9.1 Introduction

The term Ewing sarcoma family of tumors (ESFT) defines a group of small round cell neoplasms of neuroectodermal origin, which manifests as a continuum of neurogenic differentiation, with Ewing sarcoma of bone representing the least differentiated and primitive neuroectodermal tumor and peripheral neuroepithelioma the most differentiated forms. The ESFT comprise 3% of all pediatric malignancies, and are rare in the non-white population (Gurney et al. 1999).

The histogenesis of ESFT has been a source of controversy since its first description in 1921. Various hypotheses have been proposed in an attempt to identify the possible cell of origin; among these, cells of endothelial, pericytic, myeloid, mesenchymal, and neuroectodermal origin have all been suggested (Dehner 1993). The existence of either a mesenchymal stem cell or an early primitive neuroectodermal cell that has retained its ability for multilineage differentiation is the currently accepted hypothesis. It is now well accepted that the ESFT constitute a single group of neurally derived neoplasms that share unique immunocytochemical, cytogenetic, and molecular markers (de Alava and Gerald 2000; Dehner 1993). Despite aggressive treatment, 30–40% of patients with localized disease and 80% of patients with metastatic disease die due to disease progression (Coterill et al. 2000).
9.2 Epidemiology

ESFT of bone is the second most common bone malignancy in children, accounting for approximately 40% of all bone cancers (Gurney et al. 1999). Its average annual incidence rate is 2.9 per million; it peaks in the 2nd decade of life (5 per million), and it is extremely rare during the first 5 years of age (0.6 per million) (Gurney et al. 1999).

Approximately 200–250 new cases of ESFT are diagnosed in the United States each year, including a small percentage of tumors that arise in the soft tissue (extraosseous Ewing sarcoma). The disease occurs slightly more commonly in males than females with a ratio of 1.3:1. ESFT is predominantly seen in Caucasians, and is rarely diagnosed in African-Americans (Gurney et al. 1999). The protective etiology of this phenomenon has yet to be elucidated. In several African countries, the ratio of ESFT to osteosarcoma is very similar to that of US blacks. The incidence of ESFT is also lower in Hispanic and Asian populations (Parkin et al. 1993).

A number of studies have evaluated predisposing factors in ESFT. Parental farming exposure, history of inguinal hernia, and family history of gastric cancer or melanoma have been reported as associated with increased risk of developing ESFT (Gurney et al. 1999). These findings have been based on retrospective analysis, small sample size and selection bias, and have not been reproducible in all studies. Therefore, at this time, there is no convincing evidence that ESFT is associated with any disease, familial predisposition syndrome, or environmental factors. However, ESFT has been observed as second malignancy in irradiated and non-irradiated sites in a rare number of patients. Most of these cases were associated with retinoblastoma, but also have been reported in non-Hodgkin’s lymphoma, leukemia, Hodgkin’s disease and Wilms’ tumor (Spunt et al. 2004).

9.3 Pathogenesis

Understanding the pathogenesis of ESFT has been hampered by the still unknown cell of origin of this tumor. A variable expression of neuronal immunohistochemical markers and ultrastructural features, and the ability of ESFT cells to differentiate along a neural pathway in vitro, point to a neuroectodermal origin (Dehner 1993). However, since ESFT can arise in bone and soft tissue, and these tumors can also show mesenchymal and epithelial features, it has been postulated that the cell of origin for ESFT is more likely to be a primitive cell that has the capacity to differentiate along a number of cell types. Torchia et al. (2003) recently demonstrated in an experimental system that bone marrow-derived stromal cells transduced with EWS/ETS fusion proteins (described below) recapitulate some of the features of ESFT, namely they exhibit a block in osteogenic and adipogenic differentiation and express neural markers.

Despite the limitation of not knowing the cell of origin, a number of advances in our understanding of the basic biology of ESFT have been made over the last few years. This progress can be attributed to the identification of recurring chromosomal translocations in this tumor type involving the N-terminus transactivation domain of the EWS gene on chromosome 22 band q12 with the C-terminus DNA-binding domain of an ETS family of transcription factors. The ETS family fusion partner most commonly detected is FLI-1 on chromosome 11 band q24 followed by ERG on chromosome 21 band q22 and less commonly FEV, ETV1 and E1AF (Table 9.1) (de Alava and Ger-
The best characterized of the fusion proteins is EWS-FLI1. EWS is an RNA-binding protein whose function is unclear. FLI1 is a transcription factor and contains a sequence specific DNA binding domain, GGA(A/T). FLI1 plays a role in embryonic development, hematopoiesis, cell growth and differentiation, as well as tumorigenesis. The fusion product of these two genes, EWS and FLI-1, can cause neoplastic transformation in a number of in vitro and in vivo experimental systems (May et al. 1993a). Furthermore, ESFT cell lines transduced with anti-sense oligonucleotides, small interfering RNAs (siRNA) or competitive inhibitors to EWS-FLI1 demonstrate growth inhibition as well as increased susceptibility to chemotherapy induced apoptosis in culture and in mice (Prieur et al. 2004; Tanaka et al. 1997).

Although the mechanism by which EWS-FLI-1 contributes to the pathogenesis of ESFT is not completely understood, this fusion protein does bind to target genes in a sequence specific manner determined by FLI-1, but these genes are controlled by EWS regulatory domains, a more potent transcriptional activator than FLI-1 (May et al. 1993b). This aberrant gene regulation appears to result in the transforming properties of EWS-FLI1. Overexpression of FLI-1 and mutations in the DNA-binding domain of FLI1 do not recapitulate the transforming properties of EWS-FLI1, suggesting that the EWS-FLI-1 chimeric protein may also affect different target genes (May et al. 1993b). The critical genes modulated by EWS-FLI1 that contribute to the oncogenesis are not known. Some candidate genes and their potential role in the pathogenesis of ESFT are listed in Table 9.2.

At the molecular level, there are several in-frame EWS-FLI1 chimeric transcripts. The most common fusions involve fusion of EWS exon 7 with FLI1 exon 6 (type 1) and fusion of EWS exon 7 with FLI1 exon 5 (type 2) with a relative frequency of 60% and 25%,
respectively (Zucman et al. 1993). All chimeric products include the DNA-binding domain of FLI1 and the transactivation domain of EWS. Lin et al. (Lin et al. 1999) compared the transactivation potential of the type 1 fusion product with six other fusion types in vitro and showed that the type 1 fusion was a weaker transactivator than the other fusion types. This finding may in part explain the better outcome in patients whose tumors contain the type 1 translocation (de Alava et al. 1998; Zoubek et al. 1996).

The karyotype of ESFT cells is not restricted to the rearrangement involving chromosome 22. Using conventional cytogenetics and comparative genomic hybridization, trisomies in chromosome 8 and 12 as well as an unbalanced translocation have been repeatedly observed in ESFT (de Alava et al. 1998; Brisset et al. 2001; Gurney et al. 1999; Hattinger et al. 1999; Ozaki et al. 2001). The biologic and clinical significance of these abnormalities remains to be fully explored in a large group of patients. Individual genes frequently altered in human cancer and those that typically regulate cell proliferation and apoptosis have also been evaluated in ESFT. Of these genes, alterations in p53, primarily missense mutations, and alterations in INK4A, primarily homozygous deletions often associated with loss of p15 and ARF genes, have been detected in 7–15% and 18–30% of primary tumor samples from patients with ESFT, respectively (Kovar et al. 1993). Aberrations in either one of these genes have been correlated with poor overall survival (Abudu et al. 1999; Tsuchiya et al. 2000). Rarely have abnormalities in MDM2, RB or CDK4 been detected in ESFT.

In addition to genetic aberrations, dysregulation of growth factor and apoptotic pathways have also been implicated in the pathogenesis of ESFT. The best studied of these in ESFT is the insulin-like growth factor (IGF) signaling pathway. Insulin-like growth factors, IGFI and IGFII, primarily mediate their effects through the insulin-like growth factor I receptor (IGFIR). IGFIR are found on the surface of most, if not all, ESFT that have been studied to date (Scotlandi et al. 1996). Activated IGFIR results in a number of different responses that are mediated by two primary pathways, mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3-K) (Benini et al. 2004). Impairment of IGFIR function by antisense strategies, antibodies, or dominant negative constructs ameliorates its effects on proliferation, migration, angiogenesis, metastasis, and transformation as well as enhancing chemosensitivity of ESFT cells to conventional cytotoxic drugs (Scotlandi et al. 1996, 2002; Toretsky et al. 1999). These observations would suggest that IGF signaling plays a central role in the tumorigenesis of ESFT. The dominant role of this pathway is further substantiated by the fact that the presence of IGFIR is necessary for the transforming ability of EWS/ETS fusion proteins (Toretsky et al. 1997). Furthermore, Prieur et al. (2004) have recently shown that EWS/FLI1 binds to the promoter region of insulin-like growth factor binding protein-3 (IGF-BP-3), a negative regulator of IGF-1 signaling, and causes repression of its activity, demonstrating a direct link between IGF-1 signaling and EWS-FLI1.

Basic fibroblast growth factor (bFGF) and its receptors are also expressed in ESFT. bFGF belongs to a family of heparin-binding polypeptide growth factors that are important in neuronal development. The role of this growth factor in ESFT remains to be determined. One group of investigators has shown that both in vivo and vitro, the proliferation of ESFT cell lines can be inhibited by exogenous bFGF (Sturla et al. 2000). However, other investigators have shown the opposite effect, bFGF-induced cell proliferation (Girnita et al. 2000).

The recent development of molecular targeted therapy in cancer has led to the investigation of specific pathways for which these therapies exist. Imatinib mesylate is an inhibitor of structurally related tyrosine kinases including c-KIT, platelet-derived growth factor receptors (PDGFR) α and β, c-ABL, BCR-ABL, v-ABL and ABL-related gene (ARG). Chronic myelogenous leukemia (CML) cells, which express BCR-ABL tyrosine kinase, and gastrointestinal stromal tumor (GIST) cells, which have activating mutations in c-KIT, are highly sensitive to imatinib mesylate. These observations prompted an evaluation of c-KIT and PDGFR expression in ESFT. c-KIT and its ligand, stem cell factor (SCF), as well as PDGFRβ are expressed in some ESFT (Smithey et al. 2002; Scotlandi et al. 2003). In vitro experiments suggest that the c-KIT/SCF and PDGF pathways play a
role in cell proliferation, transformation and motility of ESFT and may serve as novel targets for therapy (Scotlandi et al. 2003; Uren et al. 2003). Whether the presence of these pathways is critical for the survival of ESFT cells remains to be determined.

Another promising target for cancer chemotherapy that has shed light on the biology of ESFT is the activation of death receptors of the TNF-receptor superfamily resulting in activation of effector caspases and ultimately apoptosis. Death inducing ligands for these receptors include TNF-related apoptosis-inducing ligand (TRAIL), Fas ligand, and tumor necrosis factor (TNF). Of these, TRAIL is the most potent inducer of apoptosis in ESFT (Kontny et al. 2001). However, not all ESFT that express death receptors are sensitive to TRAIL. Absence or downregulation of caspase 8 by hypermethylation of promoter region of this gene appears to be a frequent mechanism of TRAIL resistance. Fulda et al. (2001) have demonstrated that in the presence of a DNA demethylating agent, apoptosis can be induced in TRAIL insensitive ESFT cells. DNA demethylating agents and TRAIL are currently in clinical development (see Sect. 9.7, below, “Future Developments”).

In summary, genetic alterations, growth factor and apoptotic signaling pathways have been shown to play a role in the pathogenesis of ESFT. EWS/ETS fusion proteins have been recognized as playing a central role in this process. Several downstream targets of these fusion proteins have been identified. The contribution of each of these proteins and their targets, as well as their mechanism of action, requires further investigation. Using oligonucleotide or DNA microarrays and proteomics, it will be possible to begin to map out a signature gene/protein profile for ESFT. These techniques will be useful in validating existing hypotheses as well as identifying yet unrecognized signaling pathways in this tumor.

9.4 Pathology

9.4.1 Microscopic Features

As a group, ESFT exhibit a wide spectrum of cellular morphologic features. Tumors arising in bone (Ewing sarcoma) are usually composed of uniform small round cells with round nuclei containing fine chromatin and small nucleoli, scant clear or eosinophilic cytoplasm, and indistinct cytoplasmic membranes (Fig. 9.1). True rosette structures may be identified occasionally. Mitotic figures and necrosis are variable in frequency. A small subset of tumors is composed of

Figure 9.1
Ewing sarcoma composed of small round neoplastic cells arranged in vague nests. H&E stain, ×200
relatively larger cells with irregular nuclear contours and prominent nucleoli.

Intracytoplasmic glycogen within neoplastic cells may be demonstrated using PAS staining and diastase digestion. While of historical value, this technique lacks specificity and is not recommended currently for the diagnostic workup for ESFT. Primitive intercellular junctions, indicating epithelial differentiation, and dense core granules, indicating neural differentiation, may be identified by electron microscopy.

Most ESFT express CD99 (Fig. 9.2), which is a cell membrane protein encoded by the MIC2 gene located in the pseudoautosomal region at the end of the short arms of the X and Y chromosomes (Ambros et al. 1991). CD99 is a 32-kDa cell surface antigen with broad cellular expression whose function remains incompletely understood but is believed to be involved in T-cell regulation (Wingett et al. 1999). While expressed by most ESFT, CD99 expression is not specific for ESFT and may be detected in lymphoblastic lymphoma, embryonal rhabdomyosarcoma, and other soft tissue sarcomas (Perlman et al. 1994). Nevertheless, in the absence of conclusive molecular data, strong diffuse CD99 immunostaining constitutes a useful marker for ESFT in tumors lacking features suggestive of other round cell malignancies. CD99 is most useful as part of a panel of immunostains that also includes Myo-D1, TdT, and synaptophysin, which are helpful in ruling out the major differential diagnostic considerations of rhabdomyosarcoma, lymphoblastic lymphoma, and neuroblastoma, respectively. Distinction between ESFT and small cell osteosarcoma rests on the absence of CD99 expression and identification of osteoid deposition in the latter and on identification of ESFT-specific translocations in the former.

9.4.2 Molecular Pathology

Translocations involving the EWS gene are detected in the vast majority of ESFT, most commonly using reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH). Up to 18 types of in-frame EWS-FLI1 chimeric transcripts are possible and, of note, all contain the transactivating amino-terminal domain of EWS (exons 1–7) and the ETS-type DNA-binding domain of FLI1 (exon 9) (Fig. 9.3) (Zucman et al. 1993). The portion of the chimeric protein between these two domains is
variable in size and composition, reflecting genomic breaks in one of four EWS introns and one of six FLI1 introns. The two main fusion types, fusion of EWS exon 7 to FLI1 exon 6 (so-called type 1) and fusion of EWS exon 7 to FLI1 exon 5 (so-called type 2), account for about 85% of EWS-FLI1 fusions (Zoubek et al. 1994, 1996). All other EWS-FLI1 fusion types are designated by the exons involved, by convention.

EWS exon 7 forward and FLI1 exon 9 reverse primers should amplify all forms of EWS-FLI1, with potential amplification products of variable sizes. However, using this approach may result in false neg-
ative results in tumors harboring large fusions such as types 9–4 or 10–5, especially if sample RNA is partially degraded. An alternative approach entails using initially a FLI1 exon 6 reverse primer in combination with an EWS exon 7 forward primer. Such a primer pair will yield small RT-PCR products in more than 85% of cases with EWS-FLI1. To ensure detection of a minority of false-negative cases, a second-line reaction in which the EWS exon 7 forward primer is paired with a FLI1 exon 9 reverse primer and an ERG reverse primer is performed. Because of their frequency, type 1 and type 2 EWS-FLI1 products can be recognized by their size identity with an appropriate normal control.

Approximately 5% of ESFT harbor a complex or cryptic t(21;22)(q22;q12) that rearranges EWS with another ETS family gene, ERG. With an exon structure highly analogous to FLI1, several translocation variants of EWS-ERG have been noted (Zucman et al. 1993). It is noteworthy that enough molecular homology exists between FLI1, ERG, and FEV to allow the design of consensus reverse primers that could detect all the corresponding translocations using one assay.

Detection of translocations in ESFT using formalin-fixed paraffin-embedded tissue is possible using RT-PCR or a variety of FISH methods. A highly sensitive FISH assay utilizing a dual-color break-apart DNA probe flanking the EWS-R1 breakpoint region on chromosome 22 is commonly utilized. An intact DNA target is indicated by juxtaposition of the DNA probes whereas rearrangements of the EWS gene lead to separation of hybridization signals. Using this approach, all translocations involving EWS may be detected regardless of the translocation partner or fusion type. While sensitive, this approach lacks high specificity since it may detect other tumors that may harbor translocations involving the EWS gene (Fuller et al. 2004).

Genomic breakpoints in EWS are clustered within a 7-kb region, making Southern blotting useful in some circumstances. Although requiring a significant amount of tissue for DNA extraction, this technique can reliably detect EWS rearrangements regardless of the translocation partner or molecular variation in the fusion gene.

Common additional chromosomal abnormalities in ESFT include both numerical and structural findings. Most common are gains of chromosomes 8, 12, 20, and 1q and losses of 16q and 19q and der(16)t(1;16)(q12;q11.2) (Brisset et al. 2001; Ozaki et al. 2001). It appears that the presence and frequency of secondary chromosomal changes may be associated with worse clinical outcome. In one study, deletions at the short arm of chromosome 1 were associated with an unfavorable outcome in patients with localized disease (Hattinger et al. 1999). In another study, loss of 16q was an independent prognostic factor by multivariate analysis (Ozaki et al. 2001).

### 9.5 Clinical Features

Patients with ESFT commonly present during the 2nd decade of life; 80% of patients are younger than 18 years of age, and the median age at diagnosis is 14 years (Cotterill et al. 2000; Grier et al. 2003; Gurney et al. 1999). Males are more commonly affected than females, and the disease is very rare in African-Americans (Gurney et al. 1999).

ESFT has a tendency to involve the shaft of long tubular bones, pelvis, and ribs but practically every bone can be affected (Table 9.3). More than 50% of

<table>
<thead>
<tr>
<th>Site</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>Central axis</td>
<td>52–55%</td>
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<tr>
<td>Skull</td>
<td>2–6%</td>
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<tr>
<td>Clavicle/scapula</td>
<td>4–6%</td>
</tr>
<tr>
<td>Ribs</td>
<td>12–13%</td>
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<tr>
<td>Spine</td>
<td>6–8%</td>
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<tr>
<td>Pelvis</td>
<td>23–27%</td>
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<tr>
<td>Extremities</td>
<td>41–47%</td>
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<tr>
<td>Humerus</td>
<td>5–7%</td>
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<tr>
<td>Radius/ulna</td>
<td>1–3%</td>
</tr>
<tr>
<td>Hand</td>
<td>&lt;1%</td>
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<tr>
<td>Femur</td>
<td>16–19%</td>
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<tr>
<td>Tibia</td>
<td>7–10%</td>
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<tr>
<td>Fibula</td>
<td>6–9%</td>
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<tr>
<td>Foot</td>
<td>2–3%</td>
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the tumors arise from axial bones, with the pelvis being the most commonly involved (23–27%); one-third of the tumors originate in the lower extremities, and less than 10% in the upper extremities (Cotterill et al. 2000; Grier et al. 2003; Gurney et al. 1999; Paulussen et al. 2001a). In the long bones, diaphyseal involvement predominates over metaphyseal disease. ESFT usually presents with localized pain and a visible palpable mass, and almost one-half of patients have symptoms referable to their primary tumor for more than 3 months prior to the diagnosis. Older patients have a higher proportion of pelvic primaries and larger tumors. Pathologic fractures may be present in up to 15% of cases (Cotterill et al. 2000).

ESFT are aggressive neoplasms; systemic manifestations such as fever or anemia are present in 10–15% of the patients (Bacci et al. 2000), and approximately 20–25% of cases have clinically apparent metastatic disease at the time of diagnosis (Craft et al. 1998; Miser et al. 2004; Sandoval et al. 1996). Metastatic disease appears to be associated with older age (Kolb et al. 2003) and large tumors (Kolb et al. 2003; Paulussen et al. 1998a; Spunt et al. 2001) or pelvic primaries (Miser et al. 2004; Paulussen et al. 1998a; Sandoval et al. 1996). Isolated lung disease, usually bilateral, occurs in 25–45% of metastate cases; the majority of patients (50–60%) have extrapulmonary disease (usually bone and bone marrow) (Cotterill et al. 2000; Craft et al. 1998; Miser et al. 2004; Paulussen et al. 1998b; Sandoval et al. 1996).

### 9.5.1 Extraosseous ESFT

ESFT have been described in many different extraosseous locations, such as soft tissues (Raney et al. 1997), skin and subcutaneous tissue (Chow et al. 2000), gastrointestinal tract (Shek et al. 2001), kidney (Parham et al. 2001), or genitourinary tract (Gaona-Luviano et al. 2003). Neuroectodermal tumors of the kidney appear to encompass a group of primitive, highly malignant neoplasms that histologically and clinically are not well characterized. They may occur at any age, but the peak occurs during the 2nd and 3rd decades. Histologically, they are defined as primitive neural tumors, with varying amounts of rosettes and neuropil. Approximately half of the tumors have the histological appearance typical of ESFT, whereas atypical features are present in the remainder. The vast majority of tumors express CD99, but molecular confirmation of ESFT only occurs in one-third of them (Parham et al. 2001).

Cutaneous and subcutaneous ESFT appear to have an indolent course and an excellent outcome (Chow et al. 2000). Finally, an interesting association is the development of ESFT in the genitourinary tract after kidney transplant (Gaona-Luviano et al. 2003).

A small proportion of ESFT arise in the face. In this location, ESFT should be distinguished from esthesioneuroblastoma, an uncommon malignant neoplasm of the nasal vault, believed to arise from the olfactory epithelium. The exact cell of origin is controversial, but neuronal or neural crest origin is supported by the presence of neurofilaments. Inclusion within the ESFT has been proposed; however, these tumors do not express CD99, and molecular studies have not confirmed the presence of the typical fusion transcript, and therefore should be considered a different entity (Dulguerov et al. 2001).

### 9.5.2 Laboratory and Radiologic Evaluation

Patients with suspected ESFT should be thoroughly evaluated to define the extent of local disease and the presence of metastases. Elevations of the erythrocyte sedimentation rate and serum lactate dehydrogenase (LDH) are not uncommon. Bilateral bone marrow aspirations and biopsies should also be performed, and evaluation using molecular techniques such as RT-PCR is recommended. Important imaging studies included in the evaluation are chest radiograph, plain radiographs of primary and metastatic sites, bone
scintigraphy, CT of the chest, and MRI of the primary site with T₁- and T₂-weighted sequences.

Plain radiographs in ESFT typically show an ill-defined, permeative or focally moth-eaten, destructive intramedullary lesion. An ill-defined soft tissue mass adjacent to the primary bone lesion is very common. The lesion is often accompanied by a prominent multilayered periosteal reaction (“onion skin”); the perpendicular “sunburst” type of periosteal new bone formation can be present but is less common than in osteosarcoma. MRI is better than CT to define the intramedullary component of the primary tumor and the extent of soft tissue mass. In contrast to osteosarcoma, dynamic contrast-enhanced MRI is not a very reliable prognostic indicator (Miller et al. 2001). However, newer techniques such as positron emission tomography may be useful in the non-invasive evaluation of chemotherapy response.

### 9.5.3 Prognostic Factors

For patients with localized disease, large tumor size, trunk and pelvic primaries, older age, elevated LDH, and poor response to induction chemotherapy have been typically associated with worse outcome (Bacci et al. 2000; Cotterill et al. 2000; Grier et al. 2003). However, the relative importance of some of these factors may diminish as treatments evolve and improved regimens, with better systemic and local control rates, are being developed. Recent studies have shown that the use of more intensive chemotherapy, with the incorporation of ifosfamide and etoposide, for example, tend to decrease the adverse effect on outcome associated with large size and pelvic location (Grier et al. 2003; Marina et al. 1999). Nevertheless, high disease burden, as indicated by tumor volume or LDH, continues to be associated with an adverse prognosis, although this may be limited to patients treated without surgery (Oberlin et al. 2001). Older age is consistently associated with a worse outcome (Bacci et al. 2000; Cotterill et al. 2000; Grier et al. 2003; Kolb et al. 2003). Patients older than 14 years have a higher proportion of large tumors and pelvic primaries (Cotterill et al. 2000) and metastatic disease (Kolb et al. 2003). In contrast to other factors, the benefit of the addition of ifosfamide and etoposide is not seen in older patients (Grier et al. 2003).

The degree of histologic response appears to be one of the most relevant prognostic factors, on which future studies should build. Recent studies consistently have shown the prognostic value of a histologic response to induction chemotherapy, the significance of which applies across protocols and appears to be independent of the drugs used. Patients with good histologic responses had a significantly better outcome than those with poor responses in the consecutive REN-1, 2 and 3 Italian trials (Bacci et al. 2000; Picci et al. 1997) and in the CESS-81 (Jürgens et al. 1988) and CESS-86 (Paulussen et al. 2001a) German trials. Contrary to the above prognostic factors, treatment intensification may not have a major impact in increasing the proportion of patients with a favorable histologic response, probably because biologic factors influence the response to treatment. Despite the strong association between histologic response and outcome, there is no evidence to suggest that this phenomenon of resistance is mediated by expression of multidrug resistance proteins; 50–60% of ESFT express p-glycoprotein, but this expression does not seem to correlate with outcome (Hijazi et al. 1994). However, the role of other members of the ATP binding cassette (ABC) family of membrane transporters in drug resistance in ESFT has yet to be elucidated.

The type of fusion transcript also seems to influence the clinical behavior of ESFT. Although the biological behavior of tumors with the fusions EWS-FLI1 and EWS-ERG do not seem to differ (Ginsberg et al. 1999), the type of EWS-FLI1 fusion may affect prognosis. The hybrid transcripts resulting from the fusion of exons 7 and 6 of the EWS and FLI1 genes, respectively (type 1 fusion), seem to result in sarcomas of a less aggressive behavior than other fusion types (de Alava et al. 1998; Lin et al. 1999; Zoubek et al. 1996). Because ESFT manifests a continuum of neuroectodermal differentiation, the histological diversity could reflect different biological behaviors. However, there is no evidence to suggest that the degree of neuroectodermal differentiation in ESFT correlates with prognosis (Terrier et al. 1995).

The most important prognostic factor remains the presence of metastatic disease at diagnosis (Fig. 9.4)
Advances in the treatment of ESFT have only resulted in a very modest improvement in the outcome of patients with metastases. However, even among patients with metastatic disease, there is some heterogeneity. With an appropriately intensive treatment that includes bilateral lung radiation, the EICESS studies have shown that patients with isolated lung metastases may have a better prognosis, albeit still worse than patients with localized disease, while patients with extra-pulmonary metastases have a worse prognosis.

With the use of molecular techniques in the staging of ESFT, it is evident that a significant proportion of patients with localized ESFT (20–40%) have micrometastatic disease, measured as molecular detection of tumor cells by RT-PCR in peripheral blood or bone marrow. This proportion is higher among patients with clinically detectable metastases and seems to correlate with the pattern of metastatic spread; 90% with isolated lung metastases have a lower incidence at RT-PCR detected metastases than patients with bone or bone marrow disease. The prognostic significance of molecular microstaging for patients with localized disease is still unclear. However, recent studies suggest that the detection of circulating tumor cells or bone marrow micrometastases by molecular techniques may predict unfavorable outcome. In a large series of 172 patients, the detection of occult tumor cells was significantly associated with a worse outcome; the 2-year DFS estimates for patients with presence versus absence of bone marrow micrometastases were 43±18.4% and 76±9.2%, respectively ($p<0.007$). These data suggest that the use of molecular techniques may further define clinically relevant distinct groups of patients at diagnosis.

In the future, risk definitions will likely be based on: (1) “tumor load,” as defined by the volume of the primary tumor (>200 cm$^3$), the metastatic pattern...
(pulmonary vs. extrapulmonary), or the presence of micrometastatic disease detected by molecular techniques; and (2) “biologic factors,” defined by biological features, grade of histologic response, or type of fusion transcript (Rodriguez-Galindo et al. 2003).

9.6 Treatment

As defined by current imaging techniques, approximately 80% of patients with ESFT have localized disease at diagnosis (Cotterill et al. 2000). However, treatment with local control measures alone can cure less than 10% of the patients, suggesting that most patients have disseminated disease at diagnosis that is not detected with conventional methods. Thus, treatment of ESFT ought to achieve two major goals, local control and eradication of the systemic disease. These two components of therapy are staged in three phases: (a) induction chemotherapy, the goal of which is to achieve rapid initial cytoreduction and facilitate local control; (b) local control, using surgery, irradiation, or both, usually after 10–12 weeks of chemotherapy; and (c) continuation therapy, with the same (or similar) chemotherapy used for induction therapy.

9.6.1 Treatment of Patients with Localized Disease

The last 3 decades have witnessed a major improvement in the outcome of patients with ESFT. These advances in the treatment of ESFT derive largely from the cooperative trials, which have defined the active agents and their optimal schedules and combinations, and have explored treatment intensification and risk-stratification approaches (Rodriguez-Galindo et al. 2003).

9.6.1.1 Four-Drug Regimens

Following the early reports documenting improved outcomes for patients with ESFT receiving adjuvant chemotherapy (Jaffe et al. 1976), several prospective studies documented the efficacy of a four-drug regi-

men with vincristine, actinomycin D, cyclophosphamide, and doxorubicin (VACD) as well as the need to perform early aggressive cytoreduction with higher doses of alkylators, and early dose-intensification of doxorubicin (Table 9.4). Using different variants of the VACD regimen, along with local control measures, survival rates improved from less than 20% to 40–60% (Bacci et al. 1989; Burgert et al. 1990; Craft et al. 1997; Donaldson et al. 1998; Evans et al. 1991; Hayes et al. 1989; Jürgens et al. 1988; Nesbit et al. 1990; Razek et al. 1980; Oberlin et al. 2001). These early studies defined that large tumors [defined as >8 cm (Hayes et al. 1989) or >100 cm³ (Jürgens et al. 1988)], pelvic (and axial) locations, and poor histologic response to preoperative chemotherapy were strong prognostic indicators of outcome. Surgery for local control was seldom used, and radiation therapy alone was used in more than 75% of the patients (Burgert et al. 1990; Craft et al. 1997; Donaldson et al. 1998; Evans et al. 1991; Nesbit et al. 1990; Razek et al. 1980). In these studies, local control was suboptimal (particularly in pelvic primaries), 20–30% of patients developed local recurrences, and distant metastases occurred in 30–40% of patients. However, it soon became clear that with improvements in radiation planning (Jürgens et al. 1988; Burgert et al. 1990), and more aggressive local measures (Bacci et al. 1989), local control could be improved.

9.6.1.2 Role of Ifosfamide and Etoposide

The next generation of studies evaluated the incorporation of ifosfamide and etoposide (Table 9.5). In the CESS-86 study, patients with small extremity tumors continued to receive the VACD regimen, whereas ifosfamide replaced cyclophosphamide (VAID) in the treatment of patients with high risk disease (defined as >100 ml or axial location). Using the VAID regimen, the CESS-86 and the ET-2 studies obtained a modest improvement in the outcome for patients with high-risk disease (Craft et al. 1998; Paulussen et al. 2001a), establishing the VAID regimen as the standard for patients with localized ESFT. However, considering the nephrotoxicity associated with the high cumulative doses of ifosfamide, patients with standard risk disease may require less intensive therapies.
### Table 9.4. Treatment of localized ESFT with VACD (V vincristine, A actinomycin D, C cyclophosphamide, D doxorubicin, Sx surgery, RT radiation therapy, NR not reported, LRT lung irradiation, DFS disease-free survival, OS overall survival)

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Regimen</th>
<th>Local control (% patients)</th>
<th>Outcome</th>
<th>Failures</th>
<th>Poor prognostic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>IESS-II (1978–1982) (Burgert et al. 1990; Evans et al. 1991)</td>
<td>273</td>
<td>VACD – intense VACD – moderate</td>
<td>Sx (21 %) Sx+RT (NR) RT (NR): whole bone 45–55 Gy; boost 5–10 Gy</td>
<td>5-year DFS VAC+D: 60 % Local: 3 % Site (pelvis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES-79 (1978–1986) (Hayes et al. 1989)</td>
<td>52</td>
<td>VACD</td>
<td>Sx (21 %) Sx+RT (35 Gy) (NR) RT (NR): 35 Gy (good responders) – 55 Gy (poor responders)</td>
<td>5-year DFS: &lt;8 cm: 82 % &gt;8 cm: 64 % Local: 19 % Local+distant: 8 % Distant: 6 %</td>
<td>Leukocytosis Size ≥8 cm</td>
<td></td>
</tr>
<tr>
<td>CESS 81 (1981–1985) (Jürgens et al. 1988)</td>
<td>93</td>
<td>VACD</td>
<td>Sx (33 %) Sx+RT (36 Gy) (31 %) RT: 46–60 Gy (34 %)</td>
<td>5-year DFS: 55 % Local: 23 % Local+distant: 10 % Distant: 19 %</td>
<td>Volume (≥100 cm³) Site (axial) Poor histologic response</td>
<td></td>
</tr>
<tr>
<td>ET-1 (1978–1986) (Hayes et al. 1989)</td>
<td>120</td>
<td>VACD</td>
<td>Sx (5 %) Sx+RT (18 %) RT (77 %): long bones: 55–60 Gy Ribs: 35–40 Gy Pelvis: 40–45 Gy</td>
<td>5-year DFS: 41 % Local: 18 % Local+distant: 7 % Distant: 37 %</td>
<td>Site (pelvis)</td>
<td></td>
</tr>
<tr>
<td>REA-2 (1979–1982) (Bacci et al. 1989)</td>
<td>59</td>
<td>VACD</td>
<td>Sx (NR) Sx+RT (35–45 Gy) (NR) RT: 40–60 Gy (NR)</td>
<td>5-year DFS: 54 % Local: 5 % Local+distant: 14 % Distant: 25 %</td>
<td>Site (axial)</td>
<td></td>
</tr>
<tr>
<td>POG 8346 (1983–1988) (Donaldson et al. 1998)</td>
<td>141</td>
<td>VACD</td>
<td>Sx (15 %) Sx+RT (55.8 Gy) (11 %) RT: 55.8 Gy (74 %)</td>
<td>5-year DFS: 51 % Local: 23 % Local+distant: 40 %</td>
<td>Site (pelvis)</td>
<td></td>
</tr>
<tr>
<td>SFOP EW84 (Oberlin et al. 2001)</td>
<td>141</td>
<td>VACD</td>
<td>Sx (40 %) Sx+RT (40 Gy) (38 %) RT: (60 Gy) (22 %)</td>
<td>5-year DFS: 58 % Local: 13 % Poor histologic response</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The EICESS-92 study randomized patients with small primaries (<200 ml) to receive VAID or a combination of VAID and VACD, where cyclophosphamide replaced ifosfamide for the last half of the treatment. The 3-year DFS was 79% and 71%, respectively, showing that less intensive therapy may be equally effective for a selected group of patients (Craft et al. 2000).

Subsequent studies built on these findings and investigated the addition of etoposide to the VAID regimen. Preclinical and clinical evidence indicates that the combined administration of etoposide and alkylators has a synergistic antitumor effect, and that the efficacy of both agents improves with fractionated administration. The combined administration of ifosfamide and etoposide (IE) proved to be very active in patients with recurrent ESFT (Miser et al. 1987), and the response rate in untreated patients was 96% (Meyer et al. 1992). Two multi-institutional randomized studies investigated the impact of adding etoposide to the VACD and VAID regimens (Craft et al. 2000; Grier et al. 2003). In the European EICESS-92 study, patients with localized high-risk disease (>200 ml) were randomized to the addition of etopo-
side (VAID vs. EVAID). The early results of this study showed a modest (but not significant) benefit from the addition of etoposide (3-year DFS 62% vs. 54%, \( p=0.6 \)) (Craft et al. 2000). The first American Intergroup Ewing trial (INT-0091 – POG-8850/CCG-7881) investigated the incorporation of the ifosfamide-etoposide combination in the front line treatment of ESFT, and all patients were randomized to receive VACD with or without IE (Grier et al. 2003). In both the standard and the experimental treatment regimens, the planned courses of standard therapy (VACD) consisted of vincristine (2 mg/m², with maximal dose of 2 mg), cyclophosphamide (1.2 g/m² as a single dose), and doxorubicin (75 mg/m² as a bolus); actinomycin D (1.25 mg/m²) was substituted for doxorubicin after the cumulative dose of doxorubicin had reached 375 mg/m². In the experimental arm, standard therapy courses were alternated with ifosfamide and etoposide (ifosfamide 1.8 g/m²/day for 5 consecutive days, etoposide 100 mg/m²/day for 5 consecutive days). Among patients with metastatic disease, the addition of ifosfamide and etoposide did not prove to be advantageous; 5-year EFS were 22±5% and 22±6% for the experimental and standard arms, respectively. For patients with non-metastatic disease, however, the VACD/IE regimen was superior to the standard VACD (5-year EFS 69±3% vs. 54±4% respectively, \( p=0.005 \)) (Grier et al. 2003). The beneficial effect of the incorporation of the IE pair was more pronounced for patients with large tumors and patients with pelvic primaries.

Overall, this generation of studies resulted in a marked improvement in the outcome for patients with localized disease. However, advances in surgery and radiation techniques were factored into these developments. In contrast to earlier studies, more aggressive measures for local control were taken (radiation therapy alone was used in less than 50% of the patients), and the local failure rate decreased significantly, usually to less than 15% (Table 9.5) (Bacci et al. 1998; Craft et al. 1998; Grier et al. 2003; Paulussen et al. 2001a). An important contribution of the INT-0091 study was that it demonstrated that the benefit of more intensive chemotherapy was not limited to its systemic effects, but also to its effect on local control. Patients treated on the VACD/IE arm had fewer local failures than patients on the VACD arm (7% vs. 20%) (Grier et al. 2003).

### 9.6.1.3 Increasing Dose Intensity

In recent years, chemotherapy treatment for many solid malignancies has relied on increasing the total cumulative doses of the active agents, as well as intensifying therapy by increasing the doses per cycle (and per unit of time) (Table 9.6). The incorporation of granulocyte colony-stimulating factor (G-CSF) into treatment regimens has allowed modest dose intensification of multiagent chemotherapy by increasing the total dose per cycle (Granowetter et al. 2001; Kolb et al. 2003; Marina et al. 1999) or shortening the interval of time between treatments (Womer et al. 2000). ESFT are very sensitive to alkylating agents, which have a very steep dose-response curve. Based on this evidence, a rational approach to the treatment of ESFT has been to use treatment intensification, by which high doses of different non-cross-resistant agents, primarily alkylating agents and topoisomerase-II inhibitors, are administered at maximum frequency. This approach has been evaluated by three groups (Table 9.7) (Kolb et al. 2003; Granowetter et al. 2001; Marina et al. 1999).

St. Jude Children’s Research Hospital’s EWI-92 protocol evaluated the feasibility of an aggressive early induction with three courses of VCDIE (vincristine 1.5 mg/m², cyclophosphamide 1.5 g/m², doxorubicin 45 mg/m², ifosfamide 2 g/m² × 3, and etoposide 150 mg/m² × 3), followed by a prolonged maintenance therapy with intensification of alkylating agents and etoposide, including four courses of VDC (vincristine 1.5 mg/m², cyclophosphamide 1–1.5 g/m², and doxorubicin 60 mg/m²) and four courses of IE (ifosfamide 2 g/m² × 5, etoposide 150 mg/m² × 5). The 3-year EFS and OS for patients with localized disease were 78% and 90%, respectively. However, an important finding was that only 66% of patients completed therapy, and that intensification was feasible only in 25% of the patients (Marina et al. 1999).

The importance of dose intensification in the treatment of ESFT has also been evaluated in the second American Intergroup POG-CCG Ewing trial (POG-9354/CCG-7942), in which patients were...
Table 9.6. Cumulative doses per protocol

<table>
<thead>
<tr>
<th>Study</th>
<th>Doxorubicin</th>
<th>Cyclophosphamide</th>
<th>Etoposide</th>
<th>Ifosfamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/m²</td>
<td>mg/m²/week</td>
<td>g/m²</td>
<td>mg/m²/week</td>
</tr>
<tr>
<td><strong>St. Jude studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES79 (Hayes et al. 1989)</td>
<td>385</td>
<td>9.6</td>
<td>11.6</td>
<td>0.029</td>
</tr>
<tr>
<td>EW87 (Meyer et al. 1992)</td>
<td>315</td>
<td>5</td>
<td>95</td>
<td>0.15</td>
</tr>
<tr>
<td>EW92 (Marina et al. 1999)</td>
<td>375</td>
<td>8.15</td>
<td>12.5–16.5</td>
<td>0.24–0.36</td>
</tr>
<tr>
<td><strong>CESS studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CESS-81 (Jürgens et al. 1988)</td>
<td>480</td>
<td>12</td>
<td>14.4</td>
<td>0.36</td>
</tr>
<tr>
<td>CESS-86 (Paulussen et al. 2001a)</td>
<td>480</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EICESS-92 Arm A (Paulussen et al. 2001b)</td>
<td>420</td>
<td>10</td>
<td>12</td>
<td>0.28</td>
</tr>
<tr>
<td>EICESS-92 Arm B (Paulussen et al. 2001b)</td>
<td>420</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EICESS-92 Arm C (Paulussen et al. 2001b)</td>
<td>420</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>POG-CCG studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INT 0091 Reg A (Grier et al. 2003)</td>
<td>375</td>
<td>7.6</td>
<td>20.4</td>
<td>0.41</td>
</tr>
<tr>
<td>INT0091 Reg B (Grier et al. 2003)</td>
<td>375</td>
<td>7.6</td>
<td>9.6</td>
<td>0.19</td>
</tr>
<tr>
<td>INT00 Reg C (Miser et al. 1996)</td>
<td>450</td>
<td>8.3</td>
<td>17.6</td>
<td>0.32</td>
</tr>
<tr>
<td>POG9354 Reg A (Granowetter et al. 2001)</td>
<td>375</td>
<td>7.8</td>
<td>10.8</td>
<td>0.23</td>
</tr>
<tr>
<td>POG9354 Reg B (Granowetter et al. 2001)</td>
<td>375</td>
<td>12.5</td>
<td>12</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>MSKCC studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-6 (Kolb et al. 2003)</td>
<td>300</td>
<td>14.2</td>
<td>16.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>
randomized to receive alternating courses of VCD (vincristine 1.5 mg/m², cyclophosphamide 1.2 g/m², doxorubicin 75 mg/m²) and IE (ifosfamide 1.8 g/m² × 5, and etoposide 100 mg/m² × 5) over either 48 or 30 weeks. The cumulative doses of agents were similar in both arms, but in the 30-week arm higher doses per cycle were given (Tables 9.6, 9.7). The early results of this randomized trial demonstrate no difference in outcome between the standard and the dose-intensified arms (3-year EFS 76±4% vs. 74±4%, respectively, \( p=0.57 \)) (Granowetter et al. 2001).

An alternative approach to long-term intensification is the use of high-dose, short-term regimens. This is the approach evaluated by investigators at the Memorial Sloan Kettering Cancer Center with the P6 protocol, in a group of 68 patients (44 localized) with ESFT. The P6 protocol consists of four courses of high-dose CDV (cyclophosphamide 4.2 g/m², doxorubicin 75 mg/m², vincristine 2 mg/m²) alternated with three courses of IE (ifosfamide 1.8 g/m² and etoposide 100 mg/m², both given daily for 5 days). The 4-year EFS and OS for patients with localized disease were 82% and 89%, respectively (Kolb et al. 2003).

### Table 9.7. Intensification regimens for treatment of ESFT (V vincristine, C cyclophosphamide, D doxorubicin, I ifosfamide, E etoposide, Sx surgery, RT radiation therapy, EFS event-free survival, OS overall survival)

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Regimen</th>
<th>Local control</th>
<th>Outcome</th>
<th>Failures</th>
<th>Poor prognostic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maintenance: Sx+RT (36 Gy)</td>
<td>3-year OS: 90%</td>
<td>Metastatic:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IE ×4</td>
<td>RT (60–68.4 Gy)</td>
<td>3-year EFS: 27%</td>
<td>3-year OS: 35%</td>
<td></td>
</tr>
<tr>
<td>P-6 (1991–2001)  (Kolb et al. 2003)</td>
<td>68 (24 mets)</td>
<td>HD-VCD ×4</td>
<td>Localized: Sx</td>
<td>Local: 2%</td>
<td></td>
<td>Metastatic disease only prognostic factor Site, size, age, histologic response no prognostic value</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IE ×3</td>
<td>Sx+RT (45–50 Gy)</td>
<td>4-year EFS: 82%</td>
<td>Local + distant: 0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RT (55.8 Gy)</td>
<td>4-year OS: 89%</td>
<td>Distant: 9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reg B: VCD/IE (30 weeks)</td>
<td>Sx+RT (45 Gy)</td>
<td>Reg A: 76%</td>
<td>Local + distant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RT (55.8 Gy)</td>
<td>Reg B: 74%</td>
<td>Distant</td>
<td></td>
</tr>
</tbody>
</table>
### Drugs and Cycles

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Drug Details</th>
</tr>
</thead>
</table>
| VDC x 7 cycles* | (V) Vincristine 2 mg/m² day 1  
(D) Doxorubicin 75 mg/m² CI for 48 hours  
(C) Cyclophosphamide 1200 mg/m² day 1 (+ Mesna)  
G-CSF 5 mcg/kg/d until ANC > 750/mm³ |
| IE x 7 cycles | (I) Ifosfamide 1800 mg/m²/day days 1 - 5 (+ Mesna)  
(E) Etoposide 100 mg/m²/day days 1 - 5  
G-CSF 5 mcg/kg/d until ANC > 750/mm³ |

CI: Continuous Infusion; ANC: Absolute Neutrophil Count  
*5 cycles VDC and 2 cycles VC (total cumulative dose of doxorubicin is 375 mg/m²)

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**Figure 9.5**  
COG AEWS0031 Protocol for Localized ESFT

**Figure 9.6**  
EURO-EWING 99 Protocol

* Patients with previously irradiated central axis sites are not eligible for randomisation in R2 or elective Bu-Mel in R3.  
Following Bu-Mel, irradiation to any part of the brain or the myelon must not exceed 30 Gy.
9.6.1.4 Current Studies

An alternative to increasing dose intensity is decreasing the intervals between cycles while maintaining the same dose-per-cycle with the use of G-CSF. This interval compression has the potential advantages of allowing dose intensification of all agents (without apparent increases in toxicity), and limiting the time of recovery of partially resistant cells (Womer et al. 2000). In the United States, this is the approach taken by the current Children’s Oncology Group AEWS-0031 study (www.cancer.gov/clinicaltrials/cog-aews0031#studyidno_cdr000006823), in which patients are randomized to receive alternating cycles VDC and IE every 3 weeks (standard arm) or 2 weeks (dose-compression arm), which results in 33% dose intensification (Fig. 9.5).

It is important to consider that not all patients require the same intensified approach. Risk factors such as age, tumor size and site, pattern of metastatic disease, histologic response to preoperative chemotherapy, and presence of micrometastatic disease may be combined to define risk categories that will allow risk-adapted therapies, by which the cumulative doses of alkylating agents and etoposide can be tailored. This is the approach taken by the European EURO-EWING 99 protocol, a randomized, prospective study that incorporates several cooperative groups. In this European study, all patients receive induction chemotherapy with six cycles of VIDE (vincristine, ifosfamide, doxorubicin, and etoposide), after which they are stratified into three risk groups based on tumor volume, presence and pattern of metastatic disease, and histologic response to induction therapy (Fig. 9.6). This study has been designed to provide answers to very relevant questions: (1) patients with small (<200 ml) or chemoresponsive, localized tumors are randomized to receive consolidation with VAI or VAC, in an attempt to better define the least toxic regimen for low-risk patients; (2) non-metastatic high-risk patients [defined as large (>200 ml) tumors treated with radiation only, or tumors with poor histologic response] are randomized to receive consolidation with high-dose chemotherapy with autologous stem cell rescue, or VAI; (3) patients with pulmonary metastases are randomized to VAI consolidation therapy and whole lung radiation, or high-dose chemotherapy and autologous stem cell rescue. In addition to these important therapeutic questions, this study will also evaluate in a prospective manner the incidence and prognostic significance of detection of micrometastatic disease with molecular methods.

9.6.2 Local Control in ESFT (Fig. 9.7)

The local management of pediatric and young adult patients diagnosed with ESFT continues to be an important but controversial subject. Patients with ESFT require local therapy for cure but no randomized clinical trials are available to define the most appropriate local therapy modality for specific patient...
groups. In the absence of randomized studies to guide treatment, local therapy has been delivered based on information from prospective studies as well as historic series that have gradually incorporated the changes in treatment techniques that have evolved over time for both surgical therapy and radiation therapy. Approximately 150–200 cases of ESFT are diagnosed annually in the United States. This small group of patients may be subdivided into three general subgroups when considering selection of a local therapy modality. The most favorable group of patients has small localized tumors that are amenable to surgical resection or local radiation therapy. The overall outcome for this group of patients is good with high local tumor control rates and favorable overall survival. A less favorable group of patients with localized disease exists that often have large tumors, or tumors that are not amenable to surgical resection. These patients are often managed with radiation therapy alone, though multimodal local therapy incorporating both surgery and radiation may also be appropriate. Patients in this group experience local tumor control rates between 50% and 75% and also have comparatively worse overall survival rates. The most unfavorable patients with ESFT are those that present with overt metastatic disease at diagnosis. The majority of these patients receive local radiation therapy as definitive local therapy though surgical therapy may be employed in select cases. In this group of patients, local therapy still plays an important role though controlling metastatic disease now becomes a primary issue and overall disease-control rates are less than 40%. Though this classification overly simplifies the approach to local therapy, it highlights the need for a multimodal approach to patient evaluation involving the pediatric oncologist, orthopedic oncologist, pediatric surgeon, and radiation oncologist at the time of diagnosis. This allows full evaluation of disease extent including local disease involvement, which facilitates selection of the most appropriate local therapy at the time for local tumor control. Local therapy may be classified by the individual modalities employed. Evaluation of outcome in this fashion allows a more detailed analysis of prognostic factors that vary depending upon the approach to local tumor control.

### 9.6.2.1 Surgical Therapy

Available local tumor control data suggest a superior outcome with wide local surgical excision, defining wide local excision as removal of all gross tumor with a margin of normal surrounding tissue. Though bias exists in selecting smaller, more peripheral tumors for definitive surgical resection and no randomized study is available to guide selection of a local modality, concerns regarding late effects including secondary malignancies and loss of growth have moved oncologists to a primary surgical approach for local therapy. Cooperative group studies approach the dilemma of local control with treatment guidelines based on resectability of the primary lesion in the context of known clinical prognostic factors. With careful selection for surgical therapy, the local control results are favorable for this group of tumors, with 5-year local failure rate of less than 10% (Table 9.8). Volume or size of tumor has been noted as a prognostic factor for event free survival in multiple series (Grier et al. 2003; Hayes et al. 1989; Oberlin et al. 2001; Paulussen et al. 2001a). The effect of tumor size on local failure is less clear. The combined Cooperative Ewing’s Sarcoma Studies (CESS) and European Intergroup Cooperative Ewing’s Sarcoma Studies (EICESS) did not demonstrate a difference in local failure for patients treated with surgery with tumors <100 cc vs. ≥100 cc (6.1% vs. 5.6%, respectively) (Schuck et al. 2003). Patients undergoing wide local excision at St. Jude Children’s Research Hospital had no difference in local failure based on tumor size <8 cm or ≥8 cm (16.7% vs. 13.3%, respectively) (Krasin et al. 2004a). Histologic response to induction chemotherapy may play a role in predicting local outcome with surgical therapy. Patients that achieved a favorable histologic response (≥90% necrosis) had a 2% incidence of local failure in the EICESS experience while patients with poor histologic response may benefit from adjuvant radiation therapy (Krasin et al., in press; Schuck et al. 2003). The group of patients classified as extraosseous ESFT present another local challenge. Though treated with ESFT- or rhabdomyosarcoma-specific systemic therapy (Krasin et al., in press; Raney et al. 1997), appropriate local therapy for this group of patients is less clear. Patients
with extraosseous ESFT treated with postoperative radiotherapy at St. Jude Children's Research Hospital had an 8-year local failure rate of 8% when adjuvant radiation was delivered postoperatively. In patients managed with surgery without radiation, the cumulative incidence of local failure and event free survival appear to be inferior compared to ESFT arising in bone (Krasin et al. 2004a; Krasin et al., in press). The ability to achieve a complete surgical resection for patients with ESFT clearly predicts a favorable outcome. With modern surgical techniques this approach will likely remain a favored local approach if a non-morbid procedure is readily achievable.

### Table 9.8. Local failure rates according to treatment modality

<table>
<thead>
<tr>
<th>Study</th>
<th>Local treatment modality</th>
<th>Local failure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI/CESS (Schuck et al. 2003)</td>
<td>Surgery</td>
<td>4.7%</td>
</tr>
<tr>
<td></td>
<td>Surgery + radiation</td>
<td>11.0%</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>29.3%</td>
</tr>
<tr>
<td>Rizzoli (Bacci et al. 2004)</td>
<td>Surgery</td>
<td>8.8%</td>
</tr>
<tr>
<td></td>
<td>Surgery + radiation</td>
<td>8.9%</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>29.3%</td>
</tr>
<tr>
<td>Italian Cooperative (SE-91)</td>
<td>Surgery</td>
<td>7.1%</td>
</tr>
<tr>
<td>(Rosito et al. 1999)</td>
<td>Surgery + radiation</td>
<td>6.5%</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>6.7%</td>
</tr>
<tr>
<td>POG 8346 (Donaldson et al. 1998)</td>
<td>Surgery ± radiation</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>35%</td>
</tr>
<tr>
<td>St. Jude (Krasin et al. 2004a, b; Krasin et al., in press)</td>
<td>Surgery</td>
<td>12.5%</td>
</tr>
<tr>
<td></td>
<td>Surgery + radiation</td>
<td>10.8%</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>30.4%</td>
</tr>
</tbody>
</table>

Postoperative and, more recently, preoperative irradiation have been systematically applied to patients with marginally resected or poorly responding tumors. Despite the selection bias of unfavorable patients (positive surgical margins, large tumor size and poor histologic response to neoadjuvant chemotherapy) treated with combined local therapy, the available literature suggests that there is equivalent local control compared to surgery alone (Carrie et al. 1999; Rosito et al. 1999; Schuck et al. 2003; Shankar et al. 1999). The rationale for combining radiation therapy and surgical resection is based on the belief that high local tumor control rates can be achieved with limited resection and adjuvant radiation therapy. Specific rates of local tumor control are shown in Table 9.8. Prognostic factors are similar to those of patients managed with surgery alone though the effect of surgical margins and poor histologic response appear to be nullified by the addition of adjuvant radiation therapy (Schuck et al. 2003). The incidence of positive surgical resection margins appears to relate to the timing of surgical resection; upfront resection results in more frequent positive surgical margins, noted in an analysis of chest wall ESFT treated in cooperative group studies (77% vs. 50%) (Shamberger et al. 2003) and at St. Jude Children’s Research Hospital (59% vs. 18%) (Krasin et al. 2004a; Krasin et al., in press). The role of preoperative radiation is under evaluation in the current European Intergroup study (EURO-EWING 99) as well as the previous European Intergroup Cooperative Ewing’s Sarcoma Study (EICESS 92) (Schuck et al. 2003). The results from that study indicate that despite selection of a higher proportion of patients with large, central tumors the local failure rate for patients receiving preoperative radiation was 5.1% compared to 9.2% for those receiving postoperative radiation.
Several studies have evaluated the efficacy of low-dose adjuvant radiation therapy (<40 Gy) for resected ESFT (Arai et al. 1991; Dunst et al. 1991; Krasin et al., in press; Merchant et al. 1999). Selection of low-dose irradiation has usually been based on young age, limited tolerance of surrounding tissues to irradiation or favorable tumor characteristics such as small primary size and response to chemotherapy. Even in institutions with large numbers of pediatric cancer patients it is difficult to draw statistically supported conclusions for this rare tumor and limited experience with low-dose adjuvant irradiation. Local failure rates with low-dose adjuvant radiation are reported as 0%, 15% and 17% from Memorial Sloan-Kettering Cancer Center, St. Jude Children's Research Hospital, and the CESS 81 study respectively (Dunst et al. 1991; Krasin et al., in press; Schuck et al. 2003). Patients treated with standard doses of adjuvant radiation therapy at St. Jude Children's Research Hospital have a local failure rate of 0% at 8 years. Results from contemporary prospective studies of patients with ESFT indicate that local tumor control following a complete surgery with either a wide local excision or marginal excision should result in a local tumor control rate of approximately 90% at 5 years (Dunst et al. 1991; Schuck et al. 2003). The excellent results obtained in modern surgical series as well as our results with standard dose adjuvant irradiation suggest that radiation therapy at a dose below 40 Gy is inadequate to provide the high rates of local tumor control (Krasiń et al., in press).

9.6.2.3 Definitive Radiation Therapy

Selection of definitive irradiation as a local management approach for ESFT requires weighing the associated surgical morbidities against the efficacy and treatment effects of irradiation. Overall local failure rates for patients managed with radiation therapy are consistently higher than those in patients undergoing surgery, with recurrence rates of nearly 30% (Table 9.8). The previously noted biases in selecting a more favorable patient population for surgical resection may explain some of these differences. The patient and treatment variables that affect the local outcome of those treated with radiation are similar to those noted above but also include patient age ≥14 years and the quality of radiation planning and delivery. Local outcomes for patients treated at St. Jude Children's Research Hospital were positively influenced by age <14 years, tumor size <8 cm and radiation dose ≥40 Gy. Local failure rates at 10 years for patients with tumors <8 cm were only 11% compared to 46% for those with larger tumor size. Age also played a role in predicting local failure, particularly for patients with tumors of 8 cm whose risk of local failure doubled from 31% for patients <14 years of age to 60% for those patients 14 years of age (p=0.035) (Krasiń et al. 2004b). The role for definitive low-dose irradiation is limited and appeared to result in inferior rates of local tumor control even for tumors <8 cm (p=0.010) (Fig. 9.6) (Krasiń et al. 2004b).

The role of the quality of radiation planning and delivery cannot be overstated. Three cooperative group studies have demonstrated the importance of quality radiation therapy (CESS 81 and 86 and POG 8346). Central treatment plan review was instituted in CESS 86 following a local failure rate of 50% in CESS 81 for patients undergoing definitive irradiation; subsequent patients treated with definitive radiation therapy on CESS 86 had a local failure rate of only 13% (Dunst et al. 1991). Patients undergoing definitive irradiation on POG 8346 had an 84% incidence of local failure if a major deviation in dose or volume of treatment was noted. Even patients with a minor deviation experienced a 52% local failure rate compared to 20% for those with no deviation (p=0.005) (Donaldson et al. 1998). In light of the limited number of patients diagnosed with ESFT each year, great care must be given to ensure adequate targeting and dose delivery for this readily curable malignancy.

9.6.2.4 Role of Systemic Chemotherapy in Local Control

Clinical trials that have investigated the incorporation of new agents such as doxorubicin (Razek et al. 1980) or ifosfamide and etoposide (Grier et al. 2003), and the use of treatment intensification (Craft et al. 1998), have shown that systemic chemotherapy also contributes to local tumor control. The best example
of this impact was recently provided by the results of the American Intergroup Ewing trial (INT-0091 – POG-8850/CCG-7881), which randomized patients to receive VACD or VACD/IE (Grier et al. 2003). As discussed above, this study demonstrated that the benefit of more intensive chemotherapy was also due to its improvement on local control. Using the same local control guidelines, the 5-year cumulative incidence of local progression was 15% and 5% for the control VACD arm and the experimental VACD/IE arm, respectively ($p<0.001$) (Grier et al. 2003).

In summary, management of localized ESFT mandates a multidisciplinary approach to selection of local tumor control. With a limited number of cases annually patients should either be treated at specialized treatment centers or in cooperative group studies to ensure quality and consistency in the treatment approach. Based on current prognostic factors, appropriate local treatment may be selected for favorable patient groups to maximize local tumor control and minimize late effects. Patients with adverse prognostic factors may require intensification of systemic therapy as well as aggressive combined local therapy to maximize cure.

### 9.6.3 Treatment of Metastatic ESFT

The advances experienced in the treatment of patients with localized ESFT have not impacted the outcome of patients with metastases; using conventional treatment only 20–25% of patients can be cured (Fig. 9.4) (Table 9.9) (Cangir et al. 1990; Craft et al. 1997, 1998; Miser et al. 2004; Paulussen et al. 1998b; Sandoval et al. 1996; Rodriguez-Galindo et al. 2003).

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Regimen</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IESS I-II (Cangir et al. 1990) 1975–1983</td>
<td>122</td>
<td>VACD</td>
<td>5-year DFS: 30%</td>
<td></td>
</tr>
<tr>
<td>Reg. A: VACD</td>
<td></td>
<td>Reg. A: 32%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reg. B: VACD+IE</td>
<td></td>
<td>Reg. B: 29%</td>
<td>4-year EFS: Reg. C: 26%</td>
<td></td>
</tr>
<tr>
<td>Single arm: Reg.C: VACDIE (intensified)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1 (Craft et al. 1997) 1978–1986</td>
<td>22</td>
<td>VACD</td>
<td>5-year OS: 9%</td>
<td></td>
</tr>
<tr>
<td>ET-2 (Craft et al. 1998) 1987–1993</td>
<td>42</td>
<td>VAID</td>
<td>5-year OS: 23%</td>
<td></td>
</tr>
<tr>
<td>EICESS (Paulussen et al. 1998b) 1990–1995</td>
<td>171</td>
<td>VAID±E</td>
<td>4-year OS: 27%</td>
<td>Better outcome for isolated lung disease: Lungs: 34% Bone/bone marrow: 28% Combined: 14%</td>
</tr>
<tr>
<td>EW-92 (Marina et al. 1999)</td>
<td>19</td>
<td>VCDIE ×3</td>
<td>3-year OS: 35%</td>
<td>Intensification does not improve results High toxicity Incidence t-AML: 8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VCD/IE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Several institutions have explored the impact of maximum treatment intensification in metastatic ESFT. This approach explores the administration of very high doses of non-cross-resistant agents in a short period of time. Different regimens have been explored (Table 9.10) (Felgenhauer et al. 2000; Kolb et al. 2003; Strauss et al. 2003; Miser et al. 1996). Short and intensive therapy is feasible, and toxicity is substantial but manageable. Although the majority of patients with metastatic disease treated using these regimens have good clinical and histological responses, the final results are not better than those obtained with conventional therapy (Felgenhauer et al. 2000; Kolb et al. 2003; Strauss et al. 2003).

However, even among patients with metastatic disease, there is some heterogeneity. Patients with isolated lung metastases may have a better prognosis than patients with extrapulmonary metastases (Table 9.9), with long term survival rates approaching 40–45% (Miser et al. 2004; Paulussen et al. 1998b; Spunt et al. 2001). Among patients with lung metastases, those with unilateral disease (Paulussen et al. 1998a; Spunt et al. 2001) and those with good histologic response to induction chemotherapy (Paulussen et al. 1998a) appear to have a survival advantage. However, a complete radiological response to induction chemotherapy does not seem to correlate with outcome (Paulussen et al. 1998a; Spunt et al. 2001).

In approximately 50% of patients with isolated lung metastases, failures to therapy occur as isolated pulmonary disease again (Paulussen et al. 1998a; Spunt et al. 2001), suggesting that further response consolidation could potentially improve the outcome of these patients. In this regard, whole lung radiation (15–18 Gy) seems to provide a modest survival advantage (Paulussen et al. 1998a). Preliminary data of the European Bone Marrow Transplant Registry (EBMTR) (Ladenstein et al. 1999) (see below) suggest that an alternative approach to the treatment of patients with isolated lung metastases may be the use of consolidation with high-dose chemotherapy using a busulfan-based regimen, and autologous stem cell rescue. As discussed above, these two approaches are compared in a randomized manner in the current EURO-EWING 99 protocol.

### Table 9.10. Intensive, short-term regimens for high-risk ESFT

<table>
<thead>
<tr>
<th>Author</th>
<th>Regimen</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strauss et al.</td>
<td>VIDE ×6</td>
<td>VCR 1.4 mg/m² day 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOX 20 mg/m² day 1–3</td>
</tr>
<tr>
<td>Felgenhauer et al.</td>
<td>VACIME ×8</td>
<td>IFO 3 g/m² day 1–3</td>
</tr>
<tr>
<td>Kolb et al.</td>
<td>P6</td>
<td>ETO 150 mg/m² day 1–3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycles 1, 2, 3, 6:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VCR 0.67 mg/m² day 1–3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYC 2.1 g/m² day 1–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOX 25 mg/m² day 1–3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycles 4, 5, 7:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFO 1.8 g/m² day 1–5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ETO 100 mg/m² day 1–5</td>
</tr>
</tbody>
</table>
The results of treatment with megatherapy and HSCT for patients with high-risk ESFT must be analyzed considering the absence of randomized studies and the heterogeneity of the patients studied. The role of and indications for HSCT in ESFT have been reviewed by Kushner and Meyers (2001). Very few studies have evaluated the response of ESFT to the agents used in many megatherapy regimens. In general, the alkylating agents thiotepa, busulfan and melphalan have shown good responses in phase I and phase II studies (Schiffman et al. 1996), and most regimens use different combinations of these agents.

The experience of the European Cooperative Ewing Sarcoma Group (EICESS) has been reported recently in three studies by Paulussen et al. (1998b) and Burdach et al. (2000, 2003) (Table 9.11). In the first two studies, initial treatment was based on the CESS 81 and 86 protocols, and the megatherapy regimen was with melphalan and etoposide, with the addition of carboplatin and total body irradiation (TBI) for a subset of patients. The results were poor, with DFS rates of 23–24% for patients with metastatic disease to bone or bone marrow. More recently, the results of two sequential studies exploring high-dose therapy for patients with primary metastatic (multifocal bone) or recurrent disease have been reported (Burdach et al. 2003). In the first study, patients received treatment consolidation with total body irra-

### Table 9.11. Treatment of metastatic extrapulmonary ESFT with hematopoietic stem cell transplant (V vincristine, A actinomycin D, C cyclophosphamide, D doxorubicin, I ifosfamide, E etoposide, MEL melphalan, ETO etoposide, CBP carboplatin, TBI total body irradiation, DFS disease free survival, HSCT hematopoietic stem cell transplant, OS overall survival, t-AML therapy-related acute myeloid leukemia, TT thiotepa, BUS busulfan, NR not reported)

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients</th>
<th>Induction</th>
<th>Megatherapy</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paulussen (1998b)</td>
<td>36</td>
<td>VAID</td>
<td>MEL/ETO ±CBP±TBI</td>
<td>4-year DFS 23%</td>
<td>No benefit of HSCT</td>
</tr>
<tr>
<td>EICESS 1990–95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No influence of conditioning regimen</td>
</tr>
<tr>
<td>Burdach (2000)</td>
<td>17</td>
<td>VACD, EVAID</td>
<td>MEL+ETO+TBI±CBP</td>
<td>5-year DFS 24%</td>
<td>High incidence of t-AML</td>
</tr>
<tr>
<td>EICESS 1986–94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ladenstein (1996)</td>
<td>18</td>
<td>VACD, VAID</td>
<td>MEL/ETO ±CBP±TBI</td>
<td>4-year OS 26%</td>
<td>OS 3/6 of patients undergoing allo-HSCT</td>
</tr>
<tr>
<td>Austria 1984–1996</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyers (2001) CCG</td>
<td>23</td>
<td>P6</td>
<td>MEL+ETO +TBI</td>
<td>2-year DFS 24%</td>
<td>No benefit of HSCT</td>
</tr>
<tr>
<td>Kolb (2003) MSKCC</td>
<td>24</td>
<td>P6</td>
<td>MEL+TBI +TT+CBP</td>
<td>4-year EFS 12%</td>
<td>No benefit of HSCT</td>
</tr>
<tr>
<td>1990–2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High toxicity</td>
</tr>
<tr>
<td>1982–1992</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1978–1997</td>
<td></td>
<td></td>
<td></td>
<td>5-year OS 23%</td>
<td></td>
</tr>
<tr>
<td>Burdach (2003) EICESS</td>
<td>54</td>
<td>EVAIA</td>
<td>Toxic deaths: Tandem ME: MEL+ETO ×2</td>
<td>Hyper ME: 22%</td>
<td>Hyper ME: 23% Tandem ME: 4%</td>
</tr>
<tr>
<td>1986–2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| ETO etoposide, CBP carboplatin, TBI total body irradiation, DFS disease free survival, HSCT hematopoietic stem cell transplant, OS overall survival, t-AML therapy-related acute myeloid leukemia, TT thiotepa, BUS busulfan, NR not reported)
radiation, melphalan and etoposide (HyperME); in the second study, total body irradiation was not given, and patients underwent two courses of melphalan/etoposide and HSCT (TandemME). The EFS rate was 22±8% and 29±9% for the HyperME and TandemME protocols, respectively. Of note, the HyperME regimen resulted in a significantly higher incidence of toxic deaths (23% vs. 4%) (Burdach et al. 2003). In vitro studies have suggested a possible benefit of the administration of IL-2 after high-dose chemotherapy and autologous rescue; however, treatment with IL-2 does not seem to provide any beneficial antitumor effect in vivo (Burdach et al. 2003).

In the United States, Meyers et al. (2001) and Kushner and Meyers (2001) have reported the experience using the intensive P6 therapy (Kushner and Meyers 2001) or a similar intensive approach (Meyers et al. 2001), as induction, followed by similar consolidation with HSCT. In both studies, melphalan and TBI were used. The results were very poor, with DFS rates of less than 25% (Table 9.11).

As for any megatherapy approach, the agents used for myeloablation and eradication of residual disease have to be considered. Ladenstein et al. (1995, 1999) reported the results of the EBMTR for a selected series of patients in two consecutive studies. In a first review of the patients that underwent a HSCT between 1982 and 1992, the DFS rate for the group of 22 patients with metastatic disease to bone and bone marrow was 21% (Ladenstein et al. 1995). However, when analyzing in detail the different megatherapy regimens for the whole group of 63 patients receiving a HSCT, the results were superior for the group of patients that received the combination melphalan-busulfan, and for the patients that did not receive TBI. In a more updated review that included 111 patients with ESFT with bone and bone marrow metastases who underwent HSCT between 1978 and 1997, the advantage of the regimens that included busulfan was confirmed (Ladenstein et al. 1999). The overall survival at 5 years was 44% for the group of 18 patients that received busulfan, and only 23% for the group of 93 patients that were treated with regimens without busulfan. The use of busulfan provided a survival advantage also for patients with pulmonary disease alone (66% vs. 39%) and for patients with localized high-risk disease (75% vs. 38%) (Table 9.11). In summary, in these retrospective studies there appears to be an advantage for regimens that incorporate high doses of alkylating agents, generally busulfan and melphalan (Ladenstein et al. 1995, 1999). The administration of TBI does not seem to provide any additional benefit, and may only add toxicity (Burdach et al. 2003; Kushner and Meyers 2001; Ladenstein et al. 1995). It is still not clear whether this approach is beneficial for patients with bone/bone marrow metastases, and such an approach should be undertaken only in the context of an investigational study, preferably randomized.

Studies performed in children with advanced neuroblastoma and medulloblastoma have shown the feasibility and efficacy of the use of repeat cycles of high-dose chemotherapy with autologous HSCT. However, available data suggest that this approach may not be advantageous for patients with metastatic ESFT (Burdach et al. 2003).

Gene marking studies in patients with neuroblastoma that received an autologous HSCT have shown that the stem cell graft has tumor cells that may contribute to disease recurrence after HSCT (Rill et al. 1994). Although there are no similar studies in ESFT, several investigators have shown that ESFT cells are detectable by PCR in stem cell grafts, even after positive selection for CD34+ cells. As for other pediatric solid malignancies, the value of purging the stem cell harvest (and by which method) has not been clearly established (Ladenstein et al. 1997). Negative selection methodologies using pharmacological agents or antibodies have historically been the most commonly used methods to remove tumor cells from grafts. The efficacy of pharmacological agents depends on the differential sensitivity of tumor cells and normal hematopoietic progenitors to cytotoxic agents. However, interpatient variability and excessive toxicity to normal progenitors may result in significant delays in hematopoietic reconstitution. Furthermore, these methods have a suboptimal therapeutic ratio in solid tumors. In contrast to the negative selection strategies, positive selection methods are designed to attain a highly purified hematopoietic cell population. The most commonly used positive selection systems use the CD34+ antigen, which is expressed on primi-
tive hematopoietic progenitors that have the ability to reconstitute hematopoiesis.

In recent years, some institutions have performed allogeneic HSCT in patients with ESFT. The number of patients is low, and the results are contradictory (Burdach et al. 2000; Ladenstein et al. 1996). Nevertheless, with refinements in the conditioning techniques and immune manipulation, allogeneic HSCT may be a valid alternative for patients with solid tumors, and in particular ESFT (Koscielniak et al. 2005). These tumors are characterized by the expression of surface proteins derived from the characteristic gene fusion. These chimeric oncoproteins may attach to the HLA molecules and induce antitumor immune responses. It is therefore conceivable that there a clinically significant graft versus tumor effect could occur. Moreover, the immune response may not be restricted to the MHC molecules. Some tumor cells express low levels of class I molecules, and there may be an antitumor effect through the lytic function of NK cells. The main problem with allogeneic HSCT is its greater toxicity. A good alternative would be the induction of a mixed chimerism state that would result in a bidirectional immune tolerance. The induction of mixed chimerism has several advantages. First, the conditioning is less toxic and should result in less toxicity. Second, the development of host tolerance toward the graft is the first step towards adoptive immunotherapy with the infusion of immunocompetent cells of the donor that could maximize the graft versus tumor effect. Finally, mixed chimerism may help decrease the incidence and severity of graft versus host disease.

9.6.5 Second Malignancies

Classically, the incidence of second cancers in survivors of ESFT has not been higher than in other childhood cancers. The cumulative incidence of second neoplasms in most large series is not higher than 2% (Grier et al. 2003; Paulussen et al. 2001b), and sarcomas arising in the radiation field represent more than 75% of the cases (Bacci et al. 2004; Kuttesch et al. 1996). The development of radiation-induced sarcomas has a latency of several years, and it is dose dependent, with very low risk below 48 Gy and very high cumulative incidence (CI at 10 years of 35%) in patients receiving doses >60 Gy (Kuttesch et al. 1996; Tucker et al. 1987a). The use of alkylating agents appears to increase the risk of radiation-induced sarcomas (Tucker et al. 1987a).

In recent years, the use of protocols that include intensification of alkylators and topoisomerase-II inhibitors has resulted in a significant increase in the incidence of treatment-related leukemia and myelodysplastic syndromes (t-AML/MDS). This therapeutic strategy appears to be leukemogenic, and patients are at increased risk of developing both alkylator-related and etoposide-related t-AML/MDS (Kolb et al. 2003; Kushner et al. 1998; Miser et al. 1996; Rodriguez-Galindo et al. 2000a). The cumulative incidence of t-AML/MDS at 40 months was 8% in survivors of the P6 protocol (Kushner et al. 1998). Likewise, in the St. Jude EW92 protocol, the cumulative incidence of t-AML/MDS was 8%, compared to 0% in patients treated on the ES87 protocol, in which the same drugs were used, but much less intensively, and in which no G-CSF was given (Rodriguez-Galindo et al. 2000a) (Table 9.12). Compared to the low incidence of t-AML/MDS traditionally observed in less intensive protocols for non-metastatic patients, the risks seen in the high-dose studies represent major increases. This increased risk for t-AML/MDS appears to be related to both the increase in the total cumulative doses and dose intensification of alkylating agents and topoisomerase-II inhibitors, and growth factors may also play a role. Combinations of alkylators and topoisomerase-II inhibitors appear to be associated with a greater risk of t-MDS and t-AML than either class of drugs administered alone (Tucker et al. 1987b; Pedersen-Bjergaard et al. 1993). Moreover, even the addition of small doses of etoposide to an alkylator-based regimen can significantly increase the incidence of t-MDS and t-AML (Heyn et al. 1994). Although the risk for anthracycline-associated t-MDS/t-AML is generally low because of dose limitations imposed to circumvent cardiotoxicity, use of these agents with DNA-damaging drugs such as cisplatin or alkylators clearly increases the probability of leukemia induction (Pedersen-Bjergaard et al. 1992; Sandoval et al. 1993). Topoisomerase-II inhibitors have a synergistic effect when administered with
drugs that interact with DNA (Pedersen-Bjergaard et al. 1993). The role of G-CSF has not been defined, but may provide a survival advantage to genetically damaged stem cells that otherwise would undergo apoptosis, thus preventing the elimination of the genetic damage.

9.6.6 Recurrent ESFT

Although improved local control therapy and the development of new and more intensive chemotherapy combinations have reduced the failure rates, a large proportion of patients continue to experience relapse. Recurrences can be local, distant, or combined (Rodriguez-Galindo et al. 2002). During the earlier years of multimodal therapy, approximately 25% of all patients experienced local treatment failure, and another 20–40% experienced distant treatment failure (Bacci et al. 1989; Craft et al. 1997; Jürgens et al. 1988; Nesbit et al. 1990; Razek et al. 1980). The patterns of relapse have evolved over the past 3 decades, reflecting the effect of improvements in the multimodal treatment. A significant proportion of recurrences in the early studies were local or combined local and distant (Table 9.4) (Razek et al. 1980; Burgert et al. 1990; Craft et al. 1997; Nesbit et al. 1990; Jürgens et al. 1988). Improvements in the multidisciplinary approach have decreased the local recurrence rates to less than 10%.

Outcome after recurrence is very poor; the probability of survival at 5 years is less than 20% (Bacci et al. 2003; Rodriguez-Galindo et al. 2002; Shankar et al. 2003). However, patients experiencing late recurrences, patients with isolated local recurrences amenable to radical surgery, and patients with isolated lung recurrences appear to have a survival advantage (Bacci et al. 2003; Rodriguez-Galindo et al. 2002; Shankar et al. 2003).

9.7 Future Developments

Intensification of therapy has proven to have a limited role, and patients with high-risk disease continue to have a poor outcome. New agents and therapeutic approaches are still needed for ESFT (Rodriguez-Galindo 2004). Preclinical and clinical studies have shown that the camptothecin derivatives are among the most effective compounds for treating pediatric malignancies (Rodriguez-Galindo et al. 2000b), and their role in the treatment of ESFT is being investigated. Although phase I and II studies of topotecan and irinotecan as single agents have shown little or no activity in patients with refractory disease, recent studies suggest that their combination with alkylating agents may be more promising. Phase II studies of the combination of topotecan (0.75 mg/m²/day × 5 days) with cyclophosphamide (250 mg/m²/day ×

Table 9.12. Incidence of therapy-related leukemia as a function of the total cumulative doses and treatment intensification

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Doxorubicin (mg/m²)</th>
<th>Cyclophosphamide (g/m²)</th>
<th>Etoposide (mg/m²)</th>
<th>Ifosfamide (g/m²)</th>
<th>Incidence t-AML/MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Per week</td>
<td>Total</td>
<td>Per week</td>
<td>Total</td>
</tr>
<tr>
<td>ES-87 (Meyer et al. 1992)</td>
<td>315</td>
<td>5</td>
<td>9.5</td>
<td>0.15</td>
<td>3,000</td>
</tr>
<tr>
<td>EW-92 (Marina et al. 1999)</td>
<td>375</td>
<td>8.15</td>
<td>12.5–16.5</td>
<td>0.24–0.36</td>
<td>4,350</td>
</tr>
<tr>
<td>P-6 (Kolb et al. 2003)</td>
<td>300</td>
<td>14.2</td>
<td>16.8</td>
<td>0.8</td>
<td>1,500</td>
</tr>
<tr>
<td>First POG-CCG Regimen C</td>
<td>450</td>
<td>8.3</td>
<td>17.6</td>
<td>0.32</td>
<td>5,000</td>
</tr>
</tbody>
</table>
5 days) resulted in responses in 36% of patients with recurrent disease (Saylor et al. 2001), and in 56% of patients with untreated metastatic disease (Bernstein et al. 2001). In a recently reported phase I study, irinotecan was administered on a protracted schedule (10–15 mg/m²/day for 5 days in two consecutive weeks) in combination with temozolomide (100 mg/m²/day for 5 days) to children with refractory solid tumors. In this population of heavily pretreated patients, three responses were observed among the seven patients with refractory ESFT (Wagner et al. 2004). The use of topoisomerase-I inhibitors in combination with alkylating agents thus deserves further evaluation in ESFT.

Our better understanding of the molecular pathogenesis and biology of ESFT is leading to a new definition of potential targets for antitumor therapy. The tyrosine kinase inhibitor STI-571 (imatinib mesylate) has shown in preclinical models a high degree of specificity for BCR-ABL, the receptor for platelet-derived growth factor (PDGFR), and c-kit tyrosine kinases. The stem cell factor receptor c-kit is also expressed in ESFT cells, and its activation appears to be involved in cell survival. Ewing sarcoma cell lines express several imatinib mesylate-sensitive tyrosine kinases, including c-kit and PDGFR (Scotlandi et al. 2003; Uren et al. 2003). Immunohistochemical studies performed in primary ESFT tumors have shown that approximately 70% of tumors express c-kit, although the staining is strong and diffuse only in 30% of the cases (Scotlandi et al. 2003; Smithey et al. 2002), but there appears to be no association with outcome (Scotlandi et al. 2003). Despite these interesting preliminary data, in vitro studies have shown a modest effect of imatinib mesylate on the growth and proliferation of Ewing sarcoma cell lines. A phase II study of imatinib mesylate is currently being performed in the United States by the Children's Oncology Group in children with recurrent ESFT.

Another promising agent in the treatment of sarcomas is ecteinascidin-743 (ET-743). This novel compound is a minor groove binding, guanine-specific alkylating agent which also interacts with the microtubule network and blocks cell cycle progression at late S/G2. ET-743 has shown antitumoral activity in adults with advanced, pretreated soft tissue sarcomas (Yovine et al. 2004). In a recently completed phase I study in children, a complete response was observed in a patient with metastatic ESFT (Lau et al. 2005). A phase II study in children with recurrent sarcomas, including ESFT, is under development by the Children's Oncology Group.

The tumor necrosis factor (TNF) receptor superfamily is a main regulator of apoptosis, and in recent years members of this family have shown to have a pivotal role in inducing apoptosis of ESFT cells. The TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis by binding to two members of this family, the death receptor (DR) 4 and DR5. ESFT cells are exquisitely sensitive to TRAIL (Mitsaides et al. 2001; van Valen et al. 2003), and in this in vitro model sensitivity is dependent on the presence of DR4 and DR5 (Mitsaides et al. 2001). Therefore, the wide expression of DR4/DR5 in ESFT in vivo and the high sensitivity to TRAIL in vitro would suggest that further exploration of the TRAIL pathway as a therapeutic tool would be indicated. The tumoricidal activity of TRAIL in animal models of cancer is well documented (Walczak et al. 1999). However, TRAIL is expressed in a wide range of normal tissues. Although early reports suggested that TRAIL would induce apoptosis only in transformed and malignant cells, recent reports have demonstrated that TRAIL can elicit apoptosis in normal tissues as well, such as hepatocytes and brain tissue (Jo et al. 2000; Nitsch et al. 2000), a finding that limits its therapeutic use. However, further investigation of this pathway is under way. It has been shown that interferons can induce expression of TRAIL (Fanger et al. 1999), and recent studies have documented strong synergistic in vitro and in vivo antitumor effect when type I interferons (α or β) are given with ifosfamide (Sanceau et al. 2002) or with type II interferon (γ) (Abadie et al. 2004). Furthermore, in these preclinical models, the antitumor effect correlates with TRAIL induction, which would suggest that TRAIL contributes to the triggering of apoptosis in ESFT cells in an autocrine or paracrine manner.

Inhibition of histone deacetylation has proven to result in a significant antitumor effect in preclinical
and clinical models. Acetylation and deacetylation of histones alter higher order chromatin structure by influencing histone interaction with DNA. Deacetylated histones are associated with cell growth, whereas acetylated histones are associated with cell growth arrest, differentiation, and apoptosis (Peart et al. 2003). Transcription factors may also be acetylated, and the acetylation status influences their interaction with DNA. In this regard, chimeric transcription factors present in a variety of tumor systems might cause transcriptional repression of growth regulatory target genes by recruitment of transcriptional corepressors and their associated histone deacetylase (HDAC) activity (Peart et al. 2003). This is particularly relevant in ESFT, since it has been shown that the EWS-FLI1 chimeric transcription factor suppresses transforming growth factor β type II receptor (TGF-βR-II) transcription (Hahm et al. 1999). This is important, since studies indicate that restoration of TGFβ pathway in ESFT cells inhibits their growth (Hahm et al. 1999). In the xenograft model, the HDAC inhibitor MS-27–275 was able to induce an increase in TGFβR-II mRNA and restore TGFβ signaling, and this correlated with growth inhibition (Jaboin et al. 2002). Moreover, in this same model, p21^WAF/CIP1 was induced in most cell lines irrespective of the p53 status (Jaboin et al. 2002). Finally, HDAC inhibitors appear to interfere with angiogenesis, an effect that deserves further investigation (Jaboin et al. 2002). The HDAC inhibitor depsipetide is currently under phase I investigation by the Children’s Oncology Group in children with refractory solid tumors.

The insulin-like growth factor-I pathway (IGF-1/IGF-1R) is actively involved in the cell transformation induced by EWS-FLI1 and inhibition of apoptosis induced by chemotherapy (Benini et al. 2004; Toretsky et al. 1999). Studies have shown that the inhibition of the IGF-1R or of some downstream elements such as MAPK, PI3-K or Akt may provide effective antitumor activity and potentiate the effects of chemotherapeutic agents (Benini et al. 2004; Toretsky et al. 1999). Inhibition of the activation of downstream elements of PI3-K, such as mTOR, is also a rational target for antitumor therapy. Rapamycin and its derivatives such as CCI-779 might have a role in the treatment of ESFT; in vitro studies have shown that rapamycin may block cell line proliferation by promoting cell cycle arrest at the G1 phase, downregulation of EWS-FLI-1 proteins, and concomitant restoration of expression of TGFβ R-II in ESFT cells (Mateo-Lozano et al. 2003).

EWS-FLI1 may promote cell cycle progression accompanied by the suppression of the expression of cyclin-dependent kinase inhibitor p27^kip1 in ESFT cells (Matsumoto et al. 2001). Matsunobu et al. analyzed the prognostic relevance of p27^kip1 in patients with ESFT, and found that low expression levels of p27 protein correlated with poor outcome (Matsunobu et al. 2004). In the in vitro model, overexpression of p27 using an adenoviral vector inhibited cell growth and induced apoptosis, and treatment of mice bearing ESFT xenografts with intratumoral injections of p27-expressing adenovirus resulted in significant growth inhibition (Matsunobu et al. 2004). Therefore, mechanisms of increasing expression of p27 could potentially have a role in the treatment of ESFT. In this regard, it is well known that the level of p27 expression is regulated at the post-translational level, and the ubiquitin-proteasome pathway is the principal mechanism regulating p27 protein degradation (Pagano et al. 1995). In ESFT, data suggest that the EWS-FLI1 chimeric transcript might attenuate p27 protein level via activation of the proteasome-mediated degradation pathway (Matsunobu et al. 2004). Treatment of ESFT cells with a proteasome inhibitor resulted in increased expression of the p27 protein (Matsunobu et al. 2004). Proteasome inhibitors are in different phases of clinical development, and a phase I trial of the proteasome inhibitor bortezomib (PS-341) in children with refractory solid tumors has been recently completed by the Children’s Oncology Group.

Resistance of tumors to chemotherapy is often due to abnormalities in the apoptotic pathways. Caspase-8 expression is pivotal in chemotherapy-induced apoptosis in a variety of tumor systems, and decreased expression is associated with chemoresistance (Fulda et al. 2001). Decreased expression of caspase-8 protein may occur as a result of hypermethylation of caspase-8 regulatory sequences (Fulda et al. 2001). In ESFT, treatment of tumor cell lines with low caspase-8 expression with the demethylation agent
Because the EWS-FLI-1 fusion product that characterizes ESFT may induce a cytotoxic T lymphocyte response, the generation of specific cytotoxic T lymphocytes is currently under investigation (Mackall et al. 2003). Finally, because the formation of the chimeric EWS-FLI-1 gene is one of the initial transforming events in ESFT, the use of antisense oligonucleotides targeting the fusion is an attractive approach. In fact, this method has shown to be able to inhibit the growth of xenografted ESFT into nude mice (Maksimenko et al. 2003).

References


5-aza-2’-deoxycytidine reversed hypermethylation of caspase-8, resulting in restoration of caspase-8 expression and restitution of drug-induced apoptosis (Fulda et al. 2001).


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