GPOH
Gesellschaft für Pädiatrische Onkologie und Hämatologie

SFCE

EORTC
European Organization for Research and Treatment of Cancer

In collaboration with EBMT

EURO-E.W.I.N.G. 99

EUROpean **E**wing tumour **W**orking **I**nitiative of **N**ational **G**roups

Ewing Tumour Studies 19**99**

EE 99

Version 3a, 14th September 2010

Start Date: 1st March 2000

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EURO-EWING 99 Protocol Amendments.

No.	Amendment	Date	Protocol Page
1	Exclusion Criteria: Interval between date of definitive biopsy and registration >45 days	July 2001	Page 24
2	Exclusion Criteria: Interval between date of definitive biopsy and start of chemotherapy >45 days	February 2003	Page 24
3	As itemised below	August 2004	
	New UK consent forms	August 2004	Appendix B.6
	Neurotoxicity grading adjustment	August 2004	Pages: 37-38,42, 43,50,53,60,68, 70 and 77.
	SAE – Ifosfamide encephalopathy and neurotoxicity management recommendations. "*VIII.5.5 Central neurotoxicity* *Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16 for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA. It is recommended to call your appropriate trial office for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation."*	August 2004	Pages: 37-38,42,43,50, 53,60,68,70 and 77.
	SAE Warning *"In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for R2loc-randomisation due to projected busulfan toxicity."*	August 2004	Pages: 21, 22, 27-29, 35, 36, 39, 44, 47, 50, 51, 55, 58, 61, 62, 63, 66, 83, 84-86, 88, 89, 91-93.
	SAE Reporting System – Section XX6.1	August 2004	Pages; 74, 103-106.
	Names of Trial Groups and Representatives	August 2004	Page 2
	Follow-up Investigations	August 2004	Pages 94-95
	Information Sheet Amendments	August 2004	Appendix B.6
4	As itemised below	April 2005	
	Parent/Patient Information Sheet amendments	April 2005	
5	Minor administrative changes as notified to MREC 30.8.2005. Section XX.6 numbering amended. PROTOCOL REPRINT – Version 2 August 2005 – changed on front cover and on footer of all pages	August 2005	Various
8	Items listed below	September 2010	
	Footer on all pages changed to RG_09-202_EE-99 Protocol, Version 3.0, 14th September 2010.		

	Front cover updated with new version number. Contact details updated from University of Leicester to University of Birmingham.		Front Cover
	Throughout - reference to UKCCSG changed to CRCTU where appropriate.		Various
	Throughout - reference to SIAK deleted.		Various
	Reference to CCSG changed to UK where appropriate.		Various
	UK statistician changed from Claire Weston to Prof Keith Wheatley.		2
	Table of contents updated		3, 4, 5 and 6
	CRCTU (Cancer Research UK Clinical Trials Unit added to list of Abbreviations.		8
	Signature page amended to capture only Chief Investigator signature		9
	New sentence added 'NCRI Bone Clinical Study Group (formerly know as CCLG- Children's Cancer and Leukaemia Group and UKCCSG- United Kingdom Children's Cancer Study Group)'		15
	UKCCSG deleted from list.		15
	UKCCSG amended to read CCLG (formerly UKCCSG).		94
	Patient Information Sheets, Consent Forms and GP Letters deleted from protocol and will now be supplied as separate documents.		
	Contact details for Professor Lewis updated from 'St. James University Hospital' to 'Leeds General Infirmary'		120
	Footer on all pages changed to RG_09-202_EE-99 Protocol, Version 3a, 14th September 2010		

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Contents

List of Abbreviations

ALT	**AL**anine **T**ransaminase
ANC	**A**bsolute **N**eutrophil **C**ount
BM	**B**one **M**arrow
Bu-Mel	**Bu**sulfan-**Mel**phalan
CCrea	**Crea**tinine **C**learance
CCG (US)	**C**hildren's **C**ancer **G**roup **(U**nited **S**tates)
CESS	**C**ooperative **E**wing's **S**arcoma **S**tudy
CGH	**C**omparative **G**enomic **H**ybridisation
COG	**C**hildren's **O**ncology **G**roup
CRCTU	Cancer Research UK Clinical Trials Unit
CT	**C**omputerised **T**omography
CTC	**C**ommon **T**oxicity **C**riteria
EBMT	**E**uropean **O**rganisation of **B**lood and **M**arrow **T**ransplantation
ECG	**E**lectro**C**ardio**G**raphy
ECHO	**ECHO**cardiography
EFS	**E**vent-**F**ree **S**urvival
EICESS	**E**uropean **I**ntergroup **C**ooperative **E**wing's **S**arcoma **S**tudy
EORTC	**E**uropean **O**rganisation for **T**reatment and **R**esearch of **C**ancer
EORTC-STBSG	**EORTC** – **S**oft **T**issue and **B**one **S**arcoma **G**roup
FBC	**F**ull **B**lood **C**ount
FISH	**F**luorescence **I**n **S**itu **H**ybridisation
FS	**F**ractional **S**hortening
GFR	**G**lomerular **F**iltration **R**ate
GI	**G**astro**I**ntestinal
GPOH	**G**esellschaft für **P**ädiatrische **O**nkologie und **H**ämatologie
GPT	**G**lutamat-**P**yruvat-**T**ransaminase
HDT	**H**igh-**D**ose **T**herapy
IESS	**I**ntergroup **E**wing's **S**arcoma **S**tudy
IVAD	**I**fosfamide – **V**incristine – **A**ctinomycin D - **D**oxorubicin
LVEF	**L**eft **V**entricular **E**jection **F**raction
ME	**M**elphalan - **E**toposide
MMD	**M**inimal **M**etastatic **D**isease
MRD	**M**inimal **R**esidual **D**isease
MRI	**M**agnetic **R**esonance **I**maging
MUGA	**MU**lti**G**ated **A**cquisition scan
OAS	**O**ver**A**ll **S**urvival
PBPC	**P**eripheral **B**lood **P**recursor **C**ell
PNET	**P**eripheral **N**euro-**E**ctodermal **T**umour
RT-PCR	**R**everse **T**ranscript **P**olymerase **C**hain **R**eaction
SFCE	**S**ociété **F**rançaise des **C**ancers d'**E**nfants
SFOP	**S**ociété **F**rancaise d'**O**ncologie **P**édiatrique
SFOP EW	**SFOP EW**ing tumour studies
SSG	**S**candinavian **S**arcoma **G**roup
Tmp/GFR	renal tubular threshold for phosphate
Tp/Ccrea	fractional phosphate reabsorption
UDC	**U**ro**D**esoxy**C**holic **A**cid
UK-ET	**U**nited **K**ingdom **E**wing **T**umour trials
VAC	**V**incristin - **A**ctinomycin D - **C**yclophosphamide
VAI	**V**incristin - **A**ctinomycin D - **I**fosfamide
VIDE	**V**incristin - **I**fosfamide - **D**oxorubicin - **E**toposide
VOD	**V**eno-**O**cclusive **D**isease
WBC	**W**hite **B**lood **C**ount

Important Note

This document is intended to describe collaborative studies in Ewing tumours and to provide information for entering patients. The trial committee does not intend it to be used as an aide-memoire or guide for treatment of non-registered patients. Amendments may be necessary; these will be circulated to known participants in the trial, but institutions entering patients are advised to contact the appropriate study centres to confirm the correctness of the protocol in their possession. Before entering patients into one of the studies clinicians must ensure that the study protocol has received clearance from their ethical committee.

Version 3, 14th September 2010

This Protocol is approved by :

Prof Ian Lewis **Signature :** **Date :** 15th SEPT 2010

Chief Investigator

I Description, Study Objectives, Endpoints and Overview

I.1 Description, study objectives and endpoints

This is a randomised, prospective, multi-centre, international study, linking several co-operative groups, to improve outcome in patients with Ewing tumour. The treatment is stratified according to prognostic factors as determined by previous studies.

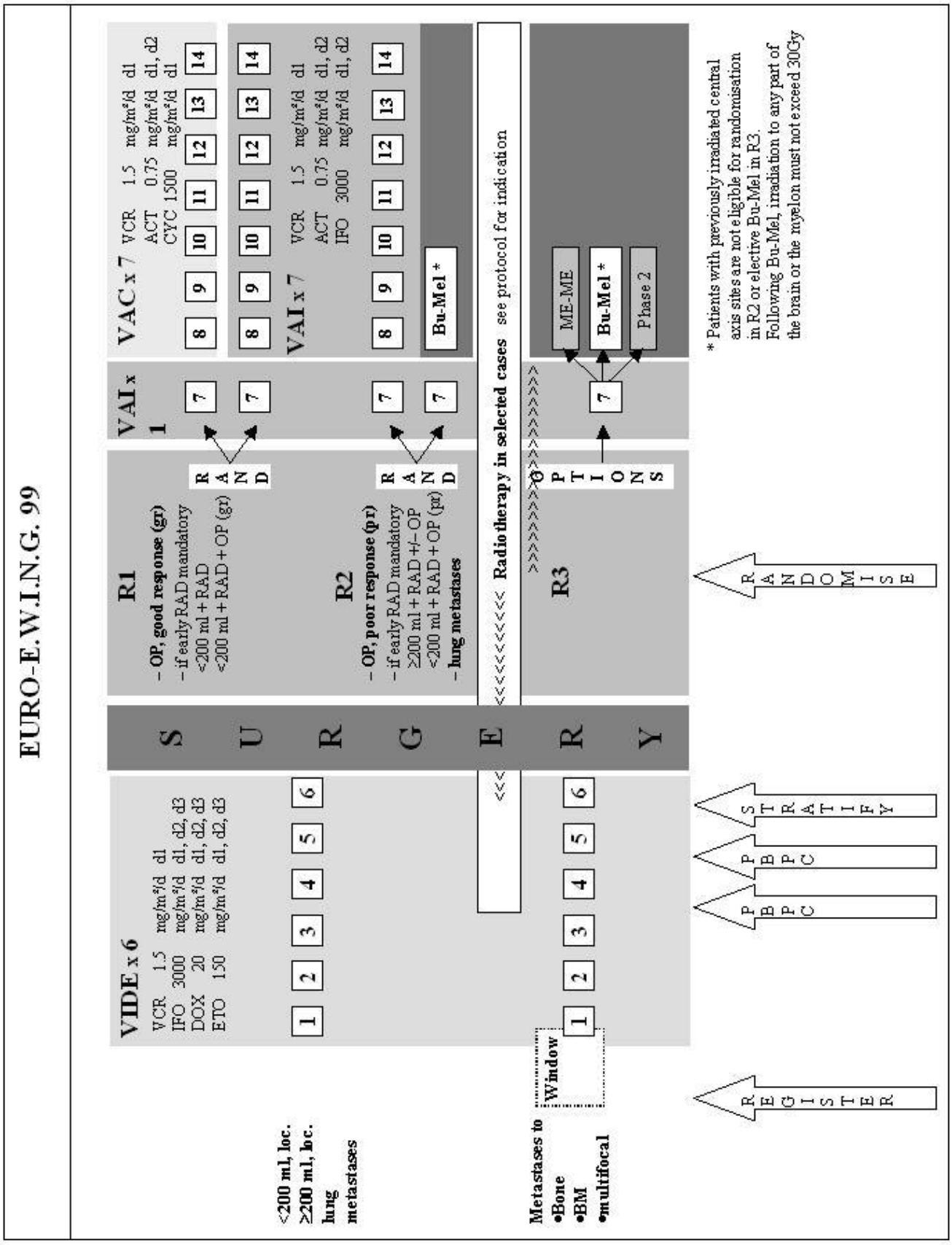
Objectives are:

1. To compare, in a randomised trial, VAI consolidation chemotherapy with VAC consolidation chemotherapy in patients with non-metastatic Ewing tumour and good histological response to standard induction VIDE chemotherapy, or in patients with localised Ewing tumour < 200 ml in volume who receive radiotherapy as primary local treatment following standard induction VIDE chemotherapy (Randomisation R1).
2. To compare, in a randomised trial, VAI consolidation chemotherapy with high-dose therapy (Busulfan-Melphalan) and PBPC rescue, A: in patients with non-metastatic Ewing tumour and poor histological response to standard induction VIDE chemotherapy, B: in patients with localised Ewing tumour ≥ 200 ml in volume who receive radiotherapy for local control following standard induction VIDE chemotherapy and Busulphan-Melphalan where applicable (Randomisation $R2_{loc}$).
3. To compare, in a randomised trial, VAI consolidation chemotherapy and whole lung irradiation with high-dose therapy (Busulfan-Melphalan) and PBPC rescue, in patients with pulmonary or pleural metastases at diagnosis (Randomisation $R2_{pulm}$).
4. To recommend and develop therapy for patients with metastases at sites other than pulmonary/pleura, - i.e., bone and/or bone marrow (R3).
5. To study the prognostic significance of EWS-Fli1 transcript type.
6. To study the frequency and prognostic value of minimal disease in bone marrow and PBPC harvest, as determined by the presence or absence of EWS-Fli1 transcript.

Primary endpoints are event-free survival (EFS) and overall survival (OAS).

Secondary endpoints are
a) feasibility, toxicity and response to VIDE induction therapy
b) feasibility, and toxicity of randomised consolidation regimens
c) EFS and OAS by prognostic group analysis

I.2 Diagram 1 – Study Overview



I.3 Randomisation / Treatment Tracks

Step 1 Consider: **Disease**		**Proceed to:**	
	Localised	▸ **Step 2a**	**page 33**
	Metastases to lung/pleura	▸ **Step 2b**	**page 43**
	Metastases to bone/bone marrow, multisystem metastases	▾ **R3**	**page 52**

Step 2a Consider: **Tumour site and modality of local treatment**	**Proceed to:**
Limb or axial + surgery (priority track)	▸ **Step 3a**
Limb or axial + primary complete surgery of primary tumour	▸ Step 3b
Limb or axial + delayed local therapy	▸ Step 3c
Limb + early radiotherapy	▸ Step 3c
Axial + early radiotherapy	▸ Step 3d

Step 2b Consider: **Tumour site and modality of local treatment**	**Proceed to:**	
Limb or axial + surgery (priority track)	▾ **R2pulm**	page 43
Limb or axial + primary complete surgery of primary tumour	▾ R2pulm	
Limb or axial + delayed local therapy	▾ R2pulm	
Limb + early radiotherapy	▾ R2pulm	
Axial + early radiotherapy	▸ off random.study, continue on VAI+lung RAD or individually tailored treatment	

Step 3a Consider: **Histological response**	**Proceed to:**	
Good	▾ **R1**	page 33
Poor	▾ **R2loc**	page 33

Step 3b Consider: **Tumour volume**	**Proceed to:**	
<200 ml	▾ R1	page 33
≥200 ml	▾ R2loc	page 33

Step 3c Consider: **Tumour volume** and response	**Proceed to:**	
< 200 ml, no / late surgery, clinical response good	▾ R1	page 33
< 200 ml, secondary surgery, histological response good	▾ R1	
< 200 ml, no / late surgery, clinical response poor	▾ R2loc	page 33
< 200 ml, secondary surgery, histological response poor	▾ R2loc	
≥ 200 ml	▾ R2loc	

Step 3d Consider: **Tumour volume (**and response in case of secondary surgery)	**Proceed to:**	
< 200 ml, no / late surgery	▾ R1	page 33
< 200 ml, histol. response good	▾ R1	
< 200 ml, histol. response poor	▸ off random. study, continue on VAI or individually tailored treatment	
≥ 200 ml	▸ off random.study, continue on VAI or individually tailored treatment	

R1 [
VAI vs. VAC
→ page 33

R2loc [[
VAI vs. Bu-Mel
→ page 33

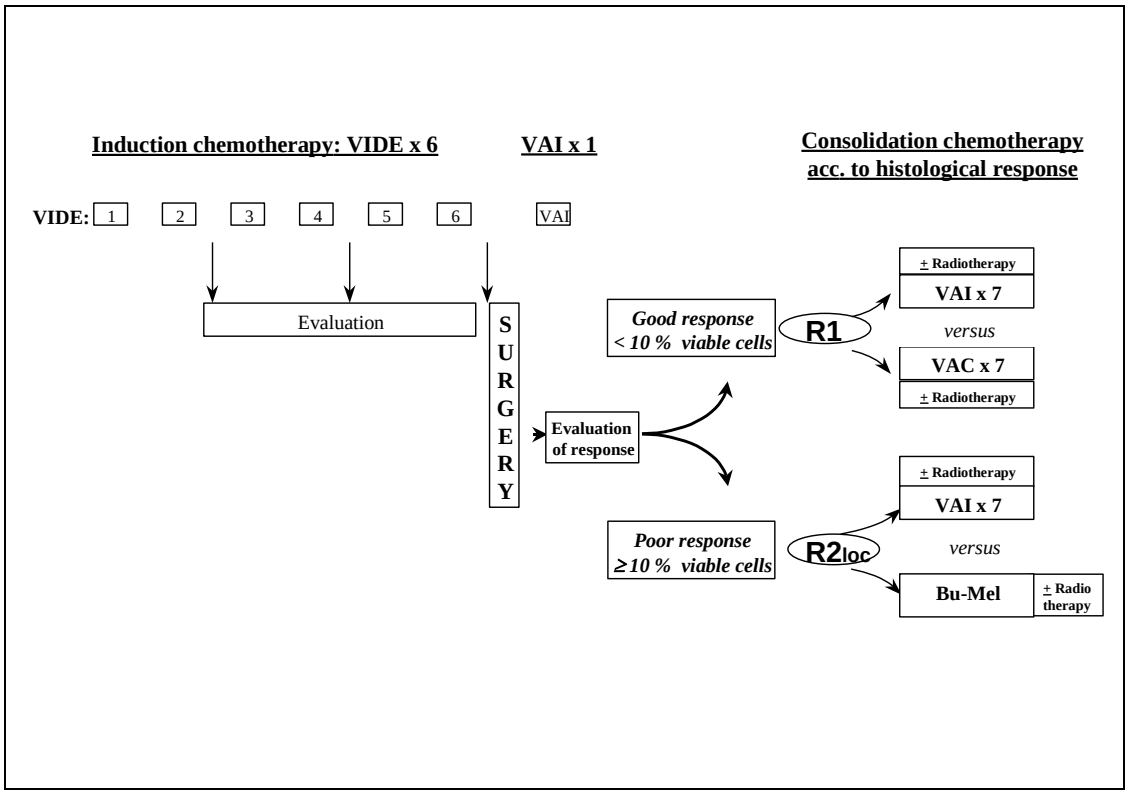
R2pulm [[
VAI + lung RAD
vs.
Bu-Mel
→ page 43

R3
Following optional window studies and 6 x VIDE + local therapy, consider Bu-Mel (or ME-ME in case of Bu-Mel contraindication).
→ page 52

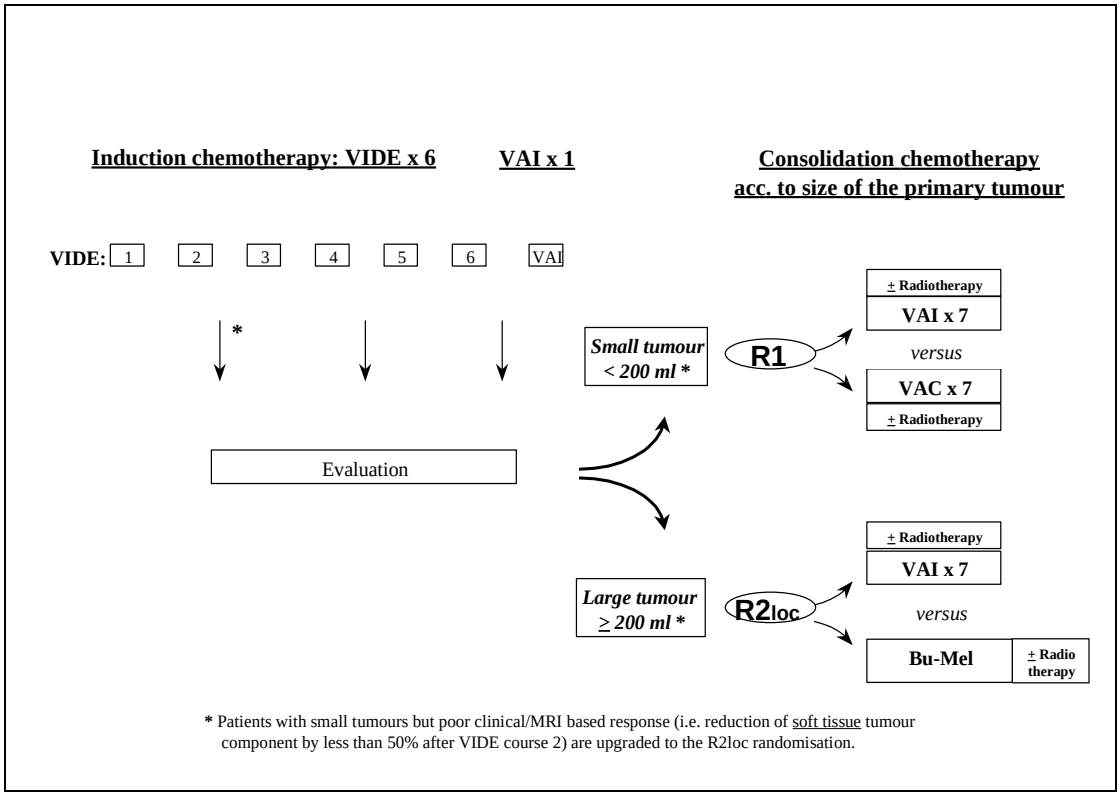
[Fax to trial office for randomisation as soon as histological response is available !

[Fax to trial office for randomisation prior to course 4 of chemotherapy whenever early radiotherapy is the only reasonable option for local treatment !

I.4 Diagram 2 – Resectable localised tumours

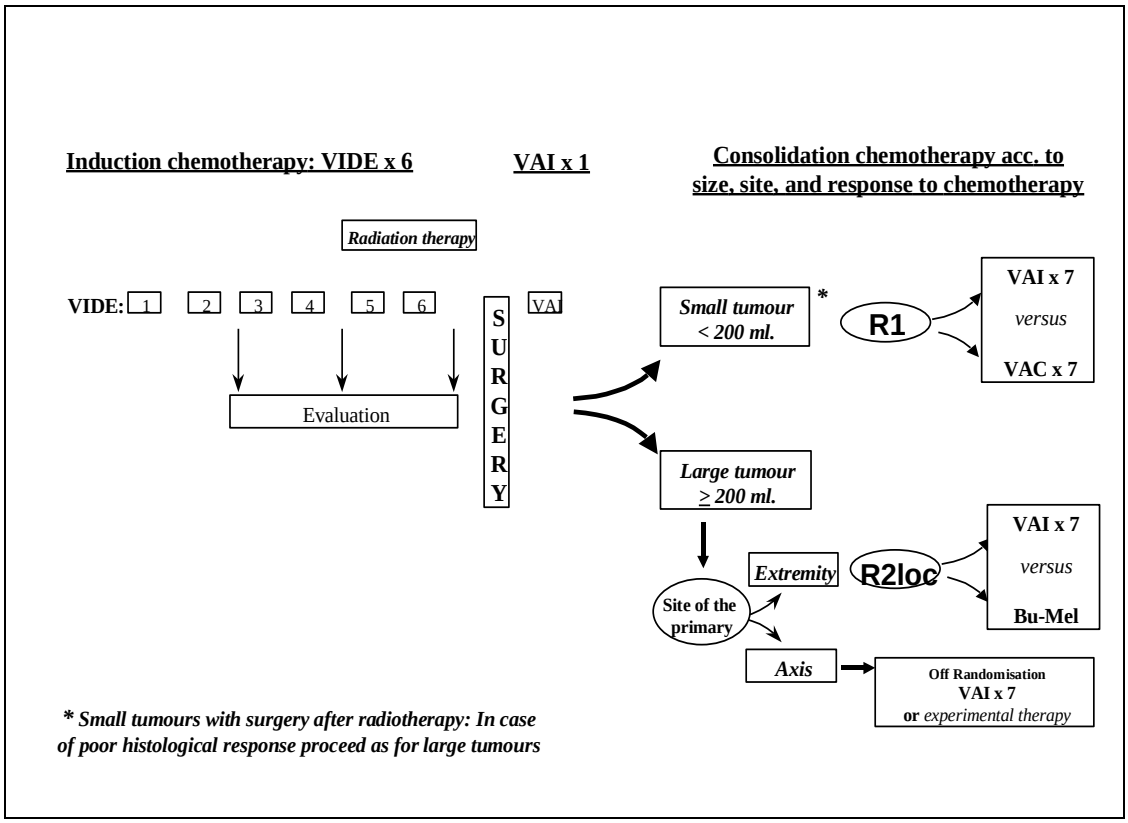


I.5 Diagram 3 – Localised tumours, resected at diagnosis or unresectable, delayed radiotherapy possible

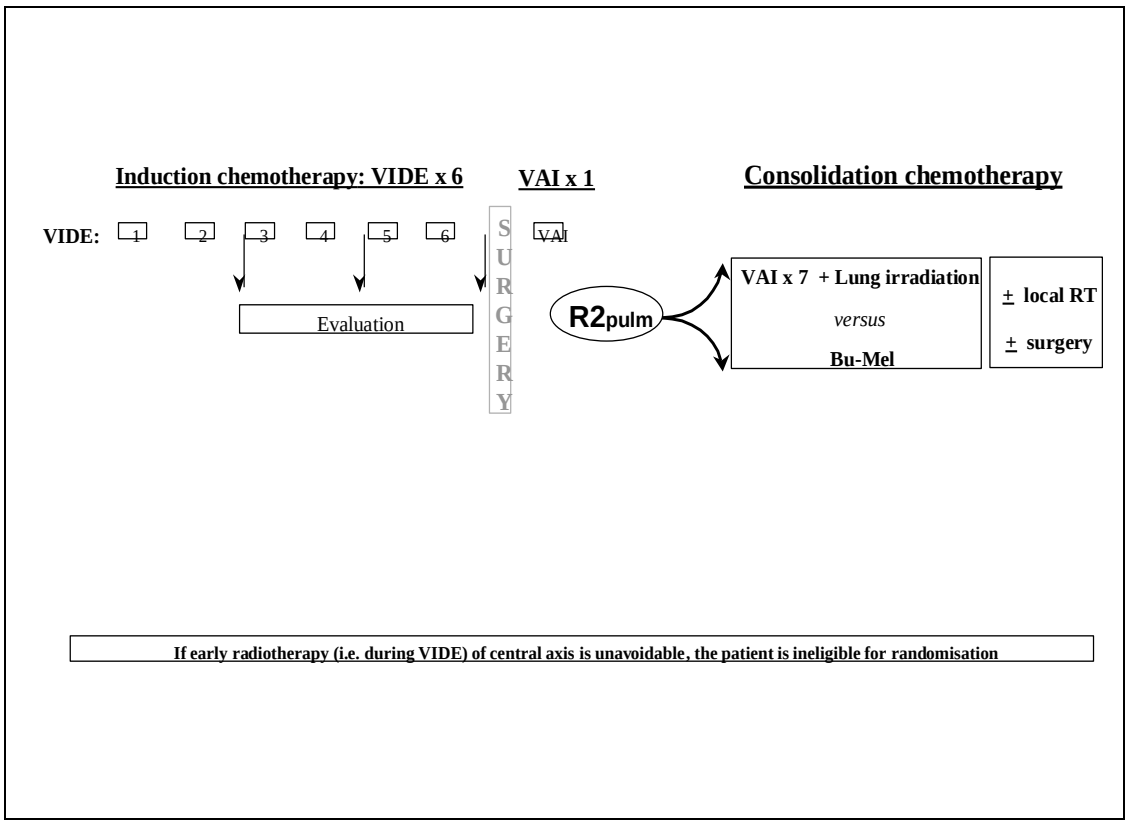


* Patients with small tumours but poor clinical/MRI based response (i.e. reduction of soft tissue tumour component by less than 50% after VIDE course 2) are upgraded to the R2loc randomisation.

I.6 Diagram 4 – Localised tumours with early radiation therapy



I.7 Diagram 5 – Tumours with pulmonary/pleural metastases



II Background and Rationale

This protocol is the result of discussions among members of European national and multinational Ewing tumour study groups who convened in Vienna (October 1996), Amsterdam (August 1997), Münster (October 1997), Amsterdam (December 1997), Paris (February 1998), Amsterdam (April 1998), Paris (September 1998), Münster, (November 1998), and Paris (March and May 1999). This version incorporates amendments agreed in Brussels (November 2003)

The groups cooperating ìn this study are :

- **NCRI Bone Clinical Study Group** (formerly known as CCLG - Children's Cancer and Leukaemia Group and UKCCSG – United Kingdom Children's Cancer Study Group)
- **GPOH**
  Gesellschaft für Pädiatrische Onkologie und Hämatologie
- **SFCE**
  Société Française des Cancers d'Enfants
- **EORTC-STBSG**
  European Organisation for Research and Treatment of Cancer - Soft Tissue and Bone Sarcoma Group
- **SSG**
  Scandinavian Sarcoma Group (Sweden)
- **COG**
  Children's Oncology Group (North America)

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

- **EBMT**
  European Organisation of Blood and Marrow Transplantation

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

II.1 Characterisation of Ewing tumours

Ewing tumours, in terms of this protocol, are characterised as tumours consisting of small, blue, round malignant cells that 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 tumour. These tumours are in more than 95 % characterised by a re-arrangement of chromosome 22, most commonly in the form of an 11;22 translocation.[1-7]

The gene rearrangement results in the production of an oncogenic transcription factor, e.g. EWS-Fli1 transcription, that shows structural variability of potential prognostic relevance depending on chromosomal breakpoint locations.[8,9]

Ewing tumours are primarily located in bony sites, and represent the second commonest primary osseous malignancy in childhood and adolescence, the annual incidence is estimated at 0.6/million population.[10] Staging procedures as presently applied identify 20-25% of cases as metastatic at diagnosis. Ewing's sarcoma was first described by James Ewing in 1921, and its responsiveness to radiation therapy was noted.[11] Without systemic treatment more than 90% of patients died from secondary metastases.[12] Hence, a Ewing tumour must be regarded as a systemic disease. Since the 1970s, aggressive cytotoxic treatment regimens have increased survival rates to 55-65% in localised, and up to 35% in primary metastatic disease.

II.2 Treatment results in localised disease

5-year survival rates of 55 to 65 % have been documented. The following drugs and/or combinations of drugs proved to be effective and are in current use: VAC (vincristine, actinomycin D, cyclophosphamide), VACA (VAC plus doxorubicin alternating with actinomycin D), the IESS II schedule (alternating the combination of ifosfamide plus etoposide and VACA), VAIA and EVAIA (ifosfamide replacing cyclophosphamide in VACA, either without or with additional etoposide). [10,12-28]

Recent publications suggest an independent prognostic impact of the type of chromosomal rearrangement. [16,29-39] Other known prognostic factors in localised disease include tumour volume, tumour site (pelvic/non-pelvic, axial/extremity, bone/soft tissue), histological response to chemotherapy, and factors related to feasibility and choice of local treatment options.[40]

In the UK ET-2 trial, increasing the dose of ifosfamide from 6 to 9 g/m²/course as compared to ET-1, and application of doxorubicin in all initial four courses ("IVAD3": ifosfamide 3g/m²/d x 3, vincristine 2 mg/m² x 1 dose, doxorubicin 20 mg/m²/d x 3 days) substantially improved event free survival for localised disease from 0.44 in ET-1 to 0.62.[41]

II.3 Treatment results in primary metastatic disease

10-20% disease-free survival has been reported in patients with primary metastatic disease who received conventional therapy.[42-44] Prognostic factors include site(s) of metastatic disease, and responsiveness to chemotherapy. In the recently completed US CCG 7881 study, patients with bone or bone marrow (BM) metastases at diagnosis had a 3-year disease free survival of less than 15% compared to 22% for any metastatic site [CCG 7951 treatment manual, 1996]. Kushner reported that patients with primary pulmonary metastases fared better than patients with primary BM involvement.[26] These findings are comparable to experiences from previous CESS and EICESS studies (FIGURE 1).[45-47]

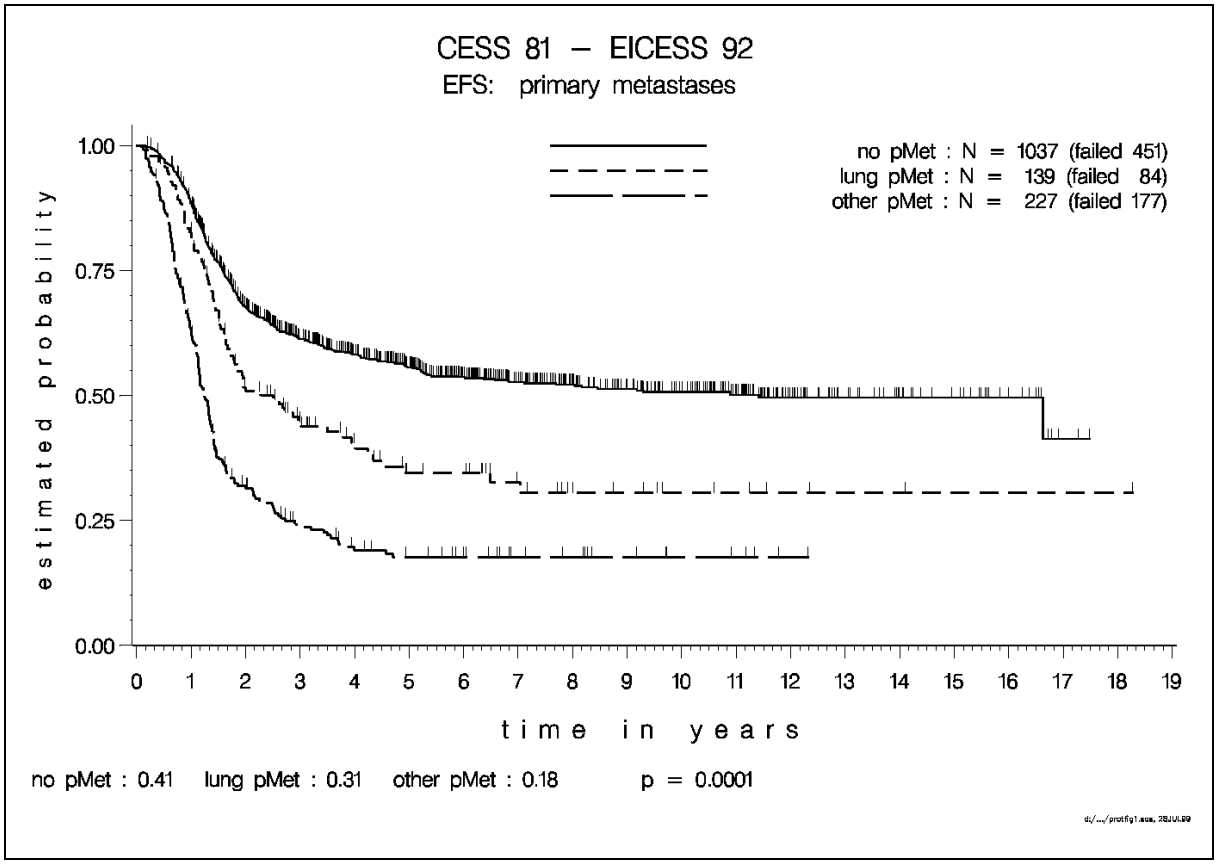


FIGURE 1: EFS according to presence and sites of primary metastases (data: 5/99)

Conventional treatment regimens induce remission, but cannot prevent relapse in primary metastatic patients. Clinically undetectable minimal residual disease leads to relapse at a median time of one to two years after completion of therapy. In order to consolidate remission by reduction of minimal residual/metastatic disease (MRD/MMD), regimens of high-dose (myeloablative) intensification with stem cell rescue have been piloted. Some patients benefit from such consolidation therapy, as evidenced by reports of up to 35% three year survivors with primary bone/BM-metastases. [20,43,44,48-54]

Several myeloablative regimens have been used. Regimens including melphalan were shown to be effective. [42,48,49,52,55,56] Promising data are available with the use of high-dose busulfan combined with melphalan or other agents. [51,57-61]

II.4 Relevance of histological response and local therapy

The relevance of the histological response of the primary tumour to initial chemotherapy both in localised and systemic disease (see FIGURE 2, FIGURE 3, and[62]) stresses the value of high intensity induction treatment in Ewing tumour patients.

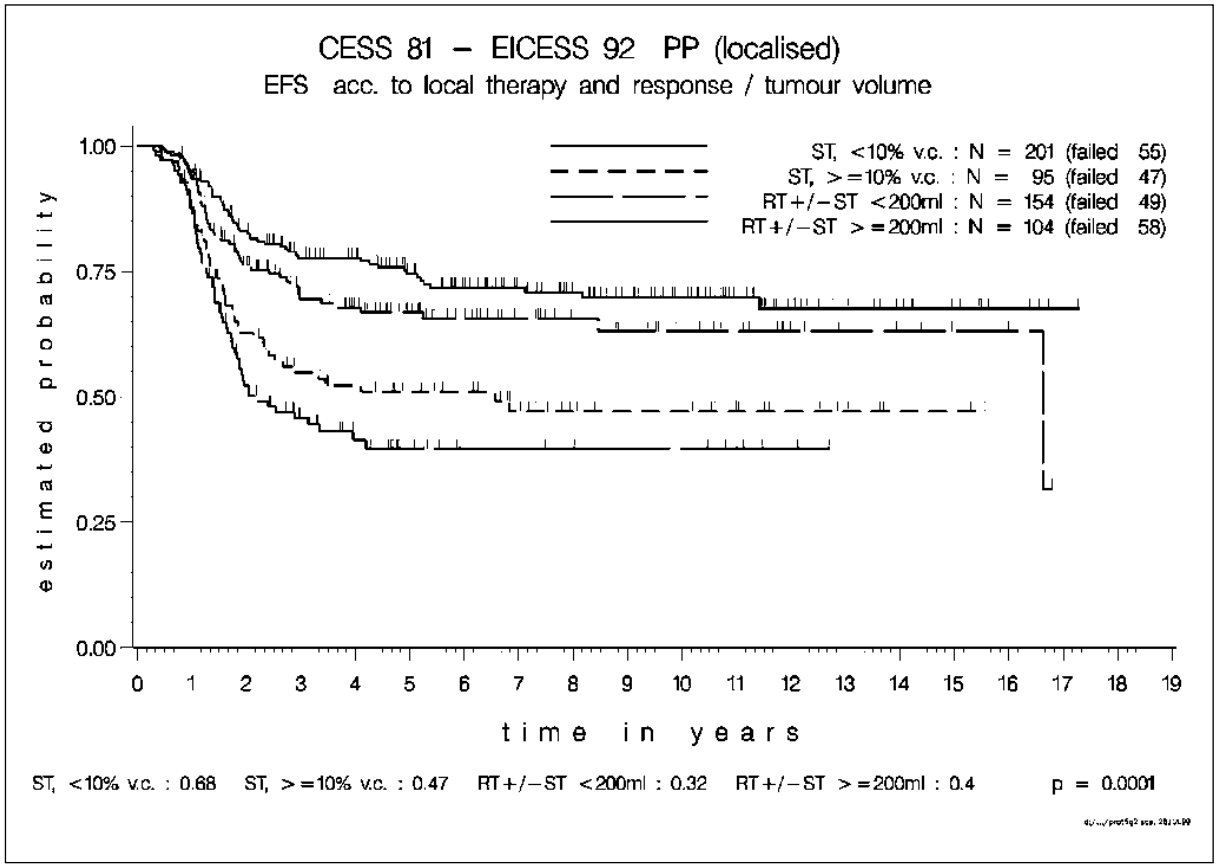


FIGURE 2: EFS according to local therapy and response / tumour volume (data: 5/99)

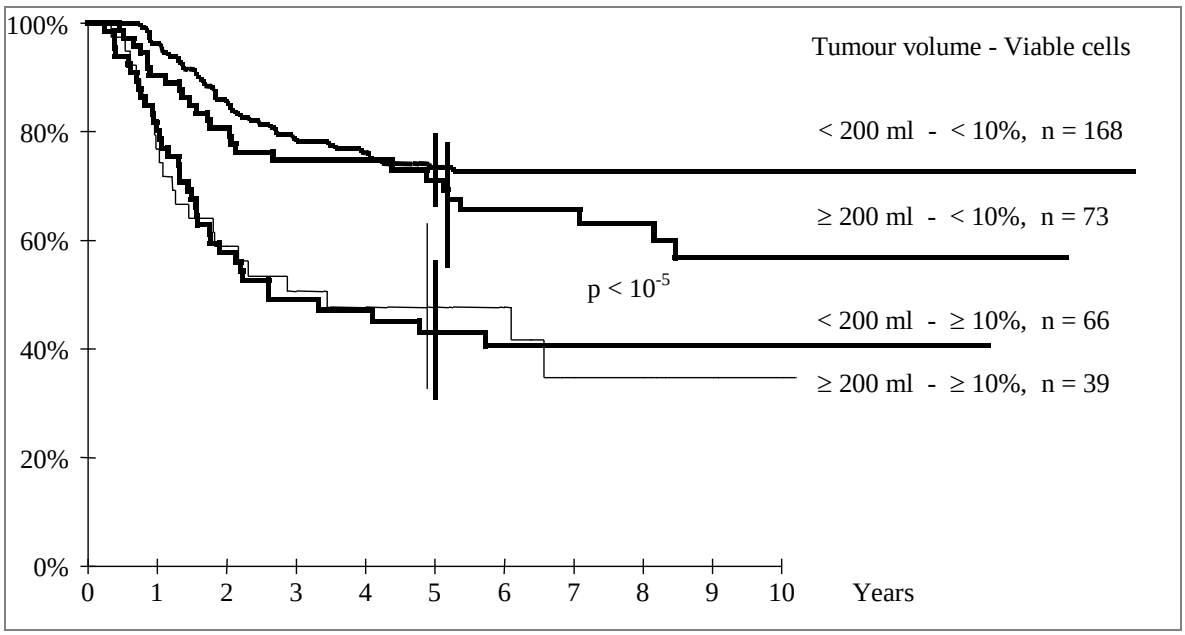


FIGURE 3: EFS according to tumour volume/histological response, patients treated with chemotherapy alone before surgery (SFOP&CESS/EICESS) (n = 346, data 8/98)

Two groups of substances appear to be the most effective agents, i.e. alkylating agents and anthracyclines. The highest known activity against Ewing tumour cells has been shown for ifosfamide and cyclophosphamide among the alkylating agents, and for the anthracycline doxorubicin. While single drug data are controversial, etoposide does seem to be efficacious in combination with alkylating agents.[24,27,63,64] The combination of vincristine, actinomycin D, and cyclophosphamide was also shown to be active. While most treatment schedules adhere to three-week application intervals, findings of good response and cure rates with the St. Jude protocol of a two-week schedule seem to indicate that increasing the dose/time intensity may be an important therapeutic principle with alkylating agents and anthracyclines.[65] In a meta-analysis covering several trials Smith and co-workers demonstrated that an induction treatment including doxorubicin with every course is superior to a scheme alternating doxorubicin with actinomycin D, even when the cumulative doses of both drugs in both schedules are identical.[66] In ET 2, a combination employing ifosfamide 9 g/m² rather than the "conventional" 6 g/m² proved highly effective, with tolerable toxicity.[41] In EICESS 92, VAIA and EVAIA for induction therapy achieved tumour response rates above those obtained with the VACA regimen in the CESS 81 study.

On the other hand, cumulative toxicity may later in the course of treatment severely affect the bone marrow, the renal tubular system, and cardiac function.

In view of these considerations, a further increase of treatment intensity during the induction treatment, in particular during the time when the primary tumour is still in place, was considered an option of further improving both the histological response and survival in Ewing tumour patients. To achieve

this goal, the drug-dose intensity of a single treatment course must be high, but must not lead to a delay of the next course, as the overall dose/time intensity would otherwise again be reduced. Local control should routinely be achieved after induction course 6. Special issues of local therapy of the primary tumour and of metastatic sites are described below.

Patients with more than 10% viable tumour cells at surgery following neoadjuvant chemotherapy (*not* radio-chemotherapy) in the previous CESS/EICESS studies had a less favourable outcome with an EFS of 0.47 after 10 years. Patients with good histological response (< 10% viable tumour cells) after chemotherapy alone had a prognosis of about 0.70 after 10 years (FIGURE 2). In a combined analysis, accumulating data on surgically treated patients from the CESS/EICESS and the SFOP EW studies, the impact of histological response was more prominent than the impact of tumour volume (FIGURE 3).

By contrast, in patients with radiotherapy as first means of local control, survival seems to be independent of histological response. In these patients, however, a correlation to initial tumour volume can be demonstrated (0.63 vs. 0.40, FIGURE 2).

II.5 Rationale and randomisation

Based on the experience of ET-1, ET-2, EW88, EW93, CESS81, CESS 86, EICESS92, and other European studies, as laid out in sections II.2 to II.4, patients will be randomised according to the following prognostic indicators:

- Presence or absence of metastatic disease (FIGURE 1)
- Site of metastases in stage IV patients: pulmonary/pleural only metastases / metastatic involvement of other sites (FIGURE 1),
- Feasibility of local therapy options and histological response to chemotherapy (in locoregional disease) (FIGURE 2, FIGURE 3),
- Initial tumour volume (in locoregional disease) of < 200ml / ≥ 200ml in patients not eligible for surgery as primary modality of local control (FIGURE 2).

Consolidation therapy will be randomly allocated as follows:

Randomisation R1 (VAI vs. VAC)

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

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

- Localised tumour – <200 ml tumour volume at diagnosis – resection after chemotherapy and early radiotherapy – **good histological response to induction chemoradiotherapy (<10% viability).**

  This randomisation should take place after course 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 (≥ 50% regression of evaluable soft tissue component).

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

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

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

Randomisation R2$_{loc}$ (VAI vs. Bu-Mel)

PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are **NOT** eligible for the R2$_{loc}$ randomisation due to anticipated busulfan toxicity, and should be treated by VAI consolidation.

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

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

- Localised tumour – extremity site – resection after chemotherapy and early radiotherapy – tumour volume ≥200 ml **or** poor histological response to induction chemotherapy (≥10% viability).

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

- Localised tumour – extremity site – tumour volume ≥200 ml, or <200 ml + poor clinical response (<50% regression of soft tissue component) – tumour unresectable – early radiotherapy – no progression under induction chemotherapy.

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

- Localised tumour – tumour volume ≥200 ml – tumour unresectable – late radiotherapy – no progression under induction chemotherapy.

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

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

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

- Localised tumour – tumour volume <200 ml – tumour unresectable — late radiotherapy – radiologically, poor response (<50 % regression) to induction chemotherapy, but no progression.

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

Randomisation R2$_{pulm}$ (VAI + whole lung irradiation vs. Bu-Mel without lung irradiation)

All patients with pulmonary/pleural metastases at diagnosis. Patients with metastases at other sites are excluded.

Randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

PLEASE NOTE:

Patients receiving early radiotherapy to central axial sites are **NOT** eligible for the R2$_{pulm}$ randomisation due to anticipated busulfan toxicity, and should be treated by VAI consolidation plus lung irradiation, or are eligible for experimental phase I/II protocols.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for the R2$_{pulm}$ randomisation due to anticipated busulfan toxicity, and should be treated by VAI consolidation plus lung irradiation.

When the CESS/EICESS patients were retrospectively allocated according to the above criteria, this resulted in EFS curves as shown in FIGURE 4:

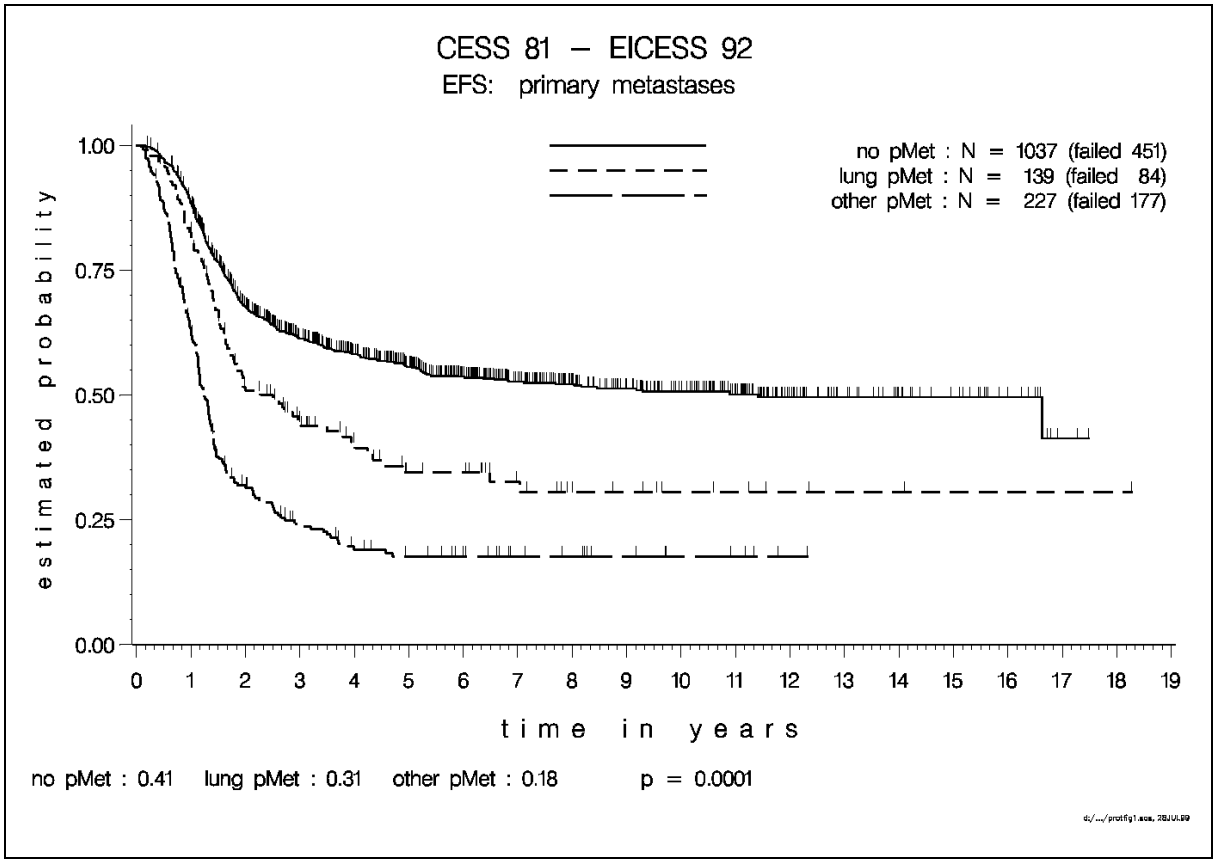


FIGURE 4: EFS in CESS/EICESS patients (4/99) when based on retrospective allocation according to EURO-E.W.I.N.G. 99 randomisation criteria.

III Patient Eligibility

III.1 Eligibility

1. Histologically confirmed Ewing Tumour (Ewing's sarcoma or peripheral PNET) of bone or soft tissue
2. Age less than 50 years
3. Completed pre-treatment investigations allowing prognostic group definition
4. No previous chemotherapy
5. Informed consent according to national guidelines prior to study entry
6. Appropriate ethical committee approval

Please note: ALL PATIENTS MUST BE REGISTERED AT DIAGNOSIS
(See section IV for registration details)

III.2 Exclusions

1. Interval between date of definitive biopsy and registration > 45 days
2. Interval between date of definitive biopsy and start of chemotherapy > 45 days
3. Abnormal cardiac function, including fractional shortening <29%, or ejection fraction < 40%.
4. Abnormal renal function including GFR <60ml/min/1.73 m^2
5. Any other medical, psychiatric or social reason incompatible with the protocol treatment.

NOTE: Patients registered in this study may be eligible for additional, e.g. supportive care, studies at the discretion of the appropriate group. All such studies must have approval by an ethical committee and the international study committee.

IV Registration

Institutions enrolling patients must contact the national Study Centre by phone, fax or e-mail for every patient within 45 days of definitive biopsy. See appendix A.1 for addresses of the appropriate study centres. The registration and randomisation forms must be transmitted immediately by FAX, unless national guidelines allow otherwise.

The information requested at enrolment include:

- Histological diagnosis
- Results of staging procedures:
  - Tumour site and volume
  - Presence or absence of lung and/or other metastases.

A *study policy commitment form* must be completed by the enrolling institution (see Appendices XXIV.2 and XXV.5).

For details on diagnostic procedures, see section VII.

Treatment should be started within two weeks, and must be started within 45 days after definitive diagnostic biopsy.

V Initial Disease Description

1. **Localised disease. Tumour volume < 200ml.**

2. **Localised disease. Tumour volume ≥ 200ml.**
   Pulmonary/pleural metastases. Any tumour volume.

3. **Metastases at extrapulmonary/pleural sites. Any tumour volume.**

VI Randomisation

Randomisation will be based on initial description, local therapy and histological response to induction therapy. Stratification as detailed in section XX and randomisation will be undertaken by each study group.

Randomisation exclusion criteria

- Consent forms not signed.
- Medical contraindications to any of the treatments to be randomised:
- Ifosfamide replaced by cyclophosphamide during VIDE induction.
- Irradiation of central axial sites prior to busulfan.
- Patient unavailable for follow-up.

PLEASE NOTE:

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are **NOT** eligible for the $R2_{loc}$ or the $R2_{pulm}$ randomisation due to anticipated busulfan toxicity, and should be treated by VAI consolidation

Randomisation R1 (VAI vs. VAC)

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

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

- Localised tumour – <200 ml tumour volume at diagnosis – resection after chemotherapy and early radiotherapy – **good histological response to induction chemoradiotherapy (<10% viability).**

  This randomisation should take place after course 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 (≥ 50% regression of evaluable soft tissue component).

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

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

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

Randomisation $R2_{loc}$ (VAI vs. Bu-Mel)

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

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

- Localised tumour – extremity site – resection after chemotherapy and early radiotherapy – tumour volume ≥200 ml **or** poor histological response to induction chemotherapy (≥10% viability).

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

- Localised tumour – extremity site – tumour volume ≥200 ml, or <200 ml + poor clinical response (<50% regression of soft tissue component) – tumour unresectable – early radiotherapy – no progression under induction chemotherapy.

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

- Localised tumour – tumour volume ≥200 ml – tumour unresectable – late radiotherapy – no progression under induction chemotherapy.

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

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

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

- Localised tumour – tumour volume <200 ml – tumour unresectable — late radiotherapy – radiologically, poor response (<50 % regression) to induction chemotherapy, but no progression.

  This randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

PLEASE NOTE:

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are **NOT** eligible for the R2loc randomisation due to anticipated busulfan toxicity, and should be treated by VAI consolidation.

Randomisation R2pulm (VAI + whole lung irradiation vs. Bu-Mel without lung irradiation)

All patients with pulmonary/pleural metastases at diagnosis. Patients with metastases at other sites are excluded.

Randomisation can take place at any time following course 4 of VIDE induction chemotherapy, before consolidation therapy.

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, or are eligible for experimental phase I/II protocols.

PLEASE NOTE:

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are **NOT**

eligible for the R2pulm randomisation due to anticipated busulfan toxicity, and should be treated by VAI consolidation plus lung irradiation.

VII Investigations at Diagnosis

VII.1 Tumour diagnosis

a) Biopsy of primary tumour

Open or needle core.

b) Histopathology, see appendix A.2: Pathology guidelines

c) Cytogenetics and molecular pathology

Fresh tissue frozen for RT-PCR analysis (see appendix A.3)

VII.2 Staging

a) Imaging of the primary tumour

Plain radiograph

MRI or CT scan with estimation of tumour volume (TV):

TV = a x b x c x F,

where a, b, and c represent the maximum tumour dimensions in three planes,

with $F = \pi / 6 = 0.52$ for spherical tumours,

or $F = \pi / 4 = 0.785$ for cylindrical tumours

b) Skeletal system

Minimum requirements:

- 99mTc whole body bone scan
- MRI of sites suspicious in bone scan

(Indication for additional biopsies: see section VII.6)

c) Bone marrow (from sites distant from primary tumour or known metastases!)

Minimum requirements:

- Aspirates from ≥ 2 sites; biopsy from ≥ 1 site: conventional cytology/histology
- RT-PCR for tumour specific chromosome 22 rearrangement (see Molecular Biological Studies, appendix A.3)

d) Lung/pleura

Minimum requirements:

- Plain chest radiograph
- Chest CT scan

(Indication for biopsies: see section VII.5)

VII.3 Organ function/ baseline investigations

a) Full blood count (FBC)

b) Urea + electrolytes, magnesium, phosphate, calcium, bicarbonate

c) Liver function tests including alkaline phosphatase, lactate dehydrogenase

d) Serum/plasma creatinine, glomerular filtration rate (GFR)/creatinine clearance (C_{crea})

e) ECG, cardiac function estimation (ECHO or MUltiGated Acquisition scan (MUGA))

f) Lung function tests (to be repeated prior to Bu-Mel)

g) Viral titres

h) Screening for hereditary thrombophilic conditions as advised by national groups.

VII.4 Definitive diagnosis

The definitive diagnosis must be based on examination of routinely stained material plus appropriate immunohistochemical evaluation. The following abnormalities are considered confirmatory:

a) Cytogenetic/molecular analysis demonstrating chromosome 22 re-arrangement

b) CD99 (MIC-2) positivity

Representative histological material should be sent for central pathology review.

SEE APPENDIX A.2 FOR PATHOLOGY GUIDELINES.

VII.5 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. Solitary nodules of 0.5-1 cm or multiple nodules of 0.3-0.5 cm are questionable evidence of metastatic disease, and biopsy proof 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 effusions in patients with chest wall tumours are not regarded as proof for lung/pleural metastases, but are considered to represent locoregional disease.[46] The site(s), size, and number of involved sites should be documented.

VII.6 Definition of metastatic disease at extra-pulmonary sites

Bone marrow metastases are defined by 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 diagnosis of metastatic bone marrow disease.

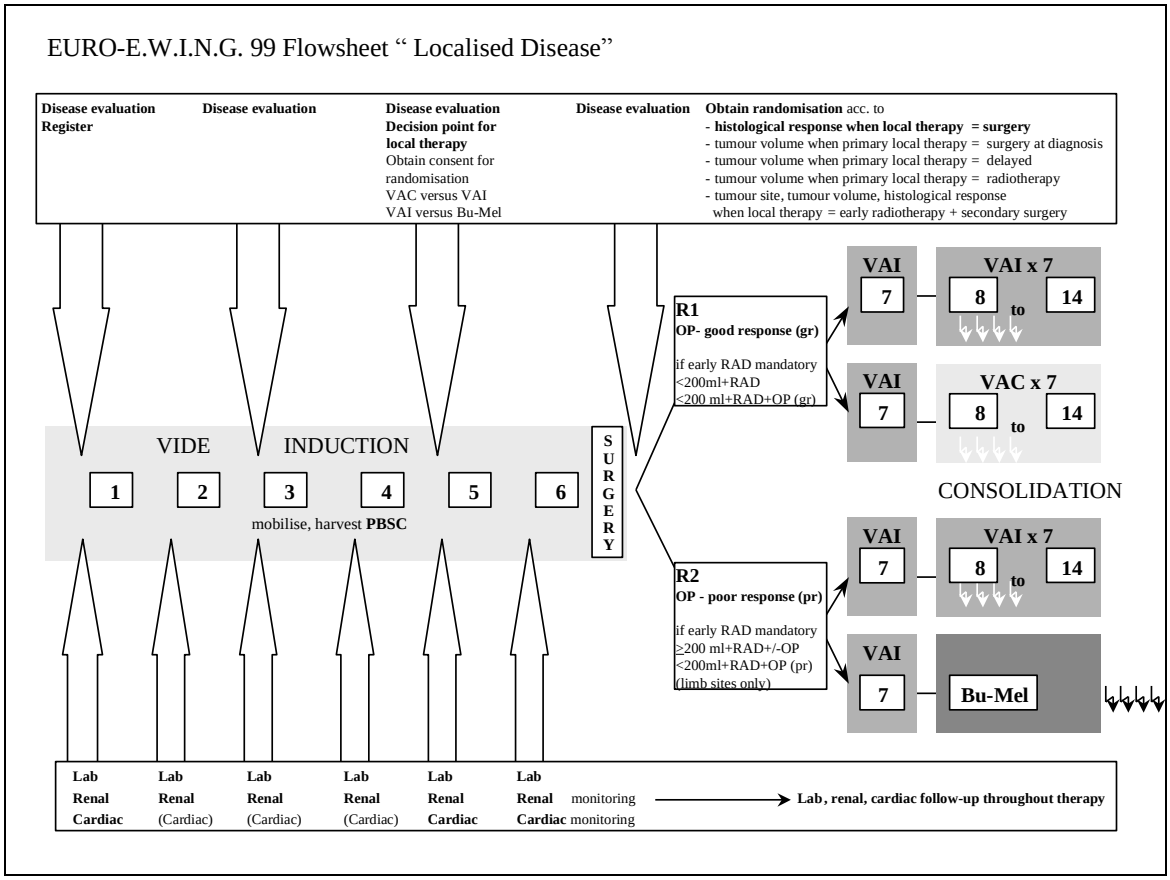
Bone metastases are considered confirmed with two or more sites of positive uptake on isotope scans in addition to the primary tumour site, unless explained by other medical conditions. A single site of positive uptake on isotope scans in addition to the primary tumour site should be confirmed by biopsy. Further imaging of sites of positive isotope uptake is recommended, e.g. CT, MR, PET.

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

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

VIII EURO-E.W.I.N.G. 99 - Localised Disease Protocol

VIII.1 Summary outline



VIII.2 Induction treatment

All patients are to receive 6 courses of VIDE chemotherapy as induction treatment.

Courses 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/m^2/d (i.v. push)	d 1	(1.5 mg/m^2/course)	(*max. single dose: 2 mg*)
IFOSFAMIDE	3.0 g/m^2/d (i.v. infusion, 1-3 h)	d1, d2, d3	(9 g/m^2/course)	plus MESNA*
DOXORUBICIN	20 mg/m^2/d (i.v. infusion, 4 h)	d1, d2, d3	(60 mg/m^2/course)	
ETOPOSIDE	150 mg/m^2/d (i.v. infusion, 1 h)	d1, d2, d3	(450 mg/m^2/course)	
*Mesna dosage	1.0 g/m^2/d 3.0 g/m^2/d	d1 d1, d2, d3	(i.v. push 1 h prior to ifosfamide) (i.v. infusion, e.g. 24 h)	

See section XI and appendix B.3 for chemotherapy guidelines and details.

VIII.3 Basic plan of VIDE courses

Day 1-3: VIDE 1 (see above)

Day 10-12: FBC, urea + electrolytes, calcium, magnesium, phosphate, bicarbonate.

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes
- GFR (calculated Creatinine clearance (C_{crea}) or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) = Renal tubular threshold for phosphate (Tm_p/GFR).

VIII.4 Additions to basic plan

VIII.4.1 Course 1 - Additions to basic plan

Complete "Investigations at diagnosis" including organ function (see section VII) *before* start of course 1!

VIII.4.2 Course 2 - Additions to basic plan

Primary tumour site disease re-evaluation following this course prior to course 3 (e.g. week 6): CT scan or MRI (with measurements). See section XV for clinical response criteria.

VIII.4.3 Course 3 - Additions to basic plan

PBPC MOBILISATION AND HARVESTING IS STRONGLY RECOMMENDED FOLLOWING VIDE 3 AND/OR 4 (see section XIV for details).

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. Send PBPC sample for EWS RT-PCR study.

VIII.4.4 Course 4 - Additions to basic plan

Day 20-22: Cardiac monitoring (Echocardiography/MUGA, ECG).

Primary tumour site disease re-evaluation: CT scan or MRI (with measurements). See section XV for clinical response criteria.

PBPC MOBILISATION AND HARVESTING IS STRONGLY RECOMMENDED FOLLOWING VIDE 3 AND/OR 4 (see section XIV for details).

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. Please collect and send PBPC sample for EWS RT-PCR study.

VIII.4.5 Decision point for primary site local therapy

- **Tumour volume < 200 ml or ≥ 200 ml. Surgical resection deemed probable or possible**

  Proceed to course 5 and 6.

  Plan local therapy. See sections XVI and XVII.

- **Tumour volume < 200 ml. Initial resection. No further local treatment or radiotherapy**

  Proceed to course 5 and 6.

  Obtain consent for randomisation VAI vs VAC (RANDOMISATION R1)

- **Tumour volume < 200 ml. Radiotherapy for anatomical/accessibility reasons ***

  Proceed to course 5 and 6.

  * Note: Patients with unresectable small tumours of extremity sites and poor clinical/MRI-based tumour regression (i.e. reduction of soft tissue tumour component by less than 50% of initial size after two courses of VIDE) are upgraded and hence eligible for the R2loc randomisation.
  Patients with small localised tumours of extremity sites, with early radiotherapy for anatomical/ accessibility reasons and with poor clinical/MRI-based response or poor histological response to radio-chemotherapy are upgraded and hence eligible for the R2loc randomisation.
  (***PLEASE NOTE: Patients upgraded to R2 loc with small localised tumours of central axial sites and early radiotherapy are ineligible for randomisation due to projected busulfan toxicity.***
  In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

  Obtain consent for randomisation VAI vs VAC (RANDOMISATION R1)

- **Tumour volume ≥ 200 ml. Radiotherapy deemed essential but can be delayed until after course 7 / High dose therapy**

  Proceed to course 5 and 6.

  Obtain consent for randomisation VAI vs Bu-Mel (RANDOMISATION R2loc)

- **Tumour volume < 200 ml* or ≥ 200 ml**. Early radiotherapy deemed essential in view of disease progression or to enable surgical resection.**

  Plan immediate local therapy. See sections XVI and XVII.

  Proceed to course 5 and 6 and VAI courses 7-14 (or alternative therapy)

  * Note: Patients with small localised tumours of extremity sites, with early radiotherapy and with poor clinical/MRI-based response or poor histological response to radio-chemotherapy are upgraded and hence eligible for the R2loc randomisation.
  Obtain consent for randomisation VAI vs Bu-Mel + PBPC RANDOMISATION R2loc
  *****PLEASE NOTE: Patients with ≥ 200 ml central axial tumours and early radiotherapy are ineligible for randomisation due to projected busulfan toxicity.***
  In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment

where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

VIII.4.6 Course 5 - Additions to basic plan

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG).

VIII.4.7 Course 6 - Additions to basic plan

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG).

Primary tumour site disease re-evaluation: CT scan or MRI (with measurements). See section XV for clinical response criteria.

VIII.5 Dose modifications during induction therapy

VIII.5.1 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 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:

  → Reduce etoposide dose by 20%

- Neutropenic sepsis grade 3 or 4:

  → Reduce etoposide dose by 20%

Further episodes of toxicity should result in reductions in etoposide dose by an additional 20%. If necessary it is advised to omit etoposide completely rather than reduce the doses of the other three drugs.

VIII.5.2 Gastrointestinal toxicity

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

  → Reduce etoposide dose by 20%

Further episodes of toxicity should result in reductions in etoposide dose by an additional 20%. If necessary it is advised to omit etoposide completely rather than reduce the dose of the other three drugs.

VIII.5.3 Nephrotoxicity

Ifosfamide:

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

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

  → Reduce etoposide dose by 30% .

See section XIII for full details on renal toxicity!

VIII.5.4 Cardiac toxicity

- Fractional shortening (FS) < 29% or left ventricular ejection fraction (LVEF) < 40%, or decrease by an absolute value of ≥ 10 percentile points from previous tests:

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

VIII.5.5 Central neurotoxicity

Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to

Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16 for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

For other possible toxicities and dose modifications see sections XI and XIII.

VIII.6 Post-VIDE course 6: Local therapy and randomisation

Wherever feasible, proceed to surgery 21 days after course 6 or on haematological recovery. The next chemotherapy course - Course 7 VAI - should be planned to commence 14 days post surgical resection. Surgical specimens should be sent for rapid histopathological assessment of response to chemotherapy including central review where deemed necessary (see pathology guidelines, appendix A.2). Results should be available within 3 weeks of surgery. For details of local therapy techniques see sections XVI and XVII.

- **Tumour volume < 200 ml or ≥ 200 ml. Surgery after VIDE performed**

  Proceed to VAI Course 7, obtain histological response data.

  Resection - tumour viability <10% - RANDOMISATION R1 - VAI vs VAC:

  Continue on VAI or VAC as randomised.

  Resection - tumour viability ≥10% - RANDOMISATION R2loc - VAI vs Bu-Mel

  Continue on VAI or Bu-Mel as randomised.

  Note*:** in case of ***incomplete resection continue treatment as randomised, and consider re-operation and/or postoperative radiotherapy (during VAC/VAI or after Bu-Mel).

- **Tumor volume < 200 ml. Local treatment by radiotherapy for anatomical reasons**

  Proceed to Course 7 VAI and local radiotherapy. These should be given concurrently commencing 21 days post course 6.

  RANDOMISATION R1 - VAI vs VAC *

  Continue on VAI or VAC as randomised, courses 8-14.

  * Note: Patients with unresectable small tumours of extremity sites and poor clinical/MRI-based tumour regression (i.e. reduction of soft tissue tumour component by less than 50% of initial size after two courses of VIDE) are upgraded to R2loc, and hence eligible for the R2loc randomisation.
  Patients with small localised tumours of extremity sites, with early radiotherapy for anatomical/ accessibility reasons and with poor clinical/MRI-based response or poor histological response to radio-chemotherapy are upgraded and hence eligible for the R2loc randomisation.
  PLEASE NOTE: Patients with small localised tumours of central axial sites and early radiotherapy upgraded to R2loc are ineligible for R2loc randomisation due to projected busulfan toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment

where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for R2loc-randomisation due to projected busulfan toxicity.

- **<u>Tumour volume ≥ 200 ml. Radiotherapy deemed essential but can be delayed until after course 7 / Bu-Mel</u>**

  Proceed to VAI Course 7.

  RANDOMISATION R2loc - VAI vs Bu-Mel

  ~~C~~ontinue on VAI or Bu-Mel as randomised.

  Patients allocated to VAI should commence radiotherapy at the start of course 8.

  Patients allocated to Bu-Mel should commence radiotherapy 8-10 weeks after high dose therapy.

- **<u>Tumour volume ≥ 200 ml with extremity tumours after early radiotherapy</u>**

  Proceed to Course 7 VAI .

  RANDOMISATION R2loc - VAI vs Bu-Mel + PBPC rescue

  ~~C~~ontinue on VAI or Bu-Mel as randomised.

- **<u>Tumour volume ≥ 200 ml with central axial tumours after early radiotherapy</u>**

  PLEASE NOTE: Group 2$_{loc}$ patients with <u>central axial</u> tumours and early radiotherapy are ineligible for randomisation due to projected busulfan toxicity.

  In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for R2loc-randomisation due to projected busulfan toxicity.

  Proceed to VAI Courses 7-14 (or alternative therapy if preferred).

After obtaining relevant information and informed consent, randomisation should take place by FAX to the appropriate study centre (see section VI).

VIII.7 VAI consolidation chemotherapy

All patients receive course 7 as VAI

Patients randomised to VAI consolidation receive Courses 8 - 14 as per course 7.

Courses 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/course)	(*max. single dose: 2 mg*)
ACTINOMYCIN D	0.75 mg/m^2/d (i.v. push)	d1, d2	(1.5 mg/m^2/course)	(*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/course)	plus MESNA*
*Mesna dosage	1.0 g/m^2/d 3.0 g/m^2/d	d1 d1, d2	(i.v. push 1 h prior to ifosfamide) (i.v. infusion, e.g. 24 h)	

See section XI and appendix B.3 for chemotherapy guidelines and details.

Day 1-2: VAI

Day 10-12: FBC, urea + electrolytes, calcium, magnesium, phosphate, bicarbonate.

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, ALT (German: GPT)
- GFR (calculated C_{crea} or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) / Renal tubular threshold for phosphate (Tmp/GFR).

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG) as indicated.

VIII.8 VAC consolidation therapy

Note: All patients receive course 7 as VAI (see above)!

Patients randomised to VAC consolidation receive courses 8 - 14 as VAC.

Courses 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/course) (*max. single dose: 2 mg*)
ACTINOMYCIN D	0.75 mg/m^2/d (i.v. push)	d1, d2	(1.5mg/m^2/course) (*max. single dose/d: 1.5 mg*)
CYCLOPHOSPHAMIDE	1500mg/m^2/d (i.v. infusion, 1-3 h)	d1	(1500 mg/m^2/course) plus MESNA
*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)

See section XI and B.3 for chemotherapy guidelines and details.

Day 1-2: VAC

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, ALT (German: GPT)
- GFR (calculated C_{crea} or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) / Renal tubular threshold for phosphate (Tmp/GFR).

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG) as indicated.

VIII.9 VAI / VAC dose modifications during consolidation therapy

VIII.9.1 Haematological/GI toxicity

In case of significant bone marrow toxicity preference should be given to 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:

  → Reduce IFO/CYC and ACT dose by 20%

- Neutropenic sepsis Grade3 or 4:

  → Reduce IFO/CYC and ACT dose by 20%

- Mucositis/GI toxicity Grade 3 or 4:

  → Reduce IFO or CYC / ACT dose by 20%

Further episodes of toxicity should result in reductions in IFO/CYC or ACT dose by an additional 20%.

VIII.9.2 Nephrotoxicity (VAI)

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

Toxicity grade*	GFR (ml/min/1.73 m^2)	Tp/C_{crea} (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 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

See section XIII for full details on renal toxicity!

VIII.9.3 Cardiac toxicity

- FS < 29% or LVEF < 40%, or decrease by an absolute value of ≥ 10 percentile points from previous tests:

  → Delay chemotherapy course for 7 days and repeat echocardiography. If FS has recovered to 29% or greater then proceed to next course. If FS remains below 29% then discuss with your national study centre whether to reduce or omit drugs (ifosfamide).

VIII.9.4 Central neurotoxicity

Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-

infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to *Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16* for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

For other possible toxicities and dose modifications see sections XI and XIII.

VIII.10 Busulfan/Melphalan + PBPC rescue consolidation therapy

<u>Note</u>: All patients receive Course 7 as VAI (see above)

VIII.10.1 Course 8 – Bu-Mel high-dose chemotherapy

Patients randomised to Bu-Mel receive busulfan-melphalan consolidation as course 8.

BUSULFAN - MELPHALAN (Bu-Mel)

		D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Busulfan per os (p.o.) 37.5 mg/m²/dose = 150 mg/m²/d (4 divided doses per day) = 600 mg/m² cumulative dose (16 divided doses total)	0 h		X	X	X	X			
	6 h		X	X	X	X			
	12 h		X	X	X	X			
	18 h		X	X	X	X			
Melphalan i.v. 140 mg/m² i.v. infusion, 30 min.							X		
Clonazepam p.o., i.v. 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

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.

NOTE: Observe contraindications detailed below!

Heparin and/or allopurinol or urodesoxycholic acid (UDCA) (d –7 to d 8)[67] may be added according to national guidelines.

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

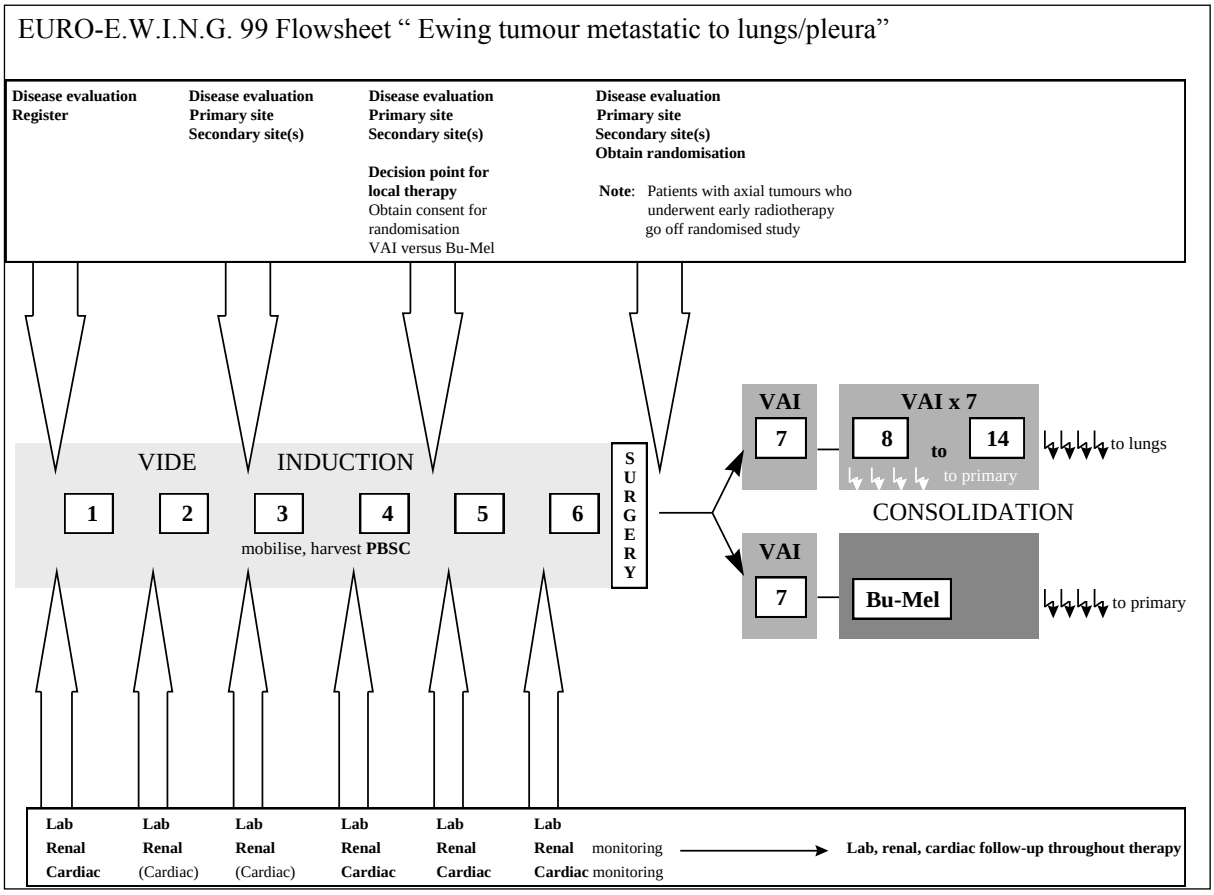
See section XI and appendix B.3 for chemotherapy guidelines and details.

VIII.10.2 Contraindications for Bu-Mel high dose therapy

Any patient who has received radiotherapy to central axial sites (e.g. chest, pelvis) is ineligible for busulfan high-dose therapy and randomisation for reasons of anticipated toxicity. **In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.** Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for Bu-Mel high dose therapy due to anticipated busulfan toxicity, and should receive the conventional treatment arm. (For alternative high dose therapy options in these patients see appendix A.4).

IX EURO-E.W.I.N.G. 99 - Pulmonary/Pleural Metastatic Disease Protocol

IX.1 Summary outline



IX.2 Induction treatment

All patients receive 6 courses of VIDE chemotherapy as induction treatment.

Courses 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/m^2/d (i.v. push)	d1	(1.5 mg/m^2/course)	(*max. single dose: 2 mg*)
IFOSFAMIDE	3.0 g/m^2/d (i.v. infusion, 1-3 h)	d1, d2, d3	(9 g/m^2/course)	plus MESNA*
DOXORUBICIN	20 mg/m^2/d (i.v. infusion, 4 h)	d1, d2, d3	(60 mg/m^2/course)	
ETOPOSIDE	150 mg/m^2/d (i.v. infusion, 1 h)	d1, d2, d3	(450 mg/m^2/course)	
*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, d3	(i.v. infusion, e.g. 24 h)	

See section XI and appendix B.3 for chemotherapy guidelines and details.

IX.3 Basic plan of VIDE courses

Day 1-3: VIDE 1 (see above)

Day 10-12: FBC, urea + electrolytes, calcium, magnesium, phosphate, bicarbonate.

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes
- GFR (calculated Creatinine clearance (C_{crea}) or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) = Renal tubular threshold for phosphate (Tm_p/GFR).

IX.4 Additions to basic plan

IX.4.1 Course 1 - Additions to basic plan

Complete "Investigations at diagnosis" including organ function (see section VII) *before* start of course 1 !

IX.4.2 Course 2 - Additions to basic plan

Primary tumour site disease re-evaluation following this course prior to course 3 (e.g. week 6): CT scan or MRI (with measurements). See section XV for clinical response criteria.

Secondary tumour site re-evaluation: CT scan chest (with measurements and number). See section XV for clinical response criteria.

IX.4.3 Course 3 - Additions to basic plan

PBPC MOBILISATION AND HARVESTING IS STRONGLY RECOMMENDED FOLLOWING VIDE 3 AND/OR 4 (see section XIV for details).

This is mandatory in patients with metastases to lungs/pleura. Send PBPC sample for EWS RT-PCR study.

IX.4.4 Course 4 - Additions to basic plan

Day 20-22: Cardiac monitoring (Echocardiography/MUGA, ECG).

Primary tumour site disease re-evaluation: CT scan or MRI (with measurements). See section XV for clinical response criteria.

Secondary tumour site re-evaluation: CT scan chest (with measurements and number). See section XV for clinical response criteria.

PBPC MOBILISATION AND HARVESTING IS MANDATORY FOLLOWING VIDE 3 AND/OR 4 (see section XIV for details).

IX.4.5 Decision point for primary site local therapy

- **Surgical resection deemed probable or possible.**

  Proceed to Course 5 and 6

  Obtain consent for

  randomisation VAI (+ lung irradiation) vs Bu-Mel, RANDOMISATION R2$_{pulm}$

- **Radiotherapy deemed essential but can be delayed until after course 7 / High dose therapy.**

  Proceed to Course 5 and 6

  Obtain consent for

  randomisation VAI (+ lung irradiation) vs Bu-Mel, RANDOMISATION R2$_{pulm}$

  PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

- **Early radiotherapy of extremity tumour deemed essential in view of disease progression or to enable surgical resection.**

  Plan immediate local therapy.

  Obtain consent for

  randomisation VAI (+ lung irradiation) vs Bu-Mel, RANDOMISATION R2$_{pulm}$

  PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

- **Early radiotherapy of central axial tumour deemed essential in view of disease progression or to enable surgical resection.**

  Plan immediate local therapy.

  PLEASE NOTE: Patients with central axial primary tumours and early radiotherapy are ineligible for randomisation due to projected busulfan toxicity.
  In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

  Proceed to VIDE courses 5&6 and VAI courses 7-14 and lung irradiation (or alternative therapy if preferred).

See local therapy guidelines, sections XVI and XVII.

IX.4.6 Course 5 - Additions to basic plan

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG).

IX.4.7 Course 6

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG).

Primary tumour site disease re-evaluation: CT scan or MRI (with measurements). See section XV for clinical response criteria.

Secondary tumour site re-evaluaton: CT scan chest (with measurements and number). See section XV for clinical response criteria.

IX.5 Dose modifications during induction therapy

IX.5.1 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 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:

  → Reduce etoposide dose by 20%

- Neutropenic sepsis grade 3 or 4:

  → Reduce etoposide dose by 20%

Further episodes of toxicity should result in reductions in etoposide dose by an additional 20%. If necessary it is advised to omit etoposide completely rather than reduce the doses of the other three drugs.

IX.5.2 Gastrointestinal toxicity

- Mucositis/gastrointenstinal (GI) toxicity grade 3 or 4:

  → Reduce etoposide dose by 20%

Further episodes of toxicity should result in reductions in etoposide dose by an additional 20%. If necessary it is advised to omit etoposide completely rather than reduce the dose of the other three drugs.

IX.5.3 Nephrotoxicity

<u>Ifosfamide</u>:

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 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:

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

  → Reduce etoposide dose by 30% .

See section XIII for full details on renal toxicity!

IX.5.4 Cardiac toxicity

- Fractional shortening (FS) < 29% or left ventricular ejection fraction (LVEF) < 40%, or decrease by an absolute value of ≥ 10 percentile points from previous tests:

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

IX.5.5 Central neurotoxicity

Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to *Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16* for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide

1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

For other possible toxicities and dose modifications see sections XI and XIII.

IX.6 Post-VIDE course 6: Local therapy of primary tumour and randomisation

Wherever feasible, proceed to surgery of the primary tumour 21 days after course 6 or on haematological recovery. The next chemotherapy course - Course 7 VAI - should be planned to commence 14 days post surgery. Surgical specimens should be sent for rapid histopathological assessment of response to chemotherapy including central review where deemed necessary (see pathology guidelines, appendix A.2). Results should be available within 3 weeks of surgery. For details of local therapy techniques see sections XVI and XVII.

- **Tumours metastatic to lung/pleura. Surgery of primary site after VIDE performed**

  Proceed to VAI Course 7.

  RANDOMISATION R2$_{pulm}$ - VAI vs Bu-Mel:

  → Continue on VAI (with lung irradiation) or Bu-Mel as randomised.

  (See section XVII for lung irradiation guidelines)

  PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity

- **Tumours metastatic to lung/pleura. Radiotherapy of primary tumour deemed essential but can be delayed until after course 7 / High dose therapy**

  Proceed to VAI Course 7.

  RANDOMISATION R2$_{pulm}$ - VAI vs Bu-Mel :

  → Continue on VAI (with lung irradiation) or Bu-Mel as randomised.

  Patients allocated to VAI should commence radiotherapy of the primary tumour at the start of course 8. (See sections XVI and XVII for local therapy and lung irradiation guidelines).

  Patients allocated to Bu-Mel + PBPC should commence radiotherapy of the primary tumour 8-10 weeks after high dose therapy. (see sections XVI and XVII).

  PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

- **Tumours metastatic to lung/pleura, with primary tumours at extremity sites after early radiotherapy**

Proceed to Course 7 .

RANDOMISATION R2pulm - VAI vs Bu-Mel:

→ Continue on VAI (with lung irradiation) or Bu-Mel as randomised.

PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.

- **Tumours metastatic to lung/pleura, with primary tumours at central axial sites after early radiotherapy**

  PLEASE NOTE: Patients with primary tumours at central axial sites and early radiotherapy are ineligible for randomisation due to projected busulfan toxicity.
  In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for randomisation due to projected busulfan toxicity.
  → Proceed to VAI Courses 7-14, and lung irradiation (or alternative therapy if preferred).

After obtaining relevant information and informed consent, randomisation should take place by FAX to the appropriate study centre (see section VI).

IX.7 VAI consolidation therapy

All patients receive Course 7 as VAI.
Patients randomised to VAI + lung irradiation consolidation receive courses 8 - 14 as per course 7.

Courses 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/course)	(*max. single dose: 2 mg*)
ACTINOMYCIN D	0.75 mg/m^2/d (i.v. push)	d1, d2	(1.5 mg/m^2/course)	(*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 /course)	plus MESNA
*Mesna dosage	1.0 g/m^2/d 3.0 g/m^2/d	d1 d1, d2	(i.v. push 1 h prior to ifosfamide) (i.v. infusion, e.g. 24 h)	

See section XI and appendix B.3 for chemotherapy guidelines and details.

Day 1-2: VAI

Day 10-12: FBC, urea + electrolytes, calcium, magnesium, phosphate, bicarbonate.

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, ALT (German: GPT)
- GFR (calculated C_{crea} or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) / Renal tubular threshold for phosphate (Tmp/GFR).

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG) as indicated.

IX.8 VAI dose modifications during consolidation therapy

IX.8.1 Haematological/GI toxicity

- Haematological recovery delayed >6 days:

  → Reduce IFO and ACT dose by 20%

- Neutropenic sepsis Grade 3 or 4:

  → Reduce IFO and ACT dose by 20%

- Mucositis/GI toxicity Grade 3 or 4:

  → Reduce IFO and ACT dose by 20%

Further episodes of toxicity should result in reductions in IFO / ACT dose by an additional 20%.

IX.8.2 Nephrotoxicity (VAI)

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

Toxicity grade*	GFR (ml/min/1.73 m^2)	Tp/C_{crea} (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 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

See section XIII for full details on renal toxicity!

IX.8.3 Cardiac toxicity

- Fractional shortening (FS) < 29% or left ventricular ejection fraction (LVEF) < 40%, or decrease by an absolute value of ≥ 10 percentile points from previous tests:

  → Delay chemotherapy course for 7 days and repeat echocardiography. If FS has recovered to 29% or greater then proceed to next course. If FS remains below 29% then discuss with your national study centre whether to reduce or omit drugs (ifosfamide).

IX.8.4 Central neurotoxicity

Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to *Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16* for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

For other possible toxicities and dose modifications see sections XI and XIII.

IX.9 Lung irradiation following VAI

In patients randomised for VAI plus bilateral whole lung irradiation, this procedure will take place after completion of all VAI courses.

See section XVII for lung irradiation guidelines.

IX.10 Busulfan/Melphalan + PBPC rescue consolidation therapy

Note: All patients receive Course 7 as VAI (see above)

IX.10.1 Course 8 – Bu-Mel high-dose chemotherapy

Patients randomised to Bu-Mel receive busulfan-melphalan consolidation as course 8.

BUSULFAN - MELPHALAN (Bu-Mel)

		D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Busulfan per os (p.o.) 37.5 mg/m²/dose = 150 mg/m²/d (4 divided doses per day) = 600 mg/m² cumulative dose (16 divided doses total)	0 h		x	x	x	x			
	6 h		x	x	x	x			
	12 h		x	x	x	x			
	18 h		x	x	x	x			
Melphalan i.v. 140 mg/m² i.v. infusion, 30 min.							x		
Clonazepam p.o., i.v. 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

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.

NOTE: Observe contraindications detailed below!

Heparin and/or allopurinol or UDCA (d –7 to d 8) may be added according to national guidelines.

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

See section XI and appendix B.3 for chemotherapy guidelines and details.

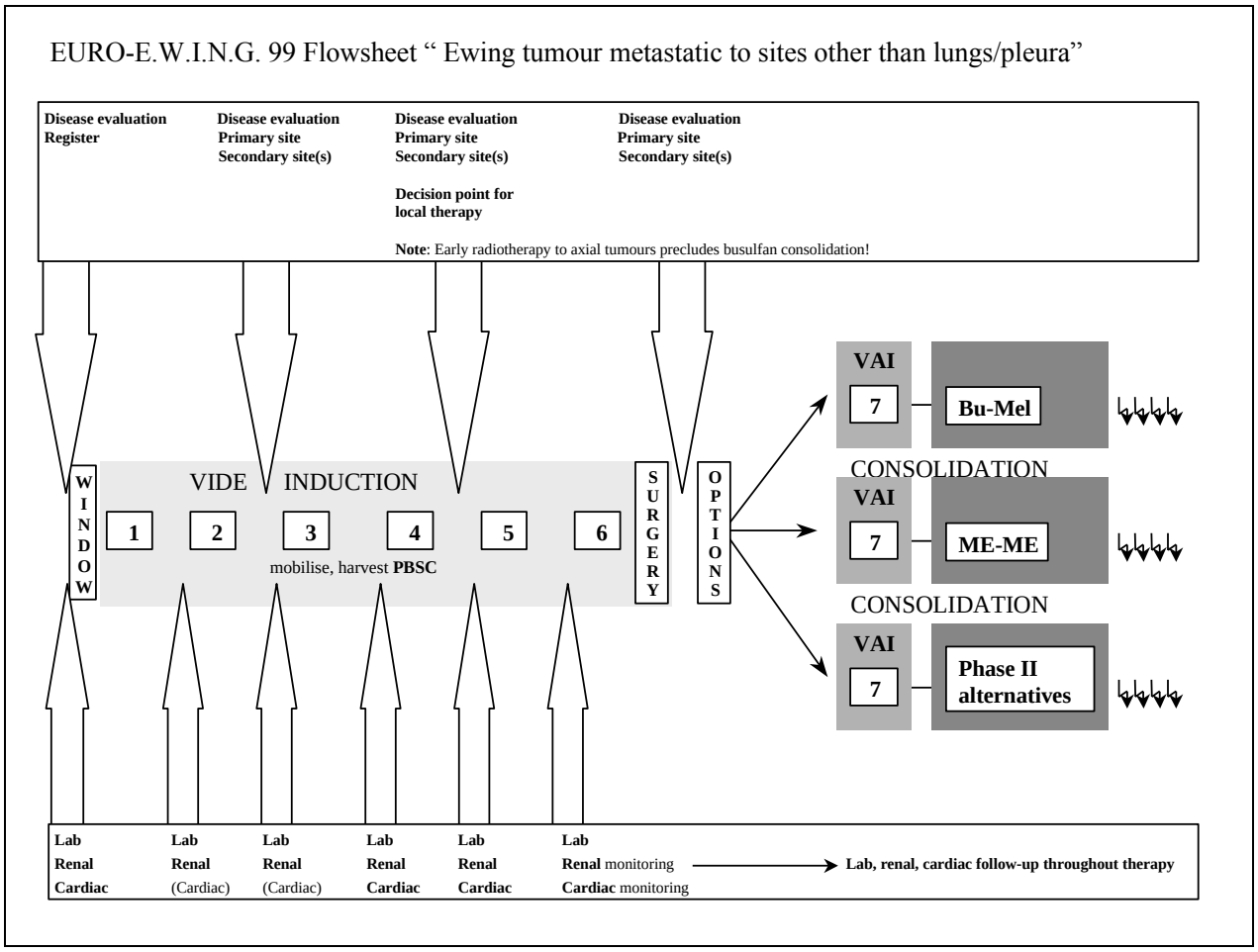
IX.10.2 Contraindications for Bu-Mel high dose therapy

Any patient who has received radiotherapy to central axial sites (e.g. chest, pelvis) is ineligible for busulfan high-dose therapy and randomisation for reasons of anticipated toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain with a dose of more than 30 Gy are NOT eligible for Bu-Mel high dose therapy due to anticipated busulfan toxicity, and should receive the conventional treatment arm. (For alternative high dose therapy options in these patients see appendix A.4).

X EURO-E.W.I.N.G. 99 - Guidelines for Patients with Metastases at Extrapulmonary Sites

X.1 Summary Outline



Patients with newly diagnosed histologically proven disseminated (stage IV) Ewing tumours with systemic metastases that are not restricted to the pulmonary or pleural region, i.e. patients with metastases to the skeleton, bone marrow, lymph nodes, etc., with or without additional pulmonary/pleural metastases may be treated following these guidelines.

Other treatment options may be available within the frame of national or international studies. It is strongly recommended that each patient should be discussed with the national study centre in order to be aware of any studies.

X.2 Upfront window studies

Patients with metastases at extrapulmonary sites are eligible for upfront courses of investigational drugs applied within national or international phase I/II studies.

X.3 Induction treatment

All patients receive 6 courses of VIDE chemotherapy as induction treatment.

Courses 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/m^2/d d1 (i.v. push)	(1.5 mg/m^2/course)	(*max. single dose: 2 mg*)
IFOSFAMIDE	3.0 g/m^2/d d1, d2, d3 (i.v. infusion, 1-3 h)	(9 g/m^2/course)	plus MESNA
DOXORUBICIN	20 mg/m^2/d d1, d2, d3 (i.v. infusion, 4 h)	(60 mg/m^2/course)	
ETOPOSIDE	150 mg/m^2/d d1, d2, d3 (i.v. infusion, 1 h)	(450 mg/m^2/course)	

*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, d3	(i.v. infusion, e.g. 24 h)

See section XI and appendix B.3 for chemotherapy guidelines and details.

X.4 Basic plan of VIDE courses

Day 1-3: VIDE 1 (see above)

Day 10-12: FBC, urea + electrolytes, calcium, magnesium, phosphate, bicarbonate.

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, liver enzymes
- GFR (calculated Creatinine clearance (C_{crea}) or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) = Renal tubular threshold for phosphate (Tm_p/GFR).

X.5 Additions to basic plan

X.5.1 Course 1 - Additions to basic plan

Complete "Investigations at diagnosis" including organ function (see section VII) *before* start of course 1 !

X.5.2 Course 2 - Additions to basic plan

Primary tumour site disease re-evaluation following this course prior to course 3 (e.g. week 6): CT scan or MRI (with measurements). See section XV for clinical response criteria.

Secondary tumour site re-evaluation: CT/MRI scans, technetium bone scans, etc., as indicated. See section XV for clinical response criteria.

X.5.3 Course 3 - Additions to basic plan

PBPC MOBILISATION AND HARVESTING IS STRONGLY RECOMMENDED FOLLOWING VIDE 3 AND/OR 4 (see section XIV for details).

Send PBPC sample for EWS RT-PCR study.

X.5.4 Course 4 - Additions to basic plan

Day 20-22: Cardiac monitoring (Echocardiography/MUGA, ECG).

Primary tumour site disease re-evaluation: CT scan or MRI (with measurements). See section XV for clinical response criteria.

Secondary tumour site re-evaluation: CT/MRI scans, technetium bone scans, etc., as indicated. See section XV for clinical response criteria.

PBPC MOBILISATION AND HARVESTING IS MANDATORY FOLLOWING VIDE 3 AND/OR 4 (see section XIV for details).

X.5.5 Decision point for primary site local therapy

Radiotherapy of central axial sites should be delayed until after BuMel high dose therapy wherever possible. Patients with inevitable early radiotherapy to central axial sites are ineligible for busulfan containing high dose therapy for reasons of anticipated toxicity, and should be treated with alternative approaches, e.g. ME-ME high dose therapy (see appendix A.4) or according to other phase I/II studies.

PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for Busulfan-Melphalan due to projected busulfan toxicity.
Please contact your appropriate national coordinator for guidance.

X.5.6 Course 5 - Additions to basic plan

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG).

X.5.7 Course 6

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG).

Primary tumour site disease re-evaluation: CT scan or MRI (with measurements).

Secondary tumour site re-evaluation: CT/MRI scans, technetium bone scans, etc., as indicated. See section XV for clinical response criteria.

X.6 Dose modifications during induction therapy

X.6.1 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 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:

  → Reduce etoposide dose by 20%

- Neutropenic sepsis grade 3 or 4:

  → Reduce etoposide dose by 20%

Further episodes of toxicity should result in reductions in etoposide dose by an additional 20%. If necessary it is advised to omit etoposide completely rather than reduce the doses of the other three drugs.

X.6.2 Gastrointestinal toxicity

- Mucositis/gastrointenstinal (GI) toxicity grade 3 or 4:

  → Reduce etoposide dose by 20%

Further episodes of toxicity should result in reductions in etoposide dose by an additional 20%. If necessary it is advised to omit etoposide completely rather than reduce the dose of the other three drugs.

X.6.3 Nephrotoxicity

<u>Ifosfamide</u>:

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 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:

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

  → Reduce etoposide dose by 30% .

See section XIII for full details on renal toxicity!

X.6.4 Cardiac toxicity

- Fractional shortening (FS) < 29% or left ventricular ejection fraction (LVEF) < 40%, or decrease by an absolute value of ≥ 10 percentile points from previous tests:

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

X.6.5 Central neurotoxicity

Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to *Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16* for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide

1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

For other possible toxicities and dose modifications see sections XI and XIII.

X.7 Local therapy following VIDE course 6

Resection of bulky tumour sites may be performed after VIDE course 6, as long as such manoeuvres will not lead to a delay in further treatment. In all other cases, local therapy should be postponed until 6-8 weeks after the completion of Bu-Mel high dose therapy and stable engraftment.

Radiotherapy of central axial sites should be delayed until after BuMel high dose therapy wherever possible. Patients with inevitable early radiotherapy are ineligible for busulfan containing high dose therapy for reasons of anticipated toxicity, and should be treated on alternative approaches, e.g. ME-ME high dose therapy (see appendix A.4).

PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to dose of more than 30 Gy are NOT eligible for Busulfan-Melphalan due to projected busulfan toxicity, and should receive alternative therapies.
Please contact your appropriate national coordinator for guidance.

X.8 Course 7 – VAI

All patients receive course 7 as VAI

Courses 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/course)	(*max. single dose: 2 mg*)
ACTINOMYCIN D	0.75 mg/m^2/d (i.v. push)	d1, d2	(1.5 mg/m^2/course)	(*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/course)	plus MESNA
*Mesna dosage	1.0 g/m^2/d 3.0 g/m^2/d	d1 d1, d2	(i.v. push 1 h prior to ifosfamide) (i.v. infusion, e.g. 24 h)	

See section XI and appendix B.3 for chemotherapy guidelines and details.

Day 1-2: VAI

Day 10-12: FBC, urea + electrolytes, calcium, magnesium, phosphate, bicarbonate.

Day 20-22 (i.e. prior to next course):

- FBC, urea & electrolytes, calcium, magnesium, phosphate, bicarbonate, alkaline phosphatase, bilirubin, ALT (German: GPT)
- GFR (calculated C_{crea} or isotopic)
- Fractional phosphate reabsorption (Tp/C_{crea}) / Renal tubular threshold for phosphate (Tmp/GFR).

Day 20-22: Cardiac monitoring (ECHO/MUGA, ECG) as indicated.

X.9 Busulfan/Melphalan + PBPC rescue consolidation therapy

Note: All patients receive Course 7 as VAI (see above)

X.9.1 Course 8 – Bu-Mel high-dose chemotherapy

Patients receive busulfan-melphalan consolidation as course 8.

BUSULFAN - MELPHALAN (Bu-Mel)

		D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Busulfan per os (p.o.) 37.5 mg/m²/dose = 150 mg/m²/d (4 divided doses per day) = 600 mg/m² cumulative dose (16 divided doses total)	0 h		X	X	X	X			
	6 h		X	X	X	X			
	12 h		X	X	X	X			
	18 h		X	X	X	X			
Melphalan i.v. 140 mg/m² i.v. infusion, 30 min.							X		
Clonazepam p.o., i.v. 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

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.

NOTE: Observe contraindications detailed below!

Heparin and/or allopurinol may be added according to national guidelines.

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

See section XI and appendix B.3 for chemotherapy guidelines and details.

X.9.2 Contraindications for Bu-Mel high dose therapy

Any patient who has received radiotherapy to central axial sites (e.g. chest, pelvis) is ineligible for busulfan high-dose therapy and randomisation for reasons of anticipated toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy. Patients expected to receive radiotherapy at any time of treatment where radiation fields contain any proportion of spinal cord or brain treated to a dose of more than 30 Gy are NOT eligible for Bu-Mel high dose therapy due to anticipated busulfan toxicity, and should receive alternative treatment.

Please contact your appropriate national coordinator for guidance.
(For alternative high dose therapy options in these patients, e.g. ME-ME, see appendix A.4).

X.10 Local therapy following Bu-Mel

Following Bu-Mel consider removal or irradiation of residual disease according to surgical/radiotherapy guidelines (sections XVI and XVII). Less acute toxicity is to be expected with the sequence of radiation ≥ 6 weeks after Bu-Mel rather than vice versa. Caution is advised with whole lung irradiation. This is to be avoided, to reduce the risk of pulmonary fibrosis, unless radiation is focused to sites of persistent disease.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

X.11 ME-ME

For details of the ME-ME high-dose regimen, see appendix A.4.

X.12 Phase II alternatives

Contact your appropriate study centre for active studies.

XI Chemotherapy Guidelines

XI.1 Chemotherapy starting rules

Chemotherapy courses should not be started unless:

- WBC $\geq 2.0*10^9$/l with absolute neutrophil count (ANC) $\geq 1.0*10^9$/l

- Platelets ≥ $80*10^9$/l.

There must not be any relevant dysfunction of inner organs, especially heart, kidney or liver prior to the start of each course!

XI.2 General guidelines

Dose/time intensity is regarded to be an essential aspect of the VIDE strategy. In case of relevant (≥ CTC grade 3) bone marrow toxicity, use of G-CSF is recommended and given priority over dose reduction. If relevant toxicity continues with further courses under G-CSF, etoposide is the first drug to be reduced by 20%. If further episodes of treatment delay and/or severe mucositis/neutropenic infections should occur, the dose of etoposide should be further reduced by another 20%. If necessary, it is advised to omit etoposide rather than reduce the doses of other drugs.

- The doses of ifosfamide and doxorubicin should be maintained if possible.
- For dose modifications in case of severe organ toxicity see section XIII.
- **In patients with body surface area (BSA) > 2 m²** the chemotherapy dose should not exceed the dose calculated for a BSA of 2 m² (observe maximum single dose for vincristine: 2.0 mg).
- The dose given to **obese patients** should be calculated based on regular body weight.
- The chemotherapy doses must be recalculated for each course of chemotherapy according to the patient's current body weight and surface area.
- **Maximum single dose of vincristine 2.0 mg!**
- **Maximum single dose of actinomycin D 1.5 mg per day!**
- **Sequence of administration according to institutional policy.**

In children under the age of one year or with a body weight of less than 10 kg, (uncommon in Ewing tumours) **the treatment strategy should be individually discussed with the national study centre.** As a guideline, the chemotherapy dose in these young infants should be reduced to 66% of the normal dose per m² body surface, or should be calculated according to kg body weight.

Dose equivalents per kg body weight*:

Actinomycin D (ACT)	0.75	mg/m² BSA	0.025	mg/kg BW
Cyclophosphamide (CYC)	1500.00	mg/m² BSA	50.00	mg/kg BW
Doxorubicin (DOX)	20.00	mg/m² BSA	0.67	mg/kg BW
Etoposide (ETO)	150.00	mg/m² BSA	5.00	mg/kg BW
Ifosfamide (IFO)	3000.00	mg/m² BSA	100.00	mg/kg BW
Melphalan (Mel)	35.00	mg/m² BSA	1.17	mg/kg BW
Vincristine (VCR)	1.50	mg/m² BSA	0.05	mg/kg BW

Busulfan (Bu) *see paragraph on Bu-Mel in appropriate protocol (sections VIII, IX, or X).*

* 1 m² body surface area (BSA) is considered equivalent to 30 kg body weight (BW)

XI.3 Drug notes

XI.3.1 Actinomycin D (ACT)

Mechanism of action: Inhibition of DNA synthesis

Maximum single dose: 1.5 mg/d

Side effects:

- Gastrointestinal irritation (nausea, vomiting, diarrhoea, ulcerative stomatitis, gastroenteritis)
- Hepatotoxicity (venoocclusive disease (VOD), particularly in young children)
- Bone marrow depression
- Alopecia
- Exanthema
- Extravasation may cause severe local and regional ulceration.

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

XI.3.2 Busulfan (Bu)

Mechanism of action: Alkylating agent

Side effects:

- Bone marrow depression
- Allergic reactions

- Alopecia (may be permanent)
- Amenorrhoea
- Aspermiogenesis
- Dysaesthesia
- Endocardial fibrosis
- Gastrointestinal irritation (nausea, vomiting, diarrhoea, stomatitis)
- Haemorrhagic cystitis (uncommon)
- Hyperpigmentation of skin
- Cataract
- Lung fibrosis (CAVE: deleterious in connection with lung irradiation!)
- Radiation recall mucositis and dermatitis if applied after radiotherapy
- Myasthenia gravis
- Mucositis
- Seizures
- VOD of the liver in up to 30% of patients in high-dose settings, usually reversible
- Irreversible transverse myelitis with tetraplegia has been observed following treatment with busulfan and subsequent radiotherapy to the spinal cord

Contraindications: Previous irradiation of central axial sites; anticipated or delivered radiotherapy delivering more than 30 Gy to any part of the spinal cord or to any part of the brain.

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.

XI.3.3 Cyclophosphamide (CYC)

Mechanism of action: Alkylating agent
Cyclophosphamide has to be activated by hepatic hydroxylation

Side effects:
- Haemorrhagic cystitis (MESNA uroprotection)
- Bone marrow depression
- Gastrointestinal irritation (nausea, vomiting, diarrhoea, stomatitis)
- Alopecia, dermatitis
- Infertility
- Imunosuppression

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

XI.3.4 Doxorubicin (Adriamycin) **(DOX)**

Mechanism of action: Inhibition of DNA synthesis

Side effects:
- Bone marrow depression
- Cardiotoxicity
- Gastrointestinal irritation (nausea, vomiting, ulceration)
- Allergic reactions with skin rash and fever
- Alopecia

Contraindications: DOX is to be discontinued following left-sided chest-wall or pulmonary irradiation, and in impaired cardiac function (see below).

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

Dose adaptation due to cardiotoxicity: If temporary deterioration of myocardial function occurs, e.g. decrease in fractional shortening < 29%, or a decrease relative to previous tests, and if this is confirmed by a repeat echocardiogram, omit doxorubicin in the next course. (If the decrease is not confirmed, i.e. if repeat investigations cannot reproduce the dysfunction, the omitted dose of DOX should be supplied instead of ACT with the first consolidation course.) If persistent deterioration of myocardial function occurs, e.g. persistent decrease in fractional shortening by an absolute value of 10 percentile points from previous tests or a persistent fractional shortening < 29%, 40% LVEF, avoid further doxorubicin. In doubt echocardiography should be complemented by radionuclide cardiac cineangiography or myocardial biopsy.

XI.3.5 Etoposide (VP16) **(ETO)**

Mechanism of action: Inhibition of DNA synthesis,
inhibition of DNA topoisomerase II

Side effects:
- Bone marrow depression
- Allergic reactions
- Hypotensive reaction due to alcohol contents in infusion fluid
- Alopecia
- Gastrointestinal irritation (nausea, vomiting, mucositis, ulcerative gastroenteritis, diarrhoea)

In allergic and/or hypotensive reactions administer hydrocortisone and antihistamines and/or catecholamines.

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

Dose adaptation due to allergic reactions: In case of uncontrollable allergic and/or hypotensive reactions omit etoposide without replacement.

XI.3.6 Etoposide phosphate **(ETOPOPHOS)**

Comparable properties to ETO, water soluble preparation, can safely be administered concentrated without the need of major dilution, and does not contain ethanol nor methanol. ETOPOPHOS may therefore be preferable in a high-dose setting.

CAVE: The Dextrane 40 content of ETOPOPHOS may lead to allergic reactions !

XI.3.7 Ifosfamide (IFO)

Mechanism of action: Alkylating agent
Ifosfamide has to be activated by hepatic hydroxylation

Side effects:

- Haemorrhagic cystitis (MESNA uroprotection)
- Nephrotoxicity: tubulopathy with glucosuria, aminoaciduria, loss of phosphate and Ca, full range of tubulopathies from subclinical changes to a full-fledged Fanconi syndrome
- Bone marrow depression
- Gastrointestinal irritation (nausea, vomiting, diarrhoea, stomatitis)
- Alopecia
- Neurotoxicity with transient somnolence and mental disturbance, seizures
- Infertility
- Imunosuppression

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

Dose adaptation due to central neurotoxicity: If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to *Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16* for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When

CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

Dose adaptation due to renal toxicity: 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 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

XI.3.8 Melphalan (Mel)

Mechanism of action: Alkylating agent

Side effects:
- Bone marrow depression
- Allergic reactions
- Alopecia
- Amenorrhoea
- Dysaesthesia
- Gastrointestinal irritation (nausea, vomiting, diarrhoea, stomatitis)
- Mucositis

Possible late effect: Lung fibrosis (uncommon)

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

XI.3.9 Mesna

Mechanism of action: Inactivation of toxic metabolites of alkylating agents in the renal tubuli and lower urinary tract

Side effects:
- Gastrointestinal irritation (nausea, vomiting, diarrhea)
- Limb pain
- Headache
- Hypotension

- Fatigue
- Allergy

XI.3.10 Vincristine (VCR)

Mechanism of action: M-phase arrest

Maximum single dose: 2.0 mg

Side effects:
- Peripheral neuropathy (areflexy, paresthesia, muscular weakness, ataxia)
- Cranial nerve palsies
- Autonomous neuropathy (constipation, paralytic ileus, urinary retention)
- Central neurotoxicity (hallucinations, epileptic seizures, SIADH)
- Arthralgia, myalgia
- Bone marrow depression
- Alopecia

In infants < 1 year or < 10 kg body weight, discuss treatment with your study centre; if in doubt, calculate dosage by kgBW, not m²BSA.

Dose adaptation due to neurotoxicity: If CTC grade 3 or 4 peripheral neurotoxicity occurs (intolerable paresthesia, marked motor loss, paralysis) discontinue vincristine without replacement.

XI.4 Cumulative drug doses

Projected cumulative drug doses (VIDE x 6 + VAI x 1 + Consolidation) are:

VIDE x 6 plus VAI x 1 plus:	VAI x 7	VAC x 7	Bu-Mel	
Cumulative ACT dose:	12	12	1.5	mg/m²
Cumulative Bu dose:			600	mg/m²
Cumulative CYC dose:		10 500		mg/m²
Cumulative DOX dose:	360	360	360	mg/m²
Cumulative ETO dose:	2 700	2 700	2 700	mg/m²
Cumulative IFO dose:	102 000	60 000	60 000	mg/m²
Cumulative Mel dose:			140	mg/m²
Cumulative VCR dose:	21	21	10.5	mg/m²

XII Supportive Care

All treatment described here, especially VIDE, is intense and aggressive and will be followed by severe bone marrow depression. Hence, treatment according to this protocol should be restricted to

institutions who are familiar with the administration of intensive aggressive combination chemotherapy and where the full range of supportive care is available.

XII.1 G-CSF

Since treatment intensity is essential in the treatment of Ewing tumours, G-CSF support is preferable to dose reduction. During VIDE induction chemotherapy a preemptive dose may be justified. The recommended dose is 5µg/kg/d as once daily s.c. injection. With CD 34 stem cell harvesting the recommended dose is 10µg/kg/d, once daily s.c..

XII.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 hypertonia.

XII.2 Antiemetic therapy

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

XII.3 Blood component therapy

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) should 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 clinical evidence of bleeding.

XII.4 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.)

XII.5 Infection prophylaxis

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

XII.6 Treatment of infections

EURO-E.W.I.N.G. 99 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.

XII.7 Psycho-social support

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. In case of paediatric cancer patients, siblings 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 both aspects of patient care.

Well trained personnel must be permanently 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
- Paediatric nurse
- Social worker
- School teacher
- Nursery school / kindergarten teacher

- Art / music teacher / therapist

Psycho-social support should encompass:

- 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.
- Building stable relationships between patient, family, and support team members
- Crisis intervention (e.g. in case of non-coping, non-compliance, etc.)
- Support with emotional aspects: coping with disease and therapy
- Play, artwork, music, ...
- Visits to the patient's home
- Psychological and social follow up (coping 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.

XIII Toxicity Monitoring

Evaluation of non-lethal toxicity is based on CTC -scored reported toxicity of all patients. The evaluation includes myelosuppression, rate of infections, capillary leak syndrome, VOD of the liver, mucosal damage, cardiac, pulmonary, renal, hepatic, central and peripheral nervous toxicity as described in the toxicity report form enclosed in the appendix. Additional investigations, e.g. lung function tests, are required in subgroups of patients, e.g. following pulmonary i

rradiation and high-dose therapy regimens.

Any serious and/or unexpected adverse event/reaction must be dealt with according to the appropriate ICH/EU guidelines. Any such event, e.g. death during treatment or unexpected life-threatening toxicity, must be reported according to SAE reporting guidelines immediately, see specific guidelines in Safety Evaluation section (XX.6).

XIII.1 Treatment stopping rules for individual patients

If any (non-lethal) adverse event as outlined above occurs in a patient, his or her further treatment according to the study protocol must be discussed with the national study centre immediately. It has to be considered how dangerous continuation of protocol therapy might be, and if there is an alternative treatment option available in the particular situation, or if treatment must be discontinued. As consequences from either decision may be deleterious to the patient, immediate discussion of the event is mandatory.

XIII.2 Routine tests during chemotherapy

Between chemotherapy cycles:

- FBC is recommended twice weekly.

Prior to next chemotherapy:

- FBC
- Serum biochemistry
- Serum and urine electrolytes including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphate
- Blood and urine glucose
- Serum alkaline phosphatase

ECG and ECHO/MUGA prior to the first course of doxorubicin, and prior to each consecutive course beyond a cumulative doxorubicin dose of 240 mg/m²BSA as a minimum requirement.

XIII.3 Renal function monitoring

XIII.3.1 Glomerular function - GFR

Serum creatinine should be monitored prior to each course of ifosfamide.

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

According to Schwartz's formula, creatinine clearance (C_{crea}) can be calculated from single serum samples: [68]

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

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

- Infants (< 1 year of age) **F** = 0.45
- Male, 1-16 years **F** = 0.55
- Female, 1-21 years **F** = 0.55
- Male, 16-21 years **F** = 0.70

Normal values [ml/min/1.73m²]:

- Infant (7 days): 45
- Infant (6 months): 60-80
- ≥ 1 year: 120

XIII.3.2 Tubular function (Tp/C_{crea} or Tm_p/GFR)

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.: [69]

Fractionated phosphate reabsorption:

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

Reference values in three age groups. "limit" refers to mean – 2 SD for T_P/C_{crea} [68]

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

XIII.3.3 Renal toxicity classification and ifosfamide dose modification

Ifosfamide:

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

Toxicity grade*	GFR (ml/min/1.73 m^2)	Tp/C_{crea} (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 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:

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

  → Reduce etoposide dose by 30% .

Ideally, GFR should be measured accurately (in addition to calculation of C_{crea}) before modifying ifosfamide or etoposide treatment.

XIII.4 Neurotoxicity monitoring (central + peripheral)

If CTC grade 3 or 4 central neurotoxicity occurs (somnolence >30% of the time, disorientation / hallucination / echolalia / perseveration / coma, or seizures on which consciousness is altered, or which are prolonged, repetitive, or difficult to control), consider the use of Methylene Blue (methylthionin) 50 mg as i.v. infusion. Prolong ifosfamide-infusion to 4-8 hours with the next application, and infuse Methylene Blue 50 mg three times daily. In the next course, apply Methylene Blue one dose of 50 mg 24 hours prior to ifosfamide. During ifosfamide infusion give three-times daily Methylene Blue infusions as described above (refer to *Nicolao and Giometto, Oncology 2003, 65[Suppl 12]:11-16* for further information). If repeated grade 3 or 4 central neurotoxicity occurs, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA. It is recommended to call your appropriate national coordinator for advice. When CYC replaces IFO for subsequent courses already during VIDE induction therapy, patients are ineligible for randomisation.

If CTC grade 3 or 4 peripheral neurotoxicity occurs (intolerable paresthesia, marked motor loss, paralysis) discontinue vincristine without replacement.

XIII.5 Doxorubicin (Adriamycin) cardiotoxicity monitoring

If temporary deterioration of myocardial function occurs, e.g. decrease in FS < 29%, LVEF < 40% (MUGA), or a decrease by an absolute value of 10 percentile points compared to previous tests, and if this is confirmed by a repeat investigation, omit doxorubicin in the next course. (If the decrease is not confirmed, i.e. if repeat investigations cannot reproduce the dysfunction, the omitted dose of DOX should be supplied instead of ACT with the first consolidation course.) If persistent deterioration of myocardial function occurs, e.g. persistent decrease in FS by an absolute value of 10 percentile points from previous tests or a persistent FS < 29%, avoid further doxorubicin. In doubt echocardiography

should be complemented by confirmatory tests, e.g. radionuclide cardiac cineangiography or myocardial biopsy.

XIV Stem Cell Harvest

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

Mobilisation should take place as early as possible in patients without bone marrow disease in order to achieve the optimum quality of progenitor cells. This should therefore preferably take place following course 2, 3 or 4 of VIDE chemotherapy. If the patient has bone marrow infiltration mobilisation should take place once the bone marrow is in remission.

$CD34^+$ cell counts are advised, to determine optimal timing for harvesting. A total of at least 6 x 10^6/kg $CD34^+$ cells should be collected providing for one transplant and one backup.[70-73] More than one collection may be necessary to collect sufficient cells. Continue G-CSF daily until and including the last day of the harvest.

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 study centre for further advice.

Stem cell harvest should be started 8-14 days after the first day of the VIDE course 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 (250-300µg/m²BSA/d) s.c. or i.v. is advised from 24 hours after the last dose of chemotherapy until completion of harvest.

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. See associated Molecular Biology studies in appendix A.3.

XV Evaluation and Classification of Clinical Response

XV.1 Methods of disease evaluation during treatment

The methods suggested below may be modified according to individual patients' needs, and additional important details (e.g. divergent response in different lesions, etc.) should be thoroughly documented and reported to the study centre.

XV.1.1 Re-imaging of primary tumour

Minimum requirements:

- MRI or CT scan with estimation of tumour volume following courses 2, 4, and 6 of VIDE, and at the end of therapy.

  Tumour volume is to be reassessed, and response of the total tumour volume to treatment will be expressed as percent reduction of total tumour volume. In patients with no soft tissue mass, this method is not applicable.

MRI scans should best be performed within 72 h before the commencement of the following course. This timing is recommended in order to avoid diagnostic errors from changes due to bone marrow depletion and atypical haematogenesis during chemotherapy.

XV.1.2 Skeletal system

Minimum requirements:

- MRI scans of known involved sites after course 2 and prior to local therapy, and at the end of therapy (and prior to high dose therapy, if applicable)
- 99mTc whole body bone scan at the end of therapy

Additional recommended investigations:

- 99mTc whole body bone scan before local therapy
- MRI/CT scans of (questionable) metastatic sites as indicated

XV.1.3 Bone marrow

Minimum requirements - all patients:

- At suspected relapse: aspirates from ≥ 2 sites, trephine biopsy from ≥ 1 site(s): conventional cytology/histology

Minimum requirements - patients with initial bone marrow involvement:

- Prior to stem cell harvest: aspirates from ≥ 2 sites: conventional cytology/histology

Additional recommended investigations – all patients:

- At local therapy: aspirates from ≥ 2 sites, trephine biopsy from one site: conventional cytology/histology

– RT-PCR for tumour specific chromosome 22 rearrangement at the time points outlined above, see Molecular Biology studies (appendix A.3).

If a patient is not entered into the molecular genetics evaluation protocol, repeat taps are encouraged only in patients with initial positive findings, according to the same time schedule.

XV.1.4 Lung/pleura

In patients with initial metastases:

- Chest CT scan after course 2, before local therapy, end of therapy

The reduction in size and/or number of pulmonary nodules is to be documented.

XV.2 Clinical response evaluation

By applying the methods of disease evaluation outlined above, clinical response will be evaluated as follows:

XV.2.1 Clinical response criteria

XV.2.1.1 Complete response (CR)

Complete disappearance of all visible disease for at least four weeks.

In patients with previous bone marrow involvement, bone marrow must be free of Ewing tumour cells at ≥ 2 sites (by light microscopy).

XV.2.1.2 Partial response (PR)

Solid tumour sites:	≥ 50% decrease, compared to baseline, of tumour volume(s) (soft tissue component; sum of all lesions) according to MRI (except: bone sites only)
Bone marrow:	free of disease (by light microscopy)
Lungs:	≥ 50% decrease, compared to baseline, of (number/size of) lesions according to CT

XV.2.1.3 Stable disease (SD)

Decrease of less than 50% or increase of less than 25%, compared to baseline, of tumour volume(s) for at least four weeks. Or persistent bone marrow involvement. No new lesions.

XV.2.1.4 Progressive disease (PD)

Any increase of more than 25%, compared to smallest measurement, in the sum of volumes of all measurable lesions. Or appearance of new lesion(s).

XV.2.2 Time schedule for clinical response evaluation

Clinical response will be evaluated in all patients:

- after course 2 of VIDE
- prior to local therapy, i.e. after course 6
- at the end of the protocol therapy,
- in addition, response will be evaluated before high dose therapy, where applicable.

XVI Surgery Guidelines and Classification of Histological Response

Definitive surgery is to follow primary chemotherapy. This rule must not be violated, unless emergency surgical procedures are mandatory at diagnosis, e.g. in case of spinal cord compression. In all cases, complete surgical removal is desirable.

Surgery of the primary tumour should be performed after course 6 of VIDE wherever possible in localised disease as well as in patients with pulmonary/pleural metastases.

Surgery is to be combined with additional radiotherapy following surgery in case of insufficient margins and/or poor histological response (≥ 10% viable tumour cells in the specimen). Post-operative radiotherapy should be applied during VAI or VAC consolidation therapy, i.e. during courses 8-12, or 6-8 weeks after stable engraftment following Bu-Mel high-dose therapy.

Wide resection should be attempted where possible (for definition, see "surgical margins" in the "disease evaluation" section above). "Debulking" manoeuvres are discouraged. The biopsy channel must completely be included in the surgical specimen.

Reconstructive surgical techniques should be applied wherever feasible.

Surgical guidelines on specific tumour sites are available through the study centres.

The surgical manoeuvre must be classified in cooperation by the surgeon and pathologist by definition of surgical margins, and of histopathological response, as outlined below (section XVI.1-2).

In case of residual lung disease after induction chemotherapy, surgical biopsies and/or removal of such lesions should be considered, consultation of the national study centre is advised.[74,75] Surgical removal of other metastases may not be possible or feasible in most cases.

XVI.1 Definition of surgical margins

All surgical procedures must be classified as defined below.[74,75]

- "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 not defined as a surgical procedure per se but is classified according to the surgical margin obtained, as outlined above.

Histopathological response must be evaluated as outlined below.

XVI.2 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:

No viable tumour cells	(Salzer-Kuntschik: grade 1)[75]
<1%	(Salzer-Kuntschik: grade 2)
<5%	
<10%	(Salzer-Kuntschik: grade 3)
<30%	

<50% (Salzer-Kuntschik: grade 4)

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

XVII Radiotherapy Guidelines

XVII.1 Introduction

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 post.

The indications for preoperative radiotherapy include clinical progression of tumour extension or anticipated marginal or intralesional resectability.

PLEASE NOTE: Patients with central axial tumours and early radiotherapy during VIDE induction therapy must NOT receive Bu-Mel high-dose therapy for anticipated toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

Postoperative radiotherapy is indicated in intralesional or marginal surgery (see below) and advised in poor histological response regardless of surgical margins.

Definitive radiotherapy is performed in inoperable lesions only. Inoperability is given in large tumours that cannot be completely resected and in tumours in critical sites where complete surgery would be mutilating or associated with a high risk of severe complications.

The study centres are available for guidance in radiotherapy planning.

XVII.2 Timing

After four courses of VIDE (as the last possible time point) all patients have to undergo local therapy planning. As routine procedure, surgery should be the first modality of local control to be applied. If after four VIDE courses the tumour is deemed unresectable, application of radiotherapy for local control should be considered. (In this case, randomisation procedures according to the specific protocol should immediately be performed, see above)

XVII.2.1 Preoperative radiotherapy

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

As an alternative approach especially in large central axial tumours, it should be discussed if radiotherapy can be withheld until after Bu-Mel high dose therapy (as this treatment option must not be applied following central axial field irradiation for reasons of toxicity).

PLEASE NOTE: Patients with central axial tumours and early radiotherapy during VIDE induction therapy must NOT receive Bu-Mel high-dose therapy for anticipated toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.2.2 Postoperative radiotherapy

Patients who are to receive postoperative irradiation are first continued on chemotherapy following surgery in order to allow recovery from surgery, wound healing and planning of radiotherapy. These patients will start radiotherapy following chemotherapy course VII if treated in the conventional VAI or VAC arms of the study. In patients where macroscopic or microscopic residual tumour is proven, postoperative irradiation may be started earlier.

In those patients who have received busulfan (Bu-Mel 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.

PLEASE NOTE: Patients with central axial tumours and early radiotherapy during VIDE induction therapy must NOT receive Bu-Mel high-dose therapy for anticipated toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.2.3 Definitive radiotherapy

The definitive radiotherapy is to start following course 6 of the induction regimen for patients in the conventional arms, or 8-10 weeks after stem cell reinfusion in patients of the Bu-Mel arm, as busulfan cannot safely be applied following irradiation of central axial fields.

PLEASE NOTE: Patients with central axial tumours and early radiotherapy during VIDE induction therapy must NOT receive Bu-Mel high-dose therapy for anticipated toxicity.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy..

XVII.3 Radiation dose

XVII.3.1 Preoperative radiotherapy

The standard target volume dose for preoperative irradiation is 54.4 Gy. In order not to compromise the dose intensity of initial preoperative chemotherapy, it is strongly advised to give preoperative irradiation in a hyperfractionated accelerated split-course scheme, without modifying chemotherapy drugs or dosages (exceptions, see above).

PLEASE NOTE: **In patients allocated to the Bu-Mel HDT consolidation arm, all radiotherapy of central axial sites should be postponed until 8-10 weeks after high-dose therapy, wherever possible. If early radiotherapy of central axial sites is mandatory, and the patient qualifies for the high-risk arm, the patient is ineligible for randomisation and is to continue on VAI or individually tailored high-dose treatment regimens, e.g. ME-ME.**

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.3.2 Postoperative radiotherapy

Doses for postoperative radiotherapy according to surgical margins:

Intralesional surgery	54.4 Gy
Marginal surgery with poor histological response (≥ 10% residual tumour cells)	54.4 Gy
Marginal surgery with good histological response (< 10% residual tumour cells)	44.8 Gy
Wide resection with poor histological response (≥ 10% residual tumour cells)	44.8 Gy according to national guidelines

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

Patients who are to receive postoperative irradiation are first continued on chemotherapy following surgery in order to allow recovery from surgery, wound healing and planning of radiotherapy. These patients will start radiotherapy following chemotherapy course 7 if treated in the conventional VAI or VAC arms of the study. In patients where macroscopic or microscopic residual tumour is proven, postoperative irradiation may be started earlier.

In those patients who have received busulfan (Bu-Mel 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 additive toxicity.

PLEASE NOTE that in patients allocated to the Bu-Mel HDT consolidation arm, all radiotherapy of central axial sites should be postponed until 8-10 weeks after high-dose treatment, wherever possible. If early radiotherapy of central axial sites is mandatory, and the patient qualifies for the high-risk arm, the patient is ineligible for randomisation and is to continue on VAI or individually tailored high-dose treatment regimens, e.g. ME-ME.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.3.3 Definitive radiotherapy

The compartment dose in definitive irradiation is 44.8 Gy with a tumour boost to at least 54.4 Gy. In individual cases, depending on the site of the tumour and the age of the patient, the boost may need to be increased (to a maximum of 64 Gy using a shrinking field technique), or reduced, which then should be discussed with the national radiotherapy reference centre. 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 to 48 Gy. These cases should be discussed with a radiotherapist experienced in the treatment of Ewing tumours in children (addresses see appendix A.1).

PLEASE NOTE that in patients allocated to the Bu-Mel HDT consolidation arm, all radiotherapy of central axial sites should be postponed until 8-10 weeks after HDT, wherever possible. If early radiotherapy of central axial sites is mandatory, and the patient qualifies for the high-risk arm, the patient is ineligible for randomisation and is to continue on VAI or individually tailored high-dose treatment regimens, e.g. ME-ME.

In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.4 Fractionation

Hyperfractionated accelerated split-course radiotherapy (1.6 Gy twice daily, at least 6 hours interfractionation interval, planned break of about 7-12 days after half of the total dose) is the preferred fractionation schedule, if normal tissue toxicity/tolerance allows this approach.

The most important theoretical advantage of this fractionation schedule is that it allows for 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 or in the planned radiotherapy break period.

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 case of severe acute reactions, omission of chemotherapeutic agents with radiosensitizing properties (actinomycin D, doxorubicin) during radiotherapy, or a break of radiotherapy should be discussed with the Study Centre / reference radiotherapist. Acute toxicity during radiotherapy has to be monitored carefully at least once weekly and is to be classified according to CTC guidelines.

Conventional fractionation (one daily fraction, five fractions per week, single dose 1.8 to 2 Gy, total dose as for hyperfractionated split-course irradiation) can be used as alternative fractionation schedule. **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 postoperative irradiation, ACT (given for consolidation chemotherapy to patients in the VAI and VAC arms) is omitted during the time of radiotherapy.

Interruptions of radiotherapy for more than one week are to be avoided. Acute toxicity during radiotherapy has to be monitored carefully at least once weekly and is to be classified according to CTC guidelines.

XVII.5 Irradiation techniques and target volume definition

(See also section XVII.9.)

Generally, radiotherapy is delivered as local radiation to the tumour extent at diagnosis with adequate safety margins. Areas of scars after biopsy or tumour resection are to be included in the radiation fields. For those patients who receive a dose of 54.8 Gy or more, a shrinking-field technique should be used, giving the boost irradiation to a smaller volume which is defined as the remaining tumour volume following induction chemotherapy plus a 2 cm safety margin. The scars after biopsy resp. after tumour resection are to be included in the radiation field. Reproducible positioning and appropriate immobilisation device will be used. Wedges and/or compensators will be used to produce homogeneous dose distributions as outlined in the reference doses.

For **tumours of the extremities** with an infiltration of muscle compartments, extended field radiotherapy with small volume boost is performed. The safety margins of the extended volume are 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 extent. In case of an 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 an adequate dose distribution. 44.8 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 applied. 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 informed. Radiation techniques with multiple fields will be used.

***PLEASE NOTE*: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.**

A **special target volume** concerns **pelvic or chest wall tumours** with non-infiltrating extension into pre-formed cavities, i.e. pelvis or chest. These tumours often show a large intrapelvic or intrathoracic mass which dramatically shrinks after chemotherapy. Irradiating the pre-treatment volume would mean that large volumes of normal tissue (bowel or bladder in the pelvis, lung in case of chest wall tumours) are in the radiation field. In these cases, the target volume in the areas of non-infiltrating tumour encompasses only the residual mass after chemotherapy at the beginning of radiotherapy and a 2 cm safety margin. For all other parts of the tumour (infiltrated muscle or bone), the above 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 in patients receiving busulfan must be avoided, 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 likely damage to the lung.

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

PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.6 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.

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

XVII.7 Chemotherapy during radiotherapy

For indications see protocols on localised/metastatic disease above!

ACT (given during VAI and VAC consolidation chemotherapy) is omitted during central axial irradiation.

During hyperfractionated accelerated split course radiotherapy, combination chemotherapy is to be continued as scheduled, and chemotherapy doses should be maintained. Special care has to be taken for monitoring acute toxicity during the first series of 22.4 Gy. If a patient undergoing radiotherapy during VIDE induction experiences severe skin or intestinal reactions following the first series of radiotherapy, DOX is to be reduced or omitted in the next series of radiotherapy.

The study centres / reference radiotherapists are available for guidance (addresses see appendix A.1).

PLEASE NOTE: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.8 Dose limits to normal tissue

The following dose limits to normal 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 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.

XVII.9 Technique of irradiation in special tumour sites

XVII.9.1 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 has to 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 in 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 with a safety margin of 2 cm. Immobilisation devices have to be used to immobilise the irradiated area reproducibly.

XVII.9.2 Pelvis

In pelvic tumours, three- to four-field techniques should be preferred to ensure an optimal dose distribution. In tumours of the iliac bone tangential opposing fields provide adequate dose distribution in most cases. To protect bladder and small bowel, individualised shielding may be necessary. Small volume boosts should be delivered if possible using four-field techniques.

Note: For target volume definition in case of non-infiltrating intrapelvic tumour masses with shrinkage after chemotherapy see section XVII.5 !

XVII.9.3 Vertebra

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

***PLEASE NOTE*: In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.**

XVII.9.4 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 Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.9.5 Chest wall

Chest wall tumours extending into the pleural cavity should be resected and are to receive postoperative irradiation where indicated (see section VII.3.2 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 (in patients < 14 years of age) or 20 Gy (in 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 44.8 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 54.4 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 in patients receiving busulfan must be avoided, as irradiation of lungs both prior to or after busulfan results to (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.

When irradiating the primary chest wall tumours 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.

Note: For target volume definition in case of non-infiltrating chest wall tumour masses with shrinkage after chemotherapy see section XVII.5!

PLEASE NOTE*: *In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.9.6 Whole lung irradiation

Note: There must not be 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 (for 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 Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.9.7 Radiation therapy for extrapulmonary metastases

Patients with metastases to extrapulmonary sites (i.e., most often 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 their protocol. All clinically detected sites should receive small volume irradiation with 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. 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 the high dose therapy. (The strategy of irradiation *after* stem cell infusion in these patients should be discussed with the national study centre.)

PLEASE NOTE*: *In patients receiving Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

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 Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

XVII.9.8 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.

XVII.10 Planning of radiotherapy

Radiotherapy reference centres are available for assistance in radiotherapy treatment planning, addresses see appendix A.1.

XVII.11 Side effects

Acute side effects may occur during radiotherapy in rapidly proliferating tissues such as skin and mucosa in the field of irradiation. These require symptomatic treatment and usually clear within 1-2 weeks following termination of radiotherapy. The irradiated skin should be kept dry using a non-perfumed powder, and mechanical irritation should be avoided. When extremities are irradiated, prophylactic physiotherapy should be initiated to avoid contractures. In patients receiving pelvic irradiation one should be aware of 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 Bu-Mel, radiation doses planned or administered involving spinal cord or brain must not exceed 30 Gy.

Side effects should be documented according to CTC scores and late effects according to RTOG/EORTC criteria, respectively.

The study centres or the reference radiotherapists are to be notified immediately about any severe (i.e. life-threatening or lethal) side effects and/or complications.

XVII.12 Radiotherapy follow-up

Irradiated patients have to be seen at least once a year following radiotherapy by the radiotherapist to record late sequelae. The results are to be documented and submitted to the study centre.

XVIII Follow-up Investigations

Long term follow-up of the study patients is mandatory for reasons of relapse and late sequelae of treatment. At the end of treatment, patients should have follow up examinations as follows:

1ST YEAR	2 monthly examination and CXR
2ND YEAR	3 monthly examination and CXR
3RD YEAR	4 monthly examination and CXR
4TH and 5TH YEAR	6 monthly examination and CXR

Ewing tumours may relapse as late as five to ten years after initial diagnosis.

In patients with radiotherapy only or incomplete surgery as local control of primary, it may be advisable to perform a MRI or CT scan at end of the treatment as baseline. Subsequent imaging is at the discretion of the clinician or as clinically indicated

CARDIAC FUNCTION

Echocardiogram and clinical review within 1-3 months of completing doxorubicin. If normal repeated at 2, 4 and 6 yearly from diagnosis, yearly if abnormal (record fractional shortening [LV-SF] and ejection fraction and any cardiac medication).

Radionuclide imaging maybe used particularly in adult practice but it is semi-invasive and involves radiation exposure.

RENAL EVALUATION

At end of treatment assess with GFR (by plasma radioisotope clearance method if possible). If abnormal may need repeating depending on local practice.

At least measurement of serum creatinine, electrolytes, Ca and PO_4 yearly is advisable.

Tubular function

- Measure – serum Na, K, Ca, Mg, PO_4 HCO_3 and alkaline phosphatase

  early morning urine for PO4, creatinine and osmolality

- Calculate TRP:- renal threshold for phosphate =

  {serum PO_4 – ([urine PO_4 x serum creatinine] ÷ urine creatinine)}

- if abnormal repeat plasma and urine samples yearly and calculate TRP
- record medication/supplements eg K+, PO_4, Vit D etc.

LUNG FUNCTION

At end of treatment for patients following lung radiotherapy and busulfan/melphalan

- assess at least clinically but also if possible with lung function including CO diffusion.

If abnormal repeat yearly.

If normal repeat 5 yearly and at the end of puberty.

GONADAL FUNCTION

To be assessed after completion of puberty or at least 1 year from the end of treatment in post pubertal patients

- **boys/men**

  testicular size
  testosterone
  LH
  FSH
  semen analysis (optional)

- **girls/women**

  menstrual history
  LH/FSH/oestradiol
  use of HRT and /or early menopause

for both sexes record pregnancy history, infertility treatment.

Inclusion of EURO-E.W.I.N.G. 99 patients into national/multicentre long term sequelae studies where available (e.g. GPOH Late Effects Surveillance System [LESS][76]) is recommended.

XIX Relapse

In case of progress of disease or relapse, the national study centre should be contacted immediately in order to individually plan further procedures. Patients may be eligible for phase II studies. For information, please contact your appropriate study centre.

XX Statistical Issues and Safety Evaluation

XX.1 Aims

XX.1.1 Total group

- To determine feasibility, safety, toxicity, and response of the VIDE induction regimen as a pilot study for all patients.

XX.1.1 Localised Ewing tumour – Randomisation R1

- To test for equivalence in terms of EFS between the two consolidation treatment arms VAI *versus* VAC.
- To test for equivalence in terms of OAS between the two consolidation treatment arms VAI *versus* VAC.
- To compare feasibility, safety, and toxicity between randomised consolidation regimens.

XX.1.2 Localised Ewing tumour – Randomisation R2$_{loc}$

- To test for improvement of EFS with the high dose chemotherapy (Bu-Mel or ME-ME), compared to conventional chemotherapy (VAI).
- To test for improvement of OAS with the high dose chemotherapy (Bu-Mel or ME-ME), compared to conventional chemotherapy (VAI).
- To compare feasibility, safety, and toxicity between randomised consolidation regimens.

XX.1.3 Ewing tumour with pulmonary/pleural metastases – Randomisation R2$_{pulm}$

- To estimate the difference in terms of EFS between VAI plus whole lung irradiation and Bu-Mel (without whole lung irradiation).
- To estimate the difference in terms of OAS between these randomised treatment strategies.
- To compare feasibility, safety, and toxicity between randomised consolidation regimens.

XX.2 Patient number estimates

Please note that for the figures given below, and for all of the following estimates of study accrual, a cautious assumption of the *minimum* case numbers was applied! Based on the EICESS 92 experience of CCLG (formerly UKCCSG) and GPOH, at least 160 patients per year are anticipated to be recruited in the UK, Germany, Austria, and the Netherlands. The number of patients in the French group (SFOP) is expected to be at least 45 per year. The participation of the SIAK and the EORTC-STBSG could contribute to achieve a total recruitment of at least 220 patients per year in Europe (randomised + non-randomised + lost to follow-up patients).

It is anticipated from previous studies that approximately 70% of all patients recruited will present with localised tumours (154 patients per year), and 30% of patients will present with primary metastatic disease (66 patients per year), respectively.

A rate of 25% non-randomised patients is expected, with an additional lost-to-follow up rate of 5%; hence, approximately 30% of patients are not expected to be included in the randomised question.

For the anticipated distribution of the randomised patients (70% of 220 per year = 154 per year) see **Table** below.

Table of case numbers in possible allocation/randomisation strata

	%		%*		%*		%*	Rand.	%
Localised	70%	Surgery	65%	< 10% viable cells	67%			R1	30%
				≥ 10% viable cells	33%			R2loc	15%
		early RAD + OP	20%	< 200 ml (and < 10 % viable cells)	67%			R1	9%
				≥ 200 ml (or ≥ 10% viable cells)	33%			R2loc	5%
		early RAD alone	15%	< 200 ml	67%			R1	7%
				≥ 200 ml axial	17%	early RAD	10%	none	<1%
						delayed RAD	40%	R2loc	3%
				≥ 200 ml extremity	16%	early RAD	10%	R2loc	<1%
						delayed RAD	40%	R2loc	3%
Metastatic	30%	Lung	50%	no progression	90%	early RAD	10%	-	1%
						delayed RAD	90%	R2pulm	12%
				progression	10%			-	2%
		Other	50%					-	15%
Total								R1	46%
								R2loc	24%
								R2pulm	12%
								none	18%

% : absolute percentage, % * : conditional percentage

R1 : Randomisation VAI / VAC
R2loc: Randomisation VAI / Bu-Mel for high risk localised patients
R2pulm: Randomisation VAI + LuRad / Bu-Mel for lung metastatic patients

Randomisation	%	N	N'	EFS
R1	46%	101 pts / y	72 pts / y	73% [64-81%]
R2loc	24%	53 pts / y	36 pts / y	54% [43-64%]
R2pulm	12 %	26 pts / y	19 pts / y	45% [27-62%]
none	18%	40 pts / y		

N : Expected number of patients eligible for randomisation if recruitment is 220/year
N' : Expected number of patients for analysis, subtracting non-randomised patients (25%) and patients lost to follow-up (5%).
EFS : 3-year EFS observed in the previous German EICESS 92 study.

XX.3 EFS estimates – experience in former studies

The 3-year EFS and corresponding 95%-CI of the different risk groups observed in the previous German, French, and British studies were as follows:

Germany:

		3-year EFS [95%CI] (cut-off date 5/99)
R1:	CESS 81 - EICESS 92:	74% [70-79%]
	EICESS 92:	73% [64-81%]
R2loc:	CESS 81 - EICESS 92:	50% [43-57%]
	EICESS 92:	54% [43-64%]
R2pulm:	CESS 81 - EICESS 92:	48% [34-62%]
	EICESS 92:	45% [27-62%]
R2loc+pulm:	CESS 81 - EICESS 92:	50% [43-56%]
	EICESS 92:	51% [42-60%]

(See figure 4)

France:

		3-year EFS [95%CI]
R1:	SURG±RAD:	80% [68-88%]
	RAD only:	71% [52-91%]
R2loc:	SURG±RAD:	36% [20-55%]
	RAD only:	23% [0-49%]

United Kingdom:

(EICESS 92)	3-year EFS [95%CI]
R1:	76% [50-90%]
R2loc:	44% [23-64%]
R2pulm:	22% [6-43%]

XX.4 Power considerations and sample size estimates

Calculation of sample sizes was based on the 3-year EFS rates and recruitment observed in the previous EICESS 92 study.

R1 randomisation: Assuming p_0=70% for the reference treatment, a one-sided test size of 5%, a power of 80% and a maximum allowable difference of Δ=10%, 548 patients are needed globally. This number may be reached after 7.6 years, assuming an anticipated number of patients of about 72 per year (randomised and not lost to follow-up) evaluable for the R1 question.

For p_0=75% with the same parameters 506 patients are necessary (7 years).

The estimations are based on the methodology described by Com-Nougue et al.. [77]

R2loc randomisation: The two-sided test size is set at α=5% and the power at 1-β=80%. In order to detect an increase in EFS for the new treatment to p_1=70% compared to p_0=55% for the reference treatment (relative risk of failure = 1/1.68, Δ=15%) the total number of patients to be considered for this randomised question would be approximately 328 (about 124 events are necessary), under the assumption of exponential survival. [78]

Based on the expected patient numbers (36 patients in R2loc per year randomised and not lost to follow up), a sufficient number of patients (N=328) can be expected to be recruited within 9 years.

R2pulm randomisation: For **randomisation R2pulm**, a difference of Δ=15% from approximately p_0=40% to p_1=55% would require 326 patients (188 events) in all. With an estimated accrual of 19 patients randomised (and not lost to follow up) per year, this question could be answered in 17 years.

As the questions of R2loc and R2pulm (advantage of high-dose therapy over conventional therapy), as well as the anticipated EFS rates (see above) are comparable, it is planned to perform a pooled analysis of both R2loc and R2pulm cohorts:

Pooled R2 population (randomisation R2loc and R2pulm): The 3-year EFS rates for this combined set of high risk patients from EICESS 92 was estimated to be approximately 50%. In order to detect an absolute difference of Δ=15% (i.e. an increase to 65% EFS), with a bilateral formulation of the logrank test, α=5% and the power 1-β=80%, about 340 patients (146 events) are necessary to be recruited in all (170 patients and 73 events per group).

Assuming that 36 patients with localised disease and 19 patients with primary lung metastases are randomised and not lost to follow up (see above) the necessary number may be reached after approximately 6 years.

Please note that a cautious assumption of the *minimum* case numbers was applied for the figures given above, hence the accrual time may well be shorter.

XX.5 Biometrical methodology

XX.5.1 End point and event definitions

Event free survival and overall survival probability will be estimated according to the method of Kaplan and Meier.[80]

- Event free survival time (**EFS**) starts at d 1 of the first VIDE treatment course (t_0) and ends at the 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.
- Conditional event free survival time (**EFS_c**) refers to randomised patients only and starts at the date of randomisation (t_0) and ends at the first event (definition see below: relapse of disease, progression 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.
- Overall survival time (**OAS**) starts at d 1 of the first VIDE treatment course (t_0) and ends at the 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.
- Conditional overall survival time (**OAS_c**) refers to randomised patients only and starts at the date of randomisation (t_0) and ends at the 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.

Endpoint definition for EFS and EFS_c (primary endpoint):

Event	Date of exit
Progression	Date of progression; if uncertain, date CT started
Relapse	Date of relapse
Secondary malignancy	Date of secondary malignancy
Death, whatever the cause	Date of death

minimum time = time from t_0 to first event (EFS, EFS_c),
where t_0 = d 1 of course 1 of VIDE for total group
t_0 = date of randomisation for group of randomised patients

Endpoint definition for OAS and OAS_c (secondary endpoint):

Event	Date of exit
Death	Date of death

minimum time = time from t_0 to death (OAS, OAS_c),
where t_0 = d 1 of course 1 of VIDE for total group,
t_0 = date of randomisation for group of randomised patients

Note: Patients lost to follow-up *before* their first event (i.e., no information for more than 2 years) are censored at the date of their last follow-up.

XX.5.2 Survival analyses: Intent to treat, according to protocol

All patients randomised in the trial will be analysed in their allocated treatment group, even if there has been non-compliance or protocol violation ("intent-to-treat" strategy). For certain questions, additional "per protocol" analyses are envisaged, e.g. for regimen-related toxicity.

No patient will be excluded from analyses once he or she is entered into the study, unless the consent to data processing is withdrawn.

Comparison between randomised arms: Comparisons are performed by logrank tests and Cox's proportional hazard model.[81,82]

Analyses of prognostic factors: Variables are univariately analysed regarding impact on EFS by lifetable analysis and logrank test, or by chi-square analyses, as appropriate.[80-82]

Multivariate analyses of variables with regard to EFS times are performed by Cox analysis.[82]

Analyses of specific failure risks, e.g. local failure versus systemic failure, are performed by means of competing risk analysis.[84]

XX.5.3 Interim analyses of EFS

The final analysis will be performed three years after the inclusion of the last patient.

Three interim analyses are planned after observing 25%, 50% and 75% of the number of expected events (i.e. 31, 62, 93 events for the R2loc randomisation). Stopping rules using the spending function approach of Lan and DeMets[85] with O'Brian-Fleming[85] type spending function will be followed to conclude at each sequential analysis. The boundary proposed in such rules requires very strong evidence of an effect to terminate at the first interim test (conservative boundaries early on), whereas the criteria at the final test are rather close to those for a single sample design (that is, a design with no interim testing).

Nominal p values for overall type I error of 0.05 Lan-DeMets boundaries are:

First analysis	0.00005
Second analysis	0.0042
Third analysis	0.0194
Final analysis	0.0430

If any of these boundaries are reached at the specified time point, patient recruitment will be stopped by the IDMC, the study chairmen will be informed, and a study committee meeting will be held to discuss further continuation/modification of the trial.

XX.6 Safety: Adverse Event Reporting

XX.6.1 Definitions

Adverse Event

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 unfavorable 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.

Adverse Reaction (AR) of an investigational medicinal product (IMP):

An adverse reaction (AR) is an untoward and unintended response to an IMP which is **RELATED** to any dose administered. All adverse events judged by the reporting investigator as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The evidence of reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected Adverse Reaction (UAR)

An ‘unexpected adverse reaction’ (UAR) is an AR, the nature or severity of which is not consistent with the applicable Summary of Product Characteristics (Product Information).

Examples of UAR:

- an expected /labelled SAR with an unexpected more severe outcome (e.g. a fatal outcome),
- an increase in the rate of occurrence of an expected, serious AR is considered as unexpected.

Serious Adverse Event (SAE) or Reaction (SAR)

A serious adverse event or serious adverse reaction is:

- any untoward medical occurrence or effect that at any dose results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect

Serious Unexpected Suspected Adverse Reaction (SUSAR)

A serious adverse event where a causal relationship to the IMP cannot be excluded is a suspected SAR and when the nature or severity is not consistent with the Product Information it constitutes a serious unexpected suspected adverse reaction (SUSAR).

XX.6.2 SAE requiring immediate reporting on SAE Form 15

In compliance with EU-Directive 2001/20/EC and ICH Guideline for Good Clinical Practice (GCP) (in USA Topic E6 and E2B) SAE which require immediate reporting on SAE Form 15 must be identified by the protocol and include

- All SAE or SAR which result in death

Death is an OUTCOME of a SAE and must be reported together with the cause of death to the group trial office on SAE Form 15. If an autopsy report is available it should be forwarded to the group trial office as soon as possible. **Death due to progression** of disease is not an SAE and should be documented on Form 17 and Form 18 only.

- All SAE or SAR which are life-threatening

The term **"life-threatening"** refers to an event in which the patient was at immediate risk of death at the time of the event i.e. required immediate intervention with life-saving intensive care treatment.

- All SAE or SAR requiring unanticipated hospitalization
- or unanticipated prolongation of an existing hospitalization,

Hospitalization is defined as at least one overnight admission. Only hospitalization which are considered unanticipated (clinically unexpected) by the physician require immediate reporting on SAE Form 15 (see below for exemptions).

- All SAE or SAR 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 resulting from tumour surgery e.g. following amputation or limb salvage surgery does not constitute a SAE.

- A congenital anomaly or birth defect is reportable as a SAE or SAR

Pregnancies and their outcome should be reported as SAE in order to identify and follow-up on any abnormalites. (Births from fathers under chemotherapy are also reportable on SAE Form 15).

- 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 CTC grade 4 require immediate reporting on SAE Form 15 e.g. nephrotoxicity (GFR $\leq$ 19ml/min/1.73 m^2) or cardiac toxicity (FS<28%, LVEF < 40%).

- other **medically important** conditions

Medical judgement should be exercised in deciding whether an adverse event / reaction is serious in other situations. Important adverse events/ reactions that are 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.

- secondary malignancies

Secondary malignancies e.g. skin cancer, myelodysplastic syndrome (MDS) usually occur later, but when they occur under protocol treatment, including within 30 days of the last day of protocol treatment, they should be reported immediately on SAE Form 15 as well as being documented on the Secondary Malignancy Form.

XX.6.3 EXEMPTIONS from immediate reporting of SAE to Group Trial Office

In compliance with EU-Directive 2001/20/EC and ICH Guideline for Good Clinical Practice (GCP) (in USA Topic E6 and E2B) SAE which do not require immediate reporting on SAE Form 15 must be identified by the protocol and are:

- Neutropenia and neutropenic fever
- Infections and fever

- Haematological toxicity (haemoglobin, WBC, granulocytes, platelets)
- Gut toxicity (mucositis / stomatitis, vomiting, diarrhoea)

These are expected events under protocol treatment, and if they resolve and do not require life saving intervention (life-threatening events) will be documented on the appropriate CRF only (see XX. 6.3).

N.B. The following are NOT SAE (no underlying AE) and do not require reporting as SAEs, but should be reported on the appropriate CRF:

- Signs and symptoms of progression of disease (Form 17)
- Death due to progression of disease (Form 17 and Form 18)

Hospitalization (for no underlying AE) does NOT constitute a SAE when

- hospitalization is for procedures required by the protocol e.g. chemotherapy, surgery, routine supportive treatment, biopsy or monitoring of the study,
- hospitalization is due to signs and symptoms associated with disease progression,
- elective hospitalization is for a pre-existing condition (i.e. a condition other than the indication for the chemotherapy) that has not worsened,
- admission is for rehabilitation in a Rehabilitation Centre or Hospice or admission is for social reasons (e.g. due to anxiety of the patient or parents but otherwise treatable on an outpatient basis).

For other serious adverse reactions (late effects of chemotherapy) or secondary malignancies following the study period please complete

- Late Effects Form 20
- Secondary Malignancy Form

XX.6.4 Documentation of Adverse Event (AE) and Serious Adverse Event (SAE)

An adverse event (AE) or a serious adverse event (SAE) can occur from the time that the subject signs the informed consent form to 30 days after last day the patient receives treatment according to EURO-E.W.I.N.G. 99 protocol (including drop-outs) regardless of whether the event is drug or protocol-related.

Adverse events are graded for severity according to a modified Common Toxicity Criteria (CTC)

version 2.0. This is not the same as assessment of "seriousness" to determine whether an AE is a SAE which is based on patient/event outcome or action taken criteria (see definition of SAE).

Adverse events and serious adverse events must be documented on the appropriate Case Report Forms (CRF) i.e. on Forms 6 (VIDE Chemotherapy), Form 10 (Consolidation Chemotherapy) Form 12 (High-dose Chemotherapy) and Form 13 (Radiotherapy), Form 17 (disease progression) and Form 18 (Follow-up / death) as appropriate. In addition certain serious adverse events (SAE) require immediate reporting to the appropriate group (GPOH, CRCTU, EORTC, SFOP, COG) trial office on SAE Form 15 and include all SAE meeting the criteria defined in XX 6.2

XX.6.5 SAE Reporting Procedures and Time Limits

The **investigator** must fax all SAE defined by the protocol in section XX 6.2: SAE requiring immediate reporting on SAE Form 15, to their group trial office within one business day. Patient`s demographic data must be pseudanonymised (Unique Patient Code Number).

SAE reports must be sent to the trial group specific trial office and Principal Investigator, who's name, phone and fax number, and email are outlined on the group-specific form 15.

The **investigator** is responsible for assessment of seriousness, severity (CTC v. 2.0) and relatedness of the SAE. The SAE Form should be completed with as much information as possible. The investigator should not wait for full details before making the initial report.

The **investigator** must fax any relevant follow-up information of any reported SAE immediately (less than 8 days) if the event is fatal, life threatening or as soon as possible if the SAE is prolonged to their Group Trial Office.

Follow-up information includes:

- the specific condition (diagnosis if possible) or event (presenting signs and symptoms) and severity (modified CTC Version 2)
- the date and time of occurrence
- causal relationship to investigational medicinal product
- concomitant (supportive) therapy (important to identify possible interactions)
- post-mortem reports (if applicable)

- other relevant information e.g. medical history, which could have played a role in the development of the SAE, family history
- additional laboratory tests required to investigate or follow-up SAE
- treatment of SAE; duration of SAE; outcome
- any action taken to the investigational medicinal product (de-challenge, re-challenge information)

The investigator must monitor the SAE until the condition resolves, stabilizes or its cause is identified.

All group trial offices (GPOH, CRCTU, EORTC, SFOP, COG) must forward all SAE which they received from their investigators to the

EWING INTERGOUP SAFETY DESK (E.I.S.D.)

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within 1 business day where the German Coordinating Principal Investigator or his delegate will review the SAE again for seriousness, relatedness and assess the SAR for expectedness according to the applicable Summary of Product Characteristics (Product Information). If the Coordinating Principal Investigator or his delegate disagrees with the assessment of seriousness and relatedness, this opinion will be added and will not replace the assessment of the investigator. The E.I.S.D. will determine whether the SAE falls into the category of serious unexpected suspected adverse reaction (SUSAR).

The **E.I.S.D.** will fax all SAE with an assessment of seriousness, relatedness and expectedness within 3 business days to the Coordinating Principal Investigator of each participating country and the group trial offices.
The **E.I.S.D.** will ensure that the all other relevant safety information including the annual safety report is prepared and forwarded to the Coordinating Principal Investigator of each participating country and to the group trial offices.

The **Coordinating Principal Investigator of each country** ensures that the competent authorities (CA), main Ethics Committee (EC) and investigators participating within his/her own country are informed of all serious unexpected suspected adverse reaction (SUSAR) and all other relevant safety information in accordance with definitions and time limits set by the EU-Directive 2001/20/EC as implemented into National Laws (in USA ICH GCP Topic E6 and E2B):

- All relevant information about SUSAR that are fatal or life-threatening must be reported as soon as possible to the CA, main EC and to all investigators involved in the clinical trial and in any case no later than seven days after knowledge by the group trial office of such a case. Relevant follow-up information is subsequently communicated within an additional eight days.
- All other SUSAR shall be reported to the CA, main EC and to all investigators as soon as possible but within a maximum of fifteen days of first knowledge by the group trial office.
- An annual safety report with a line listing of all suspected serious adverse reactions (SAR) including SUSAR, an aggregate summary tabulation of suspected SAR which occurred in the concerned trial and a report of the subjects' safety, must be forwarded to the CA and main EC.

In countries where EORTC also has patients, a written agreement between the Coordinating Principal Investigator of each country and EORTC should be obtained to clarify who is taking over the responsibility of informing competent authorities, Ethics Committee/-s and investigators of this country to avoid double notification of safety information.

XX.6.6 Stopping rules for the study protocol due to toxic deaths

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

All deaths related to treatment, such as toxic deaths, regardless of length of interval from treatment to death will be included and a description/detailed documentation of all toxicity is essential. Note that higher toxicity in high-dose chemotherapy must be expected 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; then a full analysis will 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; then a full analysis will 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 SFOP group), the limit percentage has been fixed 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 To conclude that $p_{obs} \geq 1\%$ with $\alpha = 10^{-3}$				High-dose chemotherapy 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.

XX.6.7 Stopping rules due to interim EFS analyses

See section XX.5.3.

XXI Organisational and Administrative Issues

XXI.1 Institutional commitment

All institutions participating in the study must declare their commitment to do so according to group guidelines. They must undertake to register all patients with Ewing tumour during the period of the study.

XXI.2 Study period

Patient enrolment will start on 1st September 1999. The study period is expected to extend over 6 years, depending on the actual patient enrolment rate.

XXI.3 Protocol organisation

One common international protocol will be used by all co-operative groups. This finalised master protocol in the English language will be held at the co-ordination centre. The national/group study centres may provide translations of the English master protocol.

Each co-operative group study centre will be responsible for distribution of protocols to institutions within their group.

Addenda may be added independently by any groups to address local needs, provided they have no bearing on the essential aims of the international protocol.

Subsequent to finalisation, any amendments to the protocol must be agreed by all co-operative groups. The co-ordination centre of the protocol will issue a revised version of the protocol if and when required.

XXI.4 Study forms

One common set of forms will be used by all the co-operative groups. The English language master version of the study forms will be held at the co-ordination centre, translations are within the responsibility of the national study centres.

The study centre of each co-operative group will be responsible for distribution of forms to institutions within that co-operative group.

Additional forms may be produced independently by any co-operative group for the collection of data additional to that required for the international study.

Subsequent to finalisation, amendments to the forms must be agreed by all the co-operative groups. The co-ordination centre will be responsible for issuing amended forms.

XXI.5 Documentation and data handling

After a patient and/or his/her guardian has given consent to data handling, he/she may be registered to Euro-EWING 99. For this procedure, and for all other documentation, the forms supplied in the appendix of this protocol must be completed and forwarded to the national Study Centre. All forms must indicate the institution, name and signature of the physician responsible.

Each co-operative group shall hold the database for its own patients, and shall be responsible for data quality according to local practice.

The content of the database shall be identical to the data collected on the study core data forms.

It was decided that the master database for the entire study will be held at the Intergroup Data Centre in Leicester since this is the only centre within the study not linked to a trial office.

A complete data set will be transferred by computer disk at least 3-monthly to the master database at the Intergroup Data Centre.

Forms returned from the treating institutions shall be stored at the co-operative data centres.

XXI.6 Confidentiality of patient data

The use of names as patient identifiers on paper forms and in each database will be according to national practice. An abbreviated patient identifier will be used for data transfer and for the master database.

National and European legal rules concerning data handling will be observed.

XXI.7 Data quality control

On receipt of forms at each data centre, common range and logical checks, agreed by the co-operative groups, will be carried out on data prior to transfer to the master database. Any amendment to the checking programs shall require mutual agreement of all the groups.

Cross checks of data entry will be carried out occasionally, between group data centres, on a sample of forms.

Data amendments shall only be carried out at the group data centres on their database. Errors noted on the master database, after receipt of the group database, shall be reported back to the centre of origin.

XXI.8 Data analysis and monitoring

Reports on the intergroup data base will be prepared twice yearly, describing accrual of the patients, group allocation, local therapy modalities and toxicity. These reports will be circulated to the principal investigators.

Results of the interim analysis of outcome and toxicity shall be reported to an international Independent Data Monitoring Committee (IDMC) as scheduled by protocol. The IDMC may recommend early stopping, continuation or extension of the study to the international study committee.

The international study committee shall meet as appropriate to consider patient accrual, eligibility, treatment, and outcome to ensure the smooth conduct of the study.

XXI.9 Documentation of adverse events

(See section XX.6.1 Adverse events in terms of the protocol)

***Any* life-threatening event must be reported immediately, i.e. within the next working day, and followed up by the treating institution, regardless of whether or not it falls within the categories listed in section XX.6.1. The information must be relayed to the other data centres, for further reporting according to GCP guidelines.**

The toxicity criteria will be the same for all participating groups.

XXI.10 Independent Data Monitoring Committee (IDMC)

An independent Data Monitoring Committee composed of three or four international experts will monitor the progress of the study on ethical and scientific grounds, compliant with EORTC guidelines.[87]

The role of the IDMC will be:

- **To review accrual rate**
- **To be involved in all interim analyses**

  Three sequential analyses are planned. These interim analyses will remain confidential.

  On the basis of these analyses, the IDMC will recommend whether the study can continue or whether it must be amended or terminated prematurely.

- **To monitor toxicity**

  Every 6 months the statisticians for the trial will circulate a report to the members of the IDMC about toxicity. The IDMC will review these interim toxicity data although this is primarily the

responsibility of the study committee. This biannual procedure is to avoid problems of major toxicity.

- **To examine other trials**

  The IDMC will review reports of related studies performed by other groups or organisations to determine whether such information materially affects the aims or preliminary findings of the trial. The results of the trial VAIA / EVAIA of the EICESS92 will also be reviewed to discuss of the continuation of the VIDE chemotherapy as front line treatment.

- **Other**

  The IDMC will be asked to review any major modification to the study proposed by the study committee prior to its implementation.

XXI.11 Follow-up data

All registered patients shall be followed up by the co-operative group data centres during and after completion of treatment according to the study. This also refers to patients off study for reasons of e.g. toxicity.

XXI.12 Institutional/local ethical approval and patient's consent

Institutional/local ethical approval must follow national practice.

Accepted national procedures for patient consent as documented are to be used.

The trial protocols must be approved by the appropriate ethical committee. According to the Austrian and French policy, the vote of one ethical committee is valid for other participating institutions. Other countries require approval by the responsible ethical committees of all participating institutions prior to patient entry.

The patient's and/or parents' written consent to participate in the study must be obtained after a full explanation has been given of the treatment options including the conventional and generally accepted methods of treatment and the manner of treatment allocation. If the patient is a minor, the treatment must be explained to and consent received from his/her guardian. Additionally the child should receive an explanation as to his/her means of understanding and should give consent as well, if he/she is able to do so. The right of a patient to refuse to participate without giving reasons must be respected. After the patient has entered the trial the physician must be free to give alternative treatment to that specified in the protocol at any stage if he/she feels it to be in the patient's best interest, but the reasons for doing so should be recorded, and the patient will need to remain in the study for the purpose of follow-up and

data analysis according to the treatment option to which he/she had been allocated. Similarly, the patient must remain free to withdraw at any time from protocol treatment or to withdraw his/her data from the study without giving reasons and without prejudicing his/her further treatment. A statement of the MRC (Medical Research Council, UK) policy on ethical considerations in the clinical study of cancer therapy including the question of informed consent is available from MRC, 20 Park Crescent, London W1N4AL, UK

and may be used to give guidance to participating investigators and to accompany applications to local ethical committees. EORTC guidelines can be obtained from the EORTC[86] (or via internet address http://www.eortc.be/).

All patients and/or parents must give written consent to inclusion in the trial, data processing and if applicable sending diagnostic material to reference institutions, which in all participating countries has to conform to the national data protection legislation.

This study will observe the rules for clinical research set out in the declaration of Helsinki in its latest form (Hong Kong, 1989),[87] the WHO and EC rules of "Good Clinical Practice" (GCP),[88] and the involved countries' laws.

XXI.13 Publication policy

Data relating to EURO-E.W.I.N.G. 99 must not be reported or published without prior consultation with the study chairmen. Any publication arising from the trials will have as its authors those who have produced the paper and acknowledgement to the intergroup members, e.g.

T. Blair, L. Jospin, G. Schröder, on behalf of Euro-EWING.

A final report of EURO-E.W.I.N.G. 99 will be provided within 5 years after the completion of the projected patient accrual. When this report will be published, it will be anonymously authored by "The EURO-E.W.I.N.G. 99 Group", and contributors will be listed with their individual contribution in an appendix.

XXII Associated Research

The EURO-E.W.I.N.G. 99 study is associated with additional research, e.g. in molecular biology. Participation in these projects is encouraged, further information is given in the appendix or is available from the study centres.

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Appendix A - International

A.1 Addresses
A.2 Pathology guidelines
A.3 Molecular biological studies
A.4 Alternative high-dose therapy in case of busulfan inapplicability: ME-ME
A.5 Experimental phase I/II studies

Appendix B - United Kingdom

B.1 Chemotherapy standardisation committee recommendations
B.2 Peripheral Blood Stem Cell mobilisation and collection
B.3 Amgen letter and faxback form
B.4 MREC committee approval – SEPARATE DOCUMENT
B.5 Biological Study
B.6 Patient's/parents information sheets and consent forms

Appendix C - FORMS, SEPARATE DOCUMENT

Appendix A - International

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APPENDIX A.2 - PATHOLOGY GUIDELINES

Procedures at biopsy

The diagnosis has to be histologically confirmed in every patient. Either open biopsy or needle core biopsy of the primary tumour must be performed in order to obtain sufficient material for histological diagnosis and concomitant studies outlined below.

The later inclusion of the biopsy channel/scar into the final local treatment must be considered.

The fresh surgical specimens should be sent rapidly to the local Department of Pathology. *Fresh tumour tissue* (deep frozen) must be saved for additional diagnostic investigations such as genetic analyses (molecular cytogenetics, FISH, CGH, electron microscopy). Imprints (touch preparations) should also be made and stored at –20° until further examination (e.g. cytology, FISH).

The diagnosis is based on the examination of routinely stained material supplemented with 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.

Immuno-histochemistry

Although CD99 (MIC-2 antigen) expression is not unique in Ewing tumours,

- CD 99 immunohistochemistry is obligatory in the diagnostic work-up of Ewing tumours, because >95% of Ewing tumours are positive.

(Note that a positive staining reaction has been reported in synovial sarcoma, myelosarcoma, precursor lymphoma, Burkitt's lymphoma, alveolar rhabdomyosarcoma, thymocytes in thymoma, and others.)

To distinguish Ewing’s Sarcoma versus atypical Ewing’s Sarcoma and peripheral neuroectodermal tumour (PNET) an obligatory imunohistochemical examination of neuronal expression has to be performed by means of at least the following antigens

- Synaptophysin
- S-100 protein
- NSE (neuron-specific enolase)

As useful markers for differential diagnosis within the group of small round cell tumours the following antigens can be used to identify myogenic, fibrogenic, and hematopoetic origin

- 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.

Additional diagnostic methods

- Molecular genetic analyses are strongly suggested for molecular tumour classification

Systematic investigations of Ewing tumours' molecular biological features are integrated part of the EURO-E.W.I.N.G. 99 concept. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) based detection of chromosomal transloactions, 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.

- Electron microscopy (optional)

A small sample should be immediately fixed in 2% glutaraldehyde.

Definitive diagnosis

The definitive diagnosis may be based on examination of routinely stained material
plus the obligatory immunohistochemistry panel as outlined above,
or plus two out of the following three investigations[75,90,91]:

a) molecular/cytogenetic analysis (chromosome 22 rearrangement)

b) CD 99 (Mic-2) positivity

c) electron microscopy

Each case should be signed by one of the EURO-E.W.I.N.G. 99 pathology board members. Special cases will be reviewed by the pathology board.

Names and addresses of the pathology board members are listed in appendix A.1, and are available from the national study centres.

(For definition of histological response at surgery following neoadjuvant treatment, see protocol section XVI)

APPENDIX A.3 - MOLECULAR BIOLOGICAL STUDIES

Background:

EWS gene rearrangements, involving the EWS gene and one of several ETS transcription factor genes (Fli1 85%, ERG 10%,<1% ETV1, E1AF, FEV), have been described in over 90% of tumours of the Ewing tumour family and are currently used to aid in the classification of these tumours. The gene rearrangement results in expression of a chimeric RNA product which varies in length dependent on the breakpoint in the genes. These encode aberrant transcription factors, which are thought to be involved in the pathogenesis of these tumours. The identification of these fusion products by reverse transcriptase polymerase chain reaction (RT-PCR) has been used for the differential diagnosis of Ewing family tumours. For patients with localised Ewing tumours two studies have suggested that specific EWS-ETS fusion types may be of prognostic significance.

RT-PCR for EWS-ETS fusion products is a sensitive and specific method for the identification of small numbers of circulating tumour cells, and may therefore be valuable to detect micrometastatic disease. Preliminary results suggest that in patients with localised Ewing tumour infiltration of bone marrow identified by RT-PCR may be of poor prognostic significance. Patients with bone metastases are almost always positive for bone marrow disease detected by RT-PCR and conventional cytological methods. Circulating tumour cells in peripheral blood has rarely been observed, and appeared not to correlate with extent of disease or with outcome in all studies performed so far.

Aims of this prospective, blinded, multicentre, quality controlled study:

- To evaluate the independent prognostic significance of individual *EWS-ETS* fusion types.
- To determine the prognostic impact of RT-PCR detectable tumour cells in the bone marrow of patients with Ewing tumours taken at diagnosis.
- To investigate the clinical significance of disease detected by RT-PCR in bone marrow samples taken at time of PBSC harvest and at suspected relapse.
- To define the frequency of RT-PCR detectable tumour cells in PBPC harvests and to evaluate their potential clinical significance.

Samples

Samples are requested from all newly diagnosed patients with Ewing tumours.

- *Fresh frozen tumour material* is requested from all patients at the time of diagnostic biopsy or primary tumour resection.

This material should be snap frozen in liquid nitrogen and stored in either liquid nitrogen or at –80°C until transported to the reference laboratory.

- *Bone marrow aspirates* (0.5-1ml samples into EDTA, from at least two sites excluding the involved side) to be taken at :-

  Time of diagnosis (essential)

  PBPC harvest

  Suspected relapse

- Two peripheral stem cell harvest samples (0.5-1ml into EDTA) to be taken at the time of leukapheresis.

Bone marrow and PBPC samples should be either

shipped immediately at room temperature to the reference centre to be received within 24h (Germany, Austria, Switzerland, France, Belgium, Netherlands)

or
placed into GT buffer (tubes will be provided to participating centres) and stored at –80°C until transported on dry ice to reference laboratory (UK).

Bone marrow and tumour pathology will be reviewed centrally.

Established reference laboratories:

- Austria

  Dr. H. Kovar, Dr. A. Zoubek
  E-mail: kovar@ccri.univie.ac.at; zoubek@ccri.univie.ac.at

- France

  Dr. O. Delattre
  E-mail: delattre@curie.fr

- Germany

  PD Dr. B. Dockhorn-Dworniczak,
  E-mail dwornib@uni-muenster.de

- Netherlands, Belgium

  Prof. Dr. Pancras C.W. Hogendoorn
  E-mail: P.C.W.Hogendoorn@pathology.MedFac.LeidenUniv.NL

- United Kingdom

  Ms S. Brownhill, Dr. S.A. Burchill
  ICRF Cancer Medicine Research Unit
  St James University Hospital
  Beckett Street
  LEEDS LS9 7TF
  United Kingdom
  Phone ++44 113 2065873 or ++44 113 2064922
  Fax ++44 113 2429886
  E-mail: S.A.Burchill@leeds.ac.uk

Details on transportation can be obtained from the reference laboratory.

Transport forms and information, consent forms and parent/patient information forms are included in the information appendix, **Appendix B5.**

APPENDIX A.4 - ALTERNATIVE HIGH-DOSE THERAPY IN CASE OF BUSULFANINAPPLICABILITY: ME-ME

In patients ineligible for Bu-Mel high-dose therapy a phase II study applying the ME-ME instead of the Bu-Mel strategy is available. This therapy is to follow 6 courses of VIDE induction and one course of VAI.

ME-ME consolidation

	D -7	D -6	D -5	D -4	D -3	D -2	D -1	D 0
Melphalan, 35 mg/m²/d i.v. infusion, 30 min.	x	x	x	x				
Etoposide phosphate, 60 mg/kg/d i.v. infusion, 4 h					x			
Stem cell re-infusion (min.3 x 10^6/kg CD 34^+)								x

NOTE: In case of contraindications for Bu-Mel, this high-dose therapy is compatible with early radiotherapy to central axial sites.

This alternative approach is co-ordinated by:

Prof. Dr. Stefan Burdach
E-mail: somagene@medizin.uni-halle.de

For full address see appendix A1

Patients treated with this approach will be analysed for the impact of aplasiogenic high-dose chemotherapy combined with involved compartment irradiation followed by myeloablative intensification on the prognosis of patients with extrapulmonary (with or without additional pulmonary) metastatic and early relapsed Ewing Tumours (relapse < 24 month from initial diagnosis). Multimodal therapy will be arrayed in three phases as (1) systemic induction, (2) involved compartment intensification and (3) systemic intensification.

1) Systemic induction treatment will be 6 courses of VIDE, with stem cell harvest. 2) Local therapy to all involved sites, i.e. primary tumour and all metastases is recommended. Involved compartment irradiation of all such sites performed with the last two conventional courses prior to HDT will usually require stem cell support of its own, and hence should be performed in institutions well experienced in these techniques. 3) A systemic intensification consolidation regimen of ME-ME high-dose therapy will then be applied.

Specifically the analysis will address four issues:

1. Can patients with high risk (extrapulmonary metastatic and early relapsed) Ewing tumours be cured of their malignancy ?
2. Does involved compartment irradiation improve results ?
3. Is long term monitoring by PCR of the EWS/Fli-1 transcript in the bone marrow predictive of relapse ?
4. Does tumour cell contamination of the grafts impact on the event free survival ?

The main measurable objective of the study is EFS. In addition, feasibility and toxicity of treatment and prediction of relapse by PCR of the EWS/Fli-1 transcript will be measured.

Patients will be entered via registration to EURO-E.W.I.N.G. 99 **and** to the ME-ME coordinator. A complete protocol will be available through the ME-ME coordinator.

APPENDIX A.5 - EXPERIMENTAL PHASE I/II STUDIES

As prognosis in group 3 Ewing tumour patients is expected to be very limited (<15% 5-year EFS), additional phase II studies are warranted. These may either be studies of "window" treatment in addition to the regular therapy (e.g. comparable to the treatment of the localised disease, with high-dose consolidation), or may be studies of treatment options replacing part of such a treatment. As patient numbers are only few, and treatment may be highly toxic, it was decided that enrolment of Ewing tumour patients into such studies should be performed via the national EURO-E.W.I.N.G. 99 study centres in close collaboration with the national phase II study centres. An update of active phase II studies will be available from your national study centre.

- For the NCRI Bone Clinical Study Group (formally known as CCLG- Children's Cancer and Leukaemia Group and UK-CCSG- United Kingdom Children's Cancer Study Group) Dr. Bruce Morland co-ordinates phase I/II studies in the field of paediatric oncology. UK participants should contact him via their EURO-E.W.I.N.G. 99 study centre for information on active studies.
- For GPOH group, Prof. Dr. Joachim Boos co-ordinates phase I/II studies in the field of paediatric oncology. German participants should contact him via their EURO-E.W.I.N.G. 99 study centre for information on active studies.
- For the SFOP group, Dr. Gilles Vassal co-ordinates phase I/II studies in the field of paediatric oncology. French participants should contact him via their EURO-E.W.I.N.G. 99 study centre for information on active studies.

For full addresses see appendix XXIV.1.

It is strongly recommended that each patient should be discussed with the national study centre in order to get access to active phase II studies.

Appendix B – United Kingdom

APPENDIX B.1 - UK CHEMOTHERAPY GUIDELINES

All patients should receive Cotrimoxazole prophylaxis as follows:

Surface Area	**Cotrimoxazole dose (given three times per week)**
0.5 - 0.75 m^2	240 mg bd
0.76 - 1.0 m^2	360 mg bd
> 1.0 m^2	480 mg bd

INDUCTION CHEMOTHERAPY – VIDE

FOR PATIENTS WITH A DOUBLE LUMEN LINE

Six course of VIDE are administered as induction chemotherapy at a minimum of 21 day intervals or when the neutrophil count is > 1 x 10^9/l and platelet count is > 80 x 10^9/l .

Glomerular Filtration rate should be measured prior to all courses. GFR must be > 60ml/min/1.73m^2

ECHO cardiography should be carried out prior to courses 1,3, 4, 5 and 6 or more frequently if cardiac problems arise. Fractional shortening must be > 28%

Day 1 T=0 hours Vincristine 1.5mg/m^2 slow IV bolus *Line A*

Maximum single dose 2mg

T=0 hours Etoposide 150mg/m^2 in 0.9% sodium chloride: *Line A*

</= 50mg in 150 ml NaCl

> 50 mg – 100mg in 250ml NaCl

> 100 mg – 200mg in 500ml NaCl

> 200 mg in 1000ml NaCl

Maximum concentration 0.4mg/ml

Infuse over 4 hours

T=4 hours Doxorubicin 20 mg/m^2 in 50 ml 0.9% sodium chloride *Line A*

Infuse over 4 hours. **Start with mesna and prehydration but down separate lumens, indicate this clearly on prescription.**

T=4 hours Prehydration Dextrose 5%/Saline 0.45% with potassium chloride 10 mmol/500ml. Total volume: 500ml/m^2 *Line B*

Rate 125ml/m^2/hr for 4 hours**. Run with mesna down the same lumen,** indicate this clearly on the presciption.

T=4 hours Mesna 600 mg/m^2 in 50ml 0.9% sodium chloride. *Line B*

Infuse over 4 hours**. Run with prehydration down the same lumen**, indicate this clearly on the presciption.

T=8 hours Mesna 3600mg/m^2 in Dextrose 2.5%/Saline 0.45% 2000ml/m^2 *Line B*

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours. **Start with ifosfamide**, indicate this clearly on the presciption.

T=8 hours Ifosfamide 3000mg/m^2 in 0.9% sodium chloride: *Line A/B*

< 2000mg in 25ml NaCL

>/= 2000mg – 4000mg in 50ml NaCL

>/= 4000mg in 100ml NaCl

Infuse over 1 hour**. Run with mesna hydration**, indicate this clearly on the presciption.

Day 2 T=0 hours Etoposide 150mg/m^2 in 0.9% sodium chloride: *Line A*

</= 50mg in 150 ml NaCl

> 50 mg – 100mg in 250ml NaCl

> 100 mg – 200mg in 500ml NaCl

> 200 mg in 1000ml NaCl

Maximum concentration 0.4mg/ml

Infuse over 4 hours. Do not stop mesna hydration, indicate this clearly on the presciption.

T=4 hours Doxorubicin 20 mg/m^2 in 50 ml 0.9% sodium chloride *Line A*

Infuse over 4 hours. **Do not stop mesna hydration**, indicate this clearly on the presription.

T=8 hours Mesna 3600mg/m^2 in Dextrose 2.5%/Saline 0.45% 2000ml/m^2 *Line B*

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours. **Start with ifosfamide**, indicate this clearly on the presciption.

T=8 hours Ifosfamide 3000mg/m^2 in 0.9% sodium chloride: *Line A/B*

< 2000mg in 25ml NaCL

>/= 2000mg – 4000mg in 50ml NaCL

>/= 4000mg in 100ml NaCl

Infuse over 1 hour**. Run with mesna hydration**, indicate this clearly on the presciption.

Day 3 T=0 hours Etoposide 150mg/m^2 in 0.9% sodium chloride: *Line A*

</= 50mg in 150 ml NaCl

> 50 mg – 100mg in 250ml NaCl

> 100 mg – 200mg in 500ml NaCl

> 200 mg in 1000ml NaCl

Maximum concentration 0.4mg/ml

Infuse over 4 hours. Do not stop mesna hydration, indicate this clearly on the presciption.

T=4 hours Doxorubicin 20 mg/m^2 in 50 ml 0.9% sodium chloride *Line A*

Infuse over 4 hours. **Do not stop mesna hydration**, indicate this clearly on the presription.

T=8 hours Mesna 3600mg/m^2 in Dextrose 2.5%/Saline 0.45% 2000ml/m^2 *Line B*

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours. **Start with ifosfamide**, indicate this clearly on the presciption.

T=8 hours Ifosfamide 3000mg/m^2 in 0.9% sodium chloride: *Line A/B*

< 2000mg in 25ml NaCL

>/= 2000mg – 4000mg in 50ml NaCL

>/= 4000mg in 100ml NaCl

Infuse over 1 hour**. Run with mesna hydration**, indicate this clearly on the presciption.

VAI CONSOLIDATION

FOR PATIENTS WITH A DOUBLE LUMEN LINE

Eight courses of VAI are administered as consolidation chemotherapy at a minimum of 21 day intervals or when the neutrophil count is > 1 x 10^9/l and platelet count is > 80 x 10^9/l .

Glomerular Filtration rate should be measured prior to all courses. GFR must be > 60ml/min/1.73m^2

Day 1 T=0 hours Prehydration Dextrose 5%/Saline 0.45% 375ml/m^2 *Line A*

Plus potassium chloride 10mmol/500ml

Rate 125ml/m^2/hr for 3 hours. **Run with mesna**,

indicate this clearly on the prescription.

T=0 hours Mesna 450 mg/m^2 in 50ml 0.9% sodium chloride. *Line A*

Infuse over 3 hours. **Run with prehydration**, indicate this clearly on the presciption.

T=3 hours Vincristine 1.5mg/m^2 slow IV bolus *Line A*

Maximum single dose 2mg

T=3 hours Actinomycin 0.75mg/m^2 slow IV bolus *Line A*

T=3 hours Mesna 3600mg/m^2 in Dextrose 2.5%/Saline 0.45%

Volume 3000ml/m^2

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours**. Start with ifosfamide,** indicate this clearly on the presciption.

T=3 hours Ifosfamide 3000mg/m^2 in 0.9% sodium chloride: *Line A*

< 2000mg in 25ml NaCL

>/= 2000mg – 4000mg in 50ml NaCL

>/= 4000mg in 100ml NaCl

Infuse over 1 hour**. Run with mesna hydration**, indicate this clearly on the presciption.

Day 2 T=3 hours Actinomycin 0.75mg/m^2 slow IV bolus *Line A*

T=3 hours Mesna 3600mg/m^2 in Dextrose 2.5%/Saline 0.45% Volume 3000ml/m^2

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours**. Start with ifosfamide,** indicate this clearly on the presciption.

T=3 hours Ifosfamide 3000mg/m^2 in 0.9% sodium chloride: *Line A*

< 2000mg in 25ml NaCL

>/= 2000mg – 4000mg in 50ml NaCL

>/= 4000mg in 100ml NaCl

Infuse over 1 hour**. Run with mesna hydration**, indicate this clearly on the presciption.

VAC CONSOLIDATION

FOR PATIENTS WITH A DOUBLE LUMEN LINE

Seven courses of VAC are administered as consolidation chemotherapy at a minimum of 21 day intervals or when the neutrophil count is > 1 x 10^9/l and platelet count is > 80 x 10^9/l .

Day 1 T=0 hours Prehydration Dextrose 5%/Saline 0.45% *Line A*

Volume 375ml/m^2

Plus potassium chloride 10mmol/500ml

Rate 125ml/m^2/hr for 3 hours. **Run with mesna**,

indicate this clearly on the prescription.

T=0 hours Mesna 225 mg/m^2 in 50ml 0.9% sodium chloride. *Line A*

Infuse over 3 hours. **Run with prehydration**,

indicate this clearly on the presciption.

T=3 hours Vincristine 1.5mg/m^2 slow IV bolus *Line A*

Maximum single dose 2mg

T=3 hours Actinomycin 1.5mg/m^2 slow IV bolus *Line A*

T=3 hours Mesna 1800mg/m^2 in Dextrose 2.5%/Saline 0.45%

Volume 3000ml/m^2

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours**. Start with cycolphosphamide,** indicate this clearly on the presciption.

T=3 hours	Cyclophosphamide 1500mg/m^2 in 0.9% sodium chloride: </= 1000mg in 50ml NaCL >1000mg – 2000mg in 100ml NaCL >/= 2000mg – 3000mg in 150ml NaCl Infuse over 1 hour**.** **Run with mesna hydration**, indicate this clearly on the prescription.	*Line A*

BU-MEL CONSOLIDATION

Supportive Care

All blood products should be irradiated, leukodepleted and CMV negative

Day -7 Commence **Clonazepam** 25 mcg/kg/day orally in 2 divided doses

Continue until 48 hours after last dose of Busulfan

Commence **Allopurinol** 5 mg/kg/dose oral tds

Maximum daily dose 300 mg, stop Day +7

Day -6 Start **hydration** 2l/m2/day 2.5% Dextrose/0.45% Saline

plus potassium chloride 10mmol/500mls

Commence **Heparin** infusion (Heparin Sodium)

Loading dose = 75iu/kg bolus

If < 20kg: 10iu/kg/hr to maintain APTT at 1.2-1.5

If > 20kg: 200iu/hr to maintain APTT at 1.2-1.5

Continue Heparin until discharge or day 30 whichever earlier

Busulfan 37.5 mg/m^2 4 times per day

(= 150 mg/m^2/day)

Day -5 **Busulfan** 37.5 mg/m^2 4 times per day

(= 150 mg/m^2/day)

Day -4 **Busulfan** 37.5 mg/m^2 4 times per day

(= 150 mg/m^2/day)

Day -3 **Busulfan** 37.5 mg/m^2 4 times per day

(= 150 mg/m^2/day)

Day -2 Commence **pre-hydration** with 0.9% Saline at 125 ml/m^2/hour

Do not administer Melphalan until a good urine output has been achieved (minimum 4ml/kg/min). Minimum 4 hours prehydration

Melphalan 140mg/m2 IV infuse over 15 minutes alongside pre-hydration

Post-hydration Saline 0.9% 3000ml/m2

plus potassium chloride 10 mmol/500ml

Infuse over 24 hours

Ensure urine output >4ml/kg/hr for 2 hours post Melphalan

Day -1 Rest day

Day 0 **Stem cell** re-infusion

Monitor: **Daily weight and fluid balance**

Daily FBC and U&E

Coagulation **Baseline**

+ 4 hour from starting

Daily or 6 hours after change in dose

maximum heparin dose 5000 iu/day (initially)

NB: Do not irradiate the lungs or mediastinum prior to or after Busulfan.

IFOSFAMIDE

Dilution's specification

Ifosfamide is supplied in vials containing 1 g or 2 g.

Reconstitute in Water for Injections BP as follows:

1. 1 g vial add 12.5 ml Water for Injections BP
2. 2 g vial add 25 ml Water for Injections BP

Resultant solution of 8% (80 mg / ml) ifosfamide should not be injected directly into a peripheral vein. The solution may be infused in 5% glucose, glucose / saline, or saline

Stability

A large body of information is available on stability of ifosfamide and mesna in solution.

Ifosfamide is stable in glucose/saline solution with added potassium* and is physically and chemically compatible with mesna (1mg/ml) in these solutions at concentrations of 1 mg/ml for 24 hours at room temperature, and 72 hours at $4^{o}C$.

* **(Glucose 2.5%, sodium chloride 0.45% with potassium 20 mmol/l)**

Adverse Effects

Common

- Renal toxicity, particularly tubular dysfunction
- Myelosuppression
- Emesis
- Alopecia

Occasional

- Liver dysfunction
- Haemorrhagic cystitis if inadequate mesna prophylaxis

Rare

- Encephalopathy

Interactions

The concomitant use of ifosfamide with anticoagulants, especially warfarin, may result in an increased anti-coagulant effect.

Overdosage

The most serious consequences are haemorrhagic cystitis, and myelosuppression. If the overdose is recognised early, intravenous hydration and diuresis, together with mesna may be beneficial in ameliorating damage to the urinary tract. Methylene blue and diazepam have shown some activity in reversing ifosfamide encephalopathy

DOXORUBICIN

Dilution specification

Preparation

Doxorubicin supplied in :-

(i) Vials containing 10mg and 50mg freeze dried powder. Reconstitute with water for injection or sodium chloride 0.9% injection adding 5ml to the 10mg vial and 25ml to the 50mg vial to give a 2mg/ml solution

(ii) Vials containing 10mg and 50mg as a 2mg/ml solution in sodium chloride 0.9%

Dilution

Doxorubicin is compatible with sodium chloride 0.9% and dextrose 5%

Stability

Solutions should be protected from light during storage and administration unless the solution is freshly prepared and the concentration is greater than or equal to 0.5mg/ml. In addition Doxorubicin appears to be chemically stable in polypropylene, PVC, or EVA containers for at least 7 days, when refrigerated or stored at room temperature, protected from light, and diluted in the following: sodium chloride 0.9% at concentrations of 0.1mg/ml to 2mg/ml, dextrose 5% at concentrations of 0.1mg/ml to 1.25mg/ml. In addition, at least a 7 day expiry can be given to doxorubicin reconstituted with water for injection to a concentration of 2mg/ml, stored in polypropylene syringes at 4oC

Adverse effects

Common

- Nausea and Vomiting
- Myelosuppression
- Alopecia
- Mucositis
- Red urine
- Diarrhoea
- Severe tissue damage if extravasated

Occasional

- Increased bilirubin
- Cardiomyopathy

Rare

- Hepatocellular necrosis
- Hyperpigmentation of skin, mucous membranes, nails
- Anaphylaxis, chills, fever
- Renal damage
- Drowsiness
- Conjunctivitis

Interactions

Doxorubicin may interact with the following:-

Cardiac irradiation - increased cardiac damage

Barbiturates - increased doxorubicin elimination

Verapamil- increased doxorubicin serum levels, reversal of doxorubicin resistance, reduced absorption of verapamil

Propranolol- increased cardiotoxicity

Carbamazepine, phenytoin, sodium valproate- altered anticonvulsant serum levels

Warfarin- increased warfarin effect

Cimetidine, ranitidine- increased doxorubicin toxicity

Overdose

Doxorubicin overdosage can prove fatal. Manifestations of overdose may include acute myocardial degeneration, severe myelosuppression and delayed cardiac failure. There is no specific antidote. Symptomatic supportive measures should be implemented.

DACTINOMYCIN

Dilution specification

Reconstitute by adding 1.1 ml of water for injection without preservative to the vial. 1 ml of the solution will contain 500 micrograms of dactinomycin.

Can then be added to an infusion solution of 5% dextrose or sodium chloride 0.9%.

Dactinomycin is reported to be compatible with glass and PVC containers for infusion. No information is available on compatibility with syringes.

Stability

Lyophilised powder: store at less than 25^{o} C. The powder should be protected from light.

Reconstituted solution should be used immediately as no preservative is present. However the drug is relatively stable and can be stored at 2-6^{o} C for seven days. Following dilution in 0.9% sodium chloride for 5% glucose dactinomycin is stable for 24 hours at ambient temperature.

Adverse effects

Common

- Nausea and vomiting
- Myelosuppression
- Alopecia
- Mucositis
- Severe tissue damage in the event of extravasation

Occasional

- Liver function test abnormality
- Hepatomegaly
- Reactivation of radiation reactions, e.g. radiation mucositis up to months post irradiation

Rare

- Anaphylactoid reaction
- Radiation myelitis
- Acute veno-occlusive disease resulting in hepatorenal failure

CAUTION

Dactinomycin is a vesicant drug and great care must be taken to avoid extravasation.

Dose reduction may be necessary following radiotherapy or in the event of liver dysfunction.

Interactions

Increased risk of hepatotoxicity when administered with other hepatotoxic agents especially halogenated inhalation anaesthetics such as halothane.

Overdosage

Manifestations of overdosage have included nausea, vomiting, diarrhoea, stomatitis, gastro-intestinal ulceration, severe haemopoietic depression, electrolyte disturbances, convulsions, acute renal failure and death. There is no known antidote. Treatment should be supportive.

ETOPOSIDE

Dilution specification & stability

Intravenous

- Manufacturers recommend diluting to 0.25mg/ml, however a dilution of 0.4mg/ml is stable at room temperature for 96 hours (may precipitate if refrigerated).
- Only licensed in UK for administration in Normal Saline.
- Poor water solubility therefore formulated in polyethylene glycol solubilising agent which dissolves plastics.
- Use nylon filters + PVC bags or glass bottles

Adverse Effects

Common
- Alopecia
- Myelosuppression

Occasional
- Nausea/vomiting

Rare

- Anaphylactic reactions
- Fever
- Hypotensive reactions
- Headache
- Pruritus
- Pigmentation
- Mucositis
- Second tumours

Interactions

- No major interactions with the possible exception of warfarin, where etoposide may displace protein bound warfarin or alter it's metabolism, leading to increased prothrombin times
- For the related drug tenopiside and possibly for etoposide as well, co-administration of anticonvulsants (Phenytoin or Phenobarbitone) can also result in increase clearance

Overdosage

- Full supportive measures, including the use of growth factors should be considered.
- Dialysis and haemofiltration are not effective as etoposide is highly plasma protein bound

VINCRISTINE

Dilution specification

- Dextrose 5%, Sodium Chloride 0.9%
- Undiluted at 1 mg/ml but at this concentration there would be increased toxicity with extravasation, therefore can be administered at lower concentrations, e.g. 0.2 mg/ml.

Stability

- Solution 1 mg/ml - 2 years in vial at 2 to 8 ^{o}C
- Lyophilised powder - 3 years at 2 to 8 ^{o}C. Chemically stable for 30 days after reconstitution when stored at 2 - 8 ^{o}C.

Adverse effects

Common

- Alopecia
- Abdominal pain - cramps
- Pain in jaw, bones and joints
- Constipation

Occasional

- Peripheral neuropathy (loss of deep tendon reflexes)
- Autonomic neuropathy (paralytic ileus, urinary retention)

Rare

- Leucopenia, Thrombocytopenia, Anaemia
- Nausea and vomiting
- Raised LFTs (mild and transient)
- Convulsions
- Diplopia and Photophobia

Toxicity related to individual and cumulative dose of Vincristine

CAUTION

Vincristine is a highly vesicant drug, and great care must be taken to avoid extravasation.

DO NOT GIVE INTRATHECALLY

CYCLOPHOSPHAMIDE

Dilution specification

Cyclophosphamide is reconstituted with Water for Injections BP to produce a final concentration of 20 mg/ml. At this concentration, absorptive losses onto glass, PVC and polypropylene are thought to be negligible

Compatible with glucose 5%, Sodium chloride 0.9% and glucose/saline solutions

Stability

Cyclophosphamide appears to be chemically stable when stored at 4^{O}C. A large body of information exists on stability and compatibility's of cyclophosphamide in solution.

Adverse effects

Common

- Dose related nausea and vomiting
- Alopecia
- Chemical or haemorrhagic cystitis if administered without mesna or with inadequate hydration and micturition.

Occasional

- SIADH

Rare

Cardiotoxicity presenting as congestive cardiac failure, pericardial effusion and pericardial tamponade. Possible association with previous anthracycline therapy or mediastinal irradiation.

Interactions

Possible with previous or current exposure to hepatic enzyme inducing agents including phenytoin· Concurrent dexamethasone treatment may increase cyclophosphamide metabolism

Concurrent allopurinol administration may decrease cyclophosphamide metabolism·

Interactions

Vincristine plasma clearance can be reduced by nifedipine, cimetidine or ranitidine, and

increased by phenobarbitone. The clinical relevance of these interactions in not clear.

Overdose

Plasmapheresis and phenobarbitone have been reported to be of value in cases of systemic vincristine overdose.

BUSULFAN - HIGH DOSE

Dilution specification and stability

- Tablets should be stored at room temperature i.e. below 25^0c
- Keep dry

Adverse effects

Common

- Myelosuppression, myeloid series & stem cells particularly
- Nausea & vomiting
- Mucositis
- Amenorrhoea/sterility (male & female)

Occasional

- Seizures
- Skin hyper pigmentation
- Rashes
- Diarrhoea

Rare

- Adrenal insufficiency (reversible)
- Veno-occlusive disease of the liver in 5% children (20% adults)
- Pulmonary fibrosis (Busulfan lung)
- Secondary tumours
- Gynaecomastia

CAUTION

Adequate therapeutic levels of anti-convulsants, e.g. diazepam or clonazepam are recommended before high dose Busulfan administration

Interactions

- Phenytoin increases hepatic clearance of Busulfan
- Phenobarbitone increases hepatic clearance of Busulfan

Overdose

- Supportive measures as necessary
- Haematopoietic growth factors

MELPHALAN (HIGH DOSE INTRAVENOUS)

Dilution specifications and stability

- Store at room temperature away from direct sunlight.
- Reconstitute the 50mg freeze-dried vial using 10ml of the Solvent-Diluent provided. Shake vigorously to dissolve. The resulting solution contains 5mg in 1ml anhydrous melphalan.
- Compatible with plastic containers, administration seta and in line filters.
- Spontaneous hydrolysis is slower if diluted and given in N.saline

Adverse effects

Common

- Nausea and vomiting (severe and immediate)
- Profound myelosuppression
- Mucositis
- Alopecia
- Sterility (in boys)

Occasional

- Haemorrhagic diarrhoea
- Amenorrhoea
- Encephalopathy
- Hypersensitivity

Rare

- Veno-occlusive disease
- Pulmonary fibrosis/pneumonitis
- Secondary leukaemia
- Dermatitis

CAUTION

- Bone marrow reconstitution depends upon adequate autologous, allogenic marrow or stem cells.
- GI toxicity may be extreme.

Interactions

- Nalidixic acid has been reported to result in severe GI toxicity in children.

Overdose

- Full supportive measures.
- Melphalan is dialysable but rapidly and spontaneously breaks down into non toxic products.

APPENDIX B.2 - PERIPHERAL BLOOD STEM CELL MOBILISATION AND COLLECTION

MOBILISATION OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) FOLLOWING CHEMOTHERAPY WITH VIDE

GCSF CAN BE REPLACED ON THE BASIS INDICATED IN THE ACCOMPANYING LETTER, USING THE FORM PROVIDED

Mobilisation should take place as early as possible in patients without bone marrow disease in order to achieve the optimum quality of progenitor cells. This should therefore take place following course 3 or 4 of VIDE chemotherapy. If the patient has bone marrow infiltration mobilisation should take place once the bone marrow is in remission.

DAY 1-3 VIDE CHEMOTHERAPY

DAY 5-11 G-CSF 10mcg/kg/day s/c injection

DAY 9 Daily FBC from day 9 onwards

DAY 11-13 PBPC Harvest when WCC>$3x10^9$/l (predicted to be day 11-13)[1]

Collect minimum of $2x10^6$/kg CD34+ cells

(recommended >$3x10^6$ /kg CD34+ cells)

More than one collection may be necessary to collect sufficient cells

Continue G-CSF daily until and including the last day of the harvest

Ref 1 Picton SV et al. Sequential mobilisation of peripheral blood progenitor cells in patients with bone tumours: implications for haemopoietic support of intensive therapy. Med Pediatr Onc 1996; **27**: p238 (abstr)

APPENDIX B.3 - AMGEN LETTER AND FAXBACK FORM

Amgen Limited
240 Cambridge Science Park
Milton Road
Cambridge
CB4 4WD
Tel: +44 (0)1223 420305
Fax: +44 (0)1223 423049

21st January 2000

Professor Ian Lewis
St James's University Hospital
Beckett Street
Leeds LS9 7TF

Dear Professor Lewis

Ref: Euro E.W.I.N.G.

Following on from your discussion with Cath, I am writing to confirm that Amgen Ltd would be happy to provide 100% reimbursement to UKCCSG centres wishing to use Neupogen in the above protocol.

I understand that G-CSF reimbursement will be mentioned in the protocol and that the latter will be circulated to colleagues within UKCCSG. In order to facilitate Neupogen reimbursement, I enclose a fax back form, which you may wish to put in the protocol appendix. Once a patient has completed their course of VIDE or high dose procedure, the number of Neupogen doses used should be recorded on the form. This can then be forwarded to our customer services department who will distribute the relevant quantity to the hospital in question.

I should just clarify that the 100% Neupogen reimbursement applies to hospitals in the UK. Centres in Europe who wish to use Neupogen should contact their local Amgen office to discuss support.

I trust that the above arrangement will meet with your satisfaction and I look forward to hearing from you soon.

Kind regards

Yours sincerely

Jacqui Penny
Market Development Manager, Oncology/Haematology

c.c. Cath Af Uhr, Bonita Cho, Phil Ryan, Mark Sampson, Erika Condie
Enc.

Customer Services

To: Erika Condie

Company: Amgen Customer Services

Fax: 01223 423432

Cc: Jacqui Penny

Euro- E.W.I.N.G. 99

Patient identification (initials/no.)……..

Number of filgrastim doses administrered:

30MU Pre-filled Syringes……

30MU Vials……

48MU Pre-filled Syringes……

48MU Vials……

Clinician……

Department……

Fax Number (Clinician)……

Pharmacist……

Fax Number (Pharmacist)……...

Hospital Address……..

……

……

CSD/100

APPENDIX B.4 - MREC COMMITTEE APPROVAL

EURO-EWING 99

MREC COMMITTEE APPROVAL

DOCUMENTS SUPPLIED SEPARATELY

APPENDIX B.5 - BIOLOGICAL STUDY

EURO-EWING 99

BIOLOGICAL STUDY

CCLG Biological Study

Characterisation and detection of Ewing's sarcoma and pPNET using RT-PCR for EWS gene rearrangements

(97 BS 02)

Study Coordinators

Dr Sue Burchill

Dr Ian Lewis

ORIGINAL START DATE: 1st December 1997

PROTOCOL AMENDED: 5th January 1999

FINAL COPY: PLEASE DESTROY ALL PREVIOUS DRAFTS

Children's Cancer Trials Team
Cancer Research UK Clinical Trials Unit
School of Cancer Sciences
University of Birmingham
Edgbaston
Birmingham, B15 2TT
Tel: 0121 415 8578/ 8572
Fax: 0121 414 3700

(97 BS 02)

Molecular biological studies accompanying the Euro-EWING 99

Background:

EWS gene rearrangements, involving the EWS gene and one of several ETS transcription factor genes (FLI1 85%, ERG 10%, <1% ETV1, E1AF, FEV), have been described in over 90% of tumours of the Ewing's sarcoma family and are currently used to aid in the classification of these tumours. The gene rearrangements result in expression of chimeric RNA products which vary in length dependent on the breakpoints in the genes. The fusion genes encode aberrant transcription factors, which are thought to be involved in the pathogenesis of these tumours. The identification of the fusion products by reverse transcriptase polymerase chain reaction (RT-PCR) has been used for the differential diagnosis of tumours belonging to the Ewing's sarcoma family. For patients with localised disease, two studies have suggested that specific EWS-ETS fusion types may be of prognostic significance.

RT-PCR for EWS-ETS fusion products is a sensitive and specific method for the identification of small numbers of circulating tumour cells, and may therefore be valuable to detect micrometastatic disease. Preliminary results suggest that in patients with localised Ewing sarcomas, infiltration of bone marrow identified by RT-PCR may be a marker of poor prognosis. Patients with bone metastases are almost always positive for bone marrow disease detected by RT-PCR and conventional cytological methods. Circulating tumour cells in peripheral blood has rarely been observed, and appeared not to correlate with extent of disease or with outcomes in all studies performed so far.

Aims of this prospective, blinded, multicentre, quality controlled study:-

1) To evaluate the independent prognostic significance of individual EWS-ETS fusion types.
2) To determine the prognostic impact of tumour cells detected by RT-PCR in the bone marrow of patients with tumours of the Ewing's sarcoma family taken at diagnosis.
3) To investigate the clinical significance of disease detected by RT-PCR in bone marrow samples taken at time of PBSC harvest and at suspected relapse.
4) To define the frequency of RT-PCR detectable tumour cells in PBSC harvests and to evaluate their potential clinical significance.

Samples

Samples and completed sample information sheet (available from the reference laboratory) are requested from all newly diagnosed patients with tumours of the Ewing's sarcoma family.

- *Fresh frozen tumour material* is requested from all patients at the time of diagnosis or primary tumour resection (essential).

  This material should be snap frozen in liquid nitrogen and stored in either liquid nitrogen or at -80°C until transported to the reference laboratory.

- *Bone marrow aspirates* (1 ml samples into EDTA, from at least two sites) to be taken at:-

  i) time of diagnosis

  ii) PBSC harvest

  iii) suspected relapse

- Two *peripheral stem cell harvest samples* (1 ml into EDTA) to be taken at the time of leukapheresis.

Bone marrow and PBSC samples should be either:

- Shipped **immediately** at room temperature to the reference centre to be received within 24h (Austria, France, Germany, Switzerland).

or

- Placed into GT buffer (tubes will be provided to participating centres) and stored at -80°C until transported on dry ice to reference laboratory (UK).

Bone marrow and tumour pathology will be reviewed centrally.

Established reference laboratories:

Austria

Ms D Jugovic, Dr H Kovnar, Dr A Zoubeck
Children's Cancer Research Institute,
St Anna Kinderspital,
Kinderspitalgasse 6,
A-1090 Vienna,
Austria.
Tel: ++43 1 40470 Ext. 409 or 413
Fax: ++43 1 4087230

France

Dr O Delattre, Ms M Peter, Dr G Schleiermacher

Germany

Dr B Dockhorn-Dworniczak
Institut für Pathologie der Universität,
Domagkstrasse,
48129 Münster,
Germany.
Tel: ++49 251 8355449
Fax: ++49 251 8355481

United Kingdom

Ms S Brownhill, Dr SA Burchill

ICRF Cancer Medicine Research Unit,

St James University Hospital,

Beckett Street,

Leeds LS9 7TF

United Kingdom.

Tel: ++44 113 2065873 or ++44 113 2064922

Fax: ++44 113 2429886

Details on transportation can be obtained from the respective reference laboratory

Please send bone marrow, peripheral stem cell harvest and / or tumour samples on dry ice, with one completed form for each patient at the time of sampling:

Miss Sam Brownhill
Cancer Research Unit,
St James University Hospital,
Beckett Street,
LEEDS LS9 7TF

TEL:-0113 2064922 FAX:-0113 2429886

HOSPITAL NUMBER:-	CONTACT CLINICIAN/NURSE:-
CENTRE:-	DATE OF SAMPLE ____/____/____
IS THE PATIENT REGISTERED ON THE EICESS TRIAL? Yes ☐ No ☐	OTHER COMMENTS:-
IS THIS SAMPLE (please tick):- (i) Bone marrow ☐ (ii) Peripheral stem cell harvest ☐ (iii) Tumour ☐	

PLEASE ensure tubes are clearly and permanently labelled with centre, hospital number, date of sample and sample type.

Transport Request Form **EXAMPLE ONLY**

FAX ORDER TO: TNT supermail

FAX NO: 01274 651027

TEL NO: 01274 651222

PLEASE ARRANGE COLLECTION OF A PARCEL FROM:

[1]*Participating Centre:-*

Contact Name,

Address,

Tel No.

DATE OF COLLECTION: [1]Monday 6th Feb-98

TIME OF COLLECTION: 2 - 4 pm

TO BE DELIVERED BY NOON THE NEXT DAY TO:

MISS SAM BROWNHILL
ICRF CANCER MEDICINE RESEARCH UNIT
ST JAMES'S UNIVERSITY HOSPITAL
BECKETT STREET
LEEDS LS9 7TF

TEL: 0113 2064922

ORDER TO BE PUT ON ACCOUNT NO: 0786497

INVOICE TO BE SENT TO: MRS J HOLT, AT DELIVERY ADDRESS.

[1]To be completed by participating centre

Transport Request Form

FAX ORDER TO: TNT supermail

FAX NO: 01274 651027

TEL NO: 01274 651222

PLEASE ARRANGE COLLECTION OF A PARCEL FROM:

DATE OF COLLECTION:

TIME OF COLLECTION:

TO BE DELIVERED BY NOON THE NEXT DAY TO:

MISS SAM BROWNHILL
ICRF CANCER MEDICINE RESEARCH UNIT
ST JAMES'S UNIVERSITY HOSPITAL
BECKETT STREET
LEEDS LS9 7TF

TEL: 0113 2064922

ORDER TO BE PUT ON ACCOUNT NO: 0786497

INVOICE TO BE SENT TO: MRS J HOLT, AT DELIVERY ADDRESS.