In collaboration with

EWING 2008

EudraCT number: 2008-003658-13
Trial Code: EWING2008

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Planned period of study: 2009-2015

Start of of study: 1 January 2009

End of study: 1 July 2015

End of follow-up: 2017

Date of version: 20 April 2009

Version: 1.5

Contents

1 GENERAL OVERVIEW

1.1 Important Note

This document describes a randomised trial for patients with localised or metastatic Ewing sarcomas. The protocol provides detailed information about patients' eligibility and about the procedures for entering them into the trial. It is not intended as an aide mémoire or guide for the treatment of other patients.

Each institution willing to treat patients according to EWING 2008 must receive accreditation from the appropriate national or group office (please refer to section 1.4) according to the rules set forth by that group.

Participants are required to maintain confidentiality regarding the contents of this protocol. No part of this protocol may be reproduced or circulated (written or orally) without prior authorisation by the appropriate national coordinator or the principal coordinating investigator when appropriate.

The national coordinators, on behalf of the sponsor, are responsible for submitting the application for

- an Ethics Committee opinion
- any required authorisation by a regulatory authority
- an appropriate insurance

according to national law and institutional guidelines, and for

- organising the distribution of trial medication to the trial sites as well as ensuring that all such trial medication is appropriately labelled according to national law.

The responsibility for the administration of the protocol treatments lies with the participating trial centre. Before entering patients into the trial, any investigators must ensure that the EWING 2008 study has received ethics committee clearance, authorisation of the regulatory authority and the appropriate insurance according to national law and institutional guidelines. The institution must have received approval of being accepted as a trial centre by the appropriate national or group office (please refer to section 1.4).

This protocol is the result of discussions among members of the EURO E.W.I.N.G Study Group who convened in London (April, July, and September 2005), Paris (December 2005), Amsterdam (March 2006, January 2007). The protocol has been developed following the recommended format of the master protocol of Deutsche Krebshilfe (German Cancer Aid).

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1.6 EWING 2008 Coordinating Data Centre

EWING 2008 Coordinating Data Centre

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1.7 Contact for SAE Reporting/Safety Desk

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1.9 Committee Membership

The groups cooperating in this study are:

- **CCLG** Children's Cancer and Leukaemia Group
- **COG** Children's Oncology Group (North America)
- **Czech** Czech Pediatric Oncology
- **DCOG** Dutch Childhood Oncology Group
- **GPOH** Gesellschaft für Pädiatrische Onkologie und Hämatologie
- **GPOH Austria** Gesellschaft für Pädiatrische Onkologie und Hämatologie Austria
- Polish Society of Pediatric Oncology and Hematology
- **SFCE** Société Française des Cancers d'Enfants
- **SIAK** Schweizerisches Institut für Angewandte Krebsforschung
- **SSG** Scandinavian Sarcoma Group (Sweden)
- Slovakian Bone Sarcoma Study Group
- Slovenian Bone Sarcoma Study Group

The high dose therapy and stem cell rescue part of this study is carried out in cooperation with the

- **EBMT** European Group for Blood and Marrow Transplantation

Other groups may join this project after agreement with the intergroup study committee.

An Independent Data and Safety Monitoring Committee of two independent clinicians and an independent statistician is installed who are responsible for monitoring early data and safety and efficacy of treatment. They will review the data of interim analyses and give written recommendations to the sponsor / sponsor's delegate whether the trial can be continued as planned.

Committee members of each participating group, reference physicians, trial management staff, data management committee, and the members of the Independent Data and Safety Monitoring Committee are named in Appendix A2.

1.10 Sponsor

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Responsibility of organising sponsorship for participating groups and centres outside the European Union lies with that group or centre.

1.11 Signature Page

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Intergroup Chairmen	
Date	Professor Sir Alan W Craft, MD
Date	Prof. Dr. Heribert Jürgens Leiter der Klinischen Prüfung Deutschland
Principal Coordinating Investigator	
Date	PD Dr. Uta Dirksen
Coordinating data centre representative	
Date	Prof. Dr. Wolfgang Köpcke

1.12 Summary and Flow Chart

1.12.1 Summary

EWING 2008 is a joint protocol of European and North American Ewing sarcoma study groups. The protocol is aimed at optimising treatment and treatment results of patients with Ewing sarcomas. The EWING 2008 protocol is open to all patients diagnosed with Ewing sarcomas, localised or metastatic, who are considered eligible for neoadjuvant chemotherapy. All patients registered will receive induction chemotherapy consisting of six cycles of vincristine, ifosfamide, doxorubicin and etoposide (VIDE). The decision regarding local therapy must be made following the fifth cycle of induction treatment, with a preference for surgical intervention with or without additional radiotherapy. Preoperative radiotherapy may be considered to improve the operability of otherwise inoperable lesions. In patients with localised disease or with pulmonary metastases, local treatment should be performed following the 6^{th} cycle of VIDE chemotherapy, and should be a complete tumour resection, whenever feasible. Post-operative radiotherapy is determined by the completeness of surgery and the histological response to chemotherapy.

Standard Risk R1

Good responders (R1) (< 10% viable tumour cells) with localised disease are allocated to the standard risk arm and will receive a further eight cycles of chemotherapy composed of vincristine, actinomycin D, and cyclophosphamide (VAC) (females) or ifosfamide instead of cyclophosphamide (VAI) (males). They will be randomised to receive add-on treatment with either fenretinide, zoledronic acid, fenretinide plus zoledronic acid, or no add-on treatment.

High Risk R2

Poor responders (R2) with localised disease will continue to be randomised as in EURO-E.W.I.N.G. 99 to receive either eight cycles of VAI chemotherapy or high dose treatment with busulfan-melphalan **(R2loc)**.

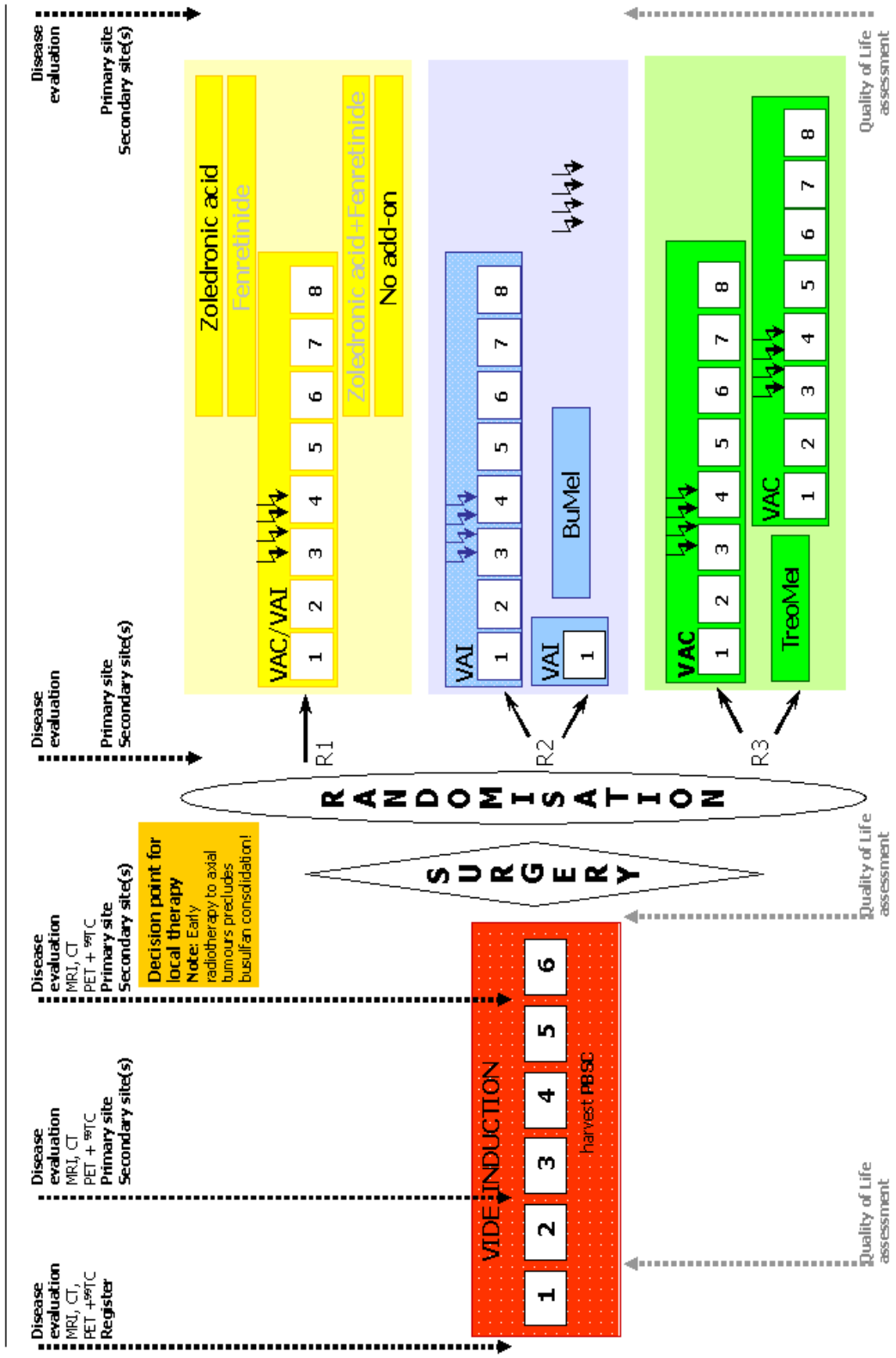
Patients with primary pulmonary metastases are also allocated to continue to be randomised as in EURO-E.W.I.N.G. 99 to receive either eight cycles of VAI chemotherapy or high dose treatment with busulfan-melphalan **(R2pulm)**.

Very High Risk R3

Patients with **disseminated disease**, i.e. dissemination to bone and/or other sites and possibly additional pulmonary dissemination **(R3)**, receive six cycles of VIDE induction chemotherapy. Patients are then randomised to either continue with eight cycles of vincristine, actinomycin D and cyclophosphamide (VAC) chemotherapy or high dose treosulfan-melphalan (TreoMel)

chemotherapy followed by autologous stem cell reinfusion followed thereafter by eight cycles of VAC chemotherapy. Local therapy in R3 patients is following VIDE induction, whenever feasible prior to high dose therapy (HDT). When long periods of immobilisation following surgery are anticipated, e.g pelvic reconstruction, surgery following HDT may be advisable. Depending on clinical response to induction chemotherapy radiotherapy prior to HDT and surgery may be an option to be considered in such patients. Any delay between VIDE and HDT for reasons of e.g. local treatment must be bridged with VAC cycles. The total number of VAC cycles is not to exceed eight cycles.

1.12.2 FLOW CHART



VIDE.....Vincristine, Ifosfamide, Doxorubicin, Etoposide
VAI.....Vincristine, Actinomycin D, Ifosfamide
VAC.....Vincristine, Actinomycin D, Cyclophosphamide
BuMel.....Busulfan, Melphalan
TreoMel..Treosulfan, Melphalan

2 SYNOPSIS

TRIAL CODE	EWING2008
EudraCT NUMBER	2008-003658-13
INTERGROUP CHAIRMEN	Prof. Dr. Heribert Jürgens; Professor Sir Alan W Craft, MD
PRINCIPAL COORDINATING INVESTIGATOR	PD Dr. Uta Dirksen
TITLE OF STUDY	EWING 2008
CONDITION	Ewing sarcoma
OBJECTIVES	**Primary objectives:** **Standard Risk R1:** in a randomised trial, to examine whether add-on treatment with fenretinide or zoledronic acid, or zoledronic acid plus fenretinide in addition to induction and maintenance chemotherapy improves event-free survival in patients with localised Ewing sarcoma and good histological response or with initial tumour volume <200ml compared to no add on treatment. **High Risk R2:** in a randomised trial, to examine whether high dose chemotherapy using busulfan-melphalan with autologous stem cell reinfusion, compared with standard chemotherapy, improves event-free survival in patients with localised Ewing sarcoma and unfavourable histological response or tumour volume>200ml (**R2loc**). In patients with pulmonary metastases high dose busulfan-melphalan chemotherapy with autologous stem cell reinfusion is randomised versus standard chemotherapy plus whole lung irradiation (**R2pulm**). **Very High Risk R3:** in a randomised trial, to examine whether the addition of high dose chemotherapy using treosulfan-melphalan followed by autologous stem cell reinfusion to eight cycles of standard adjuvant chemotherapy, compared to eight cycles of standard adjuvant chemotherapy alone, improves event-free survival in patients with primary disseminated disease. **Secondary objectives:** **Overall survival** **R1:** To investigate whether add-on fenretinide, zoledronic acid or zoledronic acid plus fenretinide improves overall survival compared to no add on treatment. **R2:** To investigate whether high dose chemotherapy with busulfan-melphalan improves overall survival. **R3:** To investigate whether high dose chemotherapy with treosulfan-melphalan improves overall survival. **Toxicity /Safety:** To evaluate short term toxicity and long term toxicity in all risk groups. **Outcome:** To analyse outcome (EFS, OS) of the entire group of patients. **Quality of life:** To describe the quality of life (QOL) longitudinally (i.e. during the course of treatment and thereafter) in patients and to determine the impact on QOL of the additional

	treatment (R1 and R3) after randomisation for consolidation treatment. Also when R2 patients have to remain consistent with EURO-E.W.I.N.G. 99, the assessment of QOL within the current trial does not violate any of the basic codes of practice defined within this treatment protocol. **Value of Positron Emission Tomography:** To examine the value of positron emission tomography in the diagnosis of Ewing sarcomas. **Time to Diagnosis:** To investigate the impact of the time to diagnosis on the presentation and outcome of the patients. **Other Secondary Endpoints: Ancillary studies** will be coordinated by a scientific advisory board. Tumour material and other biological material will be collected by reference pathologists.
SAFETY/TOXICITY	**Toxicity** is to be assessed prior to each cycle of chemotherapy. Haematological toxicity is to be assessed regularly during treatment. Anticipated adverse reactions (ARs, common toxicities), both serious and non-serious, will be recorded using a checklist which provides additional free text space for reporting other adverse events (AEs). Unanticipated serious adverse events, related or unrelated, must be reported on an SAE form within one business day. AEs are to be recorded from the first day of protocol defined therapy until three months after completion of protocol defined therapy. **Safety/toxicity of additional radiotherapy:** In patients receiving radiotherapy intervention toxicity parameters are to be evaluated prior to radiotherapy and regularly during radiotherapeutic intervention, and weekly up to 8 weeks after radiotherapy. To meet the secondary objective "toxicity of additional radiotherapy" those institutions participating in the associated Registry for the Evaluation of Late Side Effects after Radiotherapy in Childhood and Adolescence (RISK trial) will record and report relevant findings over a period of approximately 18 months post-radiotherapy. Patients receiving radiotherapy involving thoracic structures must have lung function tests. **Follow-up:** In addition late effects are to be assessed according to institutional and national guidelines. Close follow-up at 6-week intervals is recommended for the first year after end of chemotherapeutic treatment. Thereafter, the interval between follow-up investigations may be extended. After the fifth year of treatment, follow-up checks should be scheduled yearly.
STUDY DESIGN	Phase 3, open label, multi-centre, randomised controlled trial of international study groups.
DURATION OF SUBJECT PARTICIPATION	From treatment initiation until 3 months following completion of active oncological therapy.
DURATION OF TRIAL	EWING 2008 is anticipated to recruit patients for up to 6.5 years.
NUMBER OF SUBJECTS	1383

KEY INCLUSION CRITERIA	**Diagnosis:**	Histologically confirmed Ewing sarcoma of bone or soft tissue.
	Age and sex:	Either sex, age >48 months (for GPOH patients) and <50 years at the date of diagnostic biopsy. Younger or elderly patients may be reported to the appropriate office (see section 1.4) but are not included in this study.
	Registration:	≤ 45 days after diagnostic biopsy/surgery.
	Start of chemotherapy:	≤ 45 days after diagnostic biopsy/surgery.
	Informed consent:	Must be signed prior to study entry.
	Performance status:	Lansky or Karnofsky score > 50%, may be modified for handicapped patients.
	Haematological parameters:	Haemoglobin > 8 g/dl (transfusion allowed), Platelets > 80.000/µl (transfusion allowed), WBC > 2000/µl.
	Cardiac values:	LVEF > 40%, SF > 28%.
KEY EXCLUSION CRITERIA	More than one cycle of other chemotherapy prior to registration Second malignancy Pregnancy and lactation Concurrent treatment within any other clinical trial, except trials with different endpoints that due to the nature of their endpoints must run parallel to EWING 2008 e.g. trials on antiemetics, antimycotics, antibiotics, strategies for psychosocial support, etc.. Any other medical, psychiatric, or social condition incompatible with protocol treatment	
KEY TIME POINTS	**Registration:**	≤ 45 days after diagnostic biopsy.
	Reference pathology:	≤ 60 days after diagnostic biopsy.
	Radiol. response evaluation:	After VIDE 2 (earliest) or 3 (latest), After VIDE 5 (earliest) or 6 (latest), Prior to high dose chemotherapy, Prior to add-on in R1, i.e. after cycle 11 of chemotherapy, e.g. 6 VIDE + 5 VAI/VAC.
	Stem cell harvest:	After 3-4 cycles of VIDE.
	Surgery for primary tumour:	After 6 cycles of VIDE, In R3 patients prior to or after HDT.
	Randomisation: R1 and R2:	After 6 cycles of VIDE when histology is available.

	R1 and R2:	Latest after 6 cycles of VIDE when surgery is not indicated.
	R3:	Latest after 6 cycles of VIDE.
	Surgery for metastases:	After 2 cycles of consolidation treatment or after high dose treatment.
	Definitive radiotherapy:	After 6 cycles of VIDE parallel to consolidation chemotherapy, In R3 patients prior to or after HDT.
	Pre-operative radiotherapy:	Prior to surgery.
	Post-operative radiotherapy:	Concurrently with consolidation chemotherapy. In patients receiving HDT, 8-10 weeks after HDT.
	Quality of Life assessment:	After VIDE 1 (earliest) or 2(latest), After VIDE 6 (prior to local treatment), After completion of protocol treatment, After 2 years follow-up.
ENDPOINTS - REQUIREMENTS	**Primary endpoint: Event-free survival** Each investigator is responsible for the regular follow-up of all patients. The national and group offices are responsible for at least one yearly update on events. **Secondary endpoint**: **Overall survival** Each investigator is responsible for the regular follow-up of all patients. The national and group offices are responsible for at least one yearly update on events and survival. **Secondary endpoint: Safety/Toxicity** The acceptability of the safety profile of chemotherapy and radiotherapy administered throughout this trial and thereafter will be assessed by the incidence rates of toxicities (adverse reactions) recorded by checklist, and the incidence of reported unanticipated serious adverse events throughout the trial and in the follow-up period to include late toxicities. Unanticipated SAEs will be reported on an SAE Form. Other, anticipated AEs (toxicities), both serious and non-serious, will be recorded by checklist. **Secondary endpoint: Value of PET / PET-CT** PET analyses will be performed at initial diagnosis, and after the 2nd (or 3rd)) and 5th (or 6th) cycle of VIDE. The value for diagnosis and response evaluation will be assessed in comparison with standard imaging procedures such as MRI, CT scan, 99TC scintigraphy. For response evaluation, histological response will serve as an additional parameter to be compared. As FDG-PET and PET-CT may not be available in all participating institutions, PET is not mandatory and the lack of a PET investigation does not	

	violate any of the basic codes of practice defined within the treatment protocol. Also, when R2 patients have to remain consistent with EURO-E.W.I.N.G. 99, the inclusion of a PET investigation for diagnosis and follow-up within the current trial does not violate any of the basic codes of practice defined within this treatment protocol. **Secondary endpoint: Quality of life (QOL)** QOL will be assessed in R1 and R3 patients four times during protocol treatment: - after completion of the first VIDE cycle and prior to starting the 2nd cycle of induction chemotherapy, - within two weeks after completion of induction chemotherapy and before surgery or irradiation, - after completion of the protocol therapy, - two years after completion of the protocol therapy. **Secondary endpoint: Impact of the time to diagnosis on presentation and outcome** The impact of the time to diagnosis will be assessed when the last patient enrolled in the study has finished treatment.
STUDY TYPE	Phase 3, open label, randomized controlled, multi -centre, trial of international groups.
STATISTICAL ANALYSIS	Group sequential plans for all randomised risk groups (R1-R2-R3) will be conducted with 3 planned interim analyses and early stopping rules using the O'Brien and Fleming design. For the randomised questions survival probabilities will be assessed according to the method of Kaplan and Meier and will be compared by logrank tests. Safety data per cycle will be analysed using descriptive statistics. For each patient and each type of toxicity, the number of cycles with a severe (grade 3 or 4) reaction recorded will be calculated. Significant differences between the groups (gender, age, tumour volume) in terms of number of cycles with severe toxicities will be tested using Wilcoxon two sample test and Kruskal–Wallis analysis of variance, respectively.
SAMPLE SIZE	**Calculated sample size**: 1383 patients are to be randomised: R1: 626 R2loc: 284 R2pulm: 288 R3: 185
TRIAL DURATION	Anticipated accrual time for risk groups: R1: 6.5 years R2loc: 5 years R2pulm: 5 years R3: 6.5 years Duration of the entire trial: 6.5 years with additional follow-up of 2 years
PARTICIPATING CENTRES	Please refer to website.

PREVIOUS PROJECT NUMBER	Not applicable
ETHICAL CONSIDERATIONS	This study is conducted in accordance with applicable laws and regulations including, but not limited to, the ethical principles that have their origins in the Declaration of Helsinki and the International Conference on Harmonisation Guideline for Good Clinical Practice (GCP). Before any subjects are enrolled competent authorities and ethics committees concerned must grant authorization and approval of this clinical trial. Before any procedures specified in this protocol are performed, the subject or subject's parent(s)/legal guardian must sign and date the approved informed consent form according to requirements stated in national law.

3 BACKGROUND AND RATIONALE

3.1 Characterisation of Ewing sarcomas

Ewing sarcomas are characterised as tumours consisting of small, blue, round malignant cells that may exhibit varying degrees of neural differentiation. Ewing's sarcoma, malignant peripheral neuroectodermal tumour, Askin tumour and atypical Ewing's sarcoma are summarised under the term Ewing sarcoma. These tumours are characterised by a re-arrangement of chromosome 22, in more than 95% of cases in the form of an 11;22 translocation (Ambros, 1991; Aurias, 1984; Delattre, 1994; Dockhorn-Dworniczak B, 1994; Kovar, 1990; Turc Carel C, 1983; Whang PJ, 1984). The gene rearrangement results in the production of a transcription factor, in the majority EWS-FLI1 transcription, that was described to exhibit structural variability of potential prognostic relevance depending on chromosomal breakpoint locations (De Alava, 1998; Zoubek, 1996).

Most Ewing sarcomas arise in bony sites, and they represent the second commonest primary osseous malignancy in childhood and adolescence. The annual incidence is estimated at 0.6-1/million population. Staging procedures identify approximately 25% of patients as metastatic at diagnosis. Prior to the era of combination chemotherapy the prognosis of patients with Ewing sarcoma was poor with more than 90% of patients dying from secondary metastases (Patricio MB, 1991). Therefore, Ewing sarcomas must be regarded as a systemic disease. During the past 30 years the prognosis has dramatically improved owing to the introduction of multimodal treatment including combination chemotherapy, surgery and radiotherapy.

For more than 30 years, national and international groups have addressed their efforts to identifying optimal treatment strategies to improve the long term survival rate in patients with Ewing sarcoma. Actinomycin D (A), cyclophosphamide (C) and vincristine (V) were introduced in the 1970s (Jaffe, 1976). The benefit of additional anthracyclines was shown in the 1980s (Nesbit, 1990; Jürgens, 1988; Craft, 1997). Data on the value of ifosfamide as compared to cyclophosphamide are not consistent; however, a beneficial synergistic effect of ifosfamide (I) with etoposide (E) has been described by several groups (Grier, 2003; Meyer, 1992; Paulussen, 2001; Rosito, 1999).

3.2 Treatment Results in Localised Disease

The 5-year survival rate in localised ET ranges from 60 to 70%. This has been shown in the three major Ewing sarcoma trials, i.e. EICESS 92, IOR, and IESS/SSG. All of those trials have employed identical agents: A (actinomycin D), D (doxorubicin (adriamycin)), E (etoposide), C (cyclophosphamide), V (vincristine), with different doses and schedules of administration.

The EICESS 92 protocol has accomplished two parallel risk-adapted randomised trials. The risk groups were defined by stage and tumour volume. Patients with localised tumours of < 100ml were stratified into the standard risk group and patients with tumours > 100ml and/or metastatic disease were allocated to the high risk group. All patients received a four drug induction treatment of ifosfamide, vincristine, doxorubicin and actinomycin D (VAIA). Subsequently, consolidation treatment randomly allocated standard risk patients to either VAIA or VACA. In the high risk arm VAIA was compared to EVAIA with the addition of etoposide (E) (Paulussen, 2001; Schuck, 2003).

The American IESS II schedule used IE alternating with VAdriaC as the standard chemotherapy regimen with beneficial effect in patients with localised disease (Grier, 2003).

The Italian/Scandinavian ISG/SSG III trial was designed for standard risk patients. Induction treatment consisted of VAC, V, VAI and EI cycles. Patients were stratified according to histological response and allocated accordingly to different treatment arms: Good responders received 9 cycles of standard dose chemotherapy consisting of VAC, VAI, and EI. Poor responders received VAC, EC, and EI followed by high dose busulfan-melphalan (Ferrari, 2007).

All studies have identified similar risk factors. Unfavourable outcome is associated with age >15 years, large tumour volume, and poor histological response following induction treatment. Furthermore, the EICESS 92 trial has shown that surgery of the primary tumour was associated with a significantly lower local recurrence rate.

The EURO-E.W.I.N.G. 99 protocol employs VIDE induction chemotherapy (Jürgens, 2006) followed by risk-adapted randomised treatment. Patients are stratified into risk groups depending on a number of prognostic factors, including treatment-independent factors such as presence and site of metastases, discriminating between pulmonary and extrapulmonary, mainly skeletal metastases. In patients with localised disease the volume of the primary tumour and/or the histological response to induction chemotherapy are critical factors for stratification into the standard or high risk group. Standard risk patients (R1) are randomised for consolidation treatment with either VAI or VAC. High risk patients (R2) are randomised for high dose busulfan-melphalan versus VAI. Patients with extra-pulmonary metastatic disease (R3) are not randomised. In these patients high dose chemotherapy followed by autologous stem cell reinfusion or participation in a phase II study is recommended. The EURO-E.W.I.N.G. 99 trial is still ongoing and recruiting patients. Accrual into the R1 arm is above target while R2 loc has been recruiting fewer patients than expected. This discrepancy between estimated and actual recruitment appears to be primarily due to an improved histological response following the VIDE induction.

The implementation of multidrug chemotherapy using alkylating agents, topoisomerase II inhibitors and other chemotherapeutic agents that induce DNA strand breaks and the introduction of intensified doses have raised the 5-year event-free survival from 50% to 70%. The number of chemotherapy cycles in different trials varied between 12 and 16, with no obvious difference in event-free survival. The introduction of the combination of ifosfamide and etoposide and the introduction of high dose ifosfamide have significantly improved survival (Grier, 2003; Meyer, 1992). Late local treatment > 15 months from start of treatment was associated with a poor prognosis.

While type, combination and dose intensity of chemotherapy have been established to have major impact on the prognosis of Ewing sarcoma patients, the influence of treatment duration is still a matter of debate and controversy. As yet, no randomised trial has addressed the question whether long term consolidation is of benefit. In addition high cumulative doses of chemotherapeutic agents are associated with long term and late toxicity. Amongst agents commonly used in Ewing sarcoma trials, vincristine is associated with a high incidence of long lasting neuropathy (Moress, 1967), alkylating agents, with a high incidence of nephrotoxicity and infertility (Moore, 1991; Ridola, 2007), anthracyclines, with cardiotoxicity (Hudson, 2007). Thus, the identification of novel agents is essential.

3.2.1 Rationale for the R1 Gender-Stratified Standard Consolidation

The outcome of the current randomised trial in R1 patients of the EURO-E.W.I.N.G. 99 protocol is unlikely to be available before the opening of EWING 2008. Furthermore, the specific question of difference in toxicity between VAI and VAC will require a longer follow-up time for the toxicity data to mature and be collected. After VIDE induction chemotherapy and local control, R1 patients are currently randomised to either VAC or VAI chemotherapy. The study question assumes equivalency in terms of event-free and overall survival between the two arms but when the data are mature there may be a difference in toxicity outcome, in particular regarding nephrotoxicity and the risk of infertility.

Nephrotoxicity

The severity of nephrotoxicity following ifosfamide varies significantly (Skinner, 1993). The incidence of significant chronic glomerular toxicity in children ranges from 1.4% to about 30%, depending on the patient group and the measures of toxicity studied (Ashraf, 1994; Skinner, 1996). Chronic renal failure has been found to be rare, though more likely in adolescents or adults than in younger children. Clinically relevant tubular toxicity leading to Fanconi syndrome, renal tubular acidosis, or nephrogenic diabetes insipidus is detected in about 5% of patients (Rossi, 1995; Skinner, 1993, 1996). Risk factors for the development of nephrotoxicity after ifosfamide

include a higher cumulative ifosfamide dose (>60-80g/m^2) as currently used in the VAI arm of the R1 randomisation in Euro-E.W.I.N.G. 99, younger patient age at treatment (not the typical age group of Ewing sarcomas), and pre-existing renal impairment (Skinner, 2000). There are no reports describing nephrotoxicity in children after cyclophosphamide; but reversible proteinuria has been reported in an adult patient given intravenous and intraperitoneal cyclophosphamide (Lopes, 1967). Cases of an acute anti-diuretic effect leading to fatal hyponatremia have been reported in adults (Harlow, 1979).

Infertility

Ifosfamide as an alkylating agent would be expected to be gonadotoxic, but little has been published on the definite effects on fertility following ifosfamide, as opposed to cyclophosphamide for which there is extensive literature (Whitehead, 1983). An assessment of fertility in 14 females following a mean ifosfamide dose of 65g/m^2 (27-96g/m^2) showed hormone levels to be normal on day 3 of the follicular phase except for anti-Mullerian hormone which was below normal range in 4 out of 14 women tested (Gill Levitt, personal communication). There are several anecdotal reports of women with normal live births following ifosfamide chemotherapy according to Ewing protocols (JHA, 2005).

In males, the data on testicular function and hence fertility following ifosfamide are also very limited. In studies assessing males following ifosfamide treatment with a total dose of more than 60 g/m², FSH was abnormal in 8 out of 26 patients, inhibin B was abnormal in 13 out of 26, and 8 out of 13 patients had a low sperm count. One patient had fathered a child. In a smaller study that included 6 children and adolescents treated with ifosfamide-based regimens (total dose 84-126g/m^2), only 2 had a normal semen analysis, 2 were oligospermic and 2 azoospermic (Thomas, 2002).

The effect of high-dose cyclophosphamide on female fertility has been extensively investigated. Ovarian function, as defined by spontaneous menstruation and normal hormone profile without hormone supplement, was assessed in 103 women receiving high dose cyclophosphamide alone. Ovarian function was preserved in 54% (Sanders, 1988). It may thus be assumed that ovarian function is likely to be preserved at the cyclophosphamide dose of 8.4g/m^2, following VIDE x 6 chemotherapy, as used in the R1 VAC arm of EURO-E.W.I.N.G. 99. The EURO-E.W.I.N.G. 99 study is ongoing. Late effects concerning the randomised treatment arms VAC and VAI will be analysed after closing the trial. In males who received cyclophosphamide in combination with other chemotherapy agents for the treatment of solid tumours, azoospermia was permanent in 90% of men treated with cyclophosphamide doses >7.5g/m^2 (Meistrich, 1992). Considering that the current dose of cyclophosphamide in the VAC arm of R1 is >7.5g/m^2 and all patients also have VIDE x 6 induction chemotherapy one would thus expect male patients to be infertile.

The risk of significant nephrotoxicity is certain but small with ifosfamide and virtually non-existent with cyclophosphamide for both genders. The risk of females would be small or equivalent with cyclophosphamide and ifosfamide, assuming there is no direct irradiation of the ovary or uterus. Any possible advantage for preserving fertility would lie with ifosfamide. Regarding males there might be a certain chance for some patients to preserve fertility with an ifosfamide-based regimen. Thus, until the upcoming follow-up data of the EURO-E.W.I.N.G. 99 study are analysed, treatment will be administered adapted to the gender-specific risks of toxicity, using cyclophosphamide in females (VAC) and ifosfamide in males (VAI) (Ridola, 2007). It is hoped that this strategy preserves both renal and gonadal function in female patients and preserves gonadal function in some proportion of the males with an acceptable risk of renal tubular toxicity.

3.3 Rationale for a Randomised Add-onTrial in R1 Patients

The patients eligible for stratification in R1 are those with the most favourable outcome. R1 patients are expected to have an overall survival at 5 years of close to or above 70%. Still, there is a risk of relapse of about 30%. Recurrence of disease is associated with a poor outcome (Hunold, 2006; Rodriguez-Galindo, 2002).

Hence, there is clearly a need for improving survival in this young population of patients. The use of more intensive conventional chemotherapy appears to have clear limits due to toxic effects. More than 80% of relapses occur early, i.e. within the first two years following diagnosis. Therefore, the EWING 2008 trial aims to test whether add-on treatment with substances inducing apoptosis in residual tumour cells or preventing tumour angiogenesis is able to improve the event-free survival in this risk group. Amongst agents offering potential benefit without aggravating chemotherapy-related side effects are zoledronic acid and fenretinde.

3.3.1 The value of bisphosphonates in the treatment of Ewing sarcomas

Bisphosphonates are pyrophosphate analogues with replacement of the central oxygen by carbon. Variations in the side chain generate a large variety of compounds with different pharmacological patterns. According to the side chain, bisphosphonates can be classified into nitrogen-containing and non-nitrogen containing agents. All bisphosphonates are effective inhibitors of bone resorption and have been widely used for the treatment of osteoporosis, osteogenesis imperfecta, systemic osteolytic bone disease or local bone loss. Osteoclasts are the preferred target cells of bisphosphonate action. Bisphosphonates show a high affinity to hydroxyapatite. They are resorbed by activated osteoclasts and subsequently inhibit osteoclast activity (Masarachia, 1996). Non-nitrogen containing bispohosphonates are intracellularly metabolised to cytotoxic analogues of ATP leading to an early cell death of target cells. Nitrogen-containing bispohosphonates (N-BP), which are much more potent at inhibiting bone resorption *in vivo* act by inhibiting farnesyl diphosphate

synthase (FPP), a key enzyme of the mevalonate pathway. Consequently N-BP inhibits farnelysation and geranygeranylation of small G-proteins such as Ras, Rap1 and Rho (Kuroda, 2003, Nogawa 2005, Sato 2005, Yuasa 2005). The ability of inhibiting FPP is dependent on the modification of the structure and confirmation of the R2 side chain and the ability of inhibiting bone resorption correlate with the ability of inhibiting FPP. Therefore, FPP seems the major target of N-BPs.

Anti tumour effects of N-bisphosphonates

The anti-tumour effects of N-BPs are also correlated with an inhibition of FPP, as *in vitro* studies have shown that some of the effects can be reversed by replenishing tumour cells with downstream products of the mevalonate pathway, i.e. farnesol or geranylgeranol, which are required for farnelysation and geranylgeranylation of small G-proteins. Furthermore some N-BPs have been shown to inhibit angiogenesis *in vitro* and *in vivo* (Green, 2004; Giraudo, 2004; Croucher, 2003) and to lower serum levels of proangiogenic vascular endothelial growth factor and platelet derived growth factor in cancer patients (Santini, 2003 and 2006). N-BPs are widely used for the treatment of bone metastases in patients with breast cancer (Powles, 2006; Rosen, 2003), multiple myeloma (Rosen, 2003), and prostate cancer (Berry, 2006). Similar mechanisms are responsible for the induction of apoptosis of cancer cells (Mackie, 2001; Sonnemann, 2001; Ory, 2007).

Effect of N-bisphosphonates in Ewing sarcoma cells

In vitro and *in vivo* data have proven the anti-tumour activity of N-BPs against Ewing sarcoma cells:

i) N-BP pamindronate inhibits growth in eight different Ewing sarcoma cell lines via inhibition of the mevalonate pathway (Sonnemann, 2001). By contrast, non N-bisphosphonate clonodrate did not impair cell growth and viability in Ewing sarcoma cell lines.

ii) Zhou et al showed significant inhibition in the development of bone metastases after injection of zoledronic acid *in vivo*. N-BPs induced apoptosis and inhibited osseous metastases (Zhou, 2005).

iii) Zoledronic acid has a direct inhibitory effect on the growth of Ewing tumor cells in *vitro* which is induced by aopoptosis associated with caspase 3 activation and cell cycle arrest in S phase. This effect was synergistically enhanced by alkylating agents. Using an *in vivo* mouse model, zoledronic acid exerts a strong inhibitory effect on the growth of bone Ewing tumor and little effect on the growth of intramuscularly injected Ewing tumor (F. Rédini, Laboratoire Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, EA3822 - Inserm ERI7, Nantes – France, personal communication).

iv) The effects on Ewing sarcoma cells described in i) and ii) were obtained at concentrations which are not achieved *in vivo*. Serum levels of N-BPs have been reported to reach 10µM. The concentrations used in the above cited studies were 40µM. The strong affinity of BPs to bone mineral does, however, lead to much higher concentrations in bone (Gligorov, 2000).

Clinical studies with bisphosphonates and experience in children

New N-BPs have frequently been used in children with osteolytic bone disease such as osteonecrosis following chemotherapy. In a prospective clinical study pamindronate was given to 11 infants with a median age of 3.6 months (Astroem, 2006) and 41 children and adolescents (1.5-15 years (Zeitler, 2006)) over 3-6 years. In this study no adverse effects were observed. To evaluate the safety and efficacy of N-BPs in adolescents with osteoporosis 22 patients with an average age of 13.3 years (range 4.3-19 years) were treated over 1-3 years. Again, no side effects were observed (Unal, 2006). Eighteen children and adolescents between 6.2 and 17.5 years with moderate polyostotic fibrodysplasia received pamindronate for 1.2-9.1 years (average treatment duration 3.8 years), with no serious side effects (Lala, 2000).

By contrast, some adult patients treated for osseous metastases have shown osteonecrosis of the jaw after 2-3 years of treatment with novel N-BPs such as pamindronate and zoledronic acid (Sanna, 2006). In the EWING 2008 study treatment duration will be restricted to nine months. As yet, no irreversible side effects such as osteonecrosis have been reported within this time limit.

3.3.2 The value of fenretinide in the treatment of Ewing sarcomas

Fenretinide (N-(4-Hydroxyphenyl) retinamide) is a synthetic vitamin A analogue that causes apoptosis in many cancer cell lines. This is related to an increase in reactive oxygen species in Ewing sarcomas when exposed to fenretinide and the subsequent activation of the caspase enzymes. Fenretinide-induced cell death in Ewing sarcomas is dependent on activation of $p38^{MAPK}$ (Myatt*,* 2005) and increased ceramide levels (Maurer, 2000), which inhibit telomerase activity (Ogretmen, 2002). Telomerase activity is high in Ewing sarcomas (Fuchs, 2004), reflecting up-regulation driven by the *EWS-ETS* genes (Takahashi, 2003). Fenretinide-induced cell death in Ewing sarcomas is accompanied by a dose-dependent decrease in telomerase activity (Burchill, personal communication). Investigations into the effect and pharmacokinetic profile of fenretinide in Ewing sarcomas have demonstrated that:

i) Fenretinide induces cell death *in vitro* in all Ewing sarcoma cell lines examined in a dose- and time-dependent manner (Myatt, 2005).

ii) Fenretinide significantly delays Ewing sarcoma growth in preclinical mouse models (Myatt, 2005).

iii) There is no synergy or antagonism between fenretinide and conventional agent induced cell death in Ewing sarcomas (Burchill, personal communication).

iv) The effects in i) and ii) are achieved at pharmacological levels (0.6-2 micromolar; [Burchill, personal communication]) below those achieved in a phase I clinical study in children.

v) The efficacy of fenretinide-induced cell death is reduced in Ewing sarcoma cell lines incubated in hypoxia (Batra, 2004; Burchill, personal communication); death is not induced until 48 hours and requires 6-10 micromolar of fenretinide (Burchill, personal communication). Sensitivity to fenretinide may be enhanced when given in combination with inhibitors of ceramide breakdown (Batra, 2004). These observations suggest that when using the current oral formulation of fenretinide (with which it is possible to achieve up to 10 micromolar plasma levels) its efficacy in hypoxic areas of Ewing sarcomas may be reduced. Therefore we anticipate that current oral formulations of fenretinide are likely to be most useful in the minimal disease setting in Ewing sarcomas. In this setting the proportion of cells experiencing hypoxia will be smaller than in clinically detectable tumours.

Previous clinical studies with fenretinide

i) There have been a number of phase I and II studies of fenretinide in a range of tumours in adults. In children two phase I studies of fenretinide have been published (Garaventa A, 2003; Villablanca, 2006) indicating that with doses above 1400mg/m^2 mean plasma levels above 4 micromolar were achieved.

ii) There is only one published clinical study reporting the use of fenretinide in children and young adults with Ewing sarcomas. A study of fenretinide in 54 children with advanced solid tumours included five patients with Ewing sarcomas and they reported one case of stable disease (Villablanca, 2006).

In these previous studies, fenretinide has been well tolerated with minimal toxicity. An MTD for fenretinide has not been identified in adults. In children, an MTD of 2475mg/m^2 has been reported (Villablanca, 2006). Based on the available evidence, the risks to patients from taking fenretinide in this trial are thought to be minimal.

3.3.3 Rationale for the staggered introduction of fenretinide

Based on the pre-clinical and clinical data above, fenretinide is anticipated to be effective as a single agent in the minimal disease setting. However, in contrast to the long term experience in using bisphosphonates, there are very few clinical data on the feasibility, safety and efficacy of

fenretinide treatment in Ewing sarcomas. Therefore, a phase II study on the use of fenretinides has been initiated in the UK. The study was designed to perform this assessment in two ways.

i) Assessing the feasibility of achieving effective plasma levels and good compliance using the current oral formulation.
ii) Directly addressing disease response to the agent by novel biological and established clinical methods.

The dose and schedule of fenretinide chosen for this study is predicted to achieve plasma levels in the range of 4-11µM (Garaventa, 2003; Villablanca, 2006 and NCI, personal communication). This is anticipated to be in excess of the level required (2µM) to produce a response in Ewing sarcomas (Myatt, 2005). The medication is given once daily and the ability of patients to take the required number of capsules will be assessed as part of the protocol. After completion of the phase II trial and based on the recommendation of the DMC, fenretinide will then be adopted in the EWING 2008 protocol in a two by two design.

Rationale for a randomised trial in R1 patients: Conclusion

The EWING 2008 R1 trial is aimed at investigating whether maintenance treatment improves the survival rate in Ewing sarcoma patients eligible for randomisation in R1 (please refer to 7.1). Following VIDE induction all R1 patients are to receive eight cycles of VAC (females) or VAI (males) chemotherapy. Patients are randomised to additional treatment with bisphosphonates, with fenretinide, or with bisphosphonates plus fenretinide, or no additional treatment.

The protocol will be started with a randomisation for bisphosphonates. Randomised allocation of fenretinide treatment will be opened as soon as the ongoing phase II trial on fenretinide is completed as expected in 2009. The study will thus start with an initial phase, where patients are randomised to bisphosphonate treatment, and will later implement the two by two design testing both bisphosphonates and fenretinide for their impact on event-free survival.

3.4 Treatment Results in Patients with Unfavourable Localised Disease or Primary Pulmonary Metastases (R2 patients)

Based on the results from the CESS and EICESS studies (Figure 1) (Paulussen, 1993, 1998; Schuck, 2003) and other trials (Kushner, 1993; Picci, 1993; Oberlin, 2006) the EURO-E.W.I.N.G. 99 trial randomised patients with unfavourable localised disease and/or pulmonary metastases (R2) to high dose chemotherapy with busulfan-melphalan (BuMel) followed by reinfusion of autologous haematopoietic stem cells versus standard treatment with VAI. More recent analyses from other groups have confirmed the definition applied to R2 (Lin, 2006; Obata, 2006), but the question whether HD therapy might induce a favourable outcome remains to be proven. BuMel high dose treatment is contraindicated in many of the R2 patients who require radiotherapy to the

central axis. Given the importance of the study question, the EURO-E.W.I.N.G. 99 Data Monitoring Committee, despite randomisation numbers below the expected rate, has recommended to complete randomisation in this group of patients. The EURO E.W.I.N.G. steering committee has in view of the importance of the study question based on a careful review of an interim analysis strongly decided to follow this recommendation to complete the R2 randomisation within the EWING 2008 trial.

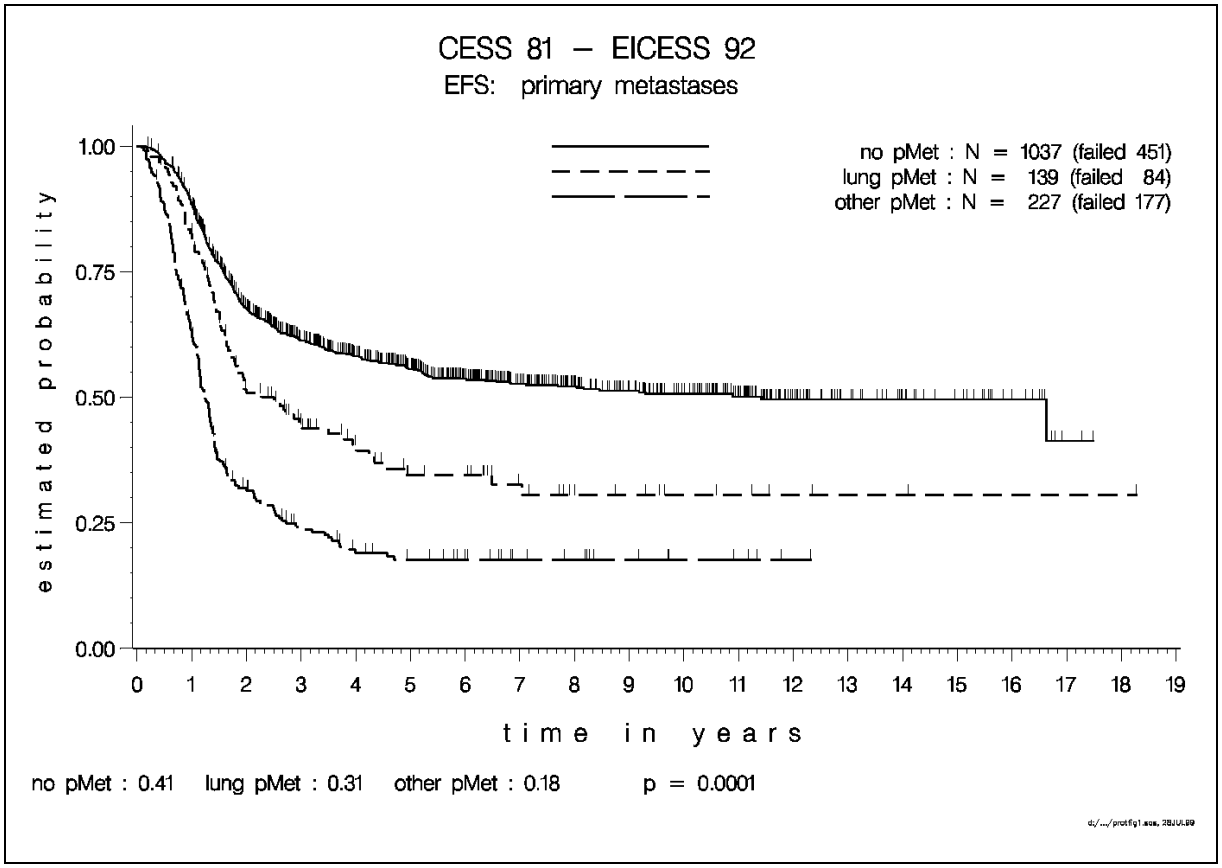


Figure 1: EFS according to presence and sites of primary metastases

3.5 Treatment Results in Patients with Disseminated Disease (R3)

Within the EURO-E.W.I.N.G. 99 trial 192 patients with primary dissemination, i.e. dissemination to bone and/or other sites and possibly additional pulmonary dissemination, were registered. In contrast to the distribution in the entire group of patients with Ewing sarcomas, the primary site in this subgroup was extremity in only 57 patients and axial/other in 135 patients (41% pelvis). The recommended treatment scheme included six cycles of VIDE induction, one cycle of VAI, and high dose chemotherapy followed by reinfusion of autologous haematopoietic stem cells. The VIDE induction cycles were completed by 168 patients (85%) and 116 patients were referred to high dose chemotherapy with busulfan (Bu), 600mg/m², and melphalan (Mel), 140mg/m², followed by reinfusion of autologous haematopoietic stem cells (SCR). The overall survival at 3 years in the total group of 188 evaluable patients was 29% (95% CI=0.04). The outcome in patients treated

with BuMel/SCR was significantly superior compared to others (Figure 2). Regarding patients who received BuMel/SCR it is noteworthy that 37 patients younger than 14 years achieved an EFS of 47% in comparison with an EFS of 22% (p=0.026) in their older counterparts >14 years. The multivariate analysis identified two major risk factors at diagnosis: primary tumour volume >200ml (p<0.001 (RR 2.25)) and >5 bone metastases (p=0.064 (RR 2.11)) (Figure 3). Thus, a subgroup of patients responding to VIDE induction has benefited from BuMel/SCR in particular in the absence of high adverse risk factors. These results must be considered biased by selection of a favourable group for busulfan-containing high dose chemotherapy: 15% of patients with disseminated Ewing sarcoma did not complete the VIDE induction mainly due to early progression. Furthermore, it has to be considered that busulfan-containing high dose chemotherapy is not compatible with radiotherapy to the central axis. Patients with large pelvic tumours (associated with a poor outcome even in patients with localised disease) who required radiotherapy were excluded from BuMel/SCR (Ladenstein, 2007).

3.5.1 Rationale for randomisation in R3 patients

The incompatibility of a busulfan-containing high dose chemotherapy regimen with radiotherapy to axial sites has prompted the search for alternative regimens. Treosulfan is a prodrug of a bifunctional alkylating cytotoxic agent and structurally related to busulfan. Treosulfan has been frequently used in high-dose treatment protocols followed by allogeneic or autologous haematopoietic stem cell transplantation/reinfusion. Published studies point out its remarkable safety with very low non-haematological toxicity in heavily pre-treated patients and in patients who are at high risk of treatment-related toxicity (Beelen, 2004; Koenigsmann, 2004). However, no study has yet been published concerning the effect of treosulfan in Ewing sarcomas. *In vitro* data have shown stronger growth inhibition by treosulfan compared to busulfan in Ewing sarcoma cell lines (Lanvers, 2006). Consequently, an amended protocol was offered to the patients within the GPOH group, and 22 patients were treated with a treosulfan-containing high dose regimen (Dirksen, 2006). The treatment was well tolerated even when given along with radiotherapy to central axial sites (Dirksen, 2006). In order to evaluate the value of high dose chemotherapy in patients with high risk Ewing sarcoma the EWING 2008 randomised R3 trial will be testing eight cycles of vincristine, actinomycin D and cyclophosphamide (VAC) chemotherapy compared to high dose chemotherapy using treosulfan-melphalan (TreoMel) followed by autologous stem cell reinfusion plus VAC consolidation chemotherapy. This treatment will be complemented by appropriate local treatment.

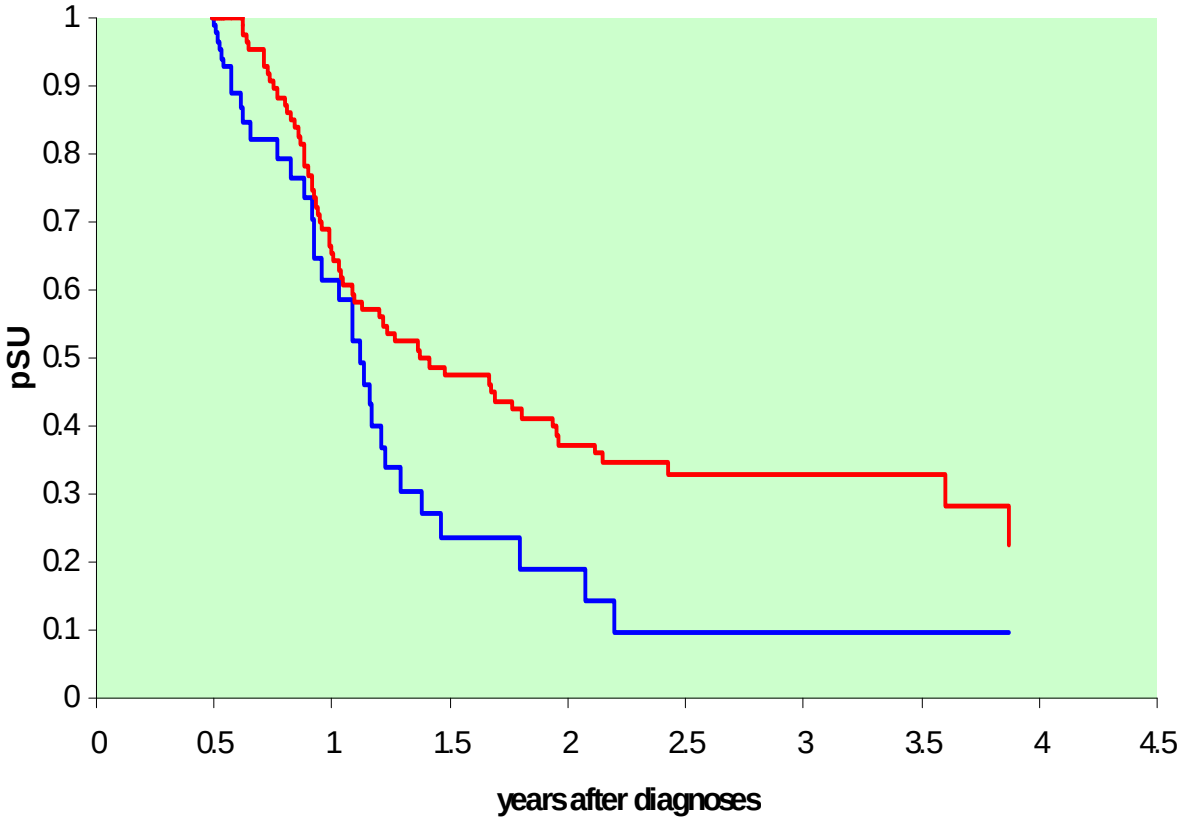


Figure 2: Outcome in patients with primary extrapulmonary metastases after 180 days with BuMel (red line) n=91; 3-yr pEFS=0.34±0.05; without BuMel (blue line) n=97; 3-yr pEFS=0.09±0.05

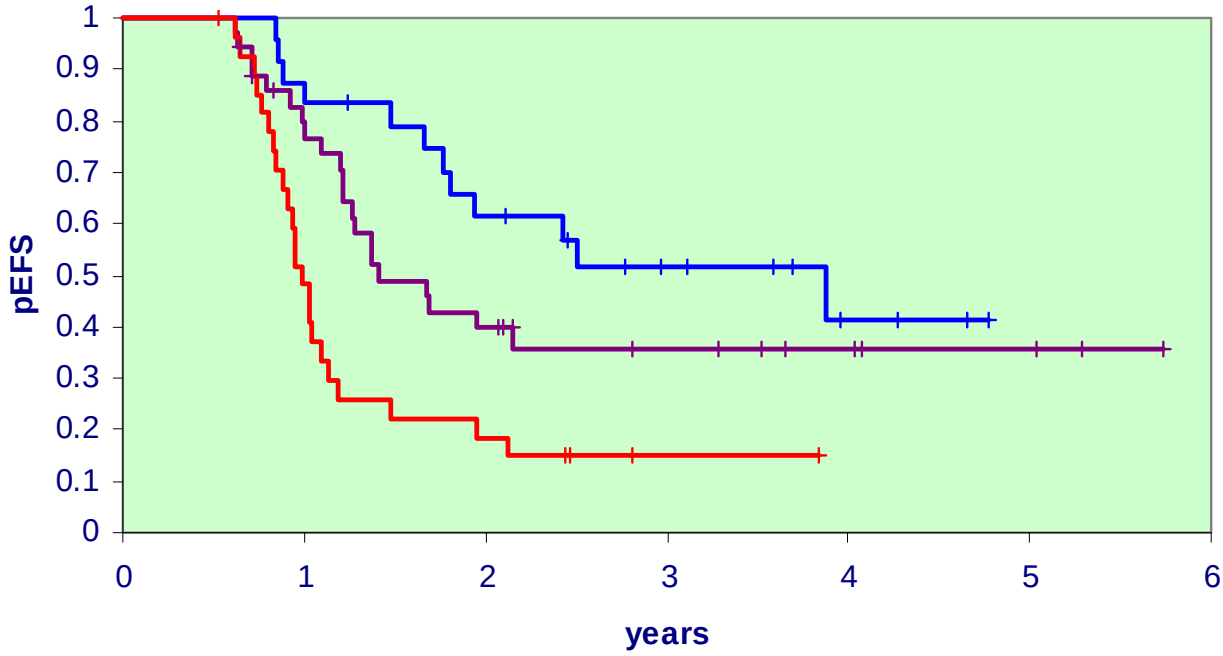


Figure 3: Event-free survival in patients with extrapulmonary metastases according to tumour volume and bony lesions. The 3-year EFS was 56% in 35 pts with <200ml and <5 bone lesions (blue line), 29% in 84 pts with <200ml and >5 bone lesions (purple line), 19% in 67 pts with >200ml and/or <5 bone lesions (red line) and extremely dismal with 7% in 47 pts with >200ml and >5 bone lesions (p<0.001).

3.6 Rationale for Integrating FDG-PET into Staging and Response Assessment

Fluoro-Deoxy-Glucose-Positron Emission Tomography (FDG-PET) is increasingly used in the diagnosis of Ewing sarcomas in many treatment centres. However, a systematic evaluation of FDG-PET results has not been performed as yet and data obtained from single centres may be considered biased and not appropriate as a rationale for routinely using PET for primary diagnosis and follow-up. Single centre analyses show superiority of FDG-PET in assessing bone/bone marrow and soft tissue involvement compared with conventional staging (Franzius, 2000; Gyorke, 2000). FDG-PET findings may lead to changing the stage and consequently the treatment group in some patients. Regarding the detection of lung metastases helical pulmonary CT was superior to FDG-PET (Franzius, 2001). Therefore, it is crucial to detect those patients for stage-adapted therapy intensification. Furthermore, there are still some patients with initially localised disease according to conventional staging who show an early relapse with bone/bone marrow involvement. It may be hypothesized, that FDG-PET is able to determine initial bone/bone marrow metastases not seen on conventional imaging.

Furthermore, FDG-PET can be used for non-invasive response assessment after neoadjuvant chemotherapy, and post-therapeutic FDG uptake has been shown to predict outcome (Franzius, 2000; Hawkins, 2002, 2005). If FDG-PET is able to determine response early in the course of chemotherapy (e.g. after 2 cycles of chemotherapy), it might be used for optimising treatment in future trials. Response assessment at the end of chemotherapy is decisive for planning local therapy. It is proposed that FDG-PET can determine response non-invasively after chemotherapy and is superior to conventional imaging in this indication. The timing of FDG-PET is therefore crucial. The EORTC PET study group recommends a minimum time interval after chemotherapy of 2 weeks before FDG-PET imaging (Young, 1999). Therefore early and late response assessment is scheduled from day 14 after the last chemotherapy application. In EWING 2008, PET will be evaluated for its value in staging and response assessment at the following time points:

1. Staging, before start of chemotherapy
2. Early response assessment, after 2-3 cycles of chemotherapy (at the earliest 2, at the latest 3 cycles of chemotherapy)
3. Late response assessment, after 5-6 cycles of chemotherapy (at the earliest 5, at the latest 6 cycles of chemotherapy)

In order to prospectively evaluate the impact of FDG-PET in staging, response assessment and determination of prognosis, FDG-PET has been integrated in the EWING 2008 trial. However, not participating in the FDG-PET study is <u>not</u> an exclusion criterion for the EWING 2008 trial. The results of FDG-PET are compared with conventional imaging, histological data and outcome. In

order to safeguard against possible artefacts and to avoid a major shift in staging results, the integration of FDG-PET results into staging is based on the following principle: all FDG-PET information used for staging must be paired with findings on conventional imaging, one other imaging modality (e.g. additional MRI or CT), or histology, if still equivocal after additional imaging.

3.7 Quality of Life

Quality of life (QOL) will be assessed in R1 and R3 patients in EWING 2008 using self-assessment and parent-proxy questionnaires. Since for Ewing sarcomas no prospective data on QOL are available regarding both short- and long-term outcome, the objectives of QOL assessment are to add information to survival data, to detect changes in psychosocial functioning over time of treatment (longitudinal comparison), and to compare QOL in patients treated in different protocol arms at one time point (cross-sectional comparison). Describing and comparing the impact of the treatment regimens on QOL will lead to a better understanding, from the patients' perspective, of the nature of treatment related side effects, both short- and long-term. These data will help define future treatment options for these patients.

The medical late effects of bone tumour therapy have been studied extensively in children (Bhatia, 2003) and young adults while the impact of these late effects on the patients' QOL has been less studied.

Like in osteosarcoma, survivors of Ewing´s sarcoma are particularly vulnerable to medical late effects because of the intensity of their treatment (surgery, chemotherapy, and irradiation) and this may lead to a significant impact on QOL. Additionally, the assessment of QOL within EWING 2008 will allow more global concerns to be addressed, for example whether QOL is affected by surgical factors, patient maturity (emotional and physical) and other characteristics such as gender and site of primary tumour (Nagarajan, 2002) and educational attainment and age (Nagarajan, 2004).

3.8 Impact of Time to Diagnosis

Due to the inconspicuousness of the early warning signs the diagnosis of a Ewing sarcoma is often delayed. As yet, the average time of delay and the putative consequences of a delayed diagnosis have not been investigated. Therefore, we aim to investigate the impact of the time to diagnosis on presentation of disease and outcome.

3.9 Ancillary studies

Trial associated ancillary studies are an important concern in the EWING 2008 trial. A panel of scientists and clinicians will coordinate the scientific projects and the distribution of biological material (please refer to 4.6).

4 TRIAL DESIGN

4.1 Trial Design

EWING 2008 is a phase 3, open label, multi-centre, randomised controlled trial of international study groups with the intention of optimising treatment and treatment results in patients with localised and advanced Ewing sarcomas.

4.2 Participating Institutions

Each national or group office (please refer to section 1.4) will have the responsibility for the accreditation of institutions wishing to participate. Participating investigators/centres must fulfil a set of basic criteria and must sign the EWING 2008 Commitment Form (please refer to Appendix B).

Criteria that are common to all groups are:

- Accreditation with one of the participating groups according to the rules set forth by that group.
- Ethical approval to participate in the trial according to the ICH-GCP guidelines and national law.
- Identification of a principal investigator responsible for the local trial site participating in EWING 2008 who fulfils the legal requirements for investigators of the country in which the institution is situated.
- Local infrastructure which provides for the investigations and treatment measures required by protocol to be performed without undue delay. This includes the shipment of fresh frozen tumour to the reference pathologist.
- Local infrastructure which provides for adequate follow-up.
- Willingness to allow monitoring.
- Willingness to comply with the protocol in all aspects of patient care, specimen handling and data management, as witnessed by signature on the commitment form (Appendix B).
- Familiarity with the chemotherapy agents under investigation and the standard of supportive care required for these patients.

4.3 Registration and Randomisation Procedures

Participating institutions are entitled to register patients with the EWING 2008 trial. Registration of institutions with the trial is managed according to the practice of each group.

Patients are to be registered with the appropriate national or group office by fax, e-mail or letter within 45 days from definitive biopsy. See section 1.4 for contact information. The registration and randomisation forms must be transmitted without delay by FAX, unless national guidelines allow otherwise.

Patients and/or their parents/legal guardians are required to give informed consent for both, registration and randomisation.

4.3.1 Randomisation

Randomisation for each group will be done centrally through the NDCs (national data centres; see 22.1) by using the method of randomly permuted blocks for each randomisation arm (R1; R2loc; R2pulm; R3). Randomisation principles are the same for each group (NDCs).

The following criteria must be fulfilled for a patient to be randomised into the trial:

- Signed informed consent to undergo randomisation.
- Essential data provided (disease extent at diagnosis, histology report including information on surgical margins if applicable).
- Completion of VIDE induction chemotherapy.
- Clinical response evaluation.

Further mandatory criteria pertaining to patients with localised disease who are stratified according to histological response to induction chemotherapy:

- Assessment of histological response of the primary tumour within 35 days of definitive surgery (assessment by reference pathologist where possible).
- No more than one additional cycle of VAI or VAC prior to surgery when needed e.g. for organisational reasons.
- No progression of disease.

Patients are excluded from randomisation:

- if any of the above criteria are missed, and in case of
- Tumour progression or
- Medical contraindication against one of the drugs used in the randomised arms.

Risk arm allocation routines are listed in chapter 7.

4.4 Expected Duration of the Trial

EWING 2008 is the continuation of the EURO-E.W.I.N.G. 99 study, which was an international collaboration of leading groups involved in clinical research on Ewing sarcomas. The group has many years of experience in conducting large international multicentre trials in this disease.

Based on the experience of the EURO-E.W.I.N.G. 99 trial and the expected accrual (see 21.3) EWING 2008 is anticipated to recruit patients for up to 6.5 years. With an additional follow-up period of 2 years the entire trial duration is estimated to be 8.5 years.

4.5 Target Population

The target population for the EWING 2008 study comprises patients aged 2 years to 50 years for the GOPH group and $\leq$ 50 years for other groups. This age group is appropriate as children and young adults have the highest incidence of Ewing sarcoma. The current study is based on well established expert knowledge of Ewing treatment in this age group, and data from previous trials do not suggest particular risks of adverse events.

4.6 Premature Termination

4.6.1 Premature termination of a study group, institution

Premature closure of an institution for this trial is to be considered if:

- Technical requirements of the protocol are not fulfilled,
- The study is not conducted in accordance with the protocol,
- The data quality does not meet the required standard,
- Data return is insufficient,
- Critical findings are quoted in a monitoring report.

Premature closure of an institution for this trial will be decided by the respective national or group coordinator after consultation with the intergroup chairmen and biometrician.

Investigators and institutions deciding not to take part any longer must inform the appropriate national or group office (please refer to section 1.4) as well as the local and the leading ethics committee immediately. The decision should be well founded. Reporting on the further follow-up of patients already on study must be guaranteed.

4.6.2 Premature termination of the trial or trial arms

Premature termination of the trial or trial arms must be considered in case of:

- Serious adverse reactions leading to substantial changes in risk–benefit considerations.
- Insufficient efficacy.
- Superiority of one treatment arm.
- New insights from other trials.
- Insufficient recruitment rate.
- Unsustainable trial organisation.

Premature termination of the trial is to be decided by the study chairmen with the study steering committee and the Independent Data and Safety Monitoring Committee.

4.7 Ancillary Studies

Trial-associated biological and scientific studies may be performed in selected subgroups of patients. All such associated projects need to be approved by a scientific advisory board. Blood, bone marrow and tumour material for approved projects will be collected by specified central institutions. Tumour will be stored sterile snap frozen in order to allow cell culturing as well as molecular studies. Furthermore, paraffin embedded tissue will be stored at reference pathology institutes. The requirements regarding the storage and shipment of blood and bone marrow depend on the investigation planned. The project's responsible scientist following approval by a scientifc advisory board will inform the participating institution briefly about the investigations planned and the material required. The scientist will provide support for the storage and shipment of required materials.

The scientific advisory board, central institutions, and reference pathology institutes are listed in Appendix A2.

5 STUDY OBJECTIVES AND ENDPOINTS

This is a randomised, prospective, multi-centre, international study, linking several co-operative groups, to improve treatment and outcome in patients with Ewing sarcoma.

The primary objective is to assess whether either of the randomised treatments is superior regarding 3-year event-free survival.

The treatment is stratified according to prognostic factors as determined by previous studies. All patients will receive VIDE chemotherapy as induction treatment (Jürgens, 2006). Disease assessment will be performed prior to treatment and after the 2nd (latest 3rd) and 5th (latest 6th) cycle of VIDE chemotherapy. Depending on the presentation at the time of diagnosis and on the histological response to induction chemotherapy, patients will be stratified into to the following risk groups:

- **Standard Risk R1**: (1) Patients with localised disease and favourable histological response at surgery after induction chemotherapy and (2) Patients with initial surgery or in whom surgery was not feasible and who have small tumours < 200ml at diagnosis.
- **High Risk localised disease R2loc**: (1) Patients with localised disease and unfavourable histological response at surgery after induction chemotherapy and (2) Patients with initial surgery or in whom surgery was not feasible and who have large tumours $\geq$ 200ml at diagnosis.
- **High Risk primary lung metastases R2pulm**: Patients with a Ewing sarcoma metastatic to the lungs and/or pleura, but not to any other sites, at the time of diagnosis.
- **Very High Risk R3**: Patients with metastatic disease not confined to the lungs and/or pleura, i.e. patients with bone metastases, bone marrow metastases or other metastases (e.g. lymph nodes, liver, CNS, etc) with and without additional pulmonary metastases at the time of diagnosis.

5.1 Primary Objectives

Standard risk (R1): In a randomised trial, to examine whether additional maintenance treatment with zoledronic acid, fenretinide, or bisphosphonates plus fenretinide, compared to no add-on treatment, improves event-free survival in patients with localised disease and a favourable risk profile.

High risk (R2): In a randomised trial, to examine whether the introduction of busulfan-containing high dose chemotherapy followed by autologous stem cell reinfusion, in comparison with a standard chemotherapy regimen, is of benefit in patients with unfavourable localised disease or pulmonary/pleural metastases.

Very high risk (R3): In a randomised trial, to examine whether after VIDE induction chemotherapy, the addition of high dose chemotherapy using treosulfan-melphalan followed by autologous stem cell reinfusion to eight cycles of VAC chemotherapy, compared to eight cycles of standard adjuvant chemotherapy alone, improves event-free survival in patients with primary disseminated disease.

5.2 Secondary Objectives

5.2.1 Overall Survival

Regarding the risk groups detailed in section 5.1, to investigate whether add-on treatment in R1, busulfan high dose treatment in R2, and the addition of treosulfan high dose treatment in R3 lead to an improvement in overall survival.

5.2.2 Toxicity

To investigate the short-term and long-term toxicity in all patients.

5.2.3 Value of Positron Emission Tomography (PET)

To determine the value of FDG-PET or PET-CT for the diagnosis and response evaluation in patients with Ewing sarcoma. As FDG-PET and PET-CT may not be available in all participating institutions, PET is not mandatory and the lack of a PET investigation does not violate any of the basic codes of practice defined within the treatment protocol. Also, when the treatment of R2 patients has to remain consistent with EURO-E.W.I.N.G. 99, the inclusion of a PET investigation for diagnosis and follow-up within the current trial does not violate any of the basic codes of practice defined within that treatment protocol.

5.2.4 Quality of Life (QOL)

Since for Ewing sarcoma patients no prospective data on QOL are available a first objective of QOL assessment is to describe QOL development longitudinally (i.e. during the course of treatment and follow-up). The second objective of QOL assessment is to determine the impact on QOL of the additional treatment after randomisation for consolidation treatment (i.e. R1 and R3) via cross-sectional comparisons. Also, when the treatment of R2 patients has to remain consistent with EURO-E.W.I.N.G. 99, the assessment of QOL within the current trial does not violate any of the basic codes of practice defined within that treatment protocol.

5.2.5 Impact of the time to diagnosis

As the average time of delay and putative consequences of a delayed diagnosis are not well documented, the impact of the time to diagnosis on the presentation of disease and the outcome of patients is investigated within this trial.

6 PATIENT SELECTION CRITERIA

6.1 Inclusion Criteria

Diagnosis: Histologically confirmed Ewing sarcoma of bone or soft tissue.

Age and sex: Either sex, age > 48 months (Germany) and < 50 years at the date of diagnostic biopsy. Patients outside this age range may be reported to the appropriate national or group office (see section 1.4), but will not be included in this study.

Registration: ≤ 45 days from diagnostic biopsy/surgery.

Start of chemotherapy: ≤ 45 days from diagnostic biopsy/surgery.

Informed consent: According to national guidelines and GCP guidelines signed prior to study entry.

Performance status: Lansky or Karnofsky score > 50%, may be modified for handicapped patients.

Cardiac: LVEF > 40% SF >28%.

6.2 Exclusion Criteria

- More than one cycle of chemotherapy prior to registration.
- Second malignancy.
- Pregnancy or lactation.
- Concurrent treatment within any other clinical trial, except trials with different endpoints that due to the nature of their endpoints must run parallel to EWING 2008, e.g. trials on antiemetics, antimycotics, antibiotics, strategies for psychosocial support, etc..
- Any other medical, psychiatric, or social condition incompatible with the protocol treatment.

7 RANDOMISATION

7.1 Randomisation for Consolidation Treatment in Patients with Localised Disease

7.1.1 R1 (+ add-on treatment)

The following patients are eligible for randomisation in the standard risk group if no progression under induction chemotherapy occurs. Randomisation should take place following VIDE 6 in all patients.

- **Localised tumour** – **any tumour volume** – resection after chemotherapy alone – **good histological response** to induction chemotherapy (<10% viability).

  This randomisation should take place after cycle 6 of VIDE induction chemotherapy, following surgery and assessment of histological response.

- **Localised tumour** – **<200 ml** tumour volume at diagnosis – tumour **unresectable** – radiologically, at least partial response to induction chemotherapy (please refer to Section 15).

  This randomisation should take place following cycle 6 of VIDE induction chemotherapy, before consolidation therapy.

- **Localised tumour** – **<200 ml tumour** volume at diagnosis – **resection at diagnosis**

  This randomisation should take place following cycle 6 of VIDE induction chemotherapy, before consolidation therapy.

7.1.2 R2loc (VAI vs. BuMel)

The following patients with localised disease are eligible for randomisation in the high risk group:

- **Localised tumour – any tumour volume –** resection after chemotherapy alone – **poor histological response** to induction chemotherapy (≥10% viability).

  This randomisation should take place after cycle 6 of VIDE induction chemotherapy, following surgery and assessment of histological response.

- **Localised tumour – extremity site –** resection after chemotherapy and early radiotherapy - **any volume** - **poor histological response** to induction chemotherapy (≥10% viability).

  This randomisation should take place after cycle 6 of VIDE induction chemotherapy, following surgery and assessment of histological response.

- **Localised tumour – extremity site –** resection after chemotherapy and early radiotherapy – **tumour volume > 200ml** - **any histological response** to induction chemotherapy.

This randomisation should take place after cycle 6 of VIDE induction chemotherapy, following surgery and assessment of histological response.

- **Localised tumour – extremity site** – tumour **unresectable** – tumour volume ≥ **200 ml** early radiotherapy or late radiotherapy – no progression under induction chemotherapy.

  This randomisation should take place following cycle 6 of VIDE induction chemotherapy, before consolidation therapy.

- **Localised tumour – extremity site** – tumour **unresectable** – tumour volume **<200 ml poor clinical response** (please refer to Section 15) **–** early radiotherapy or late radiotherapy – no progression under induction chemotherapy.

  This randomisation should take place following cycle 6 of VIDE induction chemotherapy, before consolidation therapy.

- **Localised tumour – tumour volume ≥200 ml – resection at diagnosis.**

  This randomisation should take place following cycle 6 of VIDE induction chemotherapy, before consolidation therapy.

PLEASE NOTE: Patients receiving **early radiotherapy to central axial sites** *are NOT eligible for the R2loc randomisation* due to anticipated busulfan toxicity, and should be treated by VAI consolidation.

7.2 Randomisation for Consolidation Treatment in Patients with Metastatic Disease

7.2.1 Lung metastases R2pulm (VAI vs BuMel)

- **Localised tumour – any tumour volume - any histological response - with pulmonary and/or pleural metastases at diagnosis.** Patients with metastases at other sites are excluded.

  Randomisation should take place following 6 cycles of VIDE induction chemotherapy.

PLEASE NOTE: Patients receiving **early radiotherapy to central axial sites** *are NOT eligible for the R2pulm randomisation* due to anticipated busulfan toxicity, and should be treated by VAI consolidation plus lung irradiation.

7.2.2 R3 (VAC vs. TreoMel plus VAC)

The following patients are eligible for randomisation in the high risk group

Patients with **disseminated disease**, i.e. dissemination to bone and/or other sites and possibly additional pulmonary dissemination, are eligible for randomisation in the very high risk group for patients with disseminated tumour.

Randomisation should be performed following cycle 6 of VIDE chemotherapy. Successful stem cell harvest must be assured.

8 INVESTIGATIONS AT DIAGNOSIS

A check list of required investigations is provided in Appendix B

8.1 Key Timepoints

Registration:	$\leq$ 45days following diagnostic biopsy.
Reference pathology:	≤ 60 days following diagnostic biopsy.
Response evaluation:	After cycle 2(earliest) or 3 (latest) of VIDE, After cycle 5 (earliest) or 6 (latest) of VIDE, Prior to high dose chemotherapy, Prior to add-on in R1, i.e. after cycle 11 of chemotherapy (6 VIDE + 5 VAI/VAC).
Stem cell harvest:	After 3-4 cycles of VIDE in all patients.
Surgery for primary tumour:	After 6 cycles of VIDE, in R3 patients prior to or after HDT.
Randomisation:	R1, R2: after cycle 6 of VIDE, when histology is available. R1, R2, when surgery is not indicated: at the latest following cycle 6 of VIDE. R3: at the latest following cycle 6 of VIDE.
Surgery for metastases:	After 2 cycles of consolidation treatment.
Definitive radiotherapy:	After cycle 6 of VIDE parallel to consolidation chemotherapy, in R3 patients prior to or after HDT.
Preoperative radiotherapy:	Prior to surgery.
Postoperative radiotherapy:	Concurrently with consolidation chemotherapy, in patients receiving HDT, 8-10 weeks following HDT.
Quality of Life:	After cycles 1 (earliest) or 2 (latest) of VIDE, After cycle 6 of VIDE (prior to local treatment), After completion of protocol treatment, After 2 years of follow-up.

8.2 Tumour Diagnosis

The **open biopsy of the primary tumour** is to be performed by a surgeon with expertise in tumour surgery. To obtain adequate amounts of tissue open biopsy is preferred, **only** if this is not feasible needle core biopsy (using at least five 14 gauge needles) is acceptable.
The definitive diagnosis must be based on the examination of routinely stained material plus appropriate immunohistochemical evaluation. The following abnormalities are considered confirmatory:

a) Small blue round cell tumour, PAS positive, and expression of CD99 (MIC-2) on the cell surface, other CD99 positive tumour entities excluded.

b) Cytogenetic/molecular analysis demonstrating chromosome 22 rearrangement.

Representative histological material and frozen tissue must be sent for central pathology review (Please refer to Appendix B).

8.3 Assessment before Start of Treatment

8.3.1 Basic patient information

- Height, weight and body surface area (BSA).
- Check for inclusion and exclusion criteria (please refer to Section 6).
- Menstrual history and pregnancy test if indicated.
- History (first onset of symptoms).

8.3.2 Organ function / baseline investigations

- Full blood count (FBC) and differential white blood count.
- Blood chemistry (electrolytes including magnesium, phosphate, potassium and calcium, creatinine, urea, alkaline phosphatase, lactate dehydrogenase, transaminases, bilirubin).
- Coagulation profile.
- Sex hormones, gonadotrophins.
- Urine profile including glucose, protein, creatinine, phosphate, fractional phosphate reabsorption, creatinine clearance (calculated or isotopic).
- ECG, cardiac function tests (ECHO or MUltiGated Acquisition scan (MUGA)) with determination of left ventricular ejection fraction or shortening fraction.

- Pulmonary function tests (required in patients with pulmonary metastases).
- Serum virology (according to institutional common practice).

Sperm cryopreservation is strongly recommended in male patients of reproductive age.

8.4 Imaging

Three dimensional estimation of the tumour volume is essential !

8.4.1 X-Ray

Primary tumour: X-ray in two planes showing the entire bony lesion.

Chest: Chest X-ray in two planes.

Metastatic sites: X-ray in two planes of sites suspicious to be metastases by scintigraphy or MRI or CT.

8.4.2 MRI

MRI of extremity tumours

a) Transversal image showing the entire tumour.
b) Sagittal image for alignment of the following images (c-f) parallel to the medulla.
c) Coronal T1 sequence images including the entire involved bone and adjacent joints.
d) Coronal STIR sequence images including the entire involved bone and adjacent joints.
e) Axial T2 sequence images to demonstrate the tumour's ventral and dorsal parts and its relation to vessels and nerves.
f) Sagittal T1 sequence images with fat saturation and axial T1 sequence images with fat saturation after application of gadolinium contrast.

PLEASE NOTE: c) and d) should be done with a big coil (e.g. body/heart coil), e) and f) should be done with focused coil (e.g. knee/surface coil).

MRI of pelvic tumours

a) Transversal image showing the entire tumour.
b) Sagittal image for alignment of the following images (c-f).
c) Coronal T1 sequence images including the entire involved bone and adjacent joints.
d) Coronal STIR sequence images including the entire involved bone and adjacent joints.

e) Axial T2 sequence images to demonstrate the tumour's ventral and dorsal parts and its relation to vessels and nerves.

f) Sagittal T1 with fat saturation and axial T1 with fat saturation multislice sequences after contrast application.

MRI of tumours of the spine

a) Transversal image showing the entire spine.

b) Sagittal image for alignment of the following images (c-f) parallel to the vertebra.

c) Sagittal T1 sequence images including all involved vertebra/e and soft tissue and adjacent tumour free vertebrae at both sides.

d) Sagittal STIR sequence images including all involved vertebra/e and soft tissue and adjacent tumour free vertebrae at both sides.

e) Axial T2 sequence images to demonstrate the tumour's ventral and dorsal parts and its relation to vessels and nerves, including the entire tumour extension.

f) Coronal and axial T1 with fat saturation sequence images after application of gadolinium contrast, including the entire tumour extension.

PLEASE NOTE: Precise documentation of the exact localisation of involved vertebrae is mandatory.

Other Sites

MRI or CT scan as indicated by anatomic position of the lesion.

8.4.3 Scintigraphy

99mTc bone scan, activity 500/1000 MBq. In paediatric patients the dose should be adjusted according to the guidelines of the EANM Pediatric Task Group, with a minimum of 40 MBq .

Primary Tumour:

Perfusion phase p.i., dynamic.
Early images soft tissue phase, in two dimensions 2/5 min p.i..
Late phase, 2/5 hours p.i..
Follow-up scintigraphy should be performed in a similar technique.

Whole Body Scintigraphy

Anterior and posterior images, head: anterior, posterior and on the side.

8.4.4 Computed Tomography (CT)

Chest CT: Chest CT is mandatory for detection of pulmonary metastases.

Multidetector CT or *Spiral CT* should be performed if available; otherwise single slice CT can be used.

If *PET-CT* is available, perform chest CT as diagnostic CT

1) Spiral CT scan in scan parameter 5/8 mm with reconstruction increment of 4 mm.
2) Multidetector CT scan at least with 1 mm collimation.

Spiral scan should be performed in caudo-cranial direction.

Alternatively, a single-slice CT in scan parameter 10/10 or 7/10 may be performed.

Scans should be performed after contrast application.

Documentation:

Lung window.

Soft tissue window.

8.4.5 FDG-PET or PET-CT with diagnostic chest CT and, if indicated, CT of the primary tumour

If available for initial diagnosis and follow-up, PET or PET-CT scan is to be performed in addition to bone scintigraphy at the time of diagnosis. For details please refer to Section 14.

8.5 Estimation of Tumour Volume (TV).

- In patients with multifocal disease, the primary tumour or leading structure and at least one metastasis must be measured.
  These target lesions must be described in each follow-up investigation.

TV = **a** x **b** x **c** x **F**,
where a, b, and c represent the maximum tumour dimensions in three planes,
with F = π / 6 = 0.52 for spherical tumours,
or F = π / 4 = 0.785 for cylindrical tumours

8.6 Diagnosis of Bone Metastases

The description of bone metastases must include confirmation of bone scan, PET or plain radiography findings by MRI scan or biopsy or both. To exclude any doubt - **biopsies** of suspicious sites are indicated.

Skip lesions within the compartment involved by the primary tumour are considered loco-regional extension and are NOT regarded as disseminated disease.

8.7 Diagnosis of Bone Marrow Metastases

Minimum requirements

- Aspirates from ≥ 2 sites; biopsy from ≥ 1 site: conventional cytology/histology.
- Biopsy must be taken from sites distant from the primary tumour!

Biopsy and aspirate material must be sent to a **reference pathologist** to be analysed for chromosome 22 rearrangement.

8.7.1 Definition of bone marrow metastases

Bone marrow metastases are defined as light microscopic evidence of bone marrow involvement in any aspirate or trephine biopsy sample. Molecular evidence (i.e. by RT-PCR analysis) **alone** is, by definition of this protocol, **not** considered adequate for the diagnosis of metastatic bone marrow disease. The relevance of bone marrow metastases has been addressed in the EURO-E.W.I.N.G. 99 trial. As the EURO-E.W.I.N.G. 99 trial is still recruiting and, therefore, final analyses are pending conclusions on the relevance of molecular disease are not available.

8.8 Diagnosis of Pulmonary/Pleural Metastatic Disease

Spiral CT should be performed if available, otherwise single slice CT can be used.

If PET-CT is available, perform chest CT as diagnostic CT.

8.8.1 Definition of pulmonary/pleural metastatic disease

As a rule, one pulmonary/pleural nodule of > 1 cm, or more than one nodule of > 0.5 cm are considered evidence of pulmonary/pleural metastases, as long as there is no other clear medical explanation for these lesions. In case of doubt, biopsies should be considered. A solitary nodule of 0.5-1 cm or multiple nodules of 0.3-0.5 cm are questionable evidence of metastatic disease, and confirmation by biopsy is recommended.

One solitary nodule of < 0.5 cm or several nodules of < 0.3 cm are not regarded as clear evidence of lung disease. In such cases, individual decisions regarding biopsy have to be considered. Pleural effusion in patients with chest wall tumours is not regarded as proof for lung/pleural metastases, but is considered to represent loco-regional disease (Paulussen, 1993). The site(s), size, and number of involved sites are to be recorded.

8.9 Soft Tissue Metastases

Soft tissue lesions and regional lymphnodes may be detected by whole body FDG-PET or MRI and ultrasound.

If indicated, additional abdominal CT or MRI of suspicious sites should be performed.

Lymph node metastases and soft tissue metastases as suspected by clinical examination or imaging methods should be **confirmed by biopsy.**

8.10 Timepoints of Diagnostic Procedures

8.10.1 Assessment prior to each cycle of chemotherapy

Please refer to Section 9.

8.10.2 Response evaluation under induction treatment - after 2nd and 5th VIDE

MRI or CT scan of primary site

Chest CT scan (when positive for metastases at diagnosis and in patients with poor clinical response (please refer to Section 15).

PET or PET-CT scan

- metastatic sites.

These studies are to be done

- after 2 (earliest 2, latest 3) cycles of chemotherapy and
- after 5 (earliest 5, latest 6) cycles of chemotherapy.

8.10.3 Assessment prior to surgery

Please refer to Section 8.6.2 Investigations after 5th cycle of VIDE. Imaging ought to be planned in co-operation with the surgeon!

8.10.4 Assessment after last cycle of chemotherapy

- Full blood count (FBC) and differential white blood count.
- Blood chemistry (electrolytes including magnesium, phosphate, potassium and calcium, creatinine, urea, alkaline phosphatase, lactate dehydrogenase, transaminases, bilirubin).
- Gonadotrophins and sex hormones.
- Coagulation profile.
- Urine profile including glucose, protein, creatinine, phosphate.
- Serum virology.
- ECG, cardiac function tests (ECHO or MUltiGated Acquisition scan (MUGA)) with determination of left ventricular ejection fraction, shortening fraction.
- Pulmonary function tests.
- Appropriate imaging of primary tumour site and metastases.
- Chest CT scan.

8.11 Follow-up Assessment

8.11.1 Disease-related follow-up after completion of chemotherapy

Participating institutions are to obtain follow-up information on relapse and survival indefinitely from all patients, regardless of protocol violation.

The follow-up checks may follow national guidelines or recommendations of late effects trials. Below please find a list of minimal requirements for timing of follow-up visits to ensure consistency in the detection of relapse or progression.

Disease related follow-up must extend over a minimum of 5 years.

Disease related follow-up checks must include

- Physical examination at each visit.
- Appropriate imaging of primary tumour and metastatic sites.
- Chest X-Ray, in R2pulm and R3 patients: alternating chest X- ray and chest CT.

8.11.2 Relapse

The date of relapse is defined as the date at which evidence of relapse is confirmed either radiographically or by biopsy. If relapse is detected at any site, a complete diagnostic work-up as described in Sections 8.2 to 8.5 is mandatory. For the definition of non-response, disease progression and relapse please refer to section 21.

8.11.3 Toxicity follow-up and follow-up for late effects

The follow-up checks may follow national guidelines or recommendations of late effects trials.

As a guideline the recommendations of the Late Effects Surveillance Study are listed in Section 16.6.1.

Patients who received radiotherapy should be enrolled in the Registry for the Evaluation of Late Side Effects after Radiotherapy in Childhood and Adolescence **(R**eg**i**ster zur Erfassung von **S**pätfolgen nach Strahlentherapie im **K**indes- und Jugendalter, **RISK). (Please refer to Appendix A)**

9 TREATMENT

Dose modifications are outlined in Section 10. Detailed information on chemotherapeutic agents is given in Appendix A1.

9.1 VIDE chemotherapy - Agents and Dosage

Cycles of VIDE should be given at 21 day intervals or on haematological recovery to WBC ≥ $2.0*10^9$/l with absolute neutrophil count (ANC) ≥ $1.0*10^9$/l; platelets ≥ $80*10^9$/l.

VIDE				
VINCRISTINE	1.5 mg/m²/d (i.v. push)	d1	(1.5 mg/m²/cycle)	(*max. single dose: 2 mg*)
IFOSFAMIDE	3.0 g/m²/d (i.v. infusion, 1-3 h)	d1, d2, d3	(9 g/m²/cycle)	plus MESNA*
DOXORUBICIN	20 mg/m²/d (i.v. infusion, 4 h)	d1, d2, d3	(60 mg/m²/cycle)	
ETOPOSIDE	150 mg/m²/d (i.v. infusion, 1 h)	d1, d2, d3	(450 mg/m²/cycle)	
G-CSF	5 µg/m²/d	d5 to approx. d13		
*Recommended Mesna dosage:				
	1.0 g/m²/d	d1	(i.v. push 1 h prior to ifosfamide)	
	3.0 g/m2/d	d1, d2, d3	(i.v. infusion, e.g. 24 h)	
	For oral dose refer to Appendix A1			

See Appendix A1 for chemotherapy guidelines and details.

9.1.1 Basic plan of VIDE cycles

Day 1	**V**incristine, **I**fosfamide, **D**oxorubicin, **E**toposide, Mesna bolus, Mesna, Hydration
Day 2	**I**fosfamide**, D**oxorubicin**, E**toposide**,** Mesna, Hydration
Day 3	**I**fosfamide**, D**oxorubicin, **E**toposid**e,** Mesna, Hydration
Day 4	Hydration
Day 5	**G-CSF** until leukocyte recovery
Days 5 – 19	Regular follow-up visits as needed. FBC controls according to institutional guidelines.
Days 20 - 22	PRIOR TO NEXT CYCLE Controls according to institutional guidelines <u>including</u> FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes GFR (calculated creatinine clearance (Ccrea) or isotopic) Fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tm_p/GFR). Cardiac monitoring

9.1.2 Additions to basic plan of VIDE 1-6

VIDE1	PRIOR TO START OF CYCLE 1: - complete "Investigations at diagnosis" including organ function (see Section 8.3)
VIDE 2	- Clinical response evaluation - Primary tumour site **disease re-evaluation**: CT scan or MRI (with measurements).
VIDE 3	FOLLOWING VIDE 3 AND/OR 4: - PBSC MOBILISATION AND HARVESTING. This is advised in patients with localised tumours <200 ml and mandatory in patients with localised tumours ≥200 ml or metastases to lungs/pleura only. UNLESS performed after VIDE 2: - Clinical response evaluation - Primary tumour site **disease re-evaluation**: CT scan or MRI (with measurements).
VIDE 5	- Clinical response evaluation - Primary tumour site **disease re-evaluation**: CT scan or MRI (with measurements). **PLANNING of local treatment** **Randomisation**
VIDE 6	UNLESS performed after VIDE 5: - Clinical response evaluation - Primary tumour site **disease re-evaluation**: CT scan or MRI (with measurements). **Randomisation**

9.1.3 Availability of chemotherapy agents

Vincristine, ifosfamide, doxorubicin, etoposide, and cyclophosphamide preparations from various manufacturers are commercially available in all countries participating in EWING 2008. Most of these preparations are licensed for the treatment of sarcomas. The agents will be obtained through hospital pharmacies of the investigators` institutions or external pharmacies according to local practice. The choice of the specific preparation given is left at the discretion of the treating physician. The protocol does not recommend to use or not to use preparations from specific manufacturers

9.1.4 Side effects

Side effects (Jürgens, 2006) are outlined in Section 10 and Appendix A1. Investigators are requested to refer to the latest product information for additional information.

9.1.5 Medical emergencies associated with the administration of trial medication

Investigators should refer to Section 10 and Appendix A1 where the major side effects and precautions are outlined. Furthermore, the product information will provide additional information on handling emergencies.

9.2. Local Treatment following VIDE

Whenever feasible, proceed to **surgery** 21 days after cycle 6 or on haematological recovery.

The next chemotherapy cycle - Cycle 7: VAC or VAI - should be planned to commence no later than 14 days after surgical resection.

Surgical specimens should be sent for **histopathological assessment** of response to chemotherapy including central review where deemed necessary (see Pathology Guidelines, Appendix A3). Results must be available within 3 weeks of surgery. For details of local therapy techniques see Sections 18 and 19.

After obtaining relevant information and informed consent, randomisation should be requested by FAX to the appropriate study office (see Section 1).

9.3 VAI Chemotherapy - Agents and Dosage

Cycles of VAI should be given at 21 day intervals or on haematological recovery to WBC $\geq 2.0*10^9$/l with absolute neutrophil count (ANC) $\geq 1.0*10^9$/l; platelets $\geq 80*10^9$/l.

VAI				
VINCRISTINE	1.5 mg/m^2/d (i.v. push)	d1	(1.5 mg/m^2/cycle) (*max. single dose: 2 mg*)	
ACTINOMYCIN D	0.75 mg/m^2/d (i.v. push)	d1, d2	(1.5 mg/m^2/cycle) (*max. single dose per day: 1.5 mg*)	
IFOSFAMIDE	3.0 g/m^2/d (i.v. infusion, 1-3 h)	d1, d2	(6 g/m^2/cycle)	plus MESNA*
G-CSF	5µg/m²/d		in case of poor haematological recovery	
*Recommended Mesna dosage				
	1.0 g/m^2/d	d1	(i.v. push 1 h prior to ifosfamide)	
	3.0 g/m^2/d	d1, d2	(i.v. infusion, e.g. 24 h)	
	For oral dose refer to Appendix A1			

See Appendix A1 for chemotherapy guidelines and details.

9.3.1 Basic plan of VAI

See Section 10 and Appendix A1 for chemotherapy guidelines and details.

Day 1	**V**incristine, **A**ctinomycin D, **I**fosfamide, Mesna bolus, Mesna, Hydration
Day 2	**A**ctinomycin D, **I**fosfamide, Mesna, Hydration
Day 3	Hydration
Day 4 - 9	Controls as needed according to institutional guidelines
Day 5	G-CSF 5µg/m²/d in patients with history of poor haematological recovery
Days 10 - 19	Regular check-up visits, FBC controls according to institutional guidelines
Days 20 - 22	PRIOR TO NEXT CYCLE Controls according to institutional guidelines <u>including</u> - FBC - urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes - GFR (calculated creatinine clearance (Ccrea) or isotopic) - Fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tm_p/GFR). Refer to: 10.1.3

9.3.2 Availability of chemotherapy agents

Vincristine, actinomycin D and ifosfamide preparations from various manufacturers are commercially available in all countries participating in EWING 2008. Most of these preparations are licensed for the treatment of sarcoma. The agents will be obtained through hospital pharmacies of the investigators` institutions or external pharmacies according to local practice. The choice of the specific preparation given will be left at the discretion of the treating physician. The protocol does not recommend to use or not to use preparations of specific manufacturers.

9.3.3 Side effects

Side effects are outlined in Section 10 and Appendix A1. Investigators are requested to refer to the latest product information for additional information. Dose modifications are outlined in Section 10.

9.3.4 Medical emergencies

Investigators should refer to Section 10 and Appendix A1 where the major side effects and precautions are outlined. Furthermore the product information will provide additional information on handling emergencies.

9.3.5 VAI and radiotherapy

Radiotherapy is recommended to be given concurrently with consolidation chemotherapy in order to use the synergistic effect of these treatment regimens.

PLEASE NOTE: Actinomycin D should be omitted during radiotherapy.

9.4 VAC Chemotherapy - Agents and Dosage

Cycles of VAC should be given at 21 day intervals or on haematological recovery to WBC $\geq 2.0*10^9$/l with absolute neutrophil count (ANC) $\geq 1.0*10^9$/l; platelets $\geq 80*10^9$/l.

VAC			
VINCRISTINE	1.5 mg/m^2/d (i.v. push)	d1	(1.5mg/m^2/cycle) (*max. single dose: 2 mg*)
ACTINOMYCIN D	0.75 mg/m^2/d (i.v. push)	d1, d2	(1.5mg/m^2/cycle) (max. single dose/d: 1.5 mg)
CYCLOPHOSPHAMIDE	1500mg/m^2/d (i.v. infusion, 1-3 h)	d1	(1500 mg/m^2/cycle) plus MESNA
*Recommended Mesna dosage			
	500 mg/m^2/d	d1	(i.v. push 1 h prior to cyclophosphamide)
	1500 mg/m^2/d	d1	(i.v. infusion, e.g. 24 h)
	For oral dose refer to Appendix A1		

See Appendix A1 for chemotherapy guidelines and details.

9.4.1 Basic plan of VAC

Day 1	**V**incristine, **A**ctinomycin D, **C**yclophosphamide, Mesna bolus, Mesna, Hydration
Day 2	**A**ctinomycin D
Days 3 - 9	Controls as needed according to institutional guidelines
Day 5	G-CSF 5µg/m²/d in patients with history of poor haematological recovery
Days 10 - 19	FBC controls according to institutional guidelines.
Days 20 - 22	PRIOR TO NEXT CYCLE Controls according to institutional guidelines and - FBC - urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes - GFR (calculated creatinine clearance (Ccrea) or isotopic) - Fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tm_p/GFR). Refer to: 10.1.3

9.4.2 Availability of chemotherapy agents

Vincristine, actinomycin D and cyclophosphamide preparations from various manufacturers are commercially available in all countries participating in EWING 2008. Most of these preparations are licensed for the treatment of sarcomas. The agents will be obtained through hospital pharmacies of the investigators` institutions or external pharmacies according to local practice. The choice of the specific preparation given will be left at the discretion of the responsible physician. The protocol does not recommend to use or not to use preparations of specific manufacturers.

9.4.3 Side effects and dose modification

Side effects are outlined in Section 10 and Appendix A1. Investigators are requested to refer to the latest product information for additional information.

Dose modifications are outlined in Section 10.

9.4.4 Medical emergencies

Investigators should refer to Section 10 and Appendix A1 where the major side effects and precautions are outlined. Furthermore, the product information will provide additional information on handling emergencies.

9.4.5 VAC and radiotherapy

Radiotherapy is recommended to be given concurrently with consolidation chemotherapy in order to use the synergistic effect of these treatment regimens.
PLEASE NOTE: Actinomycin D should be omitted during radiotherapy.

9.5 Busulfan–Melphalan High Dose Chemotherapy with Autologous Stem Cell Reinfusion - Agents and Dosage

Patients randomised to BuMel receive busulfan-melphalan consolidation as cycle 8.

As only the IV formulation of busulfan (Busilvex®) has obtained approval for use in high dose treatment regimens it is recommended for use within this trial.

BUSULFAN-MELPHALAN (BuMel)

		D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Busulfan IV adults: 0.8 mg/kg body weight (BW) children and adolescents: <9 kg = 1 mg/kg BW 9 - <16 kg = 1.2 mg/kg BW 16 - 23 kg = 1.1 mg/kg BW >23 - 34 kg = 0.95 mg/kg BW >34 kg = 0.8 mg/kg BW	0 h 6 h 12 h 18 h		**X** **X** **X** **X**	**X** **X** **X** **X**	**X** **X** **X** **X**	**X** **X** **X** **X**			
Melphalan IV 140 mg/m² IV infusion, 30 min.							**X**		
Clonazepam orally, IV 0.025 to 0.1 mg/kg/d		**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Stem cell re-infusion (min. 3 x 10^6/kg CD 34+)									**X**

PLEASE NOTE: In patients ≥ 60 kg body weight, calculate dosage by kgBW, not m²BSA: cumulative dose 16mg/kg, 16 divided doses, 1 mg/kgBW/dose, 4 daily doses over 4 days.

Heparin and/or allopurinol or urodesoxycholic acid (UDCA) (d –7 to d 8)[72] may be added according to institutional guidelines.

All blood products must be irradiated and leukocyte-depleted (CMV negative).

See Section 10 and Appendix A1 for chemotherapy guidelines and details.

9.5.1 Basic plan of BuMel

Day -7	Clonazepam,	Hydration
Day -6 to -3	Clonazepam, Busulfan,	Hydration
Day -2	Clonazepam, Melphalan,	Hydration
Day -1	Clonazepam,	Hydration
Day 0	Stem cell reinfusion,	Hydration
Day +5 until recovery		Hydration G-CSF 5µg/m²/d Controls according to institutional guidelines All blood products must be irradiated and leukocyte-depleted (CMV negative).

9.5.2 Availability of chemotherapy agents

Busulfan and melphalan preparations from various manufacturers are commercially available in all countries participating in EWING 2008. The agents will be obtained through hospital pharmacies of the investigators` institutions or external pharmacies according to local practice. The choice of the specific preparation given will be left at the discretion of the treating physician. The protocol does not recommend to use or not to use preparations of specific manufacturers.

9.5.3 Side effects and dose modifications

Side effects are outlined in Section 10 and Appendix A1. Investigators are requested to refer to the latest product information for additional information.

Dose modifications are outlined in Section 10.

9.5.4 Medical emergencies

Investigators should refer to Section 10 and Appendix A1 where the major side effects and precautions are outlined. Furthermore, the product information will provide additional information on handling emergencies.

9.5.5 Contraindications for BuMel high dose therapy

Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs. For patients receiving busulfan, large irradiation portals including bowel or lung have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas containing lung tissue must be avoided in patients receiving busulfan, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy.

9.6 Treosulfan-Melphalan (TreoMel) High Dose Treatment with Autologous Stem Cell Reinfusion - Agents and Dosage

Timing of TreoMel consolidation depends on the timing and type of local treatment: Local therapy in R3 patients is following VIDE induction, whenever feasible prior to high dose therapy (HDT). When long periods of immobilisation following surgery are anticipated, e.g pelvic reconstruction, surgery following HDT may be advisable.

Depending on the clinical response to induction chemotherapy radiotherapy prior to HDT and surgery may be an option to be considered in such patients. Any delay between VIDE and HDT for reasons of e.g. local treatment must be bridged with VAC cycles. The total number of VAC cycles will not exceed eight cycles.

TreoMel

	D -5	D -4	D -3	D -2	D -1	D 0
Treosulfan IV 12 g/m²/dose IV infusion, 1 hour **KEEP ph neutral!!**	**X**	**X**	**X**			
Melphalan IV 140 mg/m² IV infusion, 30 min.				**X**		
Stem cell re-infusion (min. 3 x 10^6/kg CD 34^+)						**X**

PLEASE NOTE: The cumulative treosulfan dose is 36 g/m². Treosulfan powder for infusion is to be reconstituted in prewarmed water for injection (ca. 30°C). The treosulfan solution is compatible with sterile physiological NaCl solution (saline) but **n o t** with buffered media! Prophylactic anticonvulsive treatment is not needed and hence not recommended with high dose treosulfan. Hydration 2L/m²/d using a standard hydration solution according to institutional guidelines is recommended. Urin should not be alkalised.

9.6.1 Basic plan of TreoMel

Days -5 to -3	Treosulfan,	Hydration, **Do NOT alkalise!**
Day -2	Melphalan,	Hydration
Day -1		Hydration
Day 0	Stem cell reinfusion,	Hydration
Day +5 until recovery		Hydration G-CSF 5µg/m²/d Regular controls according to institutional guidelines All blood products must be irradiated and leukocyte-depleted (CMV negative).

9.6.2 Availability of chemotherapy agents

Treosulfan from Medac is commercially available in all countries participating in EWING 2008. Treosulfan is licensed for the treatment of ovarian cancer.

The national coordinators are responsible for organising the distribution of trial medication to the trial sites as well as ensuring that all such trial medication is appropriately labelled according to national law.

In Germany, study sites will be supplied with commercial drug from MEDAC. Upon request supplies will be dispatched within one to two weeks.

9.6.3 Request for delivery of clinical supplies to study site

<table>
<tr><td colspan="4">EWING 2008
Request for Delivery of clinical supplies to Study Site</td></tr>
<tr><td>Patient ID</td><td colspan="2">Date of birth

DD/MMM/JJJJ</td><td>Centre</td></tr>
<tr><td colspan="4">Treosulfan 1000 / 5000
Active ingredient: Treosulfan (L-Threitol- 1,4-bis(methanesulfonate))
Drug distribution
For clinical trial only!
EWING 2008 clinical trial – Sponsor: University Hospital Münster, Phone +49 251 8357749</td></tr>
<tr><td colspan="4">Name of pharmacist
Please print clearly</td></tr>
<tr><td colspan="4">Full address of pharmacist
Please print clearly</td></tr>
<tr><td colspan="4">Contact details
Phone:
Fax:
E-mail:</td></tr>
<tr><td>Patient</td><td colspan="2">Weight: Kg

lb</td><td>Body surface area m²</td></tr>
<tr><td colspan="4">Request for delivery of Treosulfan to study site
Please specify:
Signature of Investigator Date DD/MMM/JJJJ</td></tr>
<tr><td colspan="3">Date drug required</td><td>DD/MMM/JJJJ</td></tr>
<tr><td colspan="3">Intention to commence treatment</td><td>DD/MMM/JJJJ</td></tr>
</table>

9.6.4 Side effects

Side effects are outlined in Section 10 and Appendix A1. Investigators are requested to refer to the latest product information for additional information.

9.6.5 Medical emergencies

Investigators should refer to Section 10 and Appendix A1 where the major side effects and precautions are outlined. Furthermore the product information will provide additional information on handling of emergencies.

9.6.6 Dose modifications for treosulfan

In children < 20 kg BW, the dose should be calculated by kg BW.

9.6.7 Contraindications for TreoMel high dose therapy

Any patient with known treosulfan incompatibility is ineligible for treosulfan high-dose therapy and randomisation for reasons of anticipated toxicity.

9.7 Zoledronic acid - Agent and Dosage

Patients randomised for zoledronic acid will receive zoledronic acid at 28 day intervals beginning with cycle 6 of VAC/VAI consolidation chemotherapy for a total period of nine months.
Patients < 18 years will receive 0.05 mg/kg BW by IV infusion 30 min-1 h.
Patients ≥ 18 years will receive a bodyweight-dependent dose:
Patients ≥40kg receive **4 mg** by IV infusion 30 min-1h
Patients 20-40 kg receive **2 mg** by IV infusion 30 min-1h
Reconstituted zoledronic acid infusions must be administered in a line separate from other drugs.

PLEASE NOTE: Maximal dose is 4mg. In order to prevent osteonecrosis of the jaw, patients must undergo an appropriate dental examination prior to treatment with zoledronic acid. Regular, six-monthly dental examination is required at the time of treatment with zoledronic acid and for a follow up period of five years after the end of treatment. While on treatment, the patients should avoid invasive dental procedures if possible (please refer to 16.7).

9.7.1 Basic plan of zoledronic acid

Cycles should be given at 28 day intervals for a period of nine months beginning with cycle 6 of VAC/VAI consolidation chemotherapy. Paralell to VAC/VAI cycles, the medication may be given at 21-day intervals. Following completion of consolidation chemotherapy, the interval must be extended to the 28 day schedule.

Day 1	PRIOR TO EACH CYCLE: - full blood count - electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes - GFR (calculated creatinine clearance (Ccrea) or isotopic) - fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tm_p/GFR) - physical examination Ensure adequate hydration of the patient. Zoledronic acid, hydration 250 ml/m² Oral calcium, paracetamol in case of flu-like symptoms
Days 2 - 6	**Oral calcium ,** paracetamol in case of flu-like symptoms
Day 7	Controls according to institutional guidelines and - calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase
Days 8 - 21/28	Regular controls according to institutional guidelines

9.7.2 Availability of chemotherapy agent

Zoledronic acid preparations from Novartis are commercially available in all countries participating in EWING 2008. Zoledronic acid is licensed for the treatment of bone metastases.

The national coordinators are responsible for organising the distribution of trial medication to the trial sites as well as ensuring that all such trial medication is appropriately labelled according to national law.

In Germany, study sites will be supplied with commercial drug from Novartis until 31 Dec 2010. Supplies will be shipped upon request.

Zoledronic acid will be provided in plastic vials containing 4 mg zoledronic acid (anhydrous) in 5mL concentrate solution for infusion and will be supplied in an open-label fashion throughout the study.

If not used immediately after dilution with infusion media, for microbiological integrity, the final solution must be placed in a refrigerator with a temperature of 2 - 8°C. The refrigerated infusion should then be equilibrated to room temperature prior to administration. The total time between dilution, storage in a refrigerator and end of infusion must not exceed 24 hours.

9.7.3 Request for delivery of clinical supplies to study site

Studie: CZOL446EDE39T

ZOMETA

Bedarfsanforderung Studienmedikation

Bitte senden Sie diesen Vordruck bei Bedarf weiterer Studienmedikation **per Fax** an:

Marguerite Kratzert Novartis Pharma GmbH Roonstraße 25 90429 Nürnberg Tel.: 0911 / 273 12 496	**Fax: 0911 / 273 15 496**

Studienmedikation	**Menge**
ZOMETA 4 mg/5 ml à	_____à 1 Durchstechflasche _____à 4 Durchstechflaschen

ZENTRUMSNUMMER: ☐

Absender: ☐

Lieferadresse:
(falls abweichend) ☐

9.7.4 Side effects and dose modifications

Side effects are outlined in Appendix A1. Investigators are requested to refer to the latest product information for additional information.

Dose modifications are not applicable.

9.7.5 Medical emergencies

Investigators should refer to Section 10 and Appendix A1 where the major side effects and precautions are outlined. Furthermore the product information will provide additional information on handling emergencies.

9.7.6 Supportive care

Calcium: daily oral calcium supplementation.

Paracetamol: to be considered in case of flu-like symptoms.

9.7.7 Contraindications for zoledronic acid therapy

Any patient who has symptoms of CTC°3 and 4 nephrotoxicity is ineligible for receiving zoledronic acid. These patients are excluded from randomisation.

9.8 Fenretinide - Agent and dosage (awaiting approval)

9.8.1 Basic plan of fenretinide

Timing: Randomisation R1, to be started concurrently with cycle 6 of VAC/VAI consolidation chemotherapy and to be continued for 6 months after end of treatment.

Cycles should be given at 28 day intervals

Day 1 MANDATORY TESTS PRIOR TO EACH CYCLE:

- FBC
- electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes, creatinine, urea, plasma proteins
- GFR (calculated creatinine clearance (Ccrea) or isotopic)
- fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tm_p/GFR)
- plasma retinol level
- ophthalmologic examination with visual acuity assessment

Fenretinide: 1860 mg/m² divided in 3 doses. High fat food should be taken with the fenretinide capsules in order to promote absorption.

Day 7

- FBC
- electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes, creatinine, urea, plasma proteins
- GFR (calculated creatinine clearance (Ccrea) or isotopic)
- fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tmp/GFR)
- plasma retinol level
- ophthalmologic examination with visual acuity assessment

Day 14

- FBC
- electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes, creatinie, urea, plasma proteins,
- GFR (calculated creatinine clearance (Ccrea) or isotopic)
- fractional phosphate reabsorption (Tp/Ccrea) = Renal tubular threshold for phosphate (Tmp/GFR)

9.8.2 Availability of chemotherapy agent

Information on the availability of fenretinide will be provided following closure of currently ongoing phase II trials.

9.8.3 Administration

Patients randomised for fenretinide receive oral capsule fenretinide at a dose of 1860 mg/m².

9.8.4 Side effects and dose modifications

Side effects are outlined in Appendix A1.

No dose modification is intended.

Toxicity*	Grade	Action
Diarrhoea	2 – 3	Continue after normalisation
Dermatotoxicity	2 – 3	Continue after normalisation, discontinue if recurrent
Gastrointestinal	2 – 3	Continue after normalisation, discontinue if recurrent
All toxicities	4	Consider to discontinue fenretinide
Nyctalopia	Any grading	Discontinue fenretinide
Pseudotumour cerebri	3 or 4	Discontinue fenretinide
Serum chemistry changes	Any grading	Continue after normalisation

* http://ctep.cancer.gov/forms/CTCAEv3.pdf

9.8.5 Medical emergencies

Investigators should refer to Section 10 and Appendix A1 for an outline of major side effects and precautions. Furthermore the product information will provide additional information on handling emergencies.

9.8.6 Supportive care

No specific supportive care is necessary.

9.8.7 Contraindications for fenretinide therapy

Patients in whom fenretinide is contraindicated are to be excluded from randomisation.

9.9 Cumulative Dose

9.9.1 Agents, abbreviations, and daily dose

Actinomycin D	A		0.75	mg/m²/d
Busulfan	Bu	adults	3.2	mg/kg/d
		children and adolescents:		
		<9 kg	4	mg/kg/d
		9 - <16 kg	4.8	mg/kg/d
		16 - 23 kg	4.2	mg/kg/d
		>23 - 34 kg	3.8	mg/kg/d
		>34 kg	3.2	mg/kg/d
Cyclophosphamide	C		1500	mg/m²/d
Doxorubicin	D		20	mg/m²/d
Etoposide	E		150	mg/m²/d
Fenretinide	Fen		1860	mg/d
Ifosfamide	I		3000	mg/m²/d
Melphalan	Mel		140	mg/m²/d
Treosulfan	Treo		12	g/m²/d
Vincristine	V		1.5	mg/m²/d
Zoledronic acid	Z	Age < 18 y	0.05	mg/kg BW
		Age ≥ 18 y >40 kg BW	4	mg/dose
		20-40 kg BW	2	mg/dose

9.9.2 Description of treatment cycles and cumulative doses

VIDE

V (Vincristine)	1.5 mg/m²	6 cycles =	9 mg/m²
I (Ifosfamide)	9000 mg/m²	6 cycles =	54,000 mg/m²
D (Doxorubicin)	60 mg/m²	6 cycles =	360 mg/m²
E (Etoposide)	450 mg/m²	6 cycles =	2700 mg/m²

VAI

V (Vincristine)	1.5 mg/m²	8 cycles =	12 mg/m²
A (Actinomycin D)	1.5 mg/m^2	8 cycles =	12 mg/m²
I (ifosfamide)	6000mg/m²	8 cycles =	48,000 mg/m²

VAC

V (Vincristine)	1.5 mg/m²	8 cycles =	12 mg/m²
A (Actinomycin D)	1.5 mg/m^2	8 cycles =	12 mg/m²
C (Cyclophosphamide)	1500 mg/m²	8 cycles =	12,000 mg/m²

BuMel

Bu (Busulfan)	Adults: 0.8 mg/kg Children and adolescents: <9 kg = 1 mg/kg 9-<16 kg = 1.2 mg/kg 16-23 kg = 1.1 mg/kg >23-34 kg= 0.95 mg/kg >34 kg = 0.8 mg/kg	1 cycle =	Adults: 12.8 mg/kg Children and adolescents: <9 kg = 16 mg/kg 9-<16 kg = 19.2 mg/kg 16-23 kg = 17.6 mg/kg >23-34 kg= 15.2 mg/kg >34 kg = 12.8 mg/kg
Melphalan	140 mg/m²	1 cycle =	140 mg/m²

TreoMel

Treosulfan	12 g/m²/d	1 cycle =	36 g/m²/d
Melphalan	140 mg/m²	1 cycle =	140 mg/m²

Zol * please refer to section 9.7

Z (Zoledronic acid)	Max. 4 mg/cycle*	9 cycles =	36 mg

Fen

Fen (Fenretinide)	1860 mg/cycle	8 cycles =	14,880 mg

10 DOSE MODIFICATIONS UNDER INDUCTION AND CONSOLIDATION TREATMENT, ALL RISK GROUPS

10.1 Dose Modifications

For detailed drug information, please refer to Appendix A1.

Symptom specific dose modifications are outlined below.

10.1.1 Haematological toxicity

Dose/time intensity is regarded as an essential aspect of induction strategy. In case of significant bone marrow toxicity preference should be given to early G-CSF support rather than dose reduction in order to maintain dose intensity.

If significant toxicity continues as defined by:

- Haematological recovery delayed >6 days or beyond 21 days:

  Reduce etoposide dose by 20% next VIDE cycle.

- Neutropenic sepsis grade 3 or 4:

  Reduce etoposide dose by 20% next VIDE cycle.

In the event of further episodes of toxicity the etoposide dose is to be reduced by an additional 20%. If necessary it is advised to omit etoposide completely rather than reducing the doses of the other three drugs. If after the omission of etoposide the toxicity of VIDE remains intolerable, the dose per VIDE course of ifosfamide may be reduced from 9 g/m²BSA/course to 6 g/m²BSA/course

10.1.2 Gastrointestinal toxicity

- Mucositis/gastrointestinal (GI) toxicity CTC° 3 or 4:

  Reduce etoposide dose by 20%

In the event of further episodes of toxicity the etoposide dose is to be reduced by an additional 20%. If necessary it is advised to omit etoposide completely rather than reducing the doses of the other three agents.

10.1.3 Nephrotoxicity/ Renal function monitoring

Glomerular Filtration Rate (GFR)

Serum creatinine should be monitored prior to each cycle of ifosfamide, cyclophosphamide, busulfan and melphalan.

Glomerular function is to be assessed according to national / group guidelines, applying either isotope clearance, or calculated creatinine clearance.

Schwartz's Formula (1-18 years) (Schwartz, 1987)

According to Schwartz's formula, creatinine clearance (Ccrea) can be calculated from single serum samples:

$$C_{crea} = \frac{F \times Height\,[cm]}{Crea_{\,serum}[mg/dl]}\,[ml/min/1.73m^2]$$

where **F** is proportional to body muscle mass, hence depending on age and gender:

Infants (< 1 year of age) **F** = 0.45

Males, 1-16 years **F** = 0.55

Females, 1-21 years **F** = 0.55

Males, 16-21 years **F** = 0.70

Normal values [ml/min/1.73m²]:

Infants (7 days): 45

Infants (6 months): 60-80

≥ 1 year: 120

Cockcroft- Gault Formula (>18 years) (Cockroft, 1976)

Females

$$\frac{1.05\,(140 - age\,(yrs))\,wt(kg)}{Crea_{\,serum}[\,\mu mol/L]}$$

Or

$$\frac{0.85\;\;(140 - age\,(yrs))\,wt(kg)}{72 \times Crea_{\,serum}[mg/dl]}$$

Males

$$\frac{1.25\,(140 - age\,(yrs))\,wt(kg)}{Crea_{\,serum}[\,\mu mol/L]}$$

Or

$$\frac{(140 - age\,(yrs))\,wt(kg)}{72 \times Crea_{\,serum}[mg/dl]}$$

PLEASE NOTE: These formulas have not been confirmed in patients receiving repeated cycles of intensive chemotherapy OR in adolescents. Renal function may be overestimated by these methods.

Tubular function (Tp/Ccrea or Tmp/GFR) (Rossi, 1994, 1995)

For tubular function, serum electrolyte and bicarbonate levels, and the calculation of fractionated phosphate reabsorption, relative amino acid reabsorption and/or fractionated Na excretion from single urine samples may be calculated according to Rossi et al.:

Fractionated phosphate reabsorption:

$$T_P/C_{crea} = Phosphate_{serum} - \frac{Phosphate_{urine} \; x \; Creatinine_{serum}}{Creatinine_{urine}} [\mu mol/ml]$$

$$T_P/C_{crea} = Phosphate_{serum} - \frac{Phosphate_{urine} \; x \; Creatinine_{serum}}{Creatinine_{urine}} 1x0{,}323[mg/dl]$$

Reference values in three age groups. "limit" refers to mean – 2 SD for T_P/Ccrea

	< 1 month		1-12 months		> 1 year	
	mean	limit	mean	limit	mean	limit
T_P/Ccrea [µmol/ml]	2.13	1.90	2.10	1.00	1.50	1.07

Ifosfamide adjustment to renal function

Classify toxicity as grade 0/1, 2, or 3/4 and adjust ifosfamide treatment as indicated if either GFR or Tp/Ccrea (Tm_p/GFR) or HCO_3 is reduced.

Toxicity grade*	GFR (ml/min/1.73 m^2)	Tp/Ccrea (Tm_p/GFR) (mmol/l)	HCO_3** (mmol/l)	Action (apply worst grade)
Grade 0/1	≥60	≥1.00	≥17.0	Continue ifosfamide dose 100%
Grade 2	40-59	0.80-0.99	14.0-16.9	Reduce ifosfamide dose by 30%
Grade 3/4	≤40	≤0.80	≤14.0	Use cyclophosphamide instead, 1500 mg/m²/d, d1

* Toxicity is scored from 0 to 4, analogous to the Common Toxicity Criteria (CTC) system, but for the purpose of modifying treatment grades 0 and 1 and grades 3 and 4 are considered together.

** Low values of HCO_3 should be re-checked when the patient is clinically stable (to rule out infection as a cause, etc.) before modifying ifosfamide dose / treatment.

Etoposide adjustment to renal function

- GFR <60 ml/min/1.73m^2:

  Reduce etoposide dose by 30%.

Zoledronic acid adjustment to renal function

Creatinine clearance	Action (apply worst grade)
≥ 60	Continue zoledronic acid dose 100 %
50-59	Reduce zoledronic acid dose by 12.5 %
40-49	Reduce zoledronic acid dose by 17.5 %
30-39	Reduce zoledronic acid dose by 25 %
< 30	Pause until recovery

10.1.4 Cardiac toxicity

- Shortening Fraction (SF) < 28% or left ventricular ejection fraction (LVEF) < 40% or decrease by an absolute value of ≥ 10 percentile points from previous tests:

  Delay chemotherapy cycle for 7 days and repeat echocardiography. If SF has recovered to 29% or greater then proceed to next cycle. If SF remains below 29% then omit DOX and substitute ACT 1.5mg/m².

  Repeat cardiac tests prior to next doxorubicin-containing cycle. If results have normalised, apply doxorubicin at normal dosage. If SF remains abnormal, refer to paragraph above.

10.1.5 Central neurotoxicity

Dose adaptation required:

Neurotoxicity CTC °3 or 4.

Methylene Blue

Treatment of ifosfamide-induced encephalopathy with methylene blue:

Adults: 50 mg (5ml ampoule of 1% solution) 4 hourly

Children: 1 mg/kg/dose 4 hourly

Additional application of clonazepam or haloperidol may be provided if indicated.

Prophylaxis of ifosfamide-induced encephalopathy:

Patients who had an episode of ifosfamide-induced encephalopathy in a previous cycle should receive one dose of methylene blue 24 hours prior to ifosfamide.

On the day of ifosfamide treatment the following dose schedule is recommended:

Adults: 50 mg (5ml ampoule of 1% solution) 6 hourly

Children: 1 mg/kg/dose 6 hourly

Prolong ifosfamide infusion to 4-8 hours with the next application, and give i.v. methylene blue 50 mg three times daily.

Repeated grade 3 or 4 central neurotoxicity:

Consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA.

It is recommended to call your appropriate national or group office (please refer to section 1.4) for advice. When ifosfamide is replaced by cyclophosphamide for subsequent cycles already during VIDE induction therapy, patients are ineligible for randomisation in R2. The randomisation in R1 and R3 is not affected.

11 STEM CELL HARVEST

Stem cell harvest is obligatory in patients with tumour volume ≥200 ml and in patients with metastases to the lungs/pleura or to extrapulmonary sites, and advised in patients with tumour volume <200 ml to accommodate the patient with a poor response to chemotherapy.

Stem cell harvest must be done in accordance with applicable national guidelines. Stem cell re-transfusion must be done in accordance with applicable national guidelines.

11.1 Time of Mobilisation

Mobilisation is recommended to take place at the earliest following the 3rd, at the latest after the 4th cycle of VIDE chemotherapy. Stem cell harvest should be started 8-14 days after the first day of the VIDE cycle according to institutional standard procedures, e.g. ≥10 $CD34^+$ cells per µl peripheral blood. Priming of peripheral blood progenitor cells with G-CSF, e.g. 10 µg/kg/d (250-300 µg/m²BSA/d) subcutaneously or IV, is advised from 24 hours after the last dose of chemotherapy until completion of harvest.

If the patient has bone marrow infiltration mobilisation should take place once the bone marrow is in remission.

11.2 Harvesting

$CD34^+$ cell counts are advised to determine optimal timing for harvesting (Engel, 1997). A total of at least 6 x 10^6/kg $CD34^+$ cells should be collected providing for one transplant and one backup (Fruehauf, 1995). More than one collection may be necessary to collect sufficient cells. Continue G-CSF daily until and including the last day of the harvest.

Please note: In Germany stem cell harvesting is to be performed exclusively in institutions holding an approval according to §13 AMG. Autologous haematopoietic stem cells must meet the following specifications:

CD34+/CD45+ cells/kg BW:	3 × 106 – 30 × 106
Viability after cryoconservation:	>70%
Volume:	55 – 110 ml
Number of products:	1 – 6

Further details for German centres are outlined in Appendix A.

11.3 Harvesting in Patients with Initial Bone Marrow Contamination

In case of initial bone marrow contamination (light microscopy) repeat bone marrow examinations are recommended prior to stem cell harvest to rule out persistent light microscopic bone marrow contamination. In case of persistent disease, delay of harvest until after clearance of the bone marrow is recommended. Contact your appropriate trial centre for further advice.

PBPC aliquots should be obtained and forwarded to the appropriate Molecular Biology reference centre to determine tumour cell content by RT-PCR for the specific patient's transcript.

CD34+ selection is recommended in all patients with initial bone marrow contamination.

12 SUPPORTIVE CARE

All treatment described here, especially VIDE, is intensive and aggressive and will be followed by severe bone marrow depression. Hence, treatment according to this protocol should be restricted to institutions familiar with the administration of intensive aggressive combination chemotherapy and where the full range of supportive care is available.

12.1 G-CSF

Treatment intensity is essential in the treatment of Ewing sarcomas.
G-CSF support is preferable to dose reduction.

VIDE: Day +5 following VIDE induction, **G-CSF** should be supplied at **5 µg/kg/d** once daily by subcutaneous injection until leukocyte recovery.

Any chemotherapy: When a previous cycle of any chemotherapy has been complicated by fever and neutropenia or prolonged hospitalisation, **G-CSF** should be started on day +1 and continued until leukocyte recovery (e.g. >5,000 x 10^6/µl or on 3 consecutive days >1,000 x 10^6/µl).

Stem cell harvest: With **$CD34^+$ stem cell harvesting, the recommended dose is 10 µg/kg/d** once daily by s.c. injection.

Stem cell reinfusion: please refer to Section 11.

12.2 Hydration

Sufficient hydration (~ 2-3 L/m²/d), with appropriate electrolyte supplementation must be provided during chemotherapy. Monitoring of blood pressure, cardiac and respiratory frequencies, body weight, and diuresis is mandatory; the application of diuretics may become necessary in case of oedema or hypertension.

12.3 Antiemetic Therapy

Antiemetic therapy should be administered according to institutional policy, e.g. ondansetron 5 mg/m²BSA (maximum single dose 8 mg) orally or IV every 12 hours for 5 days.

12.4 Blood Component Therapy

Radiation of Blood Products

Due to the risk of graft versus host reactions in patients under chemotherapy (especially in case of high dose therapy) all blood products (except fresh frozen plasma) must be irradiated with at least

20 Gy prior to transfusion, according to national policies. The use of leukocyte filters for leukocyte depletion (CMV negativity) is advised.

Red blood cells

Keep haemoglobin above 6 g/dl (haematocrit above 20 %).

Platelets

Platelet substitution is advised when platelets are <10,000/µl and/or with clinical evidence of bleeding.

12.5 Central Lines

The use of central lines is strongly recommended. (Especially in HDT patients, multi-lumen central lines are essential for PBPC sampling and supportive care).

12.6 Pneumocystis carinii Infection Prophylaxis

Pneumocystis carinii prophylaxis is mandatory according to the recommendations of the national groups.

12.7 Treatment of Infections

EWING 2008 is a very intensive protocol which is likely to result in episodes of neutropenic infection. All participating institutions must be familiar with managing such problems according to accepted general principles of supportive care and must have institutional standards.

12.8 Psycho-Social Support Recommendations

Qualified psycho-social support for patients and relatives is an integral part of the treatment strategies. Faced with a cancer diagnosis implying the risk of death or of permanent disablement and the need for long-term, aggressive multimodal therapy, patients and relatives need psychological support and crisis management. Moreover, social issues must be dealt with: housing, financial issues, unscheduled leave from work, etc.

Patients (and their families) need to continue their normal lives as much as possible and to allow their minds to turn away from the disease from time to time. Thus, school, structured and spontaneous play, artwork, music therapy, etc. should be available. Siblings of paediatric cancer patients often feel pushed back by all of the attention directed to the cancer patient and sometimes even feel guilty about being healthy. Parents may wonder if they are responsible for their child's disease (wrong food, smoking?). Thus, special attention must be paid and support

must be offered to the patient and to all family members. Close co-operation and regular exchange with the medical staff are of paramount importance in order to optimise all aspects of patient care.

12.8.1 Psycho-social support team

Well trained personnel must be available offering these services, and must be integrated in each patient's treatment strategy. The psycho-social support team should include members of the following professions:

- Clinical psychologist,
- Social worker,
- School teacher,
- Nursery school / kindergarten teacher,
- Art / music teacher / therapist.

12.8.2 Responsibility of the psycho-social team

The psycho-social support should encompass:

- Taking the social / psychological family history at first contact (intra-family relationships, coping styles, etc.),
- Help and guidance with social services, health insurance, social insurance matters, etc.,
- Help and support with practical problems during hospital stays, e.g. housing, transport, organising nannies for siblings, etc.,
- Offering assistance and aid to patients and relatives in stressful or painful situations, e.g. on the way to the operation theatre, at bone marrow taps, etc.,
- Establishing stable relationships between patient, family, and support team members,
- Crisis intervention (e.g. in case of non-coping, non-compliance, etc.),
- Support regarding emotional aspects: coping with disease and therapy,
- Play, artwork, music, ...
- Visits to the patient's home,
- Psychological and social follow-up (coping with remainders of disease and therapy, rehabilitation, occupational problems, ...),
- Psychological support in a terminal care situation.

The brief description given above can obviously not cover all issues of importance. In every patient his or her specific situation must be met by adjusted care and support which requires the permanent involvement of an experienced psycho-social team.

13 Quality of Life

QOL data will be collected for R1 and R3 trial patients in EWING 2008 via self-report and parent proxy report questionnaires as appropriate. Since for Ewing sarcoma no prospective data on QOL are available a first objective of QOL assessment is to describe the QOL development longitudinally (i.e. during the course of treatment and aftercare). The second objective of QOL assessment is to determine the impact on QOL of the additional treatment after randomisation for consolidation treatment (i.e. R1 and R3) via cross-sectional comparisons. Even if the treatment of R2 patients has to remain consistent with EURO-E.W.I.N.G. 99, the assessment of QOL within the current trial does not violate any of the basic codes of practice defined within that treatment protocol.

Describing and comparing the impact of these regimens on QOL will lead to a better understanding, from the patients' perspective, of the nature of treatment-related side effects, both short- and long-term. These data will help define future treatment options for these patients.

For patients aged 16 and over, QOL will be assessed using the EORTC QOLQ-C30 questionnaire (Aaronson, 1993; Fayers, 1995). For patients aged 15 and under, there is no paediatric QOL measure that has been validated in all participating countries. Thus, QOL for patients aged 15 and under will be assessed using either the generic PedsQOL questionnaire (Varni, 2002) or the PEDQOL questionnaire (Calaminus, 2000) according to group practice.

The reason for using two paediatric measures is that the PedsQOL questionnaire has not been validated in all European languages, whereas PEDQOL has not been validated in North America. Data have been published to suggest that these two instruments produce convergent results (Kennedy and Calaminus, 2002).

The initial QOL assessment will take place after completion of the first VIDE cycle and prior to the onset of the second cycle of induction chemotherapy (approx. protocol week 4 $\pm$ 1 week). Further assessments will be taken within 2 weeks after completion of induction chemotherapy and before surgery or irradiation, after completion of the protocol therapy and 2 years after completion of protocol therapy. Full details of QOL assessment are given in **Appendix B**.

14 PET Study

PLEASE NOTE: FDG-PET and PET-CT may not be available in all participating institutions. For this reason, PET is not mandatory and the lack of a PET investigation does not violate any of the basic codes of practice defined within the treatment protocol. Also, when the treatment of R2 patients has to remain consistent with EURO-E.W.I.N.G. 99, the inclusion of a PET investigation for diagnosis and follow up within the current trial does not violate any of the basic codes of practice defined within that treatment protocol.

14.1 Timing of FDG-PET or PET-CT Imaging

Glucose metabolism may change very early during the course of induction chemotherapy. Therefore, it is important to get baseline staging by FDG-PET or PET-CT before starting chemotherapy. Furthermore, any intervention may have an effect on glucose metabolism. Consequently, the optimal time point of the first FDG-PET or PET-CT would be before tumour biopsy. In many patients scheduling staging procedures prior to biopsy will not be feasible. In such cases, FDG-PET or PET-CT can be taken after biopsy of the primary tumour. The dates of biopsy and of FDG-PET or PET-CT must be recorded.

For follow-up investigations, the EORTC PET study group recommends a minimum time interval after chemotherapy of 2 weeks before FDG-PET or PET-CT imaging (Young, 1999). The following time schedule is recommended:

1. Staging, before start of chemotherapy.
2. Early response assessment, after 2 (earliest) to 3 (latest) cycles of chemotherapy
3. Late response assessment, after 5 (earliest) to 6 (latest) cycles of chemotherapy.

The value of FDG-PET / PET-CT for the diagnosis and for response evaluation will be determined in comparison with standard imaging procedures such as MRI, CT scan, ^{99}TC scintigraphy. For response evaluation, histological response will serve as an additional parameter to be compared.

14.2 FDG-PET Data Acquisition

FDG-PET should be performed according to the paediatric FDG-PET and PET-CT guideline of the EANM (European Association of Nuclear Medicine). FDG activity should be adapted to body weight according to the latest recommendation of the paediatric and dosimetry committees of the EANM.

Whole-body FDG-PET including arms and legs can be obtained using a stand alone full ring, dedicated PET scanner including measuring transmission of the trunk of the body and the primary tumour site. Alternatively, an in-line combined PET-CT scanner can be used for whole-body PET imaging including legs and arms with low-dose CT of the trunk of the body and the primary tumour site for attenuation correction. Pulmonary full diagnostic CT can be performed within the same examination if it has not been performed as a stand alone CT at the given time point. It is obsolete to perform a full diagnostic CT of other regions of the body unless indicated in rare situations (3-5, 9, 13). It is not sufficient to use a gamma camera based coincidence PET system. For further details please refer to Appendix B (FDG-PET examination).

15 DETERMINATION OF EFFICACY

15.1 Event-Free Survival

Event-free survival time (**EFS**) starts at the date of randomisation and ends at the date of first event or at the date of the patient's most recent consultation. Patients lost to follow-up without event are censored at the date of their last consultation.

Time of first event is defined as follows:

Event:	Date of occurrence
Non-response*:	Date of determination
Progression*:	Date of diagnosis of progression
Relapse*:	Date of diagnosis of relapse
Secondary malignancy:	Date of diagnosis of secondary malignancy
Death, whatever cause:	Date of death

*** Non-response** is defined as failure to respond to initial chemotherapy.

Progression of disease is defined as recurrent disease under active oncological therapy.

Relapse of disease is defined as recurrent disease in patients with complete clinical remission after completion of active oncological therapy.

15.2 Tumour Response

The evaluation of the primary tumour will be performed according to the RECIST criteria. The lesion(s) will be measured by imaging in at least one dimension. Measurements will be recorded in metric notation by use of a ruler or caliper. The baseline evaluation must be performed within four weeks prior to the beginning of treatment. The control measurement will be performed according to the time schedule specified below. Identical techniques and methods of assessment are to be used to describe each reported lesion at baseline.

Response is defined as follows:

Complete response (CR):	Disappearance of all tumour lesions, persistent bone changes with disappearence of intramedullary lesions and soft tissue component may be considered as CR
Partial response (PR):	At least 30% decrease of tumour volume of all measurable tumour lesions.
Stable disease (SD):	Neither PR nor progressive disease (PD)
Progressive disease (PD):	At least 20% increase in tumour volume or any new lesion.

In case of discordant response, the worst response is valid.

Each investigation consists of a physical examination, chest CT, CT or MRI of primary site, CT or MRI of known metastases.

16 SAFETY

16.1 Definitions and Explanation of Terms

An ***adverse event*** **(AE)** is any untoward medical occurrence in a patient administered an investigational medicinal product (IMP) and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavourable and unintended sign including an abnormal laboratory finding, a symptom, or a disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

An ***adverse reaction*** **(AR)** is any untoward and unintended response to an IMP, which is related to any dose administered. All **AE**s judged by either the reporting investigator or the sponsor resp. sponsor's delegate as having a reasonable causal relationship to an IMP qualify as ARs. The term AR and toxicity are synonymous. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

A ***serious adverse event (SAE) or serious adverse reaction*** **(SAR)** is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing inpatients´ hospitalisation, results in persistent or significant disability or incapacity, is a congenital anomaly or birth defect.

An ***unexpected adverse reaction*** **(UAR)** is an AR the nature or severity of which is not consistent with the applicable product information.

Examples of UARs:

- an expected / labelled SAR with an unexpected more severe clinical outcome (e.g. fatal)
- a more specific reaction than labelled (e.g. when "acute renal failure" is labelled, "interstitial nephritis" is more specific and therefore unexpected)
- an increase in the rate of occurrence of an expected AR is considered as unexpected.

A ***suspected unexpected serious adverse reaction*** (SUSAR) is an SAE where a causal relationship to the IMP is suspected, i.e. SAR, and where the nature or severity is not consistent with the product information, i.e. SUSAR.

16.2 Serious Adverse Events Requiring Immediate Reporting on SAE Form

Any of the following AEs occurring from treatment initiation until 3 months following completion of active oncological therapy must be reported.

- **AE which results in death**

  Death is an OUTCOME of an AE and must be reported together with the cause of death on the SAE form.

- **AE which is life-threatening**

  The term "*life-threatening*" refers to an AE in which the patient was at immediate risk of death at the time of the AE, i.e. required immediate intervention with life-saving intensive care treatment. It does not refer to an event which hypothetically might have caused death if it were more severe.

- **AE requiring hospitalisation**

  or

- **AE requiring prolongation of hospitalisation**

  Hospitalisation is defined as at least one overnight admission. Only AEs which are considered unanticipated (clinically unexpected) by the investigator and SAEs which are clinically expected but unexpectedly severe (CTCAE°4-5) require immediate reporting on the SAE form.

  Hospitalisation without underlying AE is not an SAE. Examples are:

  - Hospitalisation for protocol procedures e.g. chemotherapy, surgery, routine supportive treatment, biopsy or monitoring of the study.
  - Elective hospitalisation for a pre-existing condition (i.e. a condition other than the indication for the chemotherapy) that has not worsened.
  - Admission to a rehabilitation centre or hospice.
  - Hospitalisation for social reasons (e.g. due to anxiety but otherwise treatable on an outpatient basis).

- **AE or AR resulting in persistent or significant disability or incapacity.**

  Disability is defined as a substantial disruption in a person's ability to conduct normal life functions e.g. persistent blindness, deafness.

  Disability without underlying AE is not an SAE, i.e.

  - Disability resulting from tumour surgery e.g. following amputation or limb salvage surgery.

- **A congenital anomaly or birth defect**

  Pregnancy and its outcome should be reported as SAE in order to identify and follow up on outcome of pregnancy and on any congenital abnormalities, also births from fathers under chemotherapy are to be reported on the appropriate SAE form.

- **Other medically important conditions**

  Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are in the opinion of the investigator clinically unexpected and not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious and are reportable on an SAE form e.g. epileptic seizures, bronchospasmus.

- **Clinically relevant abnormal unanticipated biological or vital signs**

  Abnormal biological or vital signs commonly occur under chemotherapy but when considered clinically relevant by the physician i.e. unexpected or with severity of CTCAE grade 4 require immediate reporting on SAE form e.g. nephrotoxicity (GFR $\leq$ 19ml/min/1.73 m^2) or cardiac toxicity (FS<28%, LVEF < 40%).

- **Cancer: Secondary malignancies**

  Secondary malignancies e.g. skin cancer, myelodysplastic syndrome (MDS) usually occur later, but when they occur under protocol treatment, including 3 months of the last day of protocol defined chemotherapy, they should be reported immediately on SAE form as well as being documented on the Secondary Malignancy Form.

16.3 Protocol-Specific Exceptions for SAE Reporting

IMPORTANT NOTE: The following do not require reporting on the SAE form:

- Hospitalisation due to signs and symptoms of disease progression.
- Death due to disease progression.
- Expected hospitalisation for procedures such as blood transfusion, antibiotic treatment of neutropenic fever or CTCAE°1-3 infections, or controlled pain relief and nutritional support for gut toxicity CTCAE°1-3 or other expected toxicity CTCAE°1-3 is not to be reported on an SAE form.

Only SAEs which are considered unanticipated (clinically unexpected) by the investigator and SAEs which are clinically expected but unexpectedly severe (CTCAE°4-5) require immediate reporting on SAE form.

Be aware: SAEs which do not require immediate reporting on an SAE form, even if hospitalisation is required, are to be reported by CRF (toxicity check list); these include:

- Neutropenia and neutropenic fever (CTCAE °1-3).
- Infections and fever (CTCAE °1-3).

- Haematological toxicity: haemoglobin, WBC, granulocytes, platelets (CTCAE°1-3).
- Gut toxicity (mucositis / stomatitis, vomiting, diarrhoea) (CTCAE°1-3).
- Other expected AEs CTCAE°1-3.

16.4 Adverse Event Documentation

AEs are to be collected from the first day of protocol defined treatment until 3 months after completion of protocol-defined therapy (including maintenance chemotherapy).

AEs are graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. (http://ctep.cancer.gov/forms/CTCAEv3.pdf).

ARs both serious and non-serious are collected using a checklist CRF covering all anticipated ARs graded according to CTCAE v.3.0. The check list has a free-text box for documenting other clinically relevant AEs.

16.5 Serious Adverse Event Reporting

SAEs defined by the protocol in Section 16.1 are reportable on an SAE form to the EWING 2008 Safety Desk within one business day. The patient`s data have to be replaced by the Unique Patient Number (UPN) before forwarding the SAE form or any other information.

Address:

EWING 2008 Safety Desk
Zentrum für Klinische Studien (ZKS)
Universitätsklinikum Münster
Von-Esmarch-Str. 62
48149 Münster
Germany
Phone +49 (0)251 83 57109
Fax +49 (0)251 83 57112

The **investigator** is responsible for the assessment of seriousness, severity (CTCAE v. 3.0) and relatedness of the SAE. The SAE form should be completed with as much information as possible. Where possible, a diagnosis rather than a list of symptoms should be given. The investigator should not wait for full details before making the initial report. SAEs must be followed up until the condition resolves or stabilises. The investigator must fax any relevant follow-up information to the safety desk as soon as possible.

16.6 Serious Adverse Event Collection, Assessment and Distribution

The Intergroup Chairman for GPOH reviews each SAE again for seriousness and relatedness and assesses each SAR for expectedness according to the relevant product information.

The **EWING 2008 Safety Desk** collects and transfers all relevant safety information to the National Coordinator of each country. He/she ensures that the responsible competent authorities, ethics committees and investigators participating within his/her country are informed of all SUSARs and all other relevant safety information including Annual Safety Reports in accordance with national legal requirements.

The EWING 2008 Safety Desk informs the marketing authorization holder/s involved according to stipulation.

The Intergroup Chairmen are responsible for the ongoing safety evaluation of the trial. The EWING 2008 Safety Desk informs the Intergroup Chairmen immediately about any safety relevant information coming to its knowledge as do the Intergroup Chairmen inform the EWING 2008 Safety Desk. In case of safety relevant issues (besides SUSARs) which require expedited reporting, the EWING 2008 Safety Desk will support the Intergroup Chairmen in preparing an appropriate report in due time and distribute the report to the National Coordinators of each country.
The Intergroup Chairman from GPOH is responsible for providing the updated benefit-risk assessment of the trial for the Annual Safety Report (Part 1 of the report). The EWING 2008 Safety Desk is responsible for preparing all other parts of the Annual Safety Report, finalising it and distributing it to the National Coordinators for submission in a timely manner.
The EWING 2008 Safety Desk will provide information for the Independent Data and Safety Monitoring Committee as requested.
Relevant Product Information for the assessment of expectedness is for:

- Vincristine: Manufacturer's Product Information, see Appendix A1
- Ifosfamide: Manufacturer's Product Information, see Appendix A1
- Doxorubicin: Manufacturer's Product Information, see Appendix A1
- Etoposide: Manufacturer's Product Information, see Appendix A1
- Actinomycin D: Manufacturer's Product Information, see Appendix A1
- Cyclophosphamide: Manufacturer's Product Information, see Appendix A1
- Busulfan: Manufacturer's Product Information, see Appendix A1
- Melphalan: Manufacturer's Product Information, see Appendix A1
- Treosulfan: Investigator’s Brochure, supplied by MEDAC
- Zoledronic acid: Manufacturer's Product Information, see Appendix A1
- Fenretinide: Investigator’s Brochure, supplied following completion of Phase II

16.7 Late Effects of Chemotherapy

Late effects to be reported on follow-up forms include:

- Cardiac toxicity,

- Renal toxicity,
- Ototoxicity,
- Infertility.

In patients randomised for maintenance treatment with zoledronic acid, regular dental treatment is required, in order to prevent osteonecrosis of the joint.

16.7.1 Timepoints

The toxicity checks may follow national guidelines or recommendations of late effects trials.

Long term toxicity will be recorded on the six month follow-up form up to 5 years after diagnosis and on a yearly follow-up form thereafter and up to ten years after diagnosis. As a guideline the recommendations of the Late Effects Surveillance Study are listed below.

1st Year M O N T H	**Clinical Examination**	**Blood** FBC, Na, Cl, P, Mg, Ca, AST, ALT, γ-GT, proteine, creatinine, ESR **Urine** Phosphate, creatinine, glucose, protein	**Heart** ECG/Echo-cardiography, blood pressure	**Pulmonary function** Spirometry in patients treated with thoracic surgery and irradiation to lung or chest wall, busulfan, treosulfan high dose chemo	**Hormones** Sex Growth Thyroid	**Primary tumour** MRI CT	**Lung** X-Ray alternating with CT	**Metastatic sites**
0	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
2	**X**	**X**				**X**	**X**	**X**
4	**X**	**X**					**X**	
6	**X**	**X**	**X**	**X**		**X**	**X**	**X**
8	**X**	**X**					**X**	
10	**X**	**X**				**X**	**X**	**X**
12	**X**	**X**	**X**	**X**	**X**		**X**	
2nd Year								
2	**X**					**X**	**X**	**X**
4	**X**	**X**					**X**	
6	**X**		**X**			**X**	**X**	**X**
8	**X**	**X**					**X**	
10	**X**					**X**	**X**	**X**
12	**X**	**X**	**X**	**X**	**X**		**X**	
3rd Year								
3	**X**					**X**	**X**	**X**
6	**X**	**X**					**X**	
9	**X**					**X**	**X**	**X**
12	**X**	**X**	**X**	**X**	**X**		**X**	
4th Year								
6	**X**	**X**				**X**	**X**	**X**
12	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
5th Year and later years								
	X	**X**	**X**	**X**	**X**	**X**	**X**	**X**

17 PATHOLOGY - GUIDELINES

Before entering patients in this trial, clinicians should discuss this protocol with their pathologist and provide him/her with appropriate documents.

It is the responsibility of the clinician entering patients into the trial to ensure that the pathologist receives the protocol guidelines for the examination of biopsy material and material from tumour resection/amputation.

EWING 2008 is served by a panel of appointed pathologists. (Names and addresses are listed in Appendix A1). All biopsies must be sent to a reference pathologist to ensure uniform histopathological criteria for admission to the trial and the assessment of response to chemotherapy.

17.1 Biopsy

Biopsy sections are to be reviewed by a panel member.
PLEASE NOTE: Not all reference pathologists perform molecular pathology. A sample of fresh frozen tissue or representative paraffin-embedded material must be sent to a pathologist who performs the molecular pathology, for addresses see Appendix A1.

17.1.1 Procedures at biopsy

The diagnosis has to be histologically confirmed in every patient.
Fresh surgical specimens should be sent rapidly to the local Department of Pathology.
The diagnosis is based on the examination of routinely stained material supplemented by additional diagnostic methods as outlined below. Hematoxylin and eosin (HE) and Periodic-Acid-Schiff (PAS) are necessary for preliminary classification, followed and supplemented by immuno-histochemistry and molecular biology.
Fresh tumour tissue (deep frozen) must be saved for additional diagnostic investigations such as genetic analyses (molecular cytogenetics, Fluorescence In Situ Hybridisation (FISH), Comparative Genomic Hybridisation (CGH), electron microscopy).
Imprints (touch preparations) should also be made and stored at –20° until further examination (e.g. cytology, FISH).

17.1.2 Immunohistochemistry

CD99 immunohistochemistry is obligatory in the diagnostic work-up of Ewing sarcomas, as >95% of Ewing sarcomas show membraneous CD99 expression. Please note: CD99 (MIC-2 antigen)

expression is **not** unique in Ewing sarcomas. A positive staining reaction has been reported in synovial sarcoma, myelosarcoma, precursor lymphoma, Burkitt's lymphoma, alveolar rhabdomyosarcoma, neuroblastoma, thymocytes in thymoma, and others.
To distinguish Ewing sarcoma from atypical Ewing sarcoma and Peripheral NeuroEctodermal Tumour (PNET) an obligatory imunohistochemical examination of neuronal expression has to be performed using at least the following antigens:

- S-100
- Synaptophysin
- NSE

The following antigens are useful markers which can be used to identify myogenic, fibrogenic, and haematopoietic origin for the differential diagnosis within the group of small round cell tumours:

- Vimentin
- Desmin
- Smooth-muscle actin
- CD45 (leukocyte common antigen)

Pan B-cell antibodies, pan T-cell antibodies are recommended in suspected haematological malignancies, and TdT (terminal deoxynucleotidyl transferase) and MPO (myeloperoxidase) in tumours suspected for precursor lymphoma, Burkitt's lymphoma, and myelosarcoma.

17.1.3 Additional diagnostic methods

Molecular genetic analyses are strongly recommended to confirm the diagnosis and for molecular tumour classification.
Systematic investigations of Ewing sarcomas' molecular biological features are an integral part of the EWING 2008 concept. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) based detection of chromosomal translocations, Fluorescence In Situ Hybridisation (FISH), and/or Comparative Genomic Hybridisation (CGH) investigations of tumour samples (and bone marrow specimen and stem cell preparations) are performed in reference laboratories. A small fresh (deep frozen) tissue sample, together with a small conventional paraffin-embedded representative tissue sample, should be sent to a laboratory capable of these methods, see separate protocol below.

Conventional cytogenetics and cell culture (optional)

Fresh small tumour samples kept in RPMI should be sent to reference laboratories.

17.1.4 Definitive diagnosis

The definitive diagnosis may be based on examination of routinely stained material

plus the obligatory immunohistochemistry panel as outlined above,

or plus one of the following two investigations:

- molecular/cytogenetic analysis (chromosome 22 rearrangement)
- CD 99 (Mic-2) positivity

17.2 Surgical specimen

In order to stratify patients into the appropriate risk group, the examination of the surgical specimen is of utmost importance. Appropriate handling of the surgical specimen is of peak priority including initial handling, manipulation and dispatching the material for centralised review.

The examination of the specimen has two objectives:

1. Assessment of surgical margins
2. Assessment of response to chemotherapy

17.3 Definition of surgical margins

All surgical procedures must be classified as defined below.

- **radical / wide**
  Tumour completely removed, tumour not damaged during surgery, completely covered by intact lining of normal tissue or "capsule". This needs to be confirmed both macroscopically and microscopically. The biopsy canal must be removed en bloc with the specimen with an adequate margin.
- **marginal**
  Tumour macroscopically completely removed, tumour not damaged during surgery, but tumour tissue may reach resection margins microscopically, without clear evidence of residual tumour in situ.
- **intralesional**
  Tumour incompletely removed, or tumour damaged during surgery, or tumour tissue reaches resection margin with evidence of residual tumour in situ.

Amputation is also classified according to the surgical margin obtained, as outlined above.

Histopathological response must be evaluated as outlined below.

17.4 Histopathological response

The degree of histopathological response, i.e. the percentage of viable tumour cells in the specimen, must be determined as exactly as possible at the time of surgery following neoadjuvant treatment. Classifying response as suggested below will allow comparison with data from previous European studies (Salzer-Kuntschik, 1983):

GOOD RESPONSE

No viable tumour cells	(Salzer-Kuntschik: grade 1)
1% - **<**5% viable tumour cells	(Salzer-Kuntschik: grade 2)
≥5% - **<**10% viable tumour cells	(Salzer-Kuntschik: grade 3)

POOR RESPONSE

≥10% - <50% viable tumour cells	(Salzer-Kuntschik: grade 4)
≥ 50% viable tumour cells	(Salzer-Kuntschik: grade 5+6)

18 SURGERY - GUIDELINES

Definitive surgery is to follow primary chemotherapy, after 6 cycles of VIDE. This rule must not be violated, unless emergency surgical procedures are mandatory at diagnosis, e.g. in case of spinal cord compression. Careful planning of surgery is strongly recommended. Patients should be referred to an experienced center for their operation. The Münster office (see section 1.4) offers central guidance in planning of local therapy based on interdisciplinary tumour board discussions.

General rules:

- In all cases, adequate (wide/radical resection according to Enneking) surgical removal is desirable.
- The biopsy channel must be completely included in the surgical specimen.
- "Debulking" intralesional manoeuvres are discouraged.
- Wide excision with negative histological margins is necessary to optimise local control. Margins must be wide enough for optimal oncological control and narrow enough to maximise function.
- Reconstructive surgical techniques should be applied wherever possible, but oncological control is superior to limb preservation.
- The surgeon should consider the putative comorbidity with chemotherapy and radiotherapy. Therefore, the treatment plan for the patient should be worked out in an interdisciplinary oncology core group.
- Surgery is to be combined with additional radiotherapy following surgery in case of narrow margins and/or poor histological response ($\geq$ 10% viable tumour cells in the specimen).
- The surgical manoeuvre must be classified in cooperation by the surgeon and pathologist by definition of surgical margins and of histopathological response as outlined in Section 17.3.
- In case of residual lung disease after induction chemotherapy, surgical biopsies and/or removal of such lesions should be considered. Consultation with the national trial centre is advised.
- Surgical removal of other metastases may be considered individually.
- Combined modality local therapy should be considered standard particulary in large primaries with extensive soft tissue extension and should only be omitted when the area of original tumour extension has been included in the surgical specimen.

18.1 Tumours of the extremities

The major aim is wide tumour resection including the biopsy channel and scar. Conservation of the extremity is another major aim. The Münster office (see section 1.4) offers central guidance in planning of local therapy based on interdisciplinary tumour board discussions.

18.1.1 Femur

Distal femur

In patients with small tumours and sufficient tumour-free soft tissue implantation of an endoprosthesis (modular system) is usually feasible. In growing childen and adolescents a growing prosthesis might be used. In small children with a tumour free sciatic nerve, a rotation plasty might be considered.

Femur diaphysis

Implantation of a vascularised fibular bone might be an option for treatment in some patients. This treatment modality might be considered especially in young patients.

Implantation of an endoprosthesis is usually feasible. The use of a modular system is recommended. In children a growing endoprosthesis enables the adjustment of the prosthesis to the child`s growth. A bipolar cup allows for normal development of the pelvis in adolescence and good function in adult patients.

Proximal femur

An endoprosthesis is feasible if the biopsy was taken properly (laterally, with small longitudinal scar) and there is enough soft tissue to cover the prosthesis. Again a modular system is indicated and a growing prosthesis should be provided iin children. A total femur is feasible if there is enough soft tissue to cover. A bipolar cup allows normal development of the pelvis during growth and good function in adult patients.

18.1.2 Tibia

If the tumour initally had contact to the corresponding fibular bone the respective part of the fibula has to be considered contaminated and must be partially resected.

Proximal tibia

An implantation of a modular endoprosthesis might be feasible. In most cases a gastrocnemius flap is needed for soft tissue reconstruction.

Diaphysis of the tibia

The implantation of an autologous vascularised fibular or bone transport may be used for biological reconstruction. Alternatively, the implantation of a modular endoprosthesis is feasible.

Distal tibia

Endoprosthesis and resection arthrodesis of the tibiocalcaneous ankle are feasible. For this usually difficult location the recommendation of a reference surgeon might need to be obtained.

18.1.3 Fibula

The fibula is a bone which can usually be resected without reconstruction. If the tumour initially had contact to the corresponding tibial bone, resection of the cortical tibial bone has to be considered. If contamination of the tibial bone is radiologically suspected, resection of the tumour and reconstruction as outlined above have to be considered.

18.1.4 Humerus

Proximal humerus

Implantation of an endoprosthesis is feasible if nerves and vessels are not contaminated. Biological reconstruction, e.g. clavicula pro humero, might be considered in individual patients.

Humerus diaphysis

The implantation of an autologous vascularised fibula may be used for biological reconstruction. Alternatively implantation of a modular endoprosthesis is feasible.

18.2 Pelvis

According to the EICESS 92 and EURO-E.W.I.N.G. 99 data **postoperative radiotherapy as a rule is generally indicated in patients with a pelvic primary tumour,** even in patients with clear margins and good histological response to the induction chemotherapy.

Data from the EICESS 92 and EURO-E.W.I.N.G. 99 trial showed a clear benefit of combined modality local therapy using surgery and radiotherapy for patients with large pelvic tumours (> 200ml). In the rare case of a small pelvic tumour there was no difference in outcome when definitive radiotherapy, surgery or combined modality treatment were compared.

Pelvic surgery should only be performed in experienced centres (n>10). Limb-saving techniques avoiding external hemipelvectomy is one of the goals in pelvic surgery. Complications after pelvic sarcoma surgery can be reduced by avoiding skeletal reconstruction and choosing techniques like

hip transposition after acetabulum resection. Please contact the appropriate national or group office (see section 1.4) or a reference surgeon (see Appendix A2) for guidance.

18.3 Chest Wall, Ribs, Lung

The major aim in tumours of the chest wall, ribs, or lung - as in tumours of the extremities - is wide tumour resection. Thoracic surgery following chemotherapy should be performed in experienced centres only. The Münster office (see section 1.4) offers central guidance in the planning of local therapy based on interdisciplinary tumour board discussions.

Complete resection of the primary Ewing sarcoma of the chest wall should be performed after induction chemotherapy to obtain optimal shrinkage in order to facilitate complete resections and minimise the need for additional large field radiotherapy which is associated with a high risk of secondary morbidity in the form of growth impairment, cardiomyopathy and secondary malignancies (Shamberger, 2003).

Exploration of residual tumour lesions is essential for planning the surgical strategy. Wide rib or chest wall resection is feasible in older patients while in young patients anticipated growth impairment has to be considered for defining the extent of resection.

Following chest wall resection final exploration of the chest cavity and lung is essential. Chest wall reconstruction is indicated to achieve stabilisation and preserve respiratory mechanics. Satisfactory cosmesis is an important secondary goal. Reconstruction might be performed preferably with synthetic grafts (PTFE patch, prolene or vicryl mesh), especially in wider resections. Primary closure of soft tissues is possible in most cases.

Surgery is to be combined with additional radiotherapy following surgery in case of insufficient margins and/or poor histological response ($\geq$ 10% viable tumour cells in the specimen).

The surgical manoeuvre must be classified in cooperation by the surgeon and the pathologist for correct definition of surgical margins and histopathological response as outlined in Section 17.3.

18.3.1 Metastases to the lungs

In case of residual lung disease (initially suspected or proven metastases) or enlarged mediastinal/ hilar lymph nodes - after induction chemotherapy - surgical biopsies and/or removal of such lesions should be considered. Lung exploration and resection requires single lung ventilation and atelectasis.

19 RADIOTHERAPY - GUIDELINES

Surgery is favoured whenever feasible. Radiotherapy as an active modality for assuring local control is used as definitive radiotherapy in inoperable tumours or in combination with surgery either pre- or postoperatively.

PLEASE NOTE: Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.1 Timing

After the first reponse evaluation, following VIDE 2 or 3, interdisciplinary discussions regarding appropriate local therapy must be arranged for all patients. Every patient should be discussed by an interdisciplinary tumour board of oncologists, surgeons and radiotherapists. If indicated, appropriate images may be sent to a reference tumour board. Please contact the appropriate national or group office (see section 1.4) for contact addresses. The Münster office offers central guidance in the planning of local therapy based on interdisciplinary tumour board discussions.

Surgery should always be regarded as the first priority for local control.

- Indications for preoperative radiotherapy include clinical progression of tumour extension or anticipated marginal or intralesional resection.
- Postoperative radiotherapy is indicated in case of intralesional or marginal surgery and also in case of poor histological response regardless of surgical margins.
- Definitive radiotherapy is advised only in inoperable lesions. Inoperability is given in tumours that cannot be resected completely and in tumours of critical sites where complete surgery would result in unacceptable mutilation or is associated with a high risk of serious complications.

19.1.1 Preoperative radiotherapy

Early radiotherapy (with cycles 5 and 6, or even 3 and 4) should only be considered if the patient is expected to have a major benefit from such a procedure, e.g. in emergencies like spinal cord compression or in tumour progression under chemotherapy.

Patients scheduled for TreoMel may receive radiotherapy prior to HDT depending on the clinical response to induction chemotherapy. This should especially be considered when long periods of immobilisation following surgery are anticipated, e.g pelvic reconstruction. In these patients surgery following radiotherapy and HDT may also be an option to assure optimal safety of local control.

PLEASE NOTE: Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs. For patients receiving busulfan, large irradiation portals including bowel or lung have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas containing lung tissue must be avoided in patients receiving busulfan, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.1.2 Postoperative radiotherapy

Postoperative radiotherapy is indicated in patients with

- Poor histological response to VIDE induction chemotherapy.
- Marginal resection.
- Intralesional resection - after second look surgery (if feasible).
- Surgery at the time of diagnosis even if this was considered R0, unless a wide second look operation is feasible.
- Large pelvic tumours also in patients with wide resections.

Patients who are to receive postoperative irradiation are following surgery first continued on chemotherapy in order to allow for recovery from surgery, wound healing and planning of radiotherapy. These patients will start radiotherapy following the 2^{nd} to 4^{th} cycle of post-operative consolidation chemotherapy if treated in the conventional VAC or VAI arms of the study. In patients with macroscopic or microscopic residual disease post-operative irradiation may be started earlier. During radiotherapy actinomycin D is to be omitted.

In R3 patients scheduled for TreoMel the time interval between stem cell reinfusion following high-dose chemotherapy and the start of radiotherapy should be at least 8-10 weeks (stable engraftment provided) to avoid rebound toxicity. In patients with macroscopic or microscopic residual disease radiotherapy may be started earlier, i.e. parallel to the post-TreoMel VAC chemotherapy.

In patients given busulfan (BuMel arm) the time interval between stem cell reinfusion following high-dose chemotherapy and the start of radiotherapy should be at least 8-10 weeks (stable engraftment provided) to avoid rebound toxicity.

19.1.3 Definitive radiotherapy

Definitive radiotherapy is to start following course 6 of the VIDE induction regimen for patients in the conventional arms.

In patients scheduled for TreoMel the timing of definitive radiotherapy depends on the clinical response: patients with residual, inoperable bulky disease may receive radiotherapy prior to TreoMel parallel to VAC. In some patients, e.g. with bulky disease, definitive radiotherapy may be applied concurrently with VAC. Actinomycin D should be omitted during radiotherapy.

In patients of the BuMel arm radiotherapy should be applied 8-10 weeks after stem cell reinfusion, as busulfan cannot safely be applied following irradiation of central axial fields.

PLEASE NOTE: Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs. For patients receiving busulfan, large irradiation portals including bowel or lung have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas containing lung tissue must be avoided in patients receiving busulfan, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.2 Radiation Dose

19.2.1 Preoperative radiotherapy

The standard target volume dose for preoperative irradiation is 54.0 Gy in a normofractionated scheme with application of single doses of 1.8 Gy/day 5 days a week. Further boost techniques to increase the local dose may be applied using new technical approaches (e.g. intensity-modulated radiotherapy (IMRT), proton therapy or intraoperative radiotherapy using electrons as well as brachytherapy flabs), if feasible. Hyperfractionated accelerated irradiation (total dose of 54.4 Gy, 1.6 Gy twice daily, at least 6 hours interfractionation interval) may be an option to achieve a shorter overall duration of radiation treatment. However, in case of large irradiation portals or in case of larger bowel volumes within the radiation field normofractionated irradiation should be preferred for reasons of toxicity.

PLEASE NOTE: Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs. For patients receiving busulfan, large irradiation portals including bowel or lung have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas containing lung tissue must be avoided in patients receiving busulfan, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.2.2 Postoperative radiotherapy

Doses for postoperative radiotherapy according to surgical margins:

Intralesional surgery	45/54.0 Gy (with the option of a boost to 60.0 Gy, depending on the size and site of the primary tumour as well as the age of patient)
Marginal surgery with poor histological response (≥ 10% residual tumour cells)	54.0 Gy
Marginal surgery with good histological response (< 10% residual tumour cells)	45.0 Gy
Wide resection with poor histological response (≥ 10% residual tumour cells)	45.0 Gy according to national guidelines

In patients who show adequate surgical margins (i.e. wide or radical surgery) with good histological response, no radiotherapy is performed.

Exception: All patients with pelvic or spinal tumours must receive radiotherapy independent of surgical margins!

PLEASE NOTE: Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.2.3 Definitive radiotherapy

The compartment dose in definitive irradiation is 45.0 Gy with a tumour boost to at least 54.0 Gy. In individual cases, depending on the site of the tumour and the age of the patient, the boost dose may be adapted (increased or reduced), which should then be discussed with the national radiotherapy reference centre. In view of side effects, new technical approaches like intensity-modulated radiotherapy (IMRT), proton therapy and other high conformal therapy modalities should be considered whenever feasible and available. In children younger than 10 years with favourable prognostic factors (very small tumours < 100 ml, complete response to chemotherapy as judged by MRI and/or second look biopsy) the radiation dose may be reduced. These cases should be discussed with a radiotherapist experienced in the treatment of Ewing sarcomas in children (addresses see Appendix A1).

PLEASE NOTE: Severe toxicities leading to death have been observed in single patients who received high dose large volume radiotherapy following Bu-Mel high dose treatment.

Bu-Mel may compromise the ability to deliver effective radiation dose to central axial sites. In patients with an indication for RT, the patient will not be offered BuMel if there are critical organs such as gut/lung in the fields, unless the technique can be provided which limits the dose to critical organs. For patients receiving busulfan, large irradiation portals including bowel or lung have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas

containing lung tissue must be avoided in patients receiving busulfan, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

If radiotherapy of central axial sites involving critical organs is mandatory and the patient qualifies for the high risk arm, the patient is ineligible for randomisation and is to continue on VAI. In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.3 Fractionation

Normofractionated radiotherapy (one daily fraction, five fractions per week, single dose 1.8 to 2 Gy) is the preferred fractionation schedule. Hyperfractionated accelerated radiotherapy (1.6 Gy twice daily, at least 6 hours interfractionation interval) may be an alternative if normal tissue toxicity/tolerance allows this approach. The most important theoretical advantage of a hyperfractionated accelerated fractionation schedule is that it allows for the administration of radiotherapy in curative doses without prolonged interruption of chemotherapy. Chemotherapy therefore shall be continued during radiotherapy as planned, without breaks. Chemotherapy can be administered concurrently with radiotherapy.

Special care has to be taken when using large radiation portals, especially with a high amount of small and/or large bowel in the field. In these cases, a normofractionated treatment scheme is strongly advised.

Conventional fractionation is mandatory if the radiation field includes CNS structures (e.g. for spinal tumours) because the slow repair kinetics of CNS require long fractionation intervals.

In case of severe acute reactions, omission of chemotherapeutic agents with radiosensitizing properties during radiotherapy or a break of radiotherapy should be discussed with the trial centre/ reference radiotherapist. In general, treatment breaks should be avoided. Acute toxicity during radiotherapy has to be monitored carefully at least once weekly and is to be classified according to CTC guidelines.

PLEASE NOTE: Actinomycin D should be omitted during radiotherapy.

19.4 Irradiation Techniques and Target Volume Definition

Radiotherapy is delivered as local radiation to the tumour extent at diagnosis with adequate safety margins. Areas of scars from biopsy or tumour resection are to be included in the radiation field. For those patients who receive a dose of 54.0 Gy or more, a shrinking-field technique or new technical approaches (e.g. IMRT) should be used, giving the boost irradiation to a smaller volume which is defined as the tumour volume remaining after induction chemotherapy plus a 2 cm safety margin. Reproducible positioning and appropriate immobilisation devices must be used. Wedges and/or compensators must be used to produce homogeneous dose distributions of the reference doses.

Tumours of the extremities involving muscle compartments require extended field radiotherapy with small volume boost. The safety margins of the extended volume must be at least 3-5 cm in proximal and distal extension and 2 cm in lateral extension and in depth based on the pre-treatment tumour extension. In case of extensive intramedullary involvement or evidence of intramedullary skip lesions, whole bone irradiation is recommended. The epiphyseal plates, however, should be spared if possible. Irradiation of the whole anatomical compartment is not required if the above mentioned safety margins can be assured. An adequate strip of skin should be spared to avoid constrictive fibrosis. In most cases, opposing portals provide adequate dose distribution. 45.0 Gy are to be delivered to the extended volume. This is followed by a field reduction to the pre-treatment tumour volume with a 2 cm safety margin in proximal-distal extension and 1-2 cm safety margin in all other extensions.

For **tumours of the trunk and head&neck/skull**, safety margins of at least 2 cm in all extensions, based on the pre-treatment extent, should be kept. Smaller safety margins are allowed if otherwise non-invaded critical structures beyond well-defined anatomical borders (e.g., eye, optic chiasm) would be irradiated. In these cases, the radiotherapy reference centre should be consulted. Radiation techniques with multiple fields will be used.

Pelvic or chest wall tumours with non-infiltrating extension into pre-formed cavities, i.e. pelvis or chest require a **special target volume**. These tumours often show a large intrapelvic or intrathoracic mass which shrinks dramatically after chemotherapy. Irradiating the pre-treatment volume would mean that large volumes of healthy tissue (bowel or bladder in the pelvis, lung in case of chest wall tumours) are included in the radiation field. In these cases, the target volume in the areas of non-infiltrating tumour encompasses only the residual mass at the beginning of radiotherapy and a 2 cm safety margin. For all other parts of the tumour (infiltrated muscle or bone) the earlier mentioned more extended safety margins are to be applied.

For **patients receiving busulfan**, large irradiation portals including bowel or lung have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be

performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas containing lung tissue must be avoided in patients receiving busulfan, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

19.5 Quality of Radiation and Dose Specification

High-voltage equipment is mandatory. For extremity tumours photons of 4 to 6 MV (or cobalt 60) are recommended. For higher energies, attention must be focused on adequate skin doses in high-risk areas. For tumours of the trunk, photons of 6 to 15 MV energy are recommended. Fast electrons should not be used as the sole modality of radiation, but may be considered for small volume booster portals. New technical approaches should be considered whenever feasible and available.

Dose specification is done according to the ICRU 50 report. 3-D conformal radiotherapy planning is recommended if critical structures are situated in or near the target volume. To calculate the exact organ dose of healthy tissues at risk in the irradiation field, dose-volume histograms of critical organs are mandatory.

19.6 Chemotherapy during Radiotherapy

Actinomycin D, given during VAC and VAI consolidation chemotherapy should be omitted during central axial irradiation.

Busulfan-Melphalan: Patients with central axial tumours who are given early radiotherapy during VIDE induction therapy must NOT receive BuMel high-dose therapy for anticipated toxicity.
In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy.

For **patients receiving busulfan**, large irradiation portals including bowel or lung tissue have to be avoided. If irradiation of bowel-containing portals is mandatory, this should preferably be performed not earlier than 8-10 weeks after the application of busulfan. Any irradiation of areas containing lung tissue in patients receiving busulfan must be avoided, as the irradiation of the lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are situated in the irradiation portal, carefully planned

irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite likely damage to the lung.

It is, however, mandatory that each such case be discussed with the trial centre and a reference radiotherapist.

Doxorubicin: If a patient undergoing radiotherapy during VIDE induction experiences severe skin or intestinal reactions following the first series of radiotherapy, doxorubicin is to be reduced or omitted in the next series of radiotherapy.

Treosulfan-Melphalan: Safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

The trial centres / reference radiotherapists are available for guidance (addresses see Appendix A).

19.7 Dose Limits to Healthy Tissue

The following dose limits to healthy tissue are to be respected wherever possible:

- Spinal cord: 40 Gy (exception: tumours of the spine, allowable dose to the cord 50-54 Gy)
- Heart: 30 Gy
- Liver: 20 Gy in more than 1/3 of the organ volume.

In patients with chest wall tumours, who have received the busulfan containing chemotherapy, an irradiation of large parts of the lung should be avoided. In questionable cases, please contact the reference radiotherapists.

19.8 Technique of Irradiation in Special Tumour Sites

19.8.1 Extremities

Radiation is to be administered via opposed fields with sufficient proximal and distal safety margins according to the tumour extent at diagnosis. The surgical area must be included in the field. If necessary, a boost to the scar may be applied. To avoid constrictive fibrosis an adequate strip of skin and subcutaneous tissue may be spared at one side of the extremity throughout the whole field. The distant epiphysis may be spared when irradiating long bones as long as a safety margin of 5 cm can be assured. In tumours near joints, 45 Gy irradiation of the adjacent joint is necessary. In postoperative irradiation following implantation of prosthetic material, the prosthetic material should be included keeping a safety margin of 2 cm. Immobilisation devices must be used to immobilise the irradiated area reproducibly.

19.8.2 Pelvis

In pelvic tumours, in minimum three- to four-field techniques should be preferred to ensure optimal dose distribution. In tumours of the iliac bone tangential opposing fields provide adequate dose distribution in most cases. To protect the bladder and small bowel, individualised shielding may be necessary. Small volume boosts should be delivered using four-field techniques if possible. In some cases, patients may benefit from new high conformal therapy techniques like IMRT. These cases may be discussed with the reference radiotherapist (addresses see Appendix A).

PLEASE NOTE: For target volume definition in case of non-infiltrating intrapelvic tumour masses with shrinkage after chemotherapy see Section 19.1.3.

19.8.3 Vertebra

The target volume has to include one unaffected vertebral body above and below the affected bone. The irradiation volume must include sufficient safety margins regarding the paraspinal soft tissue component. Rotating techniques or three- or four-field techniques should be preferred. IMRT may also be an option. The maximum allowable dose to the spinal cord is 50 Gy (in conventional fractionation).

PLEASE NOTE: In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. The safety of TreoMel in patients receiving radiation to the spinal cord or brain or axial sites has not yet been established and is under evaluation in this trial.

19.8.4 Scapula

Tangential opposed fields in a prone position including a small part of lung tissue are recommended. In tumours of the upper part of the scapula, the humeral joint and the head of the humerus must usually be included.

PLEASE NOTE: In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. The safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.8.5 Chest wall

Chest wall tumours extending into the pleural cavity should be resected and are to receive postoperative irradiation where indicated (see Section 19.2.2 for indications of postoperative radiotherapy). If a patient on the conventional VAC or VAI arm presents with a tumour extending into the intra-thoracic space or infiltrating the pleural cavity, the ipsilateral hemithorax including

the ipsilateral lung is to be irradiated at a dose of 15 Gy (patients < 14 years of age) or 20 Gy (patients > 14 years). The technique is to observe the guidelines for total lung irradiation, using opposing fields. This is followed by a booster of radiation to the tumour area preferably using tangential photon portals to a total dose of 45 Gy (including the dose previously delivered to the hemithorax). The safety margins for irradiation of the primary lesion are at least 2 cm in all dimensions: dorsal/lateral, ventral/medial, as well as cranial/caudal. A small volume boost dose, possibly with electron beams, to 54.0 Gy may be delivered to areas of higher risk for local recurrence. In dorsal tumours, a sufficient dose has to be delivered to the vertebral extension of the ribs.

Any irradiation of major areas containing lung tissue must be avoided in patients receiving busulfan, as irradiation of lungs both prior to or after busulfan results in (severe) fibrosis of the involved lung. In individual cases where only minor parts of lung tissue are in the irradiation portal, carefully planned irradiation might be justified. In patients with chest wall tumours and persistent malignant effusion of the pleura, or in case of incomplete surgery, hemithorax chest wall irradiation might be considered despite the risk of damage to the lung. It is, however, mandatory that each such case be discussed with the reference centre (addresses see Appendix A).

When irradiating a primary chest wall tumour or tumour bed, lung tissue must be spared whenever possible. Tangential opposed fields and the calculation of dose–volume histograms are recommended to avoid irradiation of uninvolved parts of the lungs. Discussion of such cases with a reference centre is strongly advised (addresses see Appendix A).

PLEASE NOTE: For target volume definition in case of non-infiltrating chest wall tumour masses with shrinkage after chemotherapy see Section 19.4!

PLEASE NOTE: In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. The safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.8.6 Whole lung irradiation

PLEASE NOTE: There must be no whole lung irradiation in patients on busulfan containing regimens!

Whole lung irradiation is to be delivered to patients with pulmonary metastases at diagnosis treated in the conventional VAI + lung irradiation arm, even when complete remission is obtained with chemotherapy. Both lungs are to be irradiated using opposed photon fields ap/pa to a dose of 15 Gy (patients < 14 years of age) or 18 Gy (patients > 14 years). The daily fraction is either 1.5 Gy once daily or 1.25 Gy twice daily (lung dose). The dose calculation should be based on CT planning. In central beam calculation the lung correction factor is to be considered.

PLEASE NOTE: In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. The safety of TreoMel in patients receiving radiation to the spinal cord or brain or axial sites has not yet been established and is under evaluation in this trial.

19.8.7 Radiation therapy for extrapulmonary metastases

Patients with metastases to extrapulmonary sites (i.e. usually bone metastases) who are NOT elected to receive busulfan containing high-dose therapy may receive irradiation to extrapulmonary sites prior to high dose therapy, as specified in the protocol. All clinically detected sites should receive small volume irradiation of at least 45 Gy. If this strategy results in irradiation of more than 30% of the patient's bone marrow, local field radiotherapy shall be restricted to the most relevant areas in order to avoid extensive bone marrow irradiation. Extracranial stereotactic radiotherapy (ESRT) can be applied, if available and feasible. In addition, if a surplus of stem cells is available, reinfusion of a stem cell aliquot after such extended irradiation may be considered.

In patients receiving busulfan, NO radiotherapy to metastatic sites must be delivered prior to high dose therapy. (The strategy of irradiation after stem cell infusion in these patients should be discussed with the national trial centre.)

Isolated cerebral metastases are treated with 30 Gy (5 x 2 Gy/week) whole brain irradiation. A boost to single lesions is recommended if only one or two lesions with a maximum diameter of 2-3 cm are present. The boost dose in these cases is 20 Gy. Stereotactic radiotherapy should be used, if available.

PLEASE NOTE: In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. The safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

19.9 Irradiation for Palliation

In patients with progressive disease who are elected to receive irradiation for palliation, small volume irradiation with 12 daily fractions of 3 Gy each is recommended. Hypofractionated extracranial stereotactic radiotherapy (ESRT) with e.g. 3-4 single doses of 10-12 Gy (prescribed to the 65% isodose) with a time interval of one week between each fraction may be an alternative, whenever available and feasible. Please contact a reference radiotherapist for details (addresses see Appendix A).

19.10 Planning of Radiotherapy

Radiotherapy reference centres are available for assistance in radiotherapy treatment planning, addresses see Appendix A.

19.11 Side Effects

Acute side effects may occur during radiotherapy in rapidly proliferating tissues such as skin and mucosa in the field of irradiation. Such side effects require symptomatic treatment and usually clear within 1-2 weeks following termination of radiotherapy. The irradiated skin should be kept dry and mechanical irritation should be avoided. Local skin care should follow standard recommendations for radiotherapy. When extremities are irradiated, prophylactic physiotherapy should be initiated to avoid contractures. In patients receiving pelvic irradiation one should anticipate symptoms of radiation enteritis, e.g. spasms and diarrhoea. Mild cases usually clear with symptomatic treatment such as dietary measures. In severe cases it may be necessary to interrupt radiotherapy.

PLEASE NOTE: In patients receiving BuMel, radiation doses planned or administered involving the spinal cord or brain must not exceed 30 Gy. The safety of TreoMel in patients receiving radiation to the spinal cord or brain has not yet been established and is under evaluation in this trial.

Side effects should be documented using the CTC scoring system and late effects according to RTOG/EORTC criteria, respectively. The trial centres or the reference radiotherapists are to be notified immediately about any serious (i.e. life-threatening or lethal) side effects and/or complications.

19.12 Radiotherapy Follow-up

Irradiated patients have to be seen by the radiotherapist to record any late sequelae at least once a year following radiotherapy. The results are to be documented and submitted to the study centre. Patients treated within Germany should be enrolled into the "Registry for the evaluation of side effects after radiotherapy in childhood and adolescence". A detailed registry description as well as report forms are available online:

http://strahlentherapie.klinikum.uni-muenster.de/radtox.htm

20 PET - GUIDELINES

The following instructions on FDG-PET examination are based on the guideline of the European Association of Nuclear Medicine (EANM) concerning FDG-PET and PET/CT in paediatric patients (Stauss et al. Eur J Nucl Med Mol Imaging 2007, in press) and the latest version of the paediatric dosage card of the EANM (Lassmann et al. Eur J Nucl Med Mol Imaging 2007, in press). However, some modifications have been applied in order to adapt the examination technique to the requirements in Ewing sarcoma patients and to standardise the FDG-PET or PET-CT examinations within this trial.

20.1 Patient Preparation

A **fasting period of 6-8 h before** the FDG-PET or PET-CT examination is crucial to maintain low glucose and low insulin levels. In very **young patients < 6 years 4 h fasting** is sufficient to improve compliance. The ban regarding soft drinks, sweets, and glucose containing infusions during the fasting phase should be addressed explicitly. However, the patient ought to take water or unsweetened tea during the fasting period to **maintain good hydration**. The blood glucose level must be measured before FDG application. If it is > 120 mg/dl, FDG injection has to be postponed for several hours or FDG-PET or PET-CT has to be scheduled for another day. A thorough explanation of the scan should be provided to the patient and his/her parents by the technologist or physician (including hydration, time/duration of scanning, and details of the procedure itself).

Ideally, **i.v. access has been established outside the department of nuclear medicine in order to reduce stress for the child immediately prior to tracer injection** and thus to enhance compliance of the child when in the nuclear medicine department.

Uptake of FDG in brown adipose tissue is noted on 15-20% of PET scans in children and adolescents, which limits the study's ability to detect or rule out disease in these regions. It has been observed that brown fat uptake is encountered less frequently if the room, where the child spends the injection uptake phase, is warm. A warm blanket may also help to reduce tracer uptake in brown fat.

20.2 FDG Application

The amount of activity that needs to be administered to obtain sufficient image quality depends largely on the crystal of the PET camera, and the acquisition parameters (especially 2D or 3D mode). In children, acquisition in 3D mode (image acquisition without septa), if available, appears preferable to 2D mode due to its higher sensitivity (in combination with detectors using fast

scintillator materials). This assumption may not apply to very large patients (Body Mass Index >34). In general, the activity should be adjusted to the patient's weight and to the type of acquisition (2D or 3D) according to the latest version of the EANM dosage card (table 1). The recommended minimum activity (70 MBq) applies to commonly used positron emission tomographs. Lower activities could be administered when using systems with higher counting efficiency. Please note that the maximum activity might be limited by national regulations.

A maximum dose of 20 mg **furosemide (0.5 – 1 mg/kg BW)** can be given before or after the injection of FDG to enhance diuresis.

Weight (kg)	Dose (MBq) 2D	Dose (MBq) 3D	Weight (kg)	Dose (MBq) 2D	Dose (MBq) 3D
3	70	70	32	189	102
4	70	70	34	200	108
6	70	70	36	207	112
8	70	70	38	218	118
10	70	70	40	229	124
12	81	70	42	237	128
14	92	70	44	248	134
16	104	70	46	259	140
18	115	70	48	267	144
20	126	70	50	277	150
22	137	74	52-54	292	158
24	148	80	56-58	311	168
26	159	86	60-62	329	178
28	167	90	64-66	348	188
30	178	96	68	363	196

Table 1: Recommended dose based upon the EANM Dosage Card for 2D and 3D whole body 18F-FDG-PET (Lassmann, 2007)

20.3 Interval between FDG Injection and Data Acquisition

The patient should rest until the start of PET scanning. Standard imaging time commences at approximately 60 min post injection. It is important to use the same injection-to-scan interval at the initial and subsequent scans to assess response to treatment. Before the start of image acquisition the child should be encouraged to void.

20.4 Positioning of the Patient and Sedation

To ensure an optimum position in the scanner and to avoid movement artefacts, all patients should be comfortably immobilised during study acquisition with straps, tape, or cushions. In small children, the need for sedation has to be assessed individually and if required should be performed by an experienced physician, ideally by a paediatrician or paediatric anaesthetist. An adapted environment, an adequate attitude toward the child, a well-trained technologist for paediatric

procedures, and involved parents during the procedure all help to examine a co-operative child and may obviate the need for sedation in the majority of cases.

20.5 Data Acquisition

FDG-PET examinations have to be performed with a full ring, dedicated stand alone PET or PET-CT scanner. In Ewing sarcoma patients, a whole-body PET examination should include the entire legs and arms as the primary tumour and metastases can occur even in the distal extremities. The trunk of the body (base of the skull to middle thigh) and the primary tumour have to be scanned with transmission to allow attenuation corrected images and the calculation of standardised uptake values (SUV). In the extremities, with the exception of the primary tumour site, uncorrected emission images are sufficient.

Acquisition parameters depend largely on the detector and the type of scanner used. If transmission is measured by low-dose CT, acquisition parameters such as tube voltage and tube current time product have to be adapted to body weight and axial diameter.

Reconstruction

Attenuation corrected PET images are reconstructed iteratively.

Local evaluation of FDG-PET

FDG-PET or PET-CT images will be evaluated visually by local nuclear medicine physicians. All regions with pathological tracer uptake are to be documented and mean and maximum SUVs of the primary tumour site, the metastases, and the reference organ liver are measured (see FDG-PET documentation sheet). In case of multiple metastases, SUVs of one indicator lesion per involved organ are sufficient. The results of the FDG-PET or PET-CT examination are reported to the local paediatric oncologist. In case of stage relevant FDG-PET or PET-CT findings that were not known from conventional imaging other imaging modalities (e.g. MRI or CT) should be applied, or a biopsy should be taken if findings are still equivocal after additional imaging. In these cases please contact the study office.

20.6 Semiquantitative Analysis

SUVs will be calculated according to the following formulas:

SUV (max) = Q (max) * BW / A

SUV (mean) = Q (mean) * BW / A

with

Q (max) = maximum tissue tracer concentration [MBq/kg]

Q (mean) = mean tissue tracer concentration [MBq/kg]

BW = body weight [kg]

A = injected activity [MBq]

20.7 Reference Evaluation of FDG-PET

All FDG-PET or PET-CT images will be reviewed by the nuclear medicine reference panel. The panel will be naïve to the results of other imaging modalities for the first reading of the review and will then go on to include the results of other conventional imaging (and other modalities if available) and histological data. The results of both readings (blinded and unblinded) will be documented (see FDG-PET panel documentation sheet). In case of changes between the blinded and unblinded reading, the reason will be documented.

As FDG-PET and PET-CT may not be available in all participating institutions, PET is not mandatory and the lack of a PET investigation does not violate any of the basic codes of practice defined within the treatment protocol. Also, when treatment of R2 patients has to remain consistent with EURO-E.W.I.N.G. 99, the inclusion of a PET investigation for diagnostis and follow up within the current trial does not violate any of the basic codes of practice defined within that treatment protocol.

For details on reference evaluation, please refer to Appendix B.

21 STATISTICAL METHODS

21.1 Survival Endpoints

The primary endpoint of event-free survival (EFS) and the secondary endpoint of overall survival (OS) probability will be estimated according to the method of Kaplan and Meier (Kaplan&Meier 1958).

- Event-free survival time (**EFS**) starts at the date of randomisation and ends at the date of first event (non-response, progression of disease, relapse of disease, diagnosis of secondary malignancy, or death of the patient irrespective of its cause) or at the date of the patient's most recent consultation. Patients lost to follow-up without event are censored at the date of their last consultation.

  Non-response is defined as failure to respond to initial chemotherapy.

  Progression of disease is defined as recurrent disease under active oncological therapy.

  Relapse of disease is defined as recurrent disease in patients with complete clinical remission after completion of active oncological therapy.

- Overall survival time (**OS**) starts at the date of randomisation and ends at the date of death of the patient (irrespective of its cause) or at the date of the patient's most recent consultation. Patients lost to follow-up are censored at the date of their last consultation.

21.2 Accrual Estimates

Based on the experience of the EURO-E.W.I.N.G. 99 trial, the participating groups expect to randomise the following percentages of the total registered: R1, approx. 75% of 125-150 registered pts p.a.; R2loc and R2pulm, 55% of 65-75 registered pts p.a.(reasons for low accrual in R2 are outlined in Sections 3 and 7); R3, 65% of 40-50 registered pts p.a..

Anticipated number of randomised pts p.a., based on the EURO-E.W.I.N.G. 99 experience.

	Anticipated number of randomised pts p.a. based on the EURO-E.W.I.N.G. 99 average			Expected number of randomised pts
	R1	**R2loc**	**R2pulm**	**R3**
EORTC*	9	2	2	0
GPOH (+COG)	36	8	9	13
CCLG	17	4	4	8
SFCE	31	7	6	8
Total	**93**	**21**	**21**	**29**
Eligible for analysis p.a. (5% deducted for anticipated loss to follow-up)	**88**	**20**	**20**	**27**

*EORTC accrual estimate based on EURO-E.W.I.N.G. 99 participation, replaced in EWING 2008 by direct participation of German EORTC institutions through GPOH.

The future projection for R2pulm is conservative because it is based on the randomisation rate observed over the whole study duration of EURO-E.W.I.N.G. 99; rates in R2pulm, mainly due to rising accrual from the COG group, have increased over the last two years. With continued strong accrual it is anticipated that R2pulm will recruit up to 30 randomised pts p.a. eligible for analysis.

The estimate for high-risk R3 is based on the observation in EURO-E.W.I.N.G. 99 that approximately 75% of this group were still on study at the time of VIDE 6.

21.3 Sample Size

Randomised questions will be asked in all risks groups: R1, R2, and R3. All power calculations were based on 3-year EFS rates performing a two–sided (except R3: one side-hypothesis) logrank test with alpha=0.05 (except R3: alpha=0.025) and power=0.80. Group sequential plans with 3 interim analyses will be conducted. The Schoenfeld formula was used to compute the necessary number of events (Schoenfeld, 1981). Sample size calculations were computed with Addplan Software V. 3.1.3 (Wassmer, 2006). The accrual period is different for each risk group based on the accrual rate estimates of EURO-E.W.I.N.G. 99. For R2pulm conservative estimates were used (see 21.2). Calculations are based on a follow-up period of 2 years after the end of accrual for all groups. All patients must be observed at least until the end of this period to achieve the required power. If the accrual time is significantly shorter or longer (by >1y) than anticipated because more resp. fewer patients are randomised p.a. than anticipated, minor adjustments may be needed, with no major impact on the total sample size of the study. Due to the fact that most of the events (85-95%) in Ewing sarcoma patients occur in the first 3-5 years after diagnosis and survival reaches a nearly constant plateau thereafter, the sample size for significantly longer studies cannot be calculated with the assumption of exponential survival in this setting. Accordingly, the R2

calculations were computed with SIZE software (Shih, 1995) which allows complex modelling of the underlying survival curves.

21.3.1 Sample size for Randomisation R1

Aim: To test for the difference in terms of EFS (OS) between add-on treatment with zoledronic acid, fenretinide, or zoledronic acid plus fenretinide in a two-by-two factorial design.

The assumption of 70% 3-year EFS for the reference treatment without add-on regimen was based on the EICESS 92 estimates as survival rates of EURO-E.W.I.N.G. 99 are not available at this time. Adjustments will be made if there is major improvement in the R1 group of the EURO-E.W.I.N.G. 99 trial. No major impact on the current sample size calculation is anticipated.

The study will start with a two-arm randomisation of zoledronic acid vs. no zoledronic acid add-on treatment; zoledronic acid is available at the start of the trial while fenretinide is expected to be available for the study by the end of 2009 depending on the outcome of an ongoing fenretinide phase II trial. Following inclusion of fenretinide the randomisation allocation factor will be adjusted to obtain equal group sizes at the end of the trial. No bias for the analysis is expected from this adjustment. If fenretinide cannot be included in the study for any reason, the R1 randomisation will be continued as a simple two-arm randomisation. As the decision to include fenretinide in this trial is independent of any results in the course of the EWING 2008 trial two independent power calculations are given: one for a two-arm randomisation (Case A), and one for a two-by-two factorial design (CASE B):

Case A: Two-arm randomisation with zoledronic acid

To test for a 10% difference between zoledronic acid (3-year EFS 80%) and no zoledronic acid add-on treatment (3-year EFS 70%) a total number of **426 patients** (213 patients per group) eligible for analysis will be needed, with an expected accrual period of **5 years**. The expected number of events is 119 under the alternative hypothesis, and 146 under the null hypothesis. 5% more patients should be randomised to make up for cases lost to follow-up or missing information (**=448 patients**).

Case B: Two-by-two factorial design with fenretinide and zoledronic acid

Assuming a 10% difference for both the fenretinide and the zoledronic acid group with a 3-year EFS of 80% and an anticipated interaction of both treatments, where the combined treatment in the fenretinide plus zoledronic acid group is expected to show a 3-year EFS of 85%, the main effects of the treatments can be tested with a total number of **596 patients** (with equal group sizes: approx. 170 patients per group) eligible for analysis. The accrual period is expected to be **6.5 years.** The expected number of events is 162 under the alternative hypothesis, and 198 under

the null hypothesis. 5% more patients should be randomised to make up for cases lost to follow-up or missing information (**=626 patients**).

If no interaction occurs, and the fenretinide plus zoledronic acid group still has an expected 3-year EFS of 85%, the main effects of the treatments could be identified with a total number of **626 patients** and a slightly lower 3-year EFS of 77.5% for both the fenretinide and the zoledronic acid group.

21.3.2 Sample size for Randomisation R2

The R2 randomisation of the EURO-E.W.I.N.G. 99 trial will be continued. As of 31st October 2006, 134 R2loc patients and 143 R2pulm patients were randomised. The sample size calculations were adjusted due to the longer accrual period beginning in 1999-2001 for the R2 groups.

Sample size for Randomisation R2loc

Aim: To test for the difference in terms of EFS (OS) between conventional chemotherapy (VAI) and high dose chemotherapy (BuMel).

The 3-year EFS estimate for the baseline was calculated to be 55% referring to the EICESS 92 cohort. To detect a 15% difference between treatments a total of **270 patients** eligible for analysis will be needed. This number is expected to be reached within 5 years from the expected start of the EWING 2008 trial in January 2009; the overall accrual period including the duration of EURO-E.W.I.N.G. 99 will be approx. **14 years**. The expected number of events is 98 under the alternative hypothesis, and 120 under the null hypothesis. 5% more patients should be randomised to make up for cases lost to follow-up or missing information (=**284 patients**).

Sample size for Randomisation R2pulm (patients with pulmonary/pleural metastases)

Aim: To test for the difference in terms of EFS (OS) between VAI plus whole lung irradiation and BuMel (without whole lung irradiation).

The 3-year EFS estimate for the baseline was calculated to be 40% referring to the EICESS 92 cohort. To detect a 15% difference between treatments a total of **274 patients** eligible for analysis will be needed. This number is expected to be reached within 5 years from the expected start of the EWING 2008 trial in January 2009; the overall accrual period including the duration of EURO-E.W.I.N.G. 99 will be approx. **14 years**. The expected number of events is 144 under the alternative hypothesis, and 176 under the null hypothesis. 5% more patients should be randomised to make up for cases lost to follow-up or missing information (=**288 patients**).

21.3.3 Sample size for Randomisation R3

Aim: To test for an improvement in EFS (OAS) for high dose chemotherapy (TreoMel) as compared with standard conventional chemotherapy (VAI).

The 3-year EFS estimate for conventional chemotherapy as the reference treatment was calculated to be 15% based on the outcome of R3 patients in the EURO-E.W.I.N.G. 99 trial. To detect a 15% improvement for the experimental high dose arm a total of **176 patients** eligible for analysis will be needed. This number is to be reached within **6.5 years**. The expected number of events is 126 under the alternative hypothesis, and 155 under the null hypothesis. 5% more patients should be randomised to make up for cases lost to follow-up or missing information (=**185 patients**).

21.4 Intended Analyses

Survival analysis: All patients randomised in the trial will be included in the intent-to-treat analysis in their allocated treatment group. For certain questions additional "per protocol" analyses are intended. No patient will be excluded from analysis once he or she is entered into the study, unless informed consent is withdrawn. Event-free survival and overall survival probability will be estimated according to the method of Kaplan and Meier (1958).

Comparison between randomised arms and analyses of prognostic factors: Comparisons are performed by logrank tests. Variables are univariately analysed regarding impact on EFS by logrank test or by chi-square analysis as appropriate. Multivariate analyses of variables with regard to EFS times are performed by Cox analysis (1972). Analyses of specific failure risks are performed by means of competing risk analysis (1995). Those patients who are included in the study but cannot be randomised will be included in exploratory analyses for EFS and OS outcome.

21.5 Interim Analyses and Stopping Rule

Three interim analyses are planned after observing 25%, 50%, and 75% of the expected events in each randomisation arm before the final analyses will be done after the end of accrual and 2 years of additional follow-up. The trial will be stopped for any risk group in which the p-value for the analysis of EFS is under the nominal value that is calculated using an alpha-spending function approach by O`Brien and Fleming (1979). In such an event the study chairmen will be notified and a study committee meeting will be held to discuss the consequences.

21.6 Stopping Rules for the Study Protocol due to Toxic Deaths

Interim analyses on serious acute toxicity (grade 4 other than mucositis and haematological toxicity) and toxic deaths will take place twice a year under the supervision of the Independent Data and Safety Monitoring Committee (IDMC).

All deaths related to treatment, such as toxic deaths, regardless of the length of interval from treatment to death will be included and a description/detailed documentation of all toxicity is essential. Please note that higher toxicity must be expected with high-dose chemotherapy compared to conventional treatment (see below).

Deaths not related to the underlying malignant disease will be compared between treatment groups by logrank test and crude percentage comparison tests. If any of these tests is significant at $p<10^{-4}$, the conclusion will be that there is a relative excess of toxic deaths; a full analysis will then be considered.

Crude percentage will also be compared to the theoretically acceptable toxic death rate. If the lower boundary of the 99.9% confidence interval (binomial distribution approach) of the observed percentage is above this limit, the conclusion will be that there is an absolute excess of toxic deaths in this group; a full analysis will then be considered.

The information from such "early" interim analyses regarding an excess of toxic deaths will be forwarded to the IDMC and the study will be stopped immediately. The IDMC will decide after consultation with the study co-ordinators and statisticians whether and how the study will proceed.

Based on previous experience (<1% [10^{-4}-1.5%] of toxic deaths observed in EICESS 92, (4% [1.1-9.9%] after Busulfan-Melphalan in the SFCE group), the threshold has been set at 1% for patients on conventional chemotherapy and 5% for those on high-dose chemotherapy.

The type 1 error (α) has been fixed equal to 10^{-3} to accommodate the large number of analyses for toxicity.

Examples:

Conventional chemotherapy				High-dose chemotherapy			
To conclude that $p_{obs} \geq 1\%$ with $\alpha = 10^{-3}$				To conclude that $p_{obs} \geq 5\%$ with $\alpha = 10^{-3}$			
N	k_{lim}	p_{obs}	99.9% CI	N	k_{lim}	P_{obs}	99.9% CI
20	4	20%	[1.9-59%]	20	6	30%	[5.4-68%]
50	5	10%	[1.3-31%]	50	10	20%	[5.8-43%]
100	7	7%	[1.4-19%]	100	14	14%	[5.0-28%]
200	9	4.5%	[1.1-11%]	200	22	11%	[5.0-20%]
500	15	3%	[1.1-6.4%]	500	43	8.6%	[5.0-13%]

N : number of treated patients

k_{lim}: number of toxic deaths, leading to the conclusion of an absolute excess of toxic deaths.

22 DATA MANAGEMENT

22.1 National data centres (NDCs)

The participating study groups are associated with specific data centres. Data management will be performed by that national or group data centre (NDC). Procedures of data management are fixed in common standard operating procedures (SOPs). Data will be captured by a certified Remote Data Entry system, e.g. MARVIN software (Xclinical) for GPOH institutions.

The responsibilities of an NDC include:

- Registration of patients
- Shipment of documents required at the trial sites (investigator site file, CRFs, etc.)
- Providing information on treatment according to protocol
- Collection of paper CRFs including clinical check
- Providing assessment of data quality and quantity (missing and out of border data)
- Running reminder campaigns
- Desgining a valid trial data entry system and database
- Data entry and coding
- Regular data exchange with the coordinating data centre (CDC, s. 22.2)
- Answering plausibility checklists from the CDC and correcting national/group databases
- Relaying queries to the investigators
- Randomisation of patients

22.2 Coordinating data centre (CDC)

The coordinating data centre (CDC) is located at the Institute of Medical Informatics and Biomathematics of Münster University Hospital (address see section 1.6) and is independent from the NDCs. The responsible statistician is a staff member of the Institute of Medical Informatics and Biometrics, University of Münster. All NDCs will perform regular data exchange (every 3 months) with the CDC. The CDC will provide a list of data to be collected, time points at which they are to be collected, and an exact definition of terms.

The responsibilities of the CDC include:

- Specification of stratification criteria for randomisation
- Defining and obtaining plausibility checklists for the NDCs
- Sending plausibility check lists to the NDCs following every regular data exchange
- Merging data
- Generating biannual reports for the Independent Data and Safety Monitoring Committee

- Generating biannual reports for the Study Committee
- Generating the annual safety report in collaboration with the Safety Desk
- Interim analysis as defined in the protocol
- Closing of database
- Analysis of the randomised arms (R1, R2loc, R2pulm, R3)
- Preparation of final trial report

22.3 Patient Identification

Upon registration, every patient is assigned a unique patient number (UPN) by the appropriate national or group office. The UPN in combination with the date of birth will ensure appropriate identification. The local investigator will keep a confidential list of patients connecting UPNs with the name. Some trial groups will record the name. If so, the authorisation to record the names will be included in the appropriate informed consent form.

22.4 Data Collection

All data except safety information **will be collected by remote data entry**. For this purpose, national and group offices and institutions will be linked through the Internet (https).

All safety information, i.e. SAE and SUSAR reports, must be entered on paper forms and submitted by fax.

The following rules for completing paper CRFs have to be observed:

- CRFs are to be filled in with a black ballpoint pen. Pens or pencils are not allowed.
- Script must be clear and legible.
- Mistakes are to be cancelled by a simple horizontal line and correction is to be written above or next to it. The correction has to be signed and dated.
- Data fields which cannot be completed due to missing information have to be marked and commented.
- CRFs have to be completed in a timely fashion and finally checked and signed by the investigator.
- In case of major corrections and/or missing data, the reason is to be given.
- All requested data fields should be answered completely; this applies also if there is no major change from a previous examination.
- At all times the principal investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered.

- All primary data (e.g. diagnostic findings) which are lodged in the CRF have to be signed and dated by the responsible investigator.

The following data are regarded as source data:

- All data contained in the patient's medical records.
- Pathology/reference pathology.
- Images.
- Surgical reports.

PLEASE NOTE: The patient identification on these papers must be replaced by the UPN where applicable and, if appropriate, the patient's date of birth.

The CRFs are contained in Appendix B. Groups are free to collect additional data according to their respective practice. All patients' UPN and date of birth, but no full name or address will be recorded, unless otherwise specified.

22.5 Archiving

The archiving of all study relevant documents at the trial site, at the trial offices, and at the coordinating investigator's site will be handled according to national law.

22.6 Data Protection

Personal data of all participating patients, i.e. date of birth and data regarding the disease, treatment and follow-up will be collected.

If a patient and/or his/her parents/legal guardian have given informed consent, the patient's name and address may be held at the trial office. This is needed for central treatment planning and in case of direct patient contact. The data will be stored separate from the database and handled confidentially.

All study relevant data will be stored electronically and handled confidentially. For statistical analysis and documentation, patients will be identified only by the unique patient number (UPN).

The investigators and all members of a trial centre or other persons involved in the trial are obliged to keep study data and information confidential and to grant access only to individuals who are involved in the study.

An exception to this rule applies only to representatives of the sponsor or regulatory authorities as outlined in Section 26.

All legal requirements concerning safety, confidentiality and prevention of data loss will be respected. All involved individuals are sworn to secrecy. All NDC databases will be backed up every day. Access to data is strictly limited to authorised persons. Anonymity of data in the scope of biometrical analysis is guaranteed.

23 ETHICAL, LEGAL AND REGULATORY ISSUES

This study must be conducted in accordance with the ethical principles laid down in the Declaration of Helsinki and in CPMP/ICH/135/95 Note for Guidance on Good Clinical Practice. A clinical trial may not begin before approval of the Ethics Committees and acceptance by the competent national regulatory authorities has been obtained.

23.1 National Ethics Committee and National Regulatory Authority

The national coordinator, on behalf of the sponsor, is responsible for applying for an Ethics Committee approval and for any required authorisation of the competent authority according to national law and institutional guidelines. Furthermore, the principal coordinating investigator will provide the national coordinators of the participating countries with all documents required for an Ethics Committee approval and for acceptance by the competent authority according to German law. The national coordinators will provide all further documents required by national law and for application to the responsible Ethics Committee and the competent authority (if applicable).

23.2 Patient Information and Informed Consent Form

Before signing the informed consent form the patient and/or his/her parents/legal representatives must be informed about the disease, the treatment according to the clinical trial including estimated duration, randomisation, possible side and late effects of the treatment, and the assessment required for the treatment and about alternative treatment options. The patients and/or their parents/legal representatives must have sufficient time to decide about trial participation and must have the opportunity to ask all questions they may have concerning the trial treatment before signing the consent form.

The signature of the legal representative is required for children and adolescents below legal age. Age-adapted informed assent forms for children and adolescents are provided and should be signed by underage patients. If an underage patient who is capable of forming an opinion and assessing the information refuses participation in the trial, this has to be considered by the investigator and, if appropriate, discussed with the principal investigator.

The informed consent is documented on a standard form, written in non-technical language. A master version of each informed consent form is contained in Appendix B. The consent forms may

be modified according to group or local requirements. Modified versions must be submitted by the appropriate coordinator or principal investigator to the appropriate Ethics Committee for approval.

23.3 Consent to Data Management, Storage, and Transmission and Distribution of Biological Material

Consent to trial participation, data storage, transmission and analysis and to the distribution of biological material will be obtained on a separate consent form.

The patients and/or their parents/legal representatives by the general information sheet (Appendix B) are informed about the storage of the trial based data and are informed that representatives of the sponsor e.g. monitors, auditors, and representatives of competent authorities e.g inspectors may have the right to conduct an official review of the original documents, records and other issues that are deemed to be related to the clinical trial. Furthermore, they are informed, that pseudonymised data will be stored, transmitted and analysed and may be used for scientific publications. The patients and/or parents/legal representatives have the right to know about the data kept.

FOR GERMAN PATIENTS AND OTHERS IF INDICATED:

The patients or their parents/legal representatives are additionally asked to give consent to the transmission of their personal and health related data to:

- German Childhood Cancer Registry, Mainz (Dr. P. Kaatsch)
- Registry for the Evaluation of Late Side Effects after Radiotherapy in Childhood and Adolescence **(R**eg**i**ster zur Erfassung von **S**pätfolgen nach Strahlentherapie im **K**indes- und Jugendalter, **RISK)**, Münster (Prof. Dr. N. Willich, Dr. T. Bölling)
- Late Effects Surveillance System, Erlangen (Dr. Th. Langer)
- Relapse Registry, (Trial center Münster, Sarez, Tübingen; http://transarnet.klinikum.uni-muenster)

23.4 Consent to Surgery, Radiology, Nuclear Medicine, Radiotherapy

Information about any surgical, radiological, nuclear medicine, or radiotherapy procedure will be obtained by the clinician in charge of that procedure and informed consent will be obtained separately according to national and institutional guidelines.

23.5 Withdrawal from the Trial

The patients and/or their parents/legal representatives may withdraw from the clinical trial at any time by countermanding their informed consent without giving the reason for it. The patients

and/or their parents/legal representatives are assured in writing on the informed consent form that withdrawal from the trial will not affect the medical treatment, but that medical treatment of the disease must be continued. Date of enrollment and date of and reason for (if provided) withdrawal are to be recorded in each case.

The patients and/or their parents/legal representatives have to be informed that data stored up to this time will be used further if necessary to assess the effects of the treatment to be tested, to guarantee that the interests of all patients are not impaired, in accordance with regulatory requirements.

23.6 Sponsorship

Münster University Hospital – Universitätsklinikum Münster - takes on sponsorship of the international clinical trial EWING 2008 in the legal sense as defined by the Directive 2001/20/EC of the European Parliament and of the Council of 4th April 2001. The sponsor may delegate his duties for each participating country to a national coordinator by written agreement.

The national coordinator will fulfil the transferred duties for the sponsor and warrants compliance with all statutory provisions relevant for the sponsor. The national coordinator will notify the sponsor of all duties required by the respective national regulation that were not transferred to the national coordinator. The national coordinator will fulfil these national required duties as required. In addition, all documents relevant for the trial master file will be provided. The sponsor may audit the national coordinator to ensure adherence to all legal requirements.

23.7 Amendments

Any significant change in the trial requires a protocol amendment. An investigator must not make any changes to the study without approval of the intergroup steering committee, approval of ethics committee or sponsor, or authorisation by the competent authorities except when necessary to eliminate apparent immediate hazards to the patients. A protocol change intended to eliminate an apparent immediate hazard to patients may be implemented immediately, but the change must then be documented in an amendment, reported to the ethics committee and submitted to the appropriate competent authorities in the required time frame. Application for authorisation and approval of all other substantial protocol amendments must follow the same process as the original protocol.

Substantial protocol amendments which are likely to have an impact on the treatment and/or safety of the participants or to modify the interpretation of the data or which are otherwise significant are prepared by the intergroup steering committee. The national coordinators are responsible for submitting the application for authorisation of an amendment to the competent

authorities concerned and for submitting the application for approval to the ethics committeees concerned according to national law and institutional guidelines. Furthermore, the principal coordinating investigator in Germany will provide all national coordinators of the participating countries with all essential documents required according to the ICH Guideline and the Guideline on Good Clinical Practice and German law.

23.8 Declaration of the End of the Trial

The principal coordinating investigator, on behalf of the sponsor, is responsible for informing the national coordinators of the end of trial. The national coordinator in each country is responsible for informing the ethics committees and the competent national regulatory authorities of the regular or premature termination of the trial, according to national legal requirements.

23.9 Insurance

In general, if a subject is injured as a direct result of protocol defined treatment, reasonable and necessary medical treatment for the injury will be reimbursed by the insurance unless such expenses are covered by the subject's medical insurance, a government programme, or other responsible third party. The national coordinators of the participating countries are responsible for providing insurance of indemnity in the respective country according to national law.

In Germany patients are insured by Gerling GmbH. A copy of the certificate of insurance is provided in Appendix B. The insurance conditions are provided in the investigator site file. Copies are also attached to the informed consent form and have to be handed over to the patients and/or their parents/legal guardians.

24 QUALITY CONTROL AND QUALITY ASSURANCE

24.1 Monitoring

The national coordinators are responsible for organising adequate monitoring in the respective country. Random on-site monitoring is to be provided. However, if frequent protocol violations, incomplete CRFs, unanswered queries or other problems are encountered, focused monitoring visits may be performed.

On site monitoring visits include the checking of

- Informed consent
- Inclusion and exclusion criteria
- Source data, regarding the main efficacy and safety endpoints
- Abidance by ICH-GCP and national laws

Minimum standards and reporting requirements will be defined in a monitoring manual.

24.2 Audits

The sponsor and his legal representatives reserve the right to audit selected trial sites in order to guarantee that the study is conducted in accordance with ICH-GCP. The auditor will be independent from the staff involved in the proceedings of this trial.

24.3 Inspections

According to the pertinent European legislation, inspections of trial sites may be performed by competent authorities during or after completion of the trial.

24.4 Independent Data and Safety Monitoring

An Independent Data and Safety Monitoring Committee of two independent clinicians and an independent statistician is installed who are responsible for monitoring early data and safety and efficacy of treatment. They will review the data of interim analyses and give written recommendations to the sponsor / sponsor's delegate whether the trial can be continued as planned.

25 PUBLICATION POLICY

25.1 Publication

A clinical study report will be provided to the national coordinators within one year after the last patient`s last visit. The national coordinators are responsible for transmitting the report or a summary of the report to their ethics committee and competent authority according to national law.

Publication of this randomised clinical trial will be performed on behalf of the group. Unless authorised by the Intergroup Chairmen and the Steering Group, data from individual national or group centres or research groups should not be published until the complete multicentre trial has been published in full. Additional publications using substantial EWING 2008 data must be analysed under supervision of the coordinating data centre's statistician. They must be authorised by the study chairmen and the principal coordinating investigator and will be published on behalf of the EWING 2008 group.

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27 Glossary and Abbreviations

A	Actinomycin D
AE	Adverse Event
AR	Adverse Reaction
BSA	Body Surface Area
Bu	Busulfan
BW	Body Weight
CCLG	Children's Cancer and Leukaemia Group (UK)
CDC	Coordinating Data Centre
CGH	Comparative Genomic Hybridisation
COG	Children's Oncology Group (North America)
CR	Complete Response
CRF	Case Report Form
CT	Computed Tomography
CTC	Common Terminology Criteria
CTCAE	Common Terminology Criteria for Adverse Events
Czech	Czech Pediatric Oncology
D	Doxorubicin
DCOG	Dutch Childhood Oncology Group
E	Etoposide
EANM	European Association of Nuclear Medicine
EBMT	European Group for Blood and Marrow Transplantation
EFS	Event-free Survival
EICESS 92	European Intergroup Cooperative Ewing's Sarcoma Study 1992
EORTC	European Organisation for Research and Treatment of Cancer
ESRT	Extracranial Stereotactic Radiotherapy
EU	European Union
Eval	Evaluation

FBC	Full blood count
FISH	Fluorescence In Situ Hybridisation
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular filtration rate
GPOH	Gesellschaft für Pädiatrische Onkologie und Hämatologie
GPOH Austria	Gesellschaft für Pädiatrische Onkologie und Hämatologie Austria
HDT	High dose chemotherapy
HE	Hematoxylin and Eosin
I	Ifosfamide
IDMC	Independent Data and Safety Monitoring Committee
IMP	Investigational Medicinal Product
IMRT	Intensity Modulated Radiotherapy
ZKS	Zentrum für klinische Studien (Safety Desk)
LESS	Late Effects Surveillance System
LVEF	Left ventricular ejection fraction
MDS	Myelodysplastic Syndrome
Mel	Melphalan
MRI	Magnetic Resonance Imaging
MUGA	Multigated Acquisition Scan
NDC	National Data Centre
OS	Overall Survival
p. a.	per annum, annually
PAS	Periodic Acid Schiff
PD	Progressive Disease
PET	Positron Emission Tomography
PET-CT	Positron Emission Tomography – Computer Tomography
p. i.	post injection

PNET	Peripheral NeuroEctodermal Tumour
PR	Partial Response
PTFE	PolyTetraFluoroEthylene (teflon)
Pts	Patients
QOL	Quality of Life
RISK	Registry for Radiogenic Long-Term Sequelae
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCR	Stem Cell Reinfusion
SF	Shortening Fraction
SFCE	Société Française des Cancers d'Enfants
SIAK	Schweizerisches Institut für Angewandte Krebsforschung
SOP	Standard operating procedures
SSG	Scandinavian Sarcoma Group
SUSAR	Suspected unexpected serious adverse reaction
SUV	Standardised Uptake Value
Treo	Treosulfan
UAR	Unexpected Adverse Reaction
UPN	Unique patient number
UN	United Nations
V	Vincristine
VAC	Vincristine, actinomycin D, cyclophosphamide
VAI	Vincristine, actinomycin D, ifosfamide
VIDE	Vincristine, ifosfamide, doxorubicin, etoposide
WBC	White Blood Count
WHO	World Health Organisation