi.org/10.1007/s11060-016-2295-6

[31] Brohl, A.S., Solomon, D.A., Chang, W., *et al.* (2018) The Genomic Landscape of the Ewing Sarcoma Family of Tumors Reveals Recurrent STAG2 Mutation. *PLOS Genetics*, **14**, e1007405.

[32] Enneking, W.F., Dunham, W., Gebhardt, M.C., Malawar, M. and Pritchard, D.J. (1993) A System for the Functional Evaluation of Reconstructive Procedures after Surgical Treatment of Tumors of the Musculoskeletal System. *Clinical Orthopaedics and Related Research*, **286**, 241-246. https://doi.org/10.1097/00003086-199301000-00035

[33] Shukla, N.N., Patel, J.A., Magnan, H., Zehir, A., You, D., Tang, J., *et al.* (2017) Plasma DNA-Based Molecular Diagnosis, Prognostication, and Monitoring of Patients with EWSR1 Fusion-Positive Sarcomas. *JCO Precision Oncology*, **1**, 1-11. https://doi.org/10.1200/po.16.00028

[34] Brennan, M.F., Gounder, M. and Singer, S. (2022) EZH2 in Sarcoma Biology and Therapy. *Nature Reviews Clinical Oncology*, **19**, 402-417.

[35] Patel, D., Nandu, N.S. and Reddy, A. (2020) Extraosseus Ewing's Sarcoma in Pancreas: A Review. *Cureus*, **12**, e7505. https://doi.org/10.7759/cureus.7505

[36] Xiao, Y., Zhi, Y., Cao, G., Ma, H., Gao, J. and Li, F. (2024) Primary Ewing's Sarcoma of the Uterine Cervix: A Case Report and Review of the Literature. *Journal of Cancer Research and Clinical Oncology*, **150**, Article No. 267. https://doi.org/10.1007/s00432-024-05698-2

[37] Rachapudi, S., Srinivasan, A., Gorman, B., Wilhelm, A.B., Eyzaguirre, E. and Orihuela, E. (2022) Ewing's Sarcoma of the Male External Genitalia: A Case Report and Review of the Literature. *BMC Urology*, **22**, Article No. 121. https://doi.org/10.1186/s12894-022-01072-x

[38] Wang, S., Zhu, W., Zhang, H. and Yang, X. (2022) Extraosseous Ewing Sarcoma of the Cervical Esophagus: Case Report and Literature Review. *Ear, Nose & Throat Journal*, **101**, NP203-NP208. https://doi.org/10.1177/0145561320953696

[39] Kolosov, A., Dulskas, A., Pauza, K., Selichova, V., Seinin, D. and Stratilatovas, E. (2020) Primary Ewing's Sarcoma in a Small Intestine—A Case Report and Review of the Literature. *BMC Surgery*, **20**, Article No. 113. https://doi.org/10.1186/s12893-020-00774-z

[40] Murphey, M.D., Senchak, L.T., Mambalam, P.K., Logie, C.I., Klassen-Fischer, M.K. and Kransdorf, M.J. (2013) From the Radiologic Pathology Archives: Ewing Sarcoma Family of Tumors: Radiologic-Pathologic Correlation. *RadioGraphics*, **33**, 803-831. https://doi.org/10.1148/rg.333135005

[41] NCCN (2023) Clinical Practice Guidelines in Oncology: Bone Cancer (Version 2.2023). National Comprehensive Cancer Network.

[42] Whelan, J., Le Deley, M.C., Le Teuff, G., *et al.* (2018) Results of the Euro-E.W.I.N.G.

99 Trial. *The Lancet Oncology*, **19**, 603-615.

[43] Tap, W.D., *et al.* (2020) Sarcoma Diagnosis and Management: Advanced Imaging Applications. *Cancer*, **126**, 1914-1925.

[44] Shukla, N., *et al.* (2021) Circulating Tumor DNA as a Potential Biomarker in Pediatric Sarcomas. *Frontiers in Oncology*, **11**, Article 625682.

[45] Aoki, J., Watanabe, H., Shinozaki, T., Takagishi, K., Ishijima, H., Oya, N., *et al.* (2001) FDG PET of Primary Benign and Malignant Bone Tumors: Standardized Uptake Value in 52 Lesions. *Radiology*, **219**, 774-777. https://doi.org/10.1148/radiology.219.3.r01ma08774

[46] Casey, D.L., Wexler, L.H., Fox, J.J., *et al.* (2022) Role of Imaging in Ewing Sarcoma. *Radiologic Clinics of North America*, **60**, 29-47.

[47] Gupta, A., Riedel, R.F., Shah, C., Borinstein, S.C., Isakoff, M.S., Chugh, R., *et al.* (2023) Consensus Recommendations in the Management of Ewing Sarcoma from the National Ewing Sarcoma Tumor Board. *Cancer*, **129**, 3363-3371. https://doi.org/10.1002/cncr.34942

[48] Staege, M.S., Hutter, C., Neumann, I., Foja, S., Hattenhorst, U.E., Hansen, G., *et al.* (2004) DNA Microarrays Reveal Relationship of Ewing Family Tumors to Both Endothelial and Fetal Neural Crest-Derived Cells and Define Novel Targets. *Cancer Research*, **64**, 8213-8221. https://doi.org/10.1158/0008-5472.can-03-4059

[49] Cotterill, S.J., Ahrens, S., Paulussen, M., Jürgens, H.F., Voûte, P.A., Gadner, H., *et al.* (2000) Prognostic Factors in Ewing's Tumor of Bone: Analysis of 975 Patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. *Journal of Clinical Oncology*, **18**, 3108-3114. https://doi.org/10.1200/jco.2000.18.17.3108

[50] Machado, I., Navarro, S. and Llombart-Bosch, A. (2012) The Morphological, Immunohistochemical and Genetic Features of Ewing/PNET and their Differential Diagnosis with Other Small Round Cell Tumours. *Revista Española de Patología*, **45**, 145-156.

[51] Llombart-Bosch, A., Machado, I., Navarro, S., Bertoni, F., Bacchini, P., Alberghini, M., *et al.* (2009) Histological Heterogeneity of Ewing's Sarcoma/PNET: An Immunohistochemical Analysis of 415 Genetically Confirmed Cases with Clinical Support. *Virchows Archives*, **455**, 397-411. https://doi.org/10.1007/s00428-009-0842-7

[52] Parham, D.M. and Barr, F.G. (2012) Classification of Rhabdomyosarcoma and Its Molecular Basis. *Advances in Anatomic Pathology*, **20**, 387-397. https://doi.org/10.1097/pap.0b013e3182a92d0d

[53] Khoury, J.D., Rubin, B.P. and Subbiah, V. (2022) Ewing Sarcoma: Diagnostic Challenges and Advances in Molecular Pathology. *Seminars in Diagnostic Pathology*, **39**, 277-289.

[54] Hornick, J.L. (2021) Practical Soft Tissue Pathology: A Diagnostic Approach. 2nd Edition, Elsevier.

[55] Folpe, A.L., Goldblum, J.R., Rubin, B.P., Shehata, B.M., Liu, W., Dei Tos, A.P., *et al.* (2005) Morphologic and Immunophenotypic Diversity in Ewing Family Tumors. *American Journal of Surgical Pathology*, **29**, 1025-1033. https://doi.org/10.1097/01.pas.0000167056.13614.62

[56] Ventura-Martínez, N., Cebreros-Rosas, D., Miragall-Alba, L., *et al.* (2017) Extraskeletal Ewing's Sarcoma of the Head and Neck: A Case Report and a Literature Review. *Revista Española de Cirugía Oral y Maxilofacial*, **39**, 105-112.

[57] Hung, Y.P., Shah, S.S. and Chou, Y.P. (2021) Diagnostic Utility of NKX2.2 and PAX7

in Small Round Blue Cell Tumors. *Modern Pathology*, **34**, 1054-1065.

[58] Toki, S., Wakai, S., Sekimizu, M., Mori, T., Ichikawa, H., Kawai, A., *et al.* (2018) PAX7 Immunohistochemical Evaluation of Ewing Sarcoma and Other Small Round Cell Tumours. *Histopathology*, **73**, 645-652. https://doi.org/10.1111/his.13689

[59] Slater, O. and Shipley, J. (2007) Clinical Relevance of Molecular Genetics to Paediatric Sarcomas. *Journal of Clinical Pathology*, **60**, 1187-1194. https://doi.org/10.1136/jcp.2006.040113

[60] Hameed, M. and Mandahl, N. (2020) Bone and Soft Tissue Tumours. In: WHO Classification of Tumours Editorial Board, *Soft Tissue and Bone Tumours*, International Agency for Research on Cancer.

[61] Athale, U.H., Shurtleff, S.A., Jenkins, J.J., Poquette, C.A., Tan, M., Downing, J.R., *et al.* (2001) Use of Reverse Transcriptase Polymerase Chain Reaction for Diagnosis and Staging of Alveolar Rhabdomyosarcoma, Ewing Sarcoma Family of Tumors, and Desmoplastic Small Round Cell Tumor. *Journal of Pediatric Hematology/Oncology*, **23**, 99-104. https://doi.org/10.1097/00043426-200102000-00006

[62] Dagher, R., Williams, G., Johnsson, J.R., *et al.* (2001) Approval Summary: Imatinib Mesylate in Chronic Myelogenous Leukemia. *Clinical Cancer Research*, **7**, 391-397.

[63] Oberlin, O., Rey, A., Desfachelles, A.S., Philip, T., Plantaz, D., Schmitt, C., *et al.* (2006) Impact of High-Dose Busulfan Plus Melphalan as Consolidation in Metastatic Ewing Tumors: A Study by the Société Française des Cancers de L'Enfant. *Journal of Clinical Oncology*, **24**, 3997-4002. https://doi.org/10.1200/jco.2006.05.7059

[64] Mata Fernández, C., Sebio, A., Orcajo Rincón, J., Martín Broto, J., Martín Benlloch, A., Marcilla Plaza, D., *et al.* (2024) Clinical Practice Guidelines for the Treatment of Ewing Sarcoma (Spanish Sarcoma Research Group-GEIS). *Clinical and Translational Oncology*, **27**, 824-836. https://doi.org/10.1007/s12094-024-03602-5

[65] Gong, H., Xue, B., Ru, J., Pei, G. and Li, Y. (2023) Targeted Therapy for EWS-FLI1 in Ewing Sarcoma. *Cancers*, **15**, Article 4035. https://doi.org/10.3390/cancers15164035

[66] Italiano, A., Mir, O., Mathoulin-Pelissier, S., Penel, N., Piperno-Neumann, S., Bompas, E., *et al.* (2020) Cabozantinib in Patients with Advanced Ewing Sarcoma or Osteosarcoma (CABONE): A Multicentre, Single-Arm, Phase 2 Trial. *The Lancet Oncology*, **21**, 446-455. https://doi.org/10.1016/s1470-2045(19)30825-3

[67] Zöllner, S.K., Amatruda, J.F., Bauer, S., Collaud, S., de Álava, E., DuBois, S.G., *et al.* (2021) Ewing Sarcoma—Diagnosis, Treatment, Clinical Challenges and Future Perspectives. *Journal of Clinical Medicine*, **10**, Article 1685. https://doi.org/10.3390/jcm10081685

[68] Morales, E., Olson, M., Iglesias, F., Dahiya, S., Luetkens, T. and Atanackovic, D. (2020) Role of Immunotherapy in Ewing Sarcoma. *Journal for ImmunoTherapy of Cancer*, **8**, e000653. https://doi.org/10.1136/jitc-2020-000653

[69] Brudno, J.N., Maus, M.V. and Hinrichs, C.S. (2024) CAR T Cells and T-Cell Therapies for Cancer: A Translational Science Review. *Journal of the American Medical Association*, **332**, 1924-1935.

[70] Yu, H., Ge, Y., Guo, L. and Huang, L. (2016) Potential Approaches to the Treatment of Ewing's Sarcoma. *Oncotarget*, **8**, 5523-5539. https://doi.org/10.18632/oncotarget.12566

[71] Omer, B. (2024) C7R-GD2.CART Cells for Patients with Relapsed or Refractory Neuroblastoma and Other GD2 Positive Cancers (GAIL-N). https://classic.clinicaltrials.gov/show/NCT03635632

[72] Annesley, C. (2025) EGFR806 CAR T Cell Immunotherapy for Recurrent/Refractory

Solid Tumors in Children and Young Adults.
https://classic.clinicaltrials.gov/show/NCT03618381

[73] Locatelli, F. (2025) Anti-GD2 CAR T Cells in Pediatric Patients Affected by High Risk and/or & Relapsed/Refractory Neuroblastoma or Other GD2-positive Solid Tumors. https://classic.clinicaltrials.gov/show/NCT03373097

[74] Chang, L.-J. (2023) Safety and Efficacy Evaluation of 4th Generation Safety-Engineered CAR T & Cells Targeting Sarcomas. https://trial.medpath.com/clinical-trial/ba4ea99d4aacc038/nct03356782-safety-efficacy-evaluation-car-t-cells-sarcomas

[75] He, F., Xu, J., Zeng, F., Wang, B., Yang, Y., Xu, J., *et al.* (2025) Integrative Analysis of Ewing's Sarcoma Reveals That the MIF-CD74 Axis Is a Target for Immunotherapy. *Cell Communication and Signaling*, **23**, Article No. 23. https://doi.org/10.1186/s12964-024-02020-y

[76] Samuel, G., Crow, J., Klein, J.B., Merchant, M.L., Nissen, E., Koestler, D.C., *et al.* (2020) Ewing Sarcoma Family of Tumors-Derived Small Extracellular Vesicle Proteomics Identify Potential Clinical Biomarkers. *Oncotarget*, **11**, 2995-3012. https://doi.org/10.18632/oncotarget.27678

[77] Metts, J.L., Trucco, M., Weiser, D.A., Thompson, P., Sandler, E., Smith, T., *et al.* (2023) A Phase I Trial of Metformin in Combination with Vincristine, Irinotecan, and Temozolomide in Children with Relapsed or Refractory Solid and Central Nervous System Tumors: A Report from the National Pediatric Cancer Foundation. *Cancer Medicine*, **12**, 4270-4281. https://doi.org/10.1002/cam4.5297

[78] Schafer, E.S., Rau, R.E., Berg, S.L., Liu, X., Minard, C.G., Bishop, A.J.R., *et al.* (2020) Phase 1/2 Trial of Talazoparib in Combination with Temozolomide in Children and Adolescents with Refractory/Recurrent Solid Tumors Including Ewing Sarcoma: A Children's Oncology Group Phase 1 Consortium Study (ADVL1411). *Pediatric Blood & Cancer*, **67**, e28073. https://doi.org/10.1002/pbc.28073

[79] Li, Q., Liu, Y. and Yu, Y. (2017) Antiangiogenic Therapy for Primitive Neuroectodermal Tumor with Thalidomide: A Case Report and Review of Literature. *Medicine*, **96**, e9272. https://doi.org/10.1097/md.0000000000009272

[80] Mackintosh, C., *et al.* (2020) Repositioning of Triamterene as a Synthetic Lethal Agent in BRCA2-Deficient Tumors. *Oncotarget*, **11**, 2995-3009.

[81] Chen, W.H., Zhou, C.M., Yan, Z.Y., Chen, H., *et al.* (2021) Using Machine Learning Techniques Predicts Prognosis of Patients with Ewing Sarcoma. *Journal of Orthopaedic Research*, **39**, 2519-2527. https://doi.org/10.1002/jor.24991

[82] Gupta, A.A., Riedel, R.F., Shah, C., *et al.* (2023) Consensus Recommendations in the Management of Ewing Sarcoma from the National Ewing Sarcoma Tumor Board Working Group. *Cancer*, **129**, 837-853.

[83] Strauss, S.J., Berlanga, P. and McCabe, M.G. (2024) Emerging Therapies in Ewing Sarcoma. *Current Opinion in Oncology*, **36**, 297-304. https://doi.org/10.1097/cco.0000000000001048