

# EWING'S SARCOMA

## A general summary

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## Abstract

The purpose of this article is to give an updated summary of Ewing's Sarcoma (ES). This is a rare malignancy of bone in children and adolescent. There have been many theories about from which cells these tumors actually arise, and the latest hypotheses suggest multipotent mesenchymal precursor cells.

In Norway there are 5-10 new patients presenting with ES each year, with a median age around 15 years and a small predilection of boys compared to girls (1,5:1). On world basis, ES has a strong predilection in Caucasians, and is rarely seen in Asia and Africa.

The tumors are most often osseous, sometimes extraosseous presented as a large soft tissue tumor. Most common locations are pelvis and long bones, such as femur, tibia, fibula and humerus. Pain is often the first symptom, lasting for months to years and often related to physical activity, leading to diagnosis of osteomyelitis. A palpable mass may occur, and systemic symptoms like fever, tiredness, weight loss and anemia may indicate serious disease. X-ray confirms the suspicion of an osseous tumor, and further investigation and treatment should be performed at a specialized hospital. Standard treatment of ES is multimodal, with a combination of chemotherapy, surgery and/or radiation therapy. Sometimes tumor cells respond poorly to chemotherapy, and HMAS (High dose chemotherapy with Autologous stem cell Support) may be indicated (see 6 c). Despite intensive treatment, metastases may occur during or after treatment, and the number of patients with recurrent disease is high. Therefore, there have been a lot of studies towards new treatment, including extracorporeal irradiation (ECI) and targeted therapy.

5- years-survival is currently 60-70% in localized disease and 20-30% in metastatic disease. Unfortunately, almost all patients are likely to have micro-metastases at diagnosis.

## 1) INTRODUCTION

Some years ago, I decided to write this thesis on Ewing's Sarcoma. I had a friend that had this uncommon diagnosis. I started working at The Norwegian Radiumhospital at the sarcoma/lymphoma department in 2012, and met many of these patients. Through reading their medical history and participating in treatment of these patients over the last year, I have learned a lot about this cruel type of cancer.

Ewing's sarcoma (ES) is a rare malignancy with a strong pediatric predilection, typically presenting as a bone tumor. It was first described by James Ewing in 1921 as a "diffuse endothelioma of bone", believing that it arose from the blood vessels of the bone tissue.<sup>18</sup> Dr. Ewing was appointed as the first professor of pathology at Cornell University in 1899, and had extensive interest in and experience with osseous tumors. He cofounded the American

Society for the Control of Cancer (now the American Cancer Society) in 1913. His extensive research on cancer and its treatment led to him being on the cover of Time magazine in 1931 as “Cancer Man Ewing.”<sup>25</sup>

In the early 1980s, Ewing’s sarcoma and the peripheral Primitive neuroectodermal tumor (PNET) were both found to contain the same reciprocal translocation between chromosomes 11 and 22, t(11;22). They have been grouped into a class of cancers entitled Ewing’s Sarcoma Family of Tumor (ESFT), which include additional Extraosseous Ewing’s sarcoma, neuroepithelioma, atypical Ewing’s sarcoma, and Askins tumor (tumor of the chest wall). ES and PNET show varying degrees of neuroectodermal differentiation, where the term Ewing sarcoma has been used for those tumors that lack evidence of neuroectodermal differentiation. However, based on the identification of a common genetic lesion, and similar clinical behavior and response to treatment, the World Health Organization now refers to these tumors as ES.<sup>2</sup>

ES is an aggressive cancer of bone and, to a lesser extent, soft tissue.<sup>12</sup> These tumors are defined as round cell sarcomas<sup>2</sup>, and are characterized by uniform, densely packed, glycogen-rich small “blue” cells with round nuclei, but without distinct cytoplasmic borders or prominent nucleoli.<sup>1,13</sup> Various cell types have been suggested as ES origin, the favorite candidate at present are multipotent mesenchymal precursor cells.<sup>10</sup>

ES is a very rare cancer with few patients worldwide. Close international collaboration on research and treatment protocols are necessary for developing new treatment strategies.

## 2) EPIDEMIOLOGY

The causes of ES are unknown. Bonesarcomas in general are most often seen associated with rapid growth in younger persons. It might be an explanation that rapid proliferating cells are more exposed for neoplastic transformation, which can trigger the disease.<sup>17</sup>

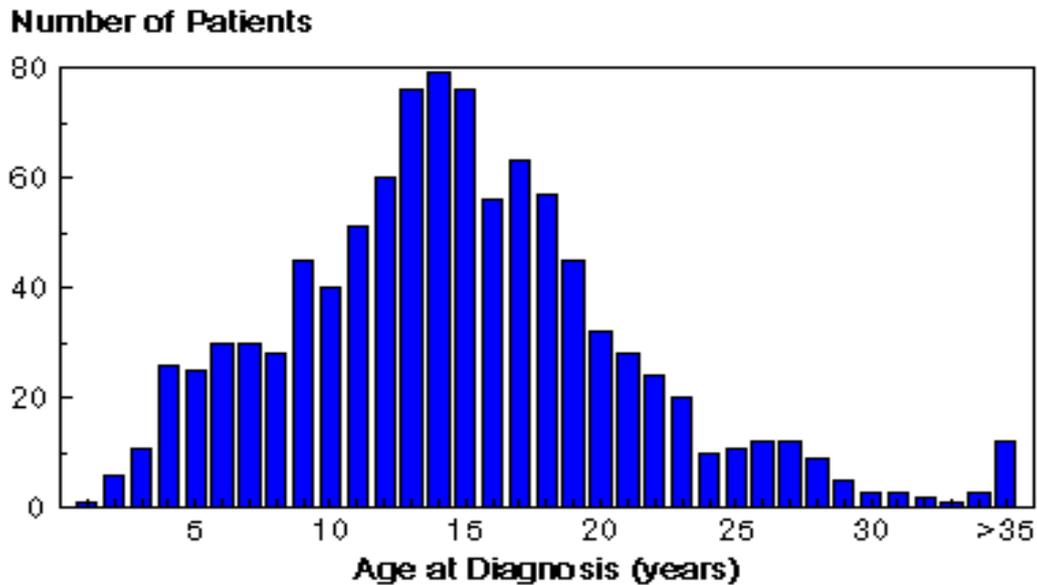
Ewing sarcoma of bone represents the second most common primary malignant tumor of bone in children and adolescents.<sup>25</sup> Overall rates of tumors of bones are approximately 40 new cases per year in Norway, and of these Ewing's sarcoma accounts for approximately 5-10%.<sup>4</sup> Median age at diagnosis is 14 to 15 years, with 95% of cases reported between the ages of 4 and 25 years.<sup>12,25</sup> In an analysis of the European Intergroup Cooperative Ewing’s Sarcoma study (EICESS) study group database, 20% of patients were over 20 years. And with advances in the molecular diagnosis of otherwise undifferentiated sarcomas, the reported incidence in young adults is increasing.<sup>12</sup>

About 25% present with metastasis at diagnosis, restricted to the lungs, bone/bone marrow, or combined. Metastases to lymph nodes and other sites may occur, but are rare.<sup>12,14</sup>

ES shows a slight male predilection (male-to-female ratio, 1.5:1).<sup>4,12,25</sup> There is a difference in the racial incidence of this tumor. Data from the Surveillance Epidemiology and End

Results (SEER) database, reporting an ES incidence 9-fold in Caucasians compared to African Americans, support the existence of an underlying genetic predisposition. <sup>1,10,12,2</sup>

Figure 1.<sup>3</sup> Age at diagnosis.



## 2) CLINICAL PRESENTATION

Two cases are presented, to illustrate common signs and symptoms.

- 1) 13 year old girl presenting with pain in her left hip lasting for 4 months. At first, the pain was related to activity, then later also at rest and at night. After 2 months she started to limp, was more tired and sleeping more than usual. She also lost her appetite, and had a total weight loss of 8 kg. She was examined by doctors several times, and pelvic X-ray taken 3 months after debut was considered negative. Later MR-imaging showed a lesion in the upper trochanter-area, suspected to be malignant.
- 2) 17 year old boy with hip pain lasting for about two years, worsening after great physical activity. 6 weeks before admittance to hospital pain got worse, with neurological outcome in the left leg. He assumed this occurred after heavy lifting. The doctor gave him painkillers and a sick leave for two weeks, and the pain was reduced. Suddenly, he got great pain again, this time without any prior physical activity. He presented with hyperesthesia and reduced sensibility in the left leg and foot, and told about episodes of weakness. Reduced appetite and morning nausea the last weeks, possibly due to strong painkillers, had resulted in weight loss of 5-8 kg. MR-imaging at local hospital showed a large tumor in the left side of pelvis, infiltrating the sacral nerves. CT-thorax shows possible multiple lung metastases.

## Signs and symptoms

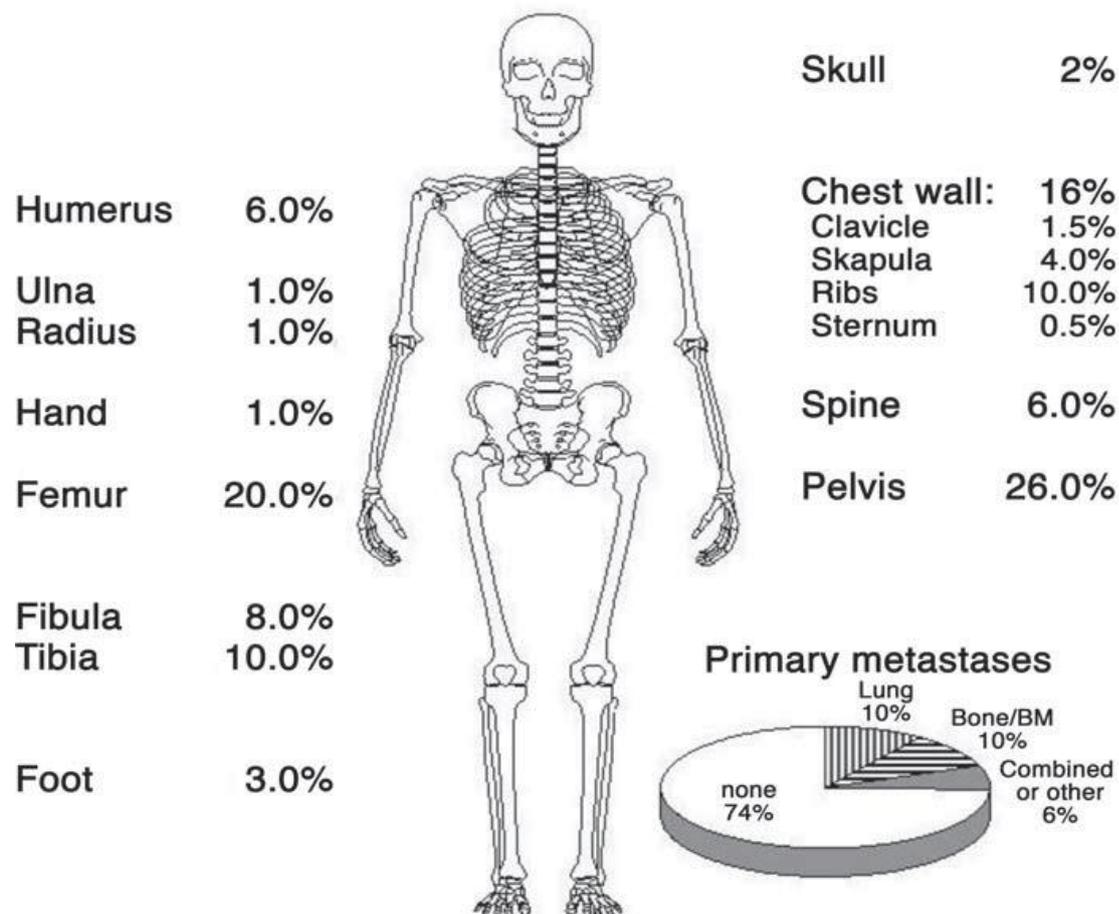
The duration of symptoms prior to diagnosis has been reported between weeks and months, with a median of 4 to 6 months.<sup>12</sup> The most common symptom is pain. The intensity varies from dull to severe, is not always related to activity, and it is often associated with athletic trauma. Pain may become very intense when the tumor is located near important nerves, like in the sacrum, pelvis or spine. Swelling or tenderness in the affected area and elevated local temperature is common, and CRP may be elevated, thereby suggesting an inflammatory condition. Common clinical differential diagnoses are osteomyelitis, tendinitis or trauma.<sup>1,25</sup> Additional clinical manifestations are chest pain (rib lesions), dental abnormalities (facial lesions), and gait disturbances (lower long-bone lesions).<sup>25</sup>

Swelling is especially seen when the long bones of the arms or legs are affected. Sometimes the tumor can interfere with movement and can weaken the bones, and about 10 % of patients present with a pathologic fracture as the initial symptom<sup>12,25</sup>. Other symptoms may include tiredness, weight loss, anemia, leukocytosis, elevated CRP, fever (remittent, about 38°C, in 20%–49% of patients) and/or increased erythrocyte sedimentation rate (43% of patients).<sup>1,2,25</sup> Systemic symptoms are observed in about 1/3 of patients, often correlating with advanced disease and metastases.<sup>1,3,4,12,25</sup>

In children and young adults with ES, skeletal tumors are most common. The tumor is often localized in the axial skeleton, with 25% of the tumors occurring in the pelvis alone. The pelvis, extremities, and ribs account for approximately 86% of cases.<sup>25</sup> The majority of long-bone lesions are metadiaphyseal (44–59%). Diaphyseal lesions account for 33–35% of cases, while ES confined to the metaphysis represents 5–15% of lesions.<sup>1,4,12,25</sup> Rarely, these tumors will arise from soft tissue and are then labeled extraosseous or extraskelatal ES (EES). The most commonly reported locations of EES include the paravertebral region (32%), lower extremities (26%) and chest wall (18%). Tumor bulk may be indiscernible for a long period of time if these tumors are deep seated, resulting in large tumor volume at diagnosis and a subsequent poorer prognosis.<sup>18,25</sup>

Important **differential diagnoses** are osteomyelitis and other benign conditions, rhabdomyosarcoma, osteosarcoma, metastatic neuroblastoma (if < 5 years of age), non-Hodgkin`s lymphoma, and metastatic bronchial carcinoma in older patients.<sup>1</sup>

Figure 2.<sup>34</sup> Most common osseous locations.



#### 4. DIAGNOSIS AND STAGING

Imaging features of osseous Ewing`s sarcoma often suggest the diagnosis, with aggressive long-bone destruction in the metadiaphysis of a child or a young adult and an associated soft-tissue mass.<sup>25</sup> EES commonly demonstrates a nonspecific radiologic appearance of a large soft-tissue mass affecting the paraspinal region or lower extremity. In case of skeletal pain, a palpable lump or a pathologic fracture, one should be referred for X-ray examination. At this point, tumor is often visible on X-ray, but it can be overlooked or mistaken for benign conditions. Early in stage, the findings may also be few. About ¼ of patients with malignant tumor of bone have already had a “negative” X-ray, in which tumor retrospectively was present.<sup>17,1</sup>

If sarcoma of bone is suspected, referral to a sarcoma center is essential. In Norway, there are two bone sarcoma centres: The Norwegian Radium Hospital (Oslo University Hospital) and at Haukeland University Hospital in Bergen.<sup>4,12</sup> Sarcoma groups at these centres include experts

in orthopedic surgery, medical-, pediatric- and radiation oncology, musculoskeletal radiology, and musculoskeletal pathology.<sup>4</sup>

**X-ray:** Radiographs are the easiest, least costly and most accessible study for detection of abnormalities of the skeleton, and will in many cases be the determining modality in diagnostic clarification. When an osseous lesion is suspected, a radiograph in 2 planes should be performed. The characteristic presentation of ES involving a long bone is a poorly marginated, lytic, destructive lesion with a fusiform outline. The periosteal reaction may be an “onion-peel” lamellation or horizontal “hair-on-end” spiculae, caused by the splitting and thickening of the cortex by tumor cells. The layering is usually continuous with reactive ossification in the form of Codman`s triangles. Subsequently, a mottled, moth-eating-appearing cortical destruction develops, with tumor extension in the spongiosa. The result may be spontaneous fracture and bulky soft tissue involvement.<sup>1,12,16,25</sup>

Figure 4.<sup>14</sup>



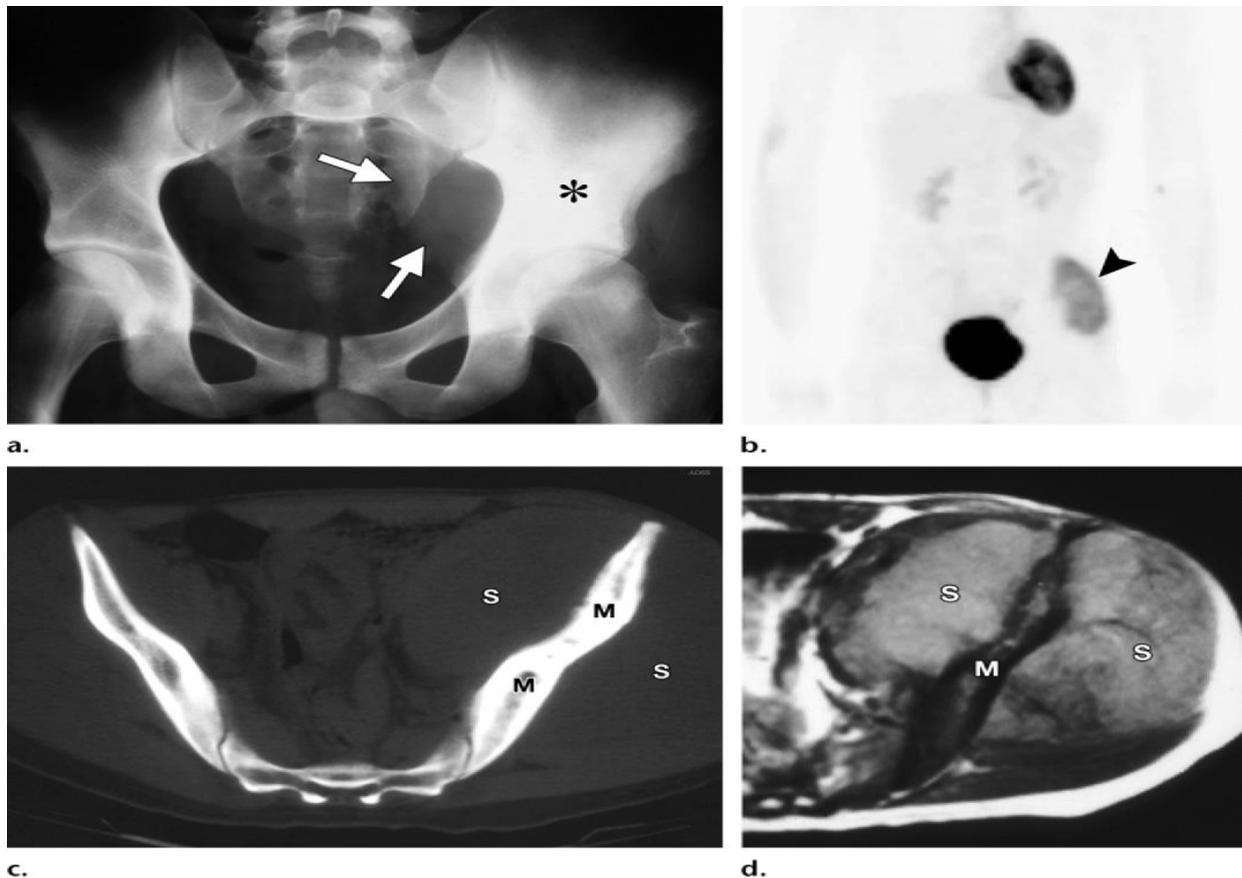
Plain radiographs of the hip and right femur. Anteroposterior views of the pelvis (A) and right femur (B). Arrows indicate soft-tissue density overlying the right iliac bone.

Magnetic resonance (**MR**) imaging is essential to assess the exact local tumor extent, and to overrule skip-lesions. Focal areas of cortical destruction are frequent, allowing continuity between the intraosseous and extraosseous components. This continuity is also commonly seen as subtle channels extending through the cortex at CT or MR imaging, a finding that reflects the underlying pathologic appearance.<sup>25</sup>

MR imaging of bone reveals marrow replacement (100%) and cortical destruction (92%), with an associated soft-tissue mass in 96% of cases. The soft-tissue mass is commonly circumferential but asymmetric around the osseous involvement. Images also determines which anatomical structures are involved and what kind of tissue the tumor is made of.<sup>17,25</sup> MR is also vital to evaluate response to neoadjuvant therapy (before surgery), direct surgical resection and detect local recurrence or metastatic disease<sup>25</sup>, and is central in planning biopsy and local therapy.

Computed tomography (CT) is not always necessary, but is indicated if MR imaging cannot be performed, and if a better view of the calcareous part of the skeleton is needed.<sup>1</sup>

Figure 5.<sup>25</sup>

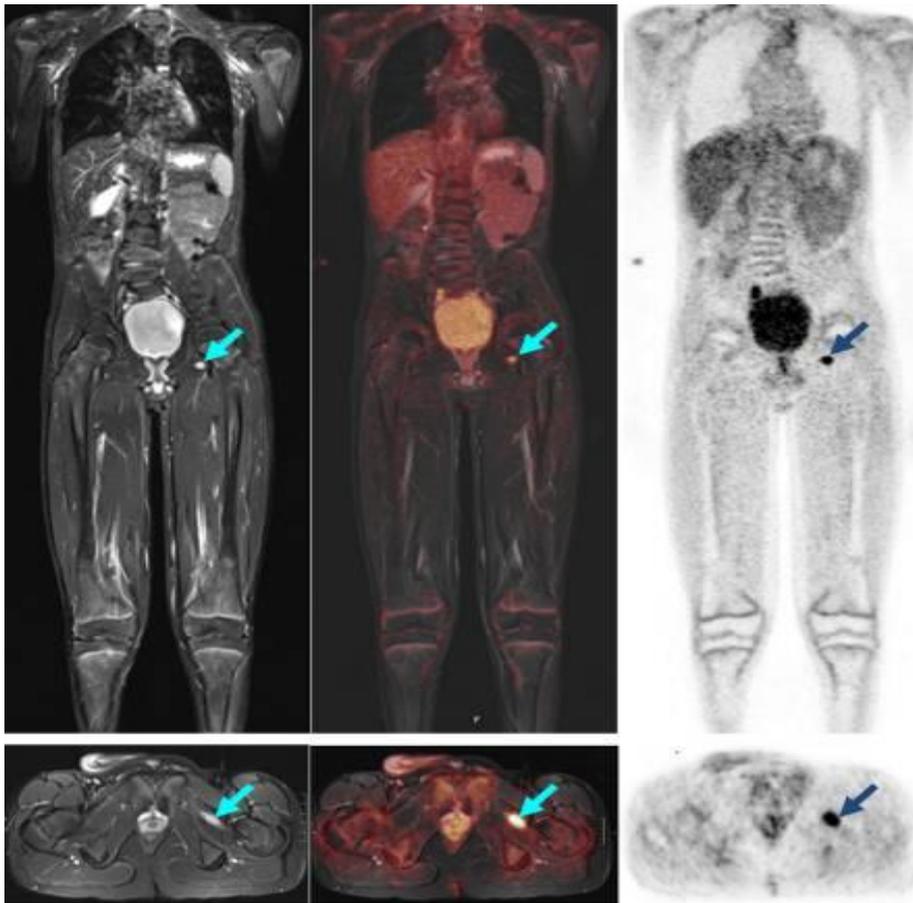


**a.** Ewing sarcoma of the pelvis with prominent sclerosis in a 20-year-old man. **(a)** Frontal radiograph shows a predominantly sclerotic lesion involving the left iliac bone (\*) with displacement of pelvic soft tissues (arrows), suggestive of an associated soft-tissue mass. **(b)** Whole-body image from fluorine 18 fluorodeoxyglucose (FDG) positron emission tomography (PET) reveals hypermetabolic activity in the lesion (arrowhead). **(c)** Axial CT image demonstrates intramedullary sclerosis (M) and a large associated soft-tissue mass (S), which is larger posteriorly and contains no calcification. **(d)** Axial T2-weighted MR image (2000/90) reveals the marrow involvement (M) with a large associated circumferential soft-tissue mass (S), which is larger posteriorly and laterally and intermediate in signal intensity.

If ES is suspected, three additional imaging tests are performed to determine whether the tumor has metastasized: CT scan of the lungs, occasionally MR imaging of central bone marrow, and a bone scintigraphy.<sup>4,2</sup> **Scintigraphy** is done both dynamic and static, and shows the extent of tumor masse in the bone. Dynamic scintigraphy shows how fast the isotope reaches the tumor, and static scintigraphy shows the uptake of the isotope over a certain period of time (two hours).<sup>17</sup>

Fluorine-18 fluorodeoxyglucose positron emission tomography (**FDG-PET**) has recently been proven to be a highly sensitive screening method for the detection of bone metastases in Ewing's sarcoma, although its exact role in the management of ES remains to be defined. In detecting bone metastases, FDG-PET may be even more sensitive than whole-body MR scans.<sup>34</sup>

Figure 6<sup>36</sup>



13-year-old boy with a history of extraosseous metastatic Ewing sarcoma of the pelvis, now showing new imaging findings (*arrows*) suspicious for soft tissue metastasis in the adductor longus muscle. On MR imaging, size regression was seen within 7 weeks. Because of an intensive increase in glucose utilization, a biopsy was performed, which revealed inflammatory changes. *Arrows* indicate the positive PET/MR finding.

The definitive diagnosis of malignant bone tumors requires **biopsy** proof. This can be achieved by ultrasound- or CT-guided fine-needle or core biopsy from the primary site. However, ES often shows extensive necrosis and it must be ascertained that the material harvested is sufficient for diagnosis, including fresh and fresh frozen material for immunohistochemical and molecular analyses. Therefore, several cores are often necessary, with frozen sections verifying representative sampling.<sup>1,12</sup> Biopsy is taken for cytogenetic and genetic analysis. Bone marrow biopsy, taken from crista iliaca, is regular procedure for metastatic investigation. Despite negative imaging studies and bone marrow biopsies, nearly all ES is likely micro-metastatic at diagnosis.<sup>6</sup>

The tumors are per definition high-grade, further histological grading is usually not performed.<sup>17</sup>

## 5. HISTOLOGY AND MICROSCOPIC EXAMINATIONS

**Microscopic examinations** contain biopsy of tumor and bone marrow, immunohistochemistry, flow cytometry (to quickly distinguish some sarcomas from lymphomas), genetic studies, cytogenetic and molecular pathological (RT-PCR and FISH) research, and sometimes electron microscopic studies.<sup>4,17</sup>

Microscopically, ES demonstrates glycogen-rich, small round-like “blue” cells with little cytoplasm. Formation of pseudorosettes may also be seen, indicating neuroectodermal differentiation.<sup>2,17</sup> Mitotic activity is low.<sup>12</sup> Immunohistochemistry shows positive vimentin (biomarker), O13, CD99 (sensitive, not specific) and FLI-1, and is negative for a panel of antibodies.<sup>13,17</sup> Diagnostic genetic studies show translocations, most frequently t(11;22)(q24;q12) with EWSR1-FLI1 gene fusion. Fluorescent in situ hybridization (FISH) or reverse transcriptase–polymerase chain reaction (RT-PCR) have greatly facilitated diagnosis and delineation of ES.<sup>12</sup>

A study on integrated multimodal genetic testing in a single institution between 2005 and 2011, showed that use of all methods capable of detecting EWSR1 rearrangements has value in the workup of suspected cases of ES.<sup>24</sup>

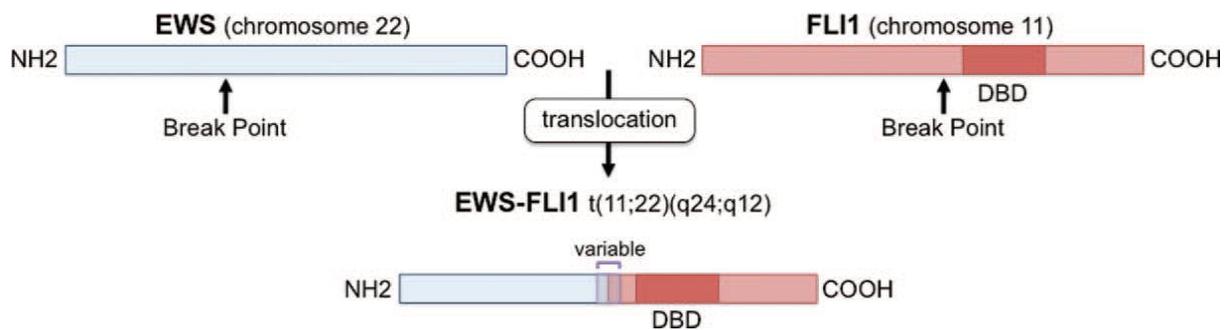
Findings in integrated clinicopathologic, cytogenetic, FISH and RT-PCR analyses of 32 pediatric patients with ES:

- Cytogenetics detected t(11;22) (n = 14) and t(21;22) (n = 1) in 15 (46.9%) patients.
- FISH detected EWSR1 rearrangements in 27 (96.4%) of 28 patients tested.
- RT-PCR was positive in 27 (84.4%) of 32 patients, including 24 EWSR1-FLI1 and 3 EWSR1-ERG. RT-PCR defined breakpoints and fusion partners in 7 cases with EWSR1 rearrangements detected by FISH.
- Sanger sequencing further delineated breakpoints in 21 (77.8%) of 27 RT-PCR positive cases.

In summary, conventional cytogenetic analysis provided a global view but had a lower detection rate and longer turnaround time than other methods. FISH is a rapid method and theoretically can detect all EWSR1 rearrangements, but it cannot identify all partners and is not completely specific for ES. RT-PCR and sequencing are more sensitive and useful in identifying fusion partners and refining breakpoints; however, these methods can be compromised by poor RNA preservation and primer design.<sup>24</sup>

The ES-defining chromosomal **translocation** fuse the 5' end of the EWSR1 Ewing sarcoma breakpoint region 1 gene on chromosome 22 to the 3' portion of a gene of the ETS transcription factor family<sup>10</sup>, including FLI1 (~90%) and ERG (>5%) and in <1% ETV1, ETV4, or FEV.<sup>12,24</sup> The function of the Ewing sarcoma gene (EWSR1) is not well-understood.<sup>12</sup> The gene FLI1 from chromosome 11 is involved in turning other genes on and off. This new fused gene, called EWS-FLI1 or EWSR1-FLI1 (Ewing sarcoma breakpoint region 1-Friend leukaemia virus integration 1, (translocation (11;22)(q24;q12))<sup>35</sup>, encodes an altered fusion protein that regulates other genes that can give rise to cancers when inappropriately expressed.<sup>2</sup>

Figure 3.<sup>10</sup>



The EWS-FLI1 fusion protein. The ETS family DNA-binding domain (DBD) is indicated. FLI1 breakpoint variability generates distinct fusion types 1 to 3.

EWSR1-FLI1 fusion proteins can be detected in the majority of ES, and substantially contributes to the malignant phenotype. Therefore, inactivation of this gene is an interesting strategy for ES therapy.<sup>5,35</sup>

There are also as mentioned several other translocations involving the EWSR1 gene, and it might occur in other malignant diseases (see differential diagnosis) with different fusion partners.<sup>17</sup>

## 6. TREATMENT

The tumors in the Ewing sarcoma family are treated similarly on the basis of their clinical presentation.<sup>2</sup> Sarcoma patients are treated after international protocols, containing guidelines for diagnostics, oncologic treatment, controls and follow ups.<sup>4</sup> In Norway, the treatment protocols are collaborations between Scandinavian Sarcoma Group ([www.ssg-org.net](http://www.ssg-org.net)) and Italian Sarcoma Group (ISG/SSG).<sup>19</sup>

It has been challenging finding effective treatment regimens for ES. There are few patients presenting with this diagnose, and therefore international cooperation is extremely important. Most of the studies are Phase II, which compare the treatment results from a group of patients getting a new treatment with the results from previous treatment regimens. Phase II trials are carried out to assess the drug's efficacy, and Phase III trials are performed to monitor side effects and compare the drug to compounds already on the market. Phase III studies randomize two groups of patients, introducing one of them to a new treatment (assumed to have a positive effect by phase II studies). The other group gets the standard treatment, and later the results from the two groups are compared.

Phase III studies are claimed to be necessary in order to make changes in current treatment regimens, but is difficult to carry out in Ewing's sarcoma due to the rarity of the tumors.<sup>37</sup>

## **a) Localized ES**

ES is highly sensitive to chemotherapy and radiotherapy. For ES isolated to one area (localized), neoadjuvant chemotherapy is used to shrink the tumor. Subsequently, the patient undergoes local treatment with surgical removal of the tumor, and/or radiation therapy. In certain cases the tumor cannot be surgically removed with adequate margins, and radiation alone may be used. The patient then receives further chemotherapy in order to kill any additional abnormal cells.<sup>1</sup> Total time of treatment is about 6 to 9 months.<sup>4</sup>

### **- Chemotherapy**

Today a combination of 6 different drugs is usually given; vincristine (V), doxorubicin(A), cyclophosphamide(C), actinomycin-D(Ac), ifosfamide(I) and etoposide(E).<sup>17,21</sup> Typically, these agents are given as the combination VACAc over 2 days followed by IE given over 5 days. The two combinations (VACAc-IE) are traditionally alternated every three weeks.<sup>2,21</sup>

Chemotherapy causes an extensive necrosis of the tumor cells. Because these cells do not produce any matrix, and disappear short after necrosis, the effective chemotherapy causes a dramatic shrinkage of the tumor, plus regresses the inflammatory response.<sup>1</sup>

The response to chemotherapy is individual. “Good responders” (GRs), have better prognosis compared to poor responders (PRs). Introduction of intensified dosing regimens has raised the 5-year survival of ES patients from 50% to 70%.<sup>12</sup>

### **- Surgery**

Orthopedic surgery is often extensive, massive and mutilating, but a necessary component of curative therapy in most primary bone tumors. Surgery is aimed at preserving function without compromising survival rates. In planning the optimal local therapy, an interdisciplinary approach involving experts experienced in this field is essential. Local treatment should be individually adapted, depending upon the site and size of the tumor, the anatomical structures near the tumor, the patient’s age, and individual preference.<sup>34</sup>

### **- Radiation**

ES is highly sensitive to radiation treatment. Historically, this was the modality of choice for the main tumor. However, radiation therapy can cause problems including chronic swelling, joint stiffness, and secondary cancers later in life. Therefore, surgical removal of the tumor was implemented to obtain local disease control without the side effects of radiation.<sup>2</sup>

Tumors removed with poor margins need local irradiation postoperatively. Some tumors are too large to remove, or are located in an axial, inoperable location. In these cases, radiation is the only local treatment.<sup>2,19</sup> According to current protocols (ISG/SSG), hyper fractionated treatment (1.5 Gray times two each day) is given in between chemotherapy.<sup>19</sup> Data analysis in large cohorts of ES patients indicated that definitive radiotherapy is associated with a higher rate of local recurrence and a significant risk for the development of secondary radiotherapy-induced malignancies, whereas on the other hand functional defects are more common following surgery.<sup>12</sup>

Radiotherapy in pelvic ES: Recent studies at the University of Münster, Germany (in 2013), show potential advantage of intensity-modulated radiotherapy (IMRT) over three-dimensional conformal radiotherapy (3D-CRT) planning in pelvic Ewing's sarcoma. Compared to 3D-CRT, IMRT showed significantly better results regarding dose conformity and bowel sparing at dose levels above 30 Gy (p=0.012). This means dose escalation in the radiotherapy of pelvic Ewing's sarcoma can be more easily achieved using IMRT.<sup>26</sup>

The use of *extracorporeal irradiation (ECI)* was first reported in 1968. The tumor-bearing bone segment is resected, treated with a single high dose fraction of radiation therapy (RT) extracorporeally and reimplanted. The potential risk of local recurrence within the reimplanted bone was of concern with this technique. A study at Royal Prince Alfred Hospital and The Children's Hospital at Westmead (Sydney, Australia), between 1996 and 2011, presented the long-term oncological outcomes of 101 patients treated with ECI. A single dose of 50 Gy was delivered to the resected bone segments. The irradiated bones were reimplanted immediately as a biological graft. Patients were treated with chemotherapy as per standard protocol. There was one local recurrence (2.86%) in Ewing's sarcoma patients, and the 5-year cumulative overall survival was 81.9%. This large series of ECI showed an excellent long-term local control. It is a good alternative reconstruction method in selected patients.<sup>23</sup> However, this technique has been tested on very few patients, and potential long-term effects have not been identified. Further studies are required to implement it in the standard treatment regimen.

## **b) Metastatic ES**

Regardless of whether metastatic disease is identified during the initial staging and patient workup, treatment of ES should include chemotherapy and local control of the primary site of disease, either by radiation therapy, surgery or both. Surgical removal of primary tumor is probably a positive prognostic factor. First-line agents of chemotherapy are the same as for clinically localized disease.<sup>18</sup>

Metastatic spread to the lungs is one of the most frequent types of secondary involvement. The metastatic deposits are multiple, sub-pleural and intrapulmonary, and may be separated from the surrounding pulmonary parenchyma. In addition to the lungs and pleura, the skeleton and the lymph nodes are sites of involvement.<sup>1</sup>

Patients who have pulmonary metastasis only (in addition to their primary tumor), seem to perform better than those with skeletal metastasis.<sup>2</sup> In these cases, metastasis surgery and total lung irradiation are performed at the end of the treatment regimen.<sup>19</sup> The contribution of surgery compared with irradiation has previously been studied (1990-2006), and suggest a possible benefit for ES patients who undergo surgical resection of lung metastases; Patients with pulmonary metastasectomy had a longer overall survival compared with those without lung resection ( $P < .0001$ ).<sup>11</sup> However, only 31 patients with lung metastases participated in this study, and only 8 patient underwent pulmonary metastasectomy. The results need to be verified in a larger study.

With regard to systemic therapy, there have been a variety of trials attempting to optimize a chemotherapeutic regimen specifically for patients with metastatic disease. These studies have investigated more intensive, time-compressed, and high-dose chemotherapy regimens, yet there has been minimal improvement in survival of patients with metastasis at presentation.<sup>6</sup> Children's Oncology Group protocol (COG) (AEWS 0031) has shown that an every-two-week interval compression regimen is superior to the every 3 week regimen in survival: 76 versus 65 percent event free survival at 4 years ( $p=0.029$ ), and 91 versus 85 percent overall survival at 4 years ( $p=0.026$ ). There was no difference in toxicity between the two regimens.<sup>2,18</sup> New time-compressed studies will be tested in a European study in near future.

Despite intensive therapy, a number of patients are not in remission at the end of induction therapy and others relapse after the treatment has finished. There is no specific treatment regimen established for recurrent cases, primarily because of the variance between cases. Treatment should be altered according to the individual treatment provided to the patient before recurrence.<sup>33</sup> The evaluation of new drug combinations is vital to treat these poorly responding or relapsing patients. There has been interest over the last decade in the use of camptothecin agents (such as topotecan and irinotecan) for metastatic disease. The current front line Children's Oncology Group trial randomizes patients with localized ES to receive topotecan in combination with cyclophosphamide to attempt to improve survival rates, as this combination has shown promise in patients with relapsed or refractory disease.<sup>6</sup> New treatment modalities are tested constantly, but not all are showing promising results.

### **c) High dose chemotherapy with autologous stem cell rescue (HMAS)**

HDT (High Dose therapy); HD-BuM cure (High dose Busulfan and Melphalan) and stem cell rescue is sometimes employed in this group of patients. Indications are metastatic disease or poor histologic or radiologic response to initial chemotherapy in localized disease (see "treatment localized disease, chemotherapy").<sup>17,19</sup> Melphalan and busulfan (BuM) are active agents against ES, however they induce great myelosuppression, and complications as neutropenic fever is common.

HMAS was first tested for lymphoma patients at the Norwegian Radiumhospital in 1987, at that time called autologous bone marrow transplant (ABMT), with stem cells from the patient's bone marrow.

Stem cell harvesting from peripheral blood started in 1994. In order to harvest an adequate number of stem cells to HMAS, peripheral blood stem cells must be mobilized from bone marrow into peripheral blood. The patient receives a mobilization course and a few days later growth factor G-CSF, which provides large production of stem cells in the bone marrow. With adequate numbers of CD34<sup>+</sup> cells and reticulocytes at blood count, stem cell harvesting starts. This takes about 5 hours. The number of CD34<sup>+</sup> cells from the blood is counted, 2.0 x 10<sup>6</sup> cells per. kilogram of body weight is required. The CD34<sup>+</sup> cells are kept in a freezer at -180<sup>0</sup>C, and high dose melphalan and busulfan (HD-BuM) are given. Two days later the stem cells are re-implanted. This treatment is offered at Radiumhospitalet and Rikshospitalet for selected patients groups <40years of age. See indications above.<sup>17</sup>

### Studies

- ISG/SSG IV, 1999-2008: The Italian Sarcoma Group and the Scandinavian Sarcoma Group designed a joint study to improve the prognosis for patients with Ewing's family tumors and synchronous *metastatic disease limited to the lungs or the pleura, or a single bone*. The program consisted of intensive five-drug combination chemotherapy, surgery and/or radiotherapy as local treatment, and consolidation treatment with high-dose busulfan/melphalan plus autologous stem cell rescue and total-lung irradiation. 102 consecutive patients were enrolled, median follow-up was 62 months. The 5-year event-free survival (EFS) probability was 0.43 (SD = 0.05) and the 5-year overall survival probability was 0.52 (SD = 0.052). Unfavorable prognostic factors were a poor histological/radiological response at the site of the primary tumor and incomplete radiological remission of lung metastases after primary chemotherapy. Long-term survival was achievable in ~50% of the patients.<sup>13</sup>
- In 1999, the Italian Sarcoma Group (ISG) and the Scandinavian Sarcoma Group (SSG) activated a joint phase II study ISG/SSG III. Treatment strategy was based on the primary use of the six drugs active against ES, VACAc-IE. For poor responder (PR) patients, treatment intensification with the addition of High Dose Therapy (HDT) with Busulfan and Melphalan was added. Three hundred patients entered the study. **Five-year event free survival was 75% for GR, 72% for PR treated with HDT and 33% for PR who did not receive HDT.** The study concluded that High-dose therapy added to the VACAc-IE regimen in PR patients is feasible and effective, and that selected groups of patients with ES can benefit from HDT.<sup>21</sup>

In the last years, several studies have shown potential benefit of HDT and peripheral blood stem cell rescue in patients with both non-metastatic and disseminated disease of ES (EW93-study and EuroEwing-99).<sup>8,21</sup> Still, most of these studies are phase II, and they only involve a small group of patients with different ages, comorbidity and previous treatment. The benefits of HMAS is not yet documented, and is currently tested in the ongoing, randomized (Phase III) Euro-Ewing study.<sup>19</sup>

## 7. PROGNOSIS AND RELAPSE

In localized disease with surgery or radiotherapy alone, 5-year survival is <10%. With treatment in multimodality trials including chemotherapy, 5-year survival is 60–70% in localized and 20–40% in metastatic disease.<sup>22</sup>

### **Prognostic factors:**

Several studies have identified tumor site and tumor volume (>8 cm<sup>3</sup>), patient age (>12 years), chemotherapy response, and the extent and site of metastasis as prognostic factors in ES. The most significant prognostic factor is the *presence/absence of metastasis* at diagnosis.<sup>9,12,18,32</sup> About 75 % of patients clinically present with localized disease.<sup>12,18</sup> In 25–30% of these patients, submicroscopic detection of tumor cells in the peripheral blood or within the bone marrow via fusion transcript RT-PCR is found. This may be of prognostic relevance, but prospective confirmation is lacking.<sup>6,12</sup>

Patients with metastatic disease have a statistically significant proportion of larger primary tumors (>8 cm<sup>3</sup>) than patients without metastatic disease (76,8 versus 54,3%), suggesting that primary tumor size may correlate with metastasis.<sup>6</sup>

### **Localized vs. metastatic ES:**

For non-metastatic ES, the histological *response to primary chemotherapy* is the main predictive factor of survival. In the French experience, patients with a good histological response have a probability of 5-year event-free survival (EFS) of 75%, compared with 40% and 20%, respectively, for intermediate or poor histological response. In the Italian experience, the reported 10-year EFS is 75% in GR patients compared with 27% in PR patients.

Those with localized extremity lesions tend to have a better prognosis than do patients with axial primary lesions, mainly because the latter tumors cannot be surgically removed with adequate margins. Primary pelvic disease is an unfavorable prognostic indicator.<sup>18</sup>

*Lung metastasis* are a negative prognostic indicator, but approximately 30% of patients with pulmonary metastasis alone will have prolonged survival (> 5years) compared with only 10% of patients with bone or bone marrow involvement.<sup>4,18</sup> A minority of patients with ES present with *regional lymph node involvement*.<sup>30</sup>

Data of patients with ESFTs followed up at different cancer centers in Turkey between 2001 and 2010 were retrospectively analyzed. The median age of 114 patients was 26 years. In patients with localized disease at presentation, the 5-year overall survival were 65%.<sup>28</sup> This study included all ESFT's, not only osseous ES, and the patients had different treatment. Other studies show 5 years overall survival 82% for patients with localized disease, and only 39% in those with metastases.<sup>14</sup>

## Relapse

### Localized ES:

Relapse occurs usually within 2 years, and is most often seen as lung metastasis. 30–40% of patients suffer from recurrent tumors either locally, as metastases, or a combination of the two.<sup>33</sup> Despite systemic chemotherapy and good local control modalities, even patients with clinically localized ES have a certain risk of relapse at distant sites (see prognosis), giving rise to the hypothesis that micrometastasis is almost universally present but undetected at initial diagnosis.<sup>12</sup>

### Metastatic ES:

Patients with metastatic ES are more likely to relapse early, and they have worse prognosis. In recent data from the Children`s Oncology Group (COG), the median time to recurrence in patients with metastatic disease at diagnosis is about one year.<sup>6</sup>

The predominant type of relapse in patients with initial metastatic disease is systemic (defined as distant recurrence only) with 73% of patients presenting with pulmonary, bone, or multisystem recurrent sites.

Despite advances in treatment, patients with metastatic or recurrent ES continue to have a bad prognosis with less than 20% overall survival.<sup>6</sup>

## Follow up

The final evaluation after completed treatment of Ewing`s sarcoma should include clinical examination, blood tests, chest X-ray, X-ray of the affected bone, CT and MR imaging of the affected skeletal part, GFR,(kidney function test) and echo Doppler examination of the heart. Patients should be followed at a sarcoma center with check-ups every third month for 3 years, every fourth month year 4 - 5 and every sixth month 6 - 10 years after treatment. These controls should include the mentioned examination methods.<sup>4</sup>

## 8. SIDE EFFECTS/LATE EFFECTS

### Chemotherapy:

- Nausea, vomiting, diarrhea, mucositis, dry mouth, altered smell and taste and constipation cause *malnutrition*. Many require intravenous nutrition.<sup>17,19</sup>
- *Hairloss*, often 2-3 weeks after chemotherapy.<sup>17,19</sup>
- *Neutropenia and thrombocytopenia*. Measurements usually twice a week, sometimes transfusion of thrombocytes is necessary. Fever may be only symptom of serious infection, in this case called neutropenic fever.<sup>17,19</sup>
- Reversible *renal toxicity*, both tubular and glomerular damage, caused by ifosfamide.<sup>4</sup>

- Irreversible *ototoxicity* with hearing loss and tinnitus, caused by cisplatin (rarely given anymore).<sup>17,19</sup>
- *CNS toxicity* caused by high dose ifosfamide, presenting with cramps, confusion, nightmares and visual disturbances.<sup>17,19</sup>
- *Infertility* caused by ifosfamide, cisplatin and doxorubicin, is common in men, and all men are offered sperm banking. Ovulation may be affected, and oocyte banking is currently being tested in Norway.<sup>4,17,19</sup>
- Anthracycline (Doxorubicin) induce *cardiotoxicity* and EF (ejection fraction) decline, associated with higher administered anthracycline dose, young age and bolus infusion.<sup>4, 27</sup>

#### Radiotherapy:

- Acute: Local irritation of skin and mucous membranes, lymph edema, nausea, vomiting and tiredness.<sup>17</sup>
- Late effects depends on dose intensity and site of radiation therapy, and may affect organs in the radiation field. Pigment changes, fibrosis and secondary cancer are some of them.<sup>17</sup> It's important to pay particular attention to growth and development in children. Actinomycin D and anthracyclines enhances the effect of radiation therapy and therefore should not be given concurrently with this.<sup>4</sup>
- Proton radiotherapy is acutely well tolerated, with mostly mild-to-moderate skin reactions. The only serious late effect reported is hematologic malignancy. Proton therapy will hopefully be offered in Norway in a couple of years, today Norwegian children have this treatment abroad.<sup>38</sup>

## 9. ADVANCES TOWARDS NEW TREATMENT STRATEGIES

New treatment strategies are actively sought, including targeted agents. An example includes the addition of agents targeted to the insulin growth factor receptor, though support for these agents for a rare disease is decreasing among drug companies.<sup>31</sup>

Targeted therapies aim to specifically eliminate tumor cells while sparing normal tissues. This can be achieved based either on a distinct presence of molecular targets in cancer versus normal cells, or on a distinct dependence of cells on the function of a target gene for survival. EWS tumor biology harbors potential for both strategies.

The presence of EWS-ETS fusion proteins fundamentally distinguishes sarcoma cells from normal tissue, and alters the presence (expression) of central target genes.<sup>10</sup>

Much effort is being invested in treating cancer with targeted therapies. Some examples are given:

- Tyrosine kinases (TKs) are overexpressed in human sarcoma tumors, and cell lines may serve as potential targets for new therapies. One TK receptor that is a promising therapeutic target is insulin-like growth factor-1 receptor (IGF1R).<sup>18</sup> Ganitumab is a

fully human monoclonal antibody against IGF1R, is well tolerated and demonstrates antitumor activity in patients with advanced recurrent ESFT.<sup>7</sup>

- Histone deacetylase (HDAC) activity has been linked to cancer development through transcriptional silencing of tumor suppressor genes. HDAC inhibitors reversing such effects have shown promising results in preclinical and clinical studies.<sup>10</sup>
- The platelet-derived growth factor receptor- $\beta$  (PDGFRB) is expressed in ES and thought to contribute to proliferation and metastasis. PDGFRB inhibition by RNAi and a specific kinase inhibitor, AG1295, has been reported to impair cell growth and chemotaxis *in vitro* and *in vivo*.<sup>10</sup>
- A ribozyme with specificity for EWSR1-FLI1 was developed and the activity *in vitro* was investigated. The data show that the fusion region of the ES specific *EWSR1-FLI1* oncogenic RNA is accessible to ribozyme mediated inactivation and cleavage *in vitro*. Thus, the ribozyme approach for the inhibition of EFST cell growth seems to be feasible for the development of new treatment strategies for EFST patients. For applications *in vivo*, systems for highly effective delivery of ribozymes to target cells are required. The *in vivo* activity of the ribozyme and the optimal length of the hybridization arms requires further evaluation.<sup>35</sup>
- Other promising therapeutic strategies include poly(ADP-ribose) polymerase (PARP) inhibitors, *mithramycin*, and a small molecule inhibitor of a protein-protein interaction, which includes EWS/FLI1. Although elevated levels of PARP in Ewing sarcoma cells were noted 20 years ago, only recently has this strategy attracted strong interest. An ongoing trial is investigating what proportion of unselected Ewing sarcoma patients would benefit from this strategy (NCT01583543).<sup>31</sup>

Other target-strategies studied are cell apoptosis, intracellular signaling kinases, angiogenesis and cell immunology.<sup>10</sup>

None of these drugs have been included as routine in treatment of ES, and international collaboration is necessary in order to progress. In December 2011, the first ENCCA (The European Network for Cancer Research in Children and Adolescents)-supported European Interdisciplinary ES Research Summit was held in Vienna, Austria. Thirty European and five North American expert scientists met to exchange and discuss their most recent, largely unpublished results, and to propose biological studies and novel promising therapeutics for the upcoming European EWING2008 and EWING2012 studies.<sup>5</sup>

## 10. CONCLUSION

Ewing's Sarcoma is a rare malignancy of bone and soft tissue with a strong predilection in children and adolescent. It is the second most common primary bone cancer in children, with approximately 5-10 new cases diagnosed each year in Norway, 225 in North America. In the majority of cases, a translocation between two genes EWS and FLI1 is implicated, giving rise to a new fused gene EWS-FLI1. The mechanisms of gene/protein regulation and intracellular signaling are increasingly examined, hoping to make new ways for possible targeted treatment. ES is highly sensitive to chemotherapy and radiation therapy, which together with surgery represents the current treatment regimen in both localized and metastatic ES.

Patients presenting with localized disease have an approximately two thirds chance of being cured. Those with isolated pulmonary metastases experience an approximately 30% long term survival, whereas those with more widespread disease, usually involving bone or bone marrow, have a less than 20% chance of cure with currently available therapy.

## Abbreviations

HMAS = High dose chemotherapy with Autologous stem cell Support  
PNET = Primitive neuroectodermal tumor  
MR = Magnetic Resonance  
CT = Computed Tomography  
ES = Ewing`s sarcoma  
SSG = Scandinavian Sarcoma Group  
ISG = Italian Sarcoma Group  
EES = Extraosseous  
ECI = Extracorporeal Irradiation  
EICESS = The European Intergroup Cooperative Ewing`s Sarcoma study  
SEER = The Surveillance Epidemiology and End Results  
FDG-PET = Fluorine-18 fluorodeoxyglucose positron emission tomography  
FISH = Fluorescent in situ hybridization  
RT-PCR = Reverse transcriptase–polymerase chain reaction  
EWSR1-FLI1 = Ewing sarcoma breakpoint region 1-Friend leukaemia virus integration 1, (translocation (11;22)(q24;q12))  
SSG = Scandinavian Sarcoma Group  
ISG = Italian Sarcoma Group  
ISG/SSG = Italian Sarcoma Group/ Scandinavian Sarcoma Group  
VACAc-IE = vincristine (V), doxorubicin (A), cyclophosphamide (C), actinomycin-D (Ac), ifosfamide (I) etoposide (E)  
GR = Good responder  
PR = Poor responder  
RT = Radiation Therapy  
IMRT = Intensity-modulated radiotherapy  
3D-CRT = Three-dimensional conformal radiotherapy  
COG = Children`s Oncology Group protocol  
HDT = High Dose therapy  
HD-BuM = High dose Busulfan and Melphalan  
ABMT = Autologous bone marrow transplant  
SD = Standard deviation  
EFS = Event free survival  
ETS = Transcription factor family, including FLI1, ERG, ETV1, ETV4, and FEV.  
TK = Tyrosine kinases  
IGF1R = insulin-like growth factor-1 receptor  
HDAC = Histone deacetylase  
PDGFRB = platelet-derived growth factor receptor-β  
ENCCA = The European Network for Cancer Research in Children and Adolescents

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