

**Scandinavian Sarcoma Group and
Oncologic Center, Lund, Sweden**

SSG XX

**A Scandinavian Sarcoma Group
treatment protocol for adult patients with
non-metastatic high-risk soft tissue sarcoma
of the extremities and trunk wall**



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Figure 1. Study population

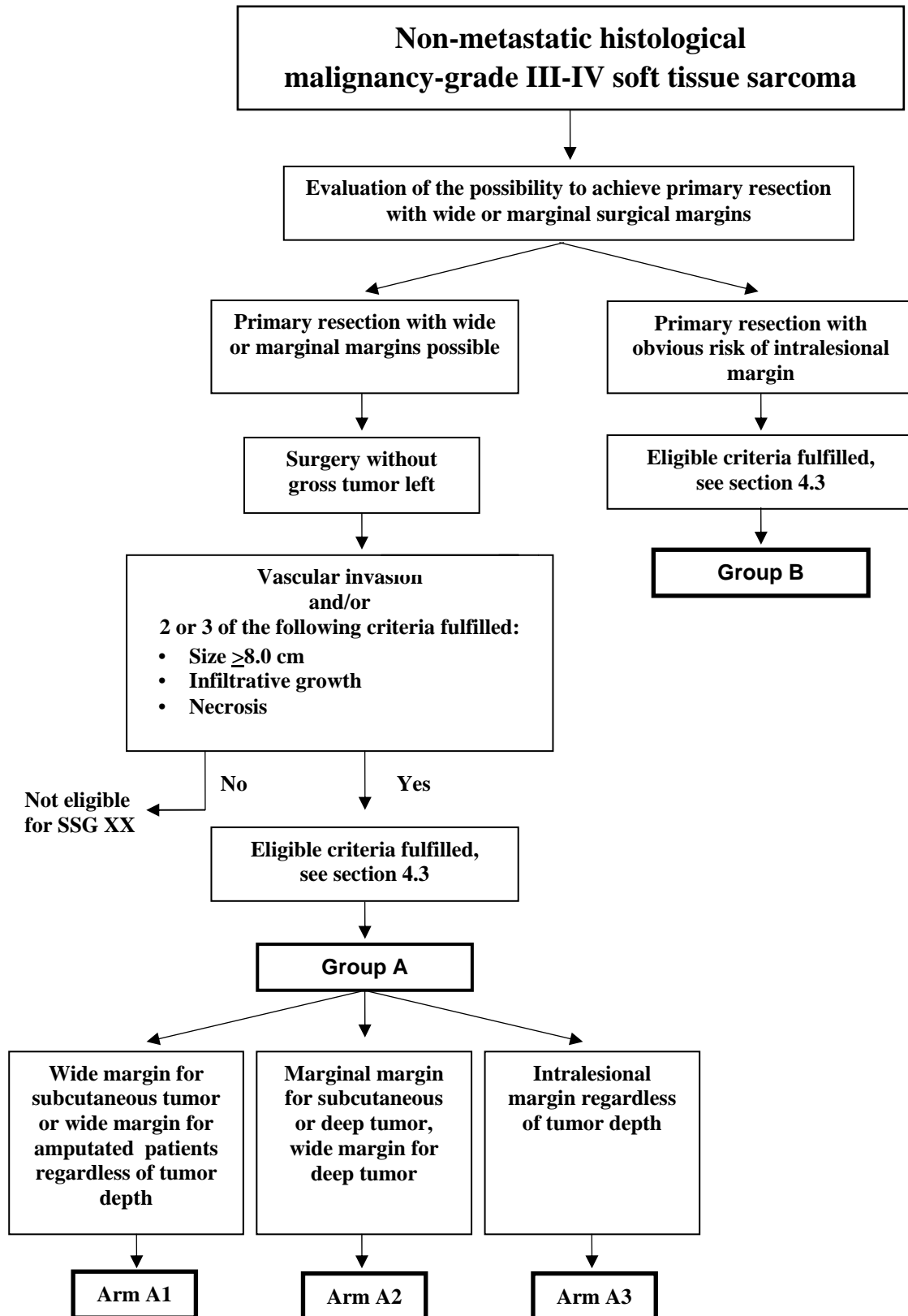


Figure 2a. Treatment schedule group A by arm 1, 2 and 3

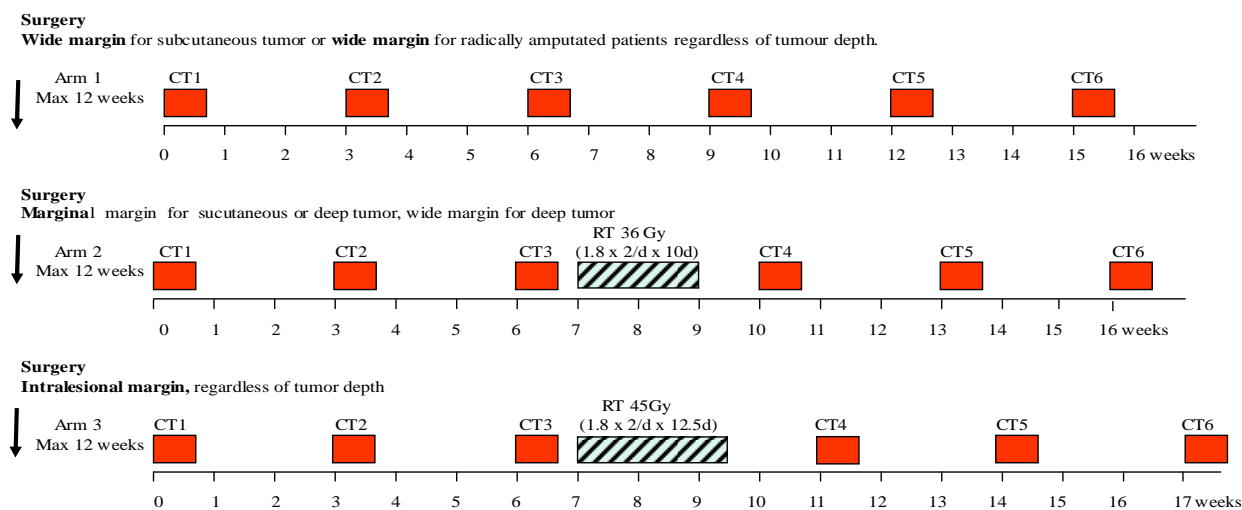
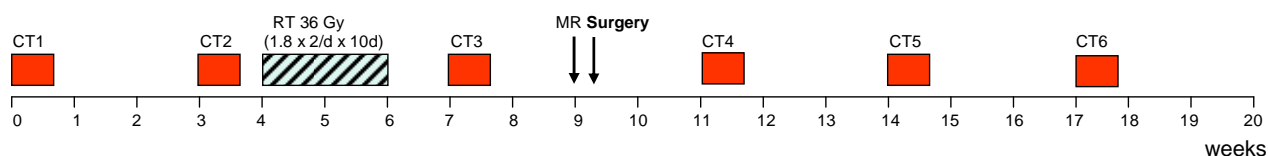


Figure 2b. Treatment schedule group B



Chemotherapy regimen

Patients <70 years of age: doxorubicin 60 mg/m² as 4-hour infusion and ifosfamide 6 g/m² (2 g/m² as 2-hour infusion, with Mesna, on 3 consecutive days). G-CSF routinely.

Patients ≥70 years of age: doxorubicin 50 mg/m² and ifosfamide 5 g/m². G-CSF routinely.

1. Preface

The Scandinavian countries (Sweden, Norway, Finland, Denmark and Iceland) have a total population of about 25 millions. The Scandinavian Sarcoma Group (SSG) was founded in 1979. The aim was to improve the prognosis for sarcoma patients in the Scandinavian countries. Uniform guidelines for diagnosis, pathology, and treatment are now used by all sarcoma centers in Scandinavia.

The first Scandinavian adjuvant chemotherapy study, carried out during 1981–86 (SSG I), was a randomized study. We reported that adjuvant doxorubicin had no effect on metastasis-free- and overall survival in patients with high-grade malignant soft tissue sarcomas (STS) of the extremities and trunk wall. SSG participated in a meta-analysis of the published results of 14 randomized clinical trials, where adjuvant chemotherapy improved metastasis-free survival and local tumor control in STS, with a trend toward better survival (Tierney et al 1995).

The second adjuvant STS protocol by SSG (SSG XIII), was a phase II non-randomized trial for a subgroup of patients with high risk to develop metastases. It was opened in 1998 and will be replaced by the present SSG XX. SSG XX is based on SSG XIII regarding use of prognostic markers to identify high risk tumors. In SSG XIII mainly postoperative radiotherapy and only postoperative chemotherapy was used. SSG XX includes a treatment group with preoperative chemo- and radiotherapy.

The following members have participated in the protocol design and comprise the SSG XX working group:

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2. Background and rationale

2.1 Chemotherapy and radiotherapy for soft tissue sarcoma

Doxorubicin and ifosfamide are active drugs in soft tissue sarcoma. In the first adjuvant trials, doxorubicin was used alone or in combination with other drugs. A meta-analysis of the 14 trials testing doxorubicin-based postoperative chemotherapy found statistically significantly reduced rates of local failures and distant metastasis, and improved disease-free survival, but only a trend toward better overall survival (Tierney et al 1995). Since the publication of the meta-analysis three additional randomized trials using more modern dose schedules have explored the benefit of doxorubicin- or ifosfamide-based chemotherapy (Brodowicz et al. 2000, Petrioli et al. 2002, Frustaci et al. 2001). The two former trials failed to demonstrate an improved survival. Frustaci et al. (2001) demonstrated a significant survival benefit at 4 years in patients treated with epirubicin and ifosfamide. Over time however, the benefit of chemotherapy in terms of overall survival rate has lost its statistical significance (Frustaci et al. 2003).

In a recently published review of STS patients treated at the M.D Anderson Cancer Center or the Memorial Sloan-Kettering Cancer Center a benefit for adjuvant doxorubicin-based chemotherapy was reported during the first year only (Cormier et al. 2004).

Both preoperative chemotherapy (Grobmyer et al. 2004) and radiotherapy are used at many centers as part of a treatment plan for patients with primary STS (Kraybill et al. 2006). Proponents for preoperative chemo-radiotherapy have suggested several potential benefits including an ability to assess response to a given chemotherapeutic regimen, earlier treatment of microscopic metastatic disease and facilitation of tumor removal. However, the value of preoperative treatment in improving survival of patients with STS remains unproven. O`Sullivan et al. (2002) randomized 94 patients to preoperative (50 Gy, 25 fractions) or postoperative (66 Gy, 33 fractions) radiotherapy and showed a slightly better overall survival in the former group after a median follow-up time of 3.3 years, but this benefit was lost after 5 years of follow-up (recurrence-free survival: 58% versus 59%) (O`Sullivan et al. 2004).

The value of radiotherapy in conjunction with surgery for local control of soft tissue sarcomas of the extremities and trunk wall has been demonstrated in multiple retrospective reviews (Lindberg et al. 1981, Suit and Spiro 1994, Trovik et al. 2001), but also in several prospective, randomized trials (Harrison et al. 1993, Pisters et al. 1996, Yang et al. 1998).

After wide and marginal surgery a dose of 50–60 Gy with daily 2 Gy fractions is generally recommended to achieve local control. For macroscopic residual tumor doses >60 Gy are recommended (Fein et al. 1995, Wolfson et al. 1998, Zagars and Ballo 2003a, Kepka et al. 2005).

The timing of radiotherapy preoperatively or postoperatively, varies between different studies. In the prospective, randomized trial by O`Sullivan et al. (2002) the timing did not affect local control. Zagars et al. (2003b) retrospectively compared disease outcome (local recurrence, metastases) in 271 patients receiving preoperative radiotherapy and 246 patients receiving postoperative radiotherapy, and found no significant differences. There was a slightly higher incidence of late radiation related complications for patients who had had postoperative radiotherapy. This finding was in accordance with the report on late radiation morbidity data of the patients in the randomized study by O`Sullivan et al. (2002). In a follow-up study the patients treated with postoperative radiotherapy tended to have greater fibrosis, joint stiffness and edema compared to those with preoperative radiotherapy (Davis et al. 2005). More

patients had wound complications in the preoperative radiotherapy group than in the postoperative group (O`Sullivan et al. 2002).

2.2 SSG XIII and rationale for SSG XX

SSG XIII was activated in 1998 and will be replaced by the present protocol. In SSG XIII adjuvant chemotherapy was administered to adult patients (≤ 70 y) with high-risk STS of the extremities and the trunk wall. The classification of a high-risk tumor was based on tumor size, vascular invasion and macroscopic and/or microscopic tumor necrosis, applying the SIN-system (Gustafson 1994, Gustafson et al. 2003). Inclusion criteria were presence of at least two of the prognostic factors: tumor size >8 cm, vascular invasion, or necrosis. Since the protocol was launched in 1998, 118 patients have been included. During the period 1998-2005 1074 primary, high-grade STS of the extremities and the trunk wall have been registered in the SSG Central Register. The identification of high risk patients eligible for adjuvant treatment may have been hampered by the difficulties of identifying vascular invasion in the SIN-system. Consistent with other investigators, we have in retrospective reviews found a considerable variation in the frequency with which this prognostic factor is identified. When present it is a strong risk factor for metastases, but a non-finding may not be informative. There is now data supporting also the prognostic importance of peripheral (pushing vs infiltrating) tumor growth pattern which provides independent prognostic information in addition to tumor size, necrosis, and vascular invasion (Engellau et al. 2005). Based on our experience with SSG XIII, and continuously improved knowledge of prognostic factors in STS, the SSG has decided to initiate a new protocol for high-risk STS with a modification of the current system of prognostication.

Based on the relative importance of the prognostic factors vascular invasion, size, necrosis, infiltrative growth pattern, and malignancy grade, we have designed a novel prognostic system for histologically high-grade malignant tumor with a good discrimination between groups with high respectively low risk for metastases (Engellau et al. 2007). Tumors with vascular invasion were thus shown to entail a high risk and following this selection, a further stratification into risk-groups is based on the presence of two of the three factors tumor size ≥ 8 cm, necrosis, and infiltrative tumor growth pattern.

In this new adjuvant protocol for high risk STS, SSG adopts an inclusion decision algorithm based on the following criteria: 1. vascular invasion and or 2. presence of at least two of the risk factors: size ≥ 8.0 cm, necrosis or infiltrative growth. The system was developed retrospectively by evaluation of 434 primary histologically high grade malignant STS and was later validated in a series of 175 patients in which patients with a high risk for metastases (>40 %) and low risk (<15 %) were separated, without an intermediate risk group remaining.

The preliminary survival data of SSG XIII are promising, and also toxicity seems moderate which have encouraged us to explore a modification of the SSG XIII protocol for adjuvant treatment.

An intention for the chemotherapy in SSG XIII was to individually adjust doses according to hematological toxicity. However, inconsistent dose modifications were used in SSG XIII, and in the current protocol predetermined doses will be given unless serious bone-marrow depression occurs. Six cycles of doxorubicin and ifosfamide will be given interpolated with hyperfractionated radiotherapy.

The current protocol includes a treatment group with preoperative chemo- and radiotherapy, for patients in whom resection of the tumor carries an obvious risk for an intralesional surgical margin. This may be the case for e.g. large, proximal lower extremity tumors. The sarcoma surgeons will present most cases on the surgical sarcoma network (Scandinavian collaboration) for discussions to decide who needs preoperative treatment. The outcome of this study group will be analyzed separately.

The purpose of the present study, SSG XX, is to evaluate the use of chemo- and radiotherapy as adjuvants in patients with high-grade STS of the extremities and trunk wall. Most patients will only be treated postoperatively (group A), those with an obvious risk for intralesional surgery will also be treated preoperatively (group B).

3. Study objectives

3.1 Primary objective

The *primary objective* is to study the risk of metastases and death for the present protocol treatment, as reflected by metastasis-free survival.

3.2 Secondary objectives

The *secondary objectives* are to study:

- cumulative incidence of local recurrence
- overall survival
- tumor progression in group B
- acute and late toxicity in relation to chemotherapy, and radiation morbidity
- secondary malignancies
- surgical margin and group allocation, in all patients, and by center.

4. Patient population

4.1 Basic criteria for inclusion in the protocol

Soft tissue sarcomas of the extremity and trunk wall with histological malignancy grade III or IV (high-grade) in the Scandinavian 4-graded system (appendix A) are considered eligible.

Patients with metastases are not eligible for this treatment protocol.

4.2 Definition of high-risk patients

4.2.1 Group A

Patients with:

- Vascular invasion (defined microscopically by the pathologist)
and/or
- Two or three of the following criteria fulfilled:
 - Size ≥ 8.0 cm (defined by the pathologist)
 - Infiltrating peripheral tumor growth pattern (defined by the pathologist)
 - Tumor necrosis (macroscopically or microscopically as defined by the pathologist).

See chapter 9. Pathology evaluation.

For definition of risk factors see appendix A5.

4.2.2 Group B

Patients with a tumor, regardless of size as measured on MR, for whom surgery carries an obvious risk of intralesional margin as discussed in section 11.1 Surgery.

4.3 Eligibility criteria (group A and group B)

- a) Age ≥ 18 years and ≤ 75 years
- b) Performance status, WHO: 0 or 1 (or higher if this is a consequence of sarcoma-related decreased motility)
- c) White blood count $\geq 3.0 \times 10^9/l$ or neutrophils $\geq 1.0 \times 10^9/l$ and thrombocytes $\geq 100 \times 10^9/l$
- d) GFR $\geq 70 \text{ml/min/1.73m}^2$
- e) ALAT and total bilirubin ≤ 2 times normal upper limit
- f) Adequate cardiac function (LVEF $\geq 50\%$)
- g) Extremity or trunk wall localization
- h) Patients fulfilling the high-risk criteria defined above, section 4.2.1 and 4.2.2.
- i) All histiotypes except: extraskeletal osteosarcoma and chondrosarcoma, Ewing, PNET, rhabdomyosarcoma, Kaposi's sarcoma, malignant mesenchymoma, clear cell sarcoma, alveolar soft part sarcoma, epithelioid sarcoma
- j) Not radiation induced sarcoma
- k) No metastases diagnosed contemporary with the diagnosis of the primary tumor
- l) At least 5 years free of another non-metastatic malignancy, except basal cell skin cancer or cervical carcinoma in situ
- m) No previous anthracycline treatment
- n) Not given any other treatment for this sarcoma including isolated limb perfusion with TNF- α and melphalan (ILP)
- o) Not pregnant or lactating
- p) Time frames:
 Group A: Not more than 12 weeks (84 days) between final surgery and start of chemotherapy. Not more than 3 weeks (21 days) between registration and start of chemotherapy. *Note that no chemotherapy may be given before registration!*
 Group B: Not more than 4 weeks (28 days) from diagnostic biopsy to start of chemotherapy subsequent to registration. Not more than 3 weeks (21 days) between registration and start of chemotherapy.
- q) Written informed consent subsequent to given oral and written patient information.

5. Treatment plan

SSG XX is a Phase II non-randomized treatment protocol for STS with high risk for distant metastases and local recurrence.

5.1 Treatment design group A

Patients in group A (arm 1–3) are treated by surgery before entering the protocol. If the primary surgery was only biopsy or intralesional surgery outside center, the patient must always have a second operation aiming to be radical. No gross tumor left is allowed for patients who are included in this protocol. *After registration in the study they receive:*

- a) three cycles with chemotherapy
- b) radiotherapy depending on arm:

- I) arm 1: patients with subcutaneous tumors operated with wide margins are not given radiotherapy, and this is also the case for radically amputated patients.
 - II) arm 2: patients with deep tumors operated with wide margins or all tumors operated with marginal margins receive radiotherapy 36 Gy, as described in 11.3.1, between chemotherapy cycle 3 and 4.
 - III) arm 3: all patients with tumors operated with intralesional margins are given 45 Gy, as described in 11.3.1, between chemotherapy cycle 3 and 4. Also patients who need a re-operation (see above) are given 45 Gy.
- c) three additional cycles of chemotherapy.

5.2 Treatment design group B

Patients in group B are considered to carry an obvious risk for intralesional surgery, and immediate surgery is therefore not performed. The patients are registered in the study when this decision is made, and they *thereafter* receive:

- a) two cycles of chemotherapy
- b) radiotherapy 36 Gy as described in 11.3.2
- c) one more cycle of chemotherapy
- d) MR evaluation two weeks after chemotherapy cycle 3, and if no progressive disease is demonstrated (PD defined as increase in largest tumor diameter $\geq 25\%$ (WHO) and contrast enhancement by MR, or metastases) the patient will proceed to:
- e) surgery; and if this is macroscopically radical (no gross tumor left)
- f) three more cycles with chemotherapy.

Note that there will be no histological response evaluation for group B in the protocol, since radiotherapy induces destruction of tumor architecture and histological chemotherapy response evaluation becomes unreliable.

In case of progressive disease or gross tumor left during surgery, the patient has reached a major event, and protocol treatment is ended. The patient is then treated individually outside of the protocol, but followed for survival.

6. Endpoints and Statistical methods

6.1 Endpoints

The *primary endpoint* is metastasis-free survival defined as time from start of treatment (surgery for group A and chemotherapy for group B) until the first of the events metastases or death of any cause.

Secondary endpoints are

- time to local recurrence from start of treatment, considering death as a competing event
- overall survival defined as time from start of treatment until death of any cause,
- proportion of patients with progression of local disease preoperatively in group B (for definition of progression, see section 5.2)
- toxicities of grade ≥ 3 (according to CTCAE scheme, version 3.0 (appendix I) in relation to chemotherapy, and RTOG/ EORTC scheme in relation to radiotherapy, appendix D8 and D9)
- frequency and type of secondary malignancies
- the proportions of wide, marginal and intralesional histopathological margin in group A and group B after final surgery
- group allocation in all patients and by center.

6.2 Sample size considerations

Most metastases have occurred within the first three years and hence it is of special interest to evaluate the primary endpoint at this time.

For patients fulfilling the high risk criteria, who are 18–75 years of age, and who have not received chemotherapy, the 3-year metastasis-free survival has been estimated to be about 50% (data from SSG Central Register). With addition of adjuvant chemotherapy the desired achievement is to increase this number to 60% or higher. About 60 patients with a grade III-IV STS localized to the trunk wall or extremities without metastases are diagnosed per year in the recruitment area. Based on the study by Engellau et al. (manuscript submitted 2007) about 50% (n=30 per year) of such patients will fulfil the high risk and other eligibility criteria of this study. Recruitment of 150 patients would take about 5 years. With an additional 2 years of follow-up, the estimated 3-year metastasis-free survival will have a 95% confidence interval of length $\pm 8\%$ (based on simulations with the above assumptions of 3-year metastasis-free survival of 60%). This is considered to be an acceptable level of certainty.

The statistical calculations are based on 150 patients in arm A who have completed the treatment plan. To account for an anticipated drop-out rate and other reasons of 5% 158 patients in arm A have to be recruited. Thus, inclusion of patients in group B will be closed when the planned number of patients in group A (n=158) have been recruited.

6.3 Analysis sets

Analyses of treatment efficacy endpoints will primarily be performed on all patients who have started the protocol treatment, i.e. the patients who have started the protocol chemotherapy (“Per Protocol set”). Analyses of chemotherapy toxicity will be based on the “Per Protocol set” and analyses of radiotherapy toxicity will be based on those patients in arms A2 and A3 and B in this set who have started radiotherapy. Patients who drop out between registration and start of protocol chemotherapy treatment will be accounted for by means of descriptive statistics, and the reasons for not starting the protocol treatment will be reported

6.4 Statistical analyses

Demographic and prognostic variables will be presented by means of descriptive statistics.

The primary endpoint, metastasis-free survival, as well as overall survival, will be illustrated by means of Kaplan-Meier curves complemented with 95% confidence bands. The cumulative incidence of local recurrence, with death as competing event (Kalbfleisch and Prentice 1980), will also be graphed by time.

Other secondary endpoints, including toxicity, will be analysed by descriptive statistical methods.

Group A and B will be analysed separately, and a pooled analysis of patients in group A and B will also be performed.

The primary and secondary endpoints will be compared with SSG register data on patients not treated with chemotherapy. In these analyses stratification and Cox proportional hazards models will be used to adjust for differences in important prognostic factors.

6.5 Timing of the analyses

A safety analysis will be performed by the SSG XX resource group after end of treatment of 20 patients. More than two treatment related deaths or more than five other serious adverse events should lead to a discussion regarding finalizing or amending the protocol.

About one year before the last patient will be included (i.e. after about 120 patients), a preliminary analysis of efficacy and toxicity endpoints will be done. This early analysis is intended for the planning of future SSG studies for high risk soft tissue sarcoma. The analysis will be finished rather close to the inclusion of the last patient of the present study, and hence the recruitment will not be jeopardized.

The main analyses will be performed 2 years after inclusion of the last patient in group A. Analyses on long-time follow-up of events and late toxicity will also be performed. The timing of this will be determined after the main analysis.

7. Pretreatment investigations

Mandatory investigations for the study (supplemented by other investigations according to clinical routine):

- Performance status, WHO (evaluated within 2 weeks prior to registration)
- Body height
- White blood counts, neutrophiles, thrombocytes, creatinine, ALAT and total bilirubin
- MR of the involved tumor site must have been done before surgery in group A and before start of chemotherapy in group B (see appendix B)
- Chest x-ray (posteroanterior and lateral views)
- CT scan of the chest (see appendix B)
- estimation of left ventricular ejection fraction (LVEF) either by cardiac ultrasound or by MUGA scan
- GFR.

8. Investigations during treatment and follow-up

8.1 During treatment

8.1.1 Before each chemotherapy cycle (group A and group B)

- Body weight
- White blood counts, neutrophiles, thrombocytes, creatinine, ALAT and total bilirubin
- In all cycles after radiotherapy scoring of acute radiotherapy side effects (according to the RTOG Acute Radiation Toxicity Scoring, see appendix D8)
- Scoring of adverse events since last chemotherapy cycle.

8.1.2 Weekly blood tests

- White blood counts, neutrophiles, thrombocytes.

8.1.3 Before chemotherapy cycle 4

- Chest x-ray (posteroanterior and lateral views)(group A)
- LVEF (group A and group B).

8.1.4 Before surgery in group B

- White blood counts with neutrophils, thrombocytes, creatinine, ALAT and total bilirubin
- Chest x-ray (posteroanterior and lateral views)
- MR of the involved tumor site.

8.2 After treatment, about 6 weeks after last given chemotherapy completion (group A and group B)

For all patients who were included in the study and had received any chemotherapy, also for those who were prematurely withdrawn from the protocol treatment:

- Performance status (WHO)
- White blood counts, thrombocytes, ALAT, total bilirubin, creatinine
- Examination of tumor site
- Scoring of acute radiotherapy side effects (see appendix D8)
- MR of the postoperative tumor site (serves as a baseline for further controls)
- Chest x-ray (posteroanterior and lateral views)
- Estimation of left ventricular ejection fraction (LVEF) either by cardiac ultrasound or by MUGA scan
- GFR.

8.3 Follow-up

For all patients who were included in the study, also for those who were prematurely withdrawn from the protocol treatment, the follow-up is mandatory.

8.3.1 Schedule

- Years 1(counted from follow-up at 6 weeks) - 2: every 3rd months.
- Years 3-5: every 6th months.
- Years 6-10: every year.

8.3.2 Investigations

- Examination of tumor site
- Scoring of late radiotherapy effects (according to the RTOG/EORTC Late Radiation Morbidity Scoring Scheme, see appendix D9)
- MR of the postoperative tumor site if recurrence is suspected clinically
- Chest x-ray (posteroanterior and lateral views)
- CT scan of the chest on suspicion of lung metastases
- Estimation of left ventricular ejection fraction (LVEF) either by cardiac ultrasound or by MUGA scan and GFR at 1, 5 and 10 years after treatment.

9. Pathology evaluation

The histological diagnosis and for group A, presence of risk factors must be determined by a local sarcoma pathologist before registration is done and any treatment starts. There will be no central review of the pathological diagnosis before registration. The SSG's panel of pathologists will routinely review the histological sarcoma diagnosis later.

9.1 Diagnostic biopsy for group A and group B

The diagnostic biopsy is best planned by the surgeon, and in case of an open biopsy it is strongly recommended that this should be performed by the surgeon responsible for the definitive surgery. The location of the diagnostic biopsy must at subsequent surgery be included *en bloc* with the specimen. If needle biopsy is done, the skin has to be tattooed for inclusion of the puncture canal in the operative specimen.

Group A

Patients in group A will have surgery without any preoperative treatment. Preoperative diagnosis sufficient for decision of surgery can be based on a fine-needle aspirate if the specimen is evaluated by an experienced cytologist. If there is not sufficient material for diagnosis, a core needle biopsy should be performed. The precise tumor type and grade is determined on the surgical specimen.

Group B

Group B includes patients who will receive preoperative chemo-radiation. It is very important to have good material for diagnosis in these circumstances since the tumor can be necrotic or otherwise affected by treatment which may prevent a reliable diagnosis. A core needle biopsy or an open surgical biopsy for a histological diagnosis should be performed for classification of tumor type and grade. Diagnosis based on fine needle aspiration biopsy alone is *not* accepted for group B.

Guidelines for handling of biopsies and surgical specimens, macroscopic and microscopic examination and definition of risk factors, see appendix A.

9.2 Pathology report

The report by the local pathologist should include the following parameters (Rubin et al. 2006) which will be partly reported at the pathology CRF *by the pathologist*:

1. Histological diagnosis (reported on CRF, group A and B)
2. Grade (reported on CRF, group A and B)
3. Size (reported on CRF, only group A)
4. Growth pattern (reported on CRF, only group A)
5. Presence of vascular invasion (reported on CRF, only group A)
6. Presence and amount of necrosis (reported on CRF, only group A)
7. Specimen type with localization and statement of which tissue involved, including removed lymph nodes (not reported on CRF)
8. Statement of the microscopic resection margins (not reported on pathology CRF).

10. Radiological evaluation

10.1 Assessment of the primary tumor

MR of the tumor site: Mandatory for all patients, unless contraindicated (e.g. pacemaker, intracranial magnetic clips). In these cases MR may be replaced by contrast enhanced CT.

Group A: The radiologist and the surgeon together decide if the quality of a previously performed MR exam (for example from a different institution) is sufficient for planning of surgery and evaluation of tumor characteristics. If the exam is considered insufficient, it must be repeated in accordance with guidelines below (Appendix B).

Group B: A previously performed MR exam from a different institution must be repeated at the tumor center where preoperative evaluation will be done if the quality is not optimal. In order to confidently evaluate therapy-induced changes, MR must be done with the same machine and examination protocol before and after preoperative chemo-radiation therapy.

10.2 Assessment to exclude detectable metastases

Chest x-ray (posteranterior and lateral views): Mandatory for all patients with the purpose to evaluate possible lung metastases and serve as a baseline for later controls.

Chest CT scan: Mandatory for all patients. Contrast enhancement is optional for the evaluation of lung metastases. If additional information about mediastinal structures is required, contrast enhancement is recommended.

Evaluation of lung lesions: No radiological criterion for pulmonary tumor spread is 100% specific. The radiologist will interpret lung lesions in discussion with the investigator. Lung metastases at diagnosis will exclude the patient from inclusion in the protocol. One pulmonary nodule of ≥ 1.0 cm, or 3 lesions ≥ 0.5 cm, is considered evidence of metastases, unless explained by other reasons. Smaller and fewer lesions are considered possible metastases, and inclusion in the protocol will be decided by the clinician and radiologist in each individual case.

11. Protocol treatment

11.1 Surgery

Surgery is carried out either at the start of the treatment (group A) or after the 3rd cycle of chemotherapy (group B). Surgery is optimally performed 3–4 weeks after radiotherapy for group B in order to avoid the fibrotic tissue response (Brian O`Sullivan, personal communication). Before definite surgery neutrophils should be $\geq 1.0 \times 10^9/l$ and thrombocytes $\geq 80 \times 10^9/l$ and should not be declining.

Preoperative chemo- and radiation therapy (group B) should be used when there is an obvious risk for surgery with an intralesional margin and amputation for a better margin is not indicated. The sarcoma surgeons will present most cases on the surgical sarcoma network (Scandinavian collaboration) to discuss the indication for preoperative treatment. The final decision will be the responsibility of the treating surgeon.

Surgery should be planned and performed in a manner that optimizes the chance for a wide surgical margin according to Enneking et al. (1980). The planning is based on clinical and radiological findings (most often MR). If an extremity tumor can not be removed with at least a marginal or an intralesional margin, without leaving macroscopic tumor tissue behind, an amputation has to be considered after preoperative treatment has been tried (group B).

No fixed guidelines can be given for either the choice or extent of local tumor surgery, or the type of reconstruction. Liberal use of soft tissue flaps, rotational or free vascularized flaps, for wound coverage is strongly recommended. This is especially important when preoperative radiotherapy has been used to reduce the risk of wound complications (Bell et al. 1991).

The definition of margins is described in appendix A.

The surgical case report form is completed by the investigator (oncologist) after information from the surgeon.

11.2 Chemotherapy

Treatment with doxorubicin and ifosfamide will be performed in accordance to the product characteristics and in an authorized form with notification to the interactions with other drugs, contraindications and to adequate medical condition.

11.2.1 Requirements for start of each cycle of chemotherapy

Bone marrow function

- a) neutrophiles $\geq 1.0 \times 10^9/l$ or white blood count $\geq 3.0 \times 10^9/l$ and neutrophiles $\geq 0.8 \times 10^9/l$
- b) thrombocytes $\geq 80 \times 10^9/l$

If these criteria are not fulfilled, the cycle must be postponed until these conditions are fulfilled, and if *not* so within a maximum of two weeks the patient is excluded from further protocol treatment.

Renal function

Serum creatinine within normal range is required. Otherwise a GFR is mandatory before start, and this must be ≥ 70 ml/min/1.73 m². If not the investigation must be repeated within a maximum of two weeks. If renal function is not satisfactory (< 70 ml/min/1.73 m²) omit ifosfamide and give doxorubicin alone. Resume ifosfamide at future courses if GFR ≥ 70 ml/min/1.73 m².

Cardiac function

Before the fourth chemotherapy cycle in group A and group B a cardiac ultrasound or MUGA-scan should be repeated. In case of cardiac dysfunction (LVEF < 50 %) the three last cycles of doxorubicin should not be administered (only ifosfamide).

Other reasons for chemotherapy treatment delay

If treatment is delayed ≥ 2 weeks due to other reasons than bone marrow depression the local investigator should take contact with the resource group for discussion.

11.2.2 Drugs, doses and infusion times

There are two infusion modalities to choose for the cytostatic drug regimen. However, drug doses and infusion times should be followed strictly. One time schedule is adjusted for an out-patient situation. If so, a double lumen central line should be used, and both drugs are given concomitantly. For those who do not use double lumen central line the ifosfamide infusion should follow doxorubicin. With this regimen the patient has to stay in hospital until day 2.

Drug doses, for patients < 70 y, and infusion times:

Day 1: Doxorubicin 60 mg/m² as a 4-hour infusion

Day 1,2,3: Ifosfamide 2 g/m² as a 2-hour infusion (6 g in total) (with Mesna).

To patients ≥ 70 y: doxorubicin 50 mg/m² and ifosfamide 5g/m² (in total) are given with the same infusion times as above.

11.2.3 Dose reduction

If febrile neutropenia occurs in spite of G-CSF (see 11.2.6) the doses in all following cycles should be reduced to doxorubicin 50 mg/m² and ifosfamide 5 g/m², respectively. G-CSF is to be given also after the following cycles.

If case of febrile neutropenia in spite of G-CSF in the elderly group (≥ 70 y), dose reduction with 20% is recommended.

11.2.4 Handling of ifosfamide toxicity

Serious bladder and/or CNS toxicity may occur when using high dose of ifosfamide. For handling of such toxicity see appendix C.

11.2.5 Treatment regimens

Alternative 1: Out-patient treatment day 1–3 (drug doses: <70 y)

Day	Time h	Drug	Dose	Fluid	Volume	Schedule
1		Prehydration		NaCl 0.9 % with KCl 10 mmol	500 ml	Over 1 hour
	T = 0	Doxorubicin	60 mg/m ²	Glucose 5%	500 ml	Over 4 hours
	T= 0	Ifosfamide + Mesna	2 g/m ² 0.4 g/m ²	NaCl 0.9 %	500 ml/m ²	Over 2 hours
	T= 2	Mesna	0.6 g/m ²	NaCl 0.9% with KCl 20 mmol	1000 ml/m ²	Over 4 hours
	T = 8	Mesna orally**	800 mg/m ²			
2		Prehydration		NaCl 0.9% with KCl 10 mmol	500 ml	Over 1 hour
	T= 0	Ifosfamide* + Mesna	2 g/m ² 0.4 g/m ²	NaCl 0.9 %	500 ml/m ²	Over 2 hours
	T= 2	Mesna	0.6 g/m ²	NaCl 0.9% with KCl 20 mmol	750-1000 ml/m ²	Over 4 hours
	T = 8	Mesna orally**	800 mg/m ²			
3		Prehydration		NaCl 0.9% with KCl 10 mmol	500 ml	Over 1 hour
	T= 0	Ifosfamide* + Mesna	2 g/m ² 0.4 g/m ²	NaCl 0.9 %	500 ml/m ²	Over 2 hours
	T= 2	Mesna	0.6 g/m ²	NaCl 0.9% with KCl 20 mmol	750-1000 ml/m ²	Over 4 hours
	T = 8	Mesna orally**	800 mg/m ²			

* Should be started with a minimum of 18 hours after start of the ifosfamide infusion on the previous day.

** The patient may leave the hospital after end of the mesna infusion and take oral mesna 800 mg/m² 2 hours later. As an alternative mesna 0.4 g/m² may be given as iv injection 4 hours after end of the mesna infusion. The total fluid volume per day during ifosfamide treatment must never be below 2000 ml/m².

To patients ≥ 70 y: reduce the drug doses to doxorubicin 50 mg/m² and ifosfamide 5g/m²

**Alternative 2: In-patient treatment day 1, out-patient treatment day 2–3
(drug doses: <70 y)**

Day	Time h	Drug	Dose	Fluid	Volume	Schedule
1		Preinfusion		NaCl 0.9 %	100 ml	Over 10 min
	T = 0	Doxorubicin	60 mg/m ²	Glucose 5%	500 ml	Over 4 hours
	T= 4	Prehydration		NaCl 0.9% with KCl 10 mmol	500 ml	Over 1 hour
	T= 5	Ifosfamide + Mesna	2 g/m ² 0.4 g/m ²	NaCl 0.9 %	500 ml/m ²	Over 2 hours
	T= 7	Mesna	0.6 g/m ²	NaCl 0.9%, with KCl 20 mmol/l	1000 ml/m ²	Over 4 hours
	T=13	Mesna orally**	800 mg/m ²			
2		Prehydration		NaCl 0.9 % with KCl 10 mmol	500 ml	Over 1 hour
	T= 0	Ifosfamide* + Mesna	2 g/m ² 0.4 g/m ²	NaCl 0.9 %	500 ml/m ²	Over 2 hours
	T= 2	Mesna	0.6 g/m ²	NaCl 0.9% with KCl 20 mmol/l	750-1000 ml/m ²	Over 4 hours
	T=8	Mesna orally**	800 mg/m ²			
3		Prehydration		NaCl 0.9 % with KCl 10 mmol	500 ml	Over 1 hour
	T= 0	Ifosfamide* + Mesna	2 g/m ² 0.4 g/m ²	NaCl 0.9 %	500 ml/m ²	Over 2 hours
	T= 2	Mesna	0.6 g/m ²	NaCl 0.9% with KCl 20 mmol/l	750-1000 ml/m ²	Over 4 hours
	T=8	Mesna orally**	800 mg/m ²			

* Should be started with a minimum of 18 hours after start of the ifosfamide infusion on the previous day.

** The patient may leave the hospital after end of the mesna infusion and take oral mesna 800 mg/m² 2 hours later. As an alternative mesna 0.4 g/m² may be given as iv injection 4 hours after end of the mesna infusion. The total fluid volume per day during ifosfamide treatment must never be below 2000 ml/m².

To patients ≥ 70 y: reduce the drug doses to doxorubicin 50 mg/m² and ifosfamide 5g/m².

11.2.6 G-CSF

G-CSF support is mandatory after each cycle (both for <70 y and ≥ 70 y) and begin at least 24 hours after end of chemotherapy and continue until WBC $\geq 5.0 \times 10^9/l$ and at least for 8 days. Pegylated filgrastim 6 mg as a single subcutaneous injection 24 hours after completion of chemotherapy is an alternative option.

11.3 Radiotherapy

11.3.1 Group A

Patients with wide margins for deep tumors and marginal margins for all tumors will receive radiotherapy (36 Gy) between chemotherapy cycle 3 and 4 (arm 2). Patients with intralesional margins will receive 45 Gy regardless of tumor depth (arm 3).

The fractionation schedule is 2 x 1.8 Gy/day, with minimum 6-hour interval between the two daily fractions, and if possible 5 treatment days per week. Due to radiosensitizing effect of doxorubicin a minimal interval between doxorubicin and radiotherapy should be 7 days.

11.3.2 Group B

Radiotherapy with 36 Gy is given after the two initial chemotherapy cycles. The fractionation schedule is 2 x 1.8 Gy/day, with at least 6 hours interval between the two daily fractions, and if possible 5 treatment days per week. Due to radiosensitizing effect of doxorubicin a minimal interval between doxorubicin and radiotherapy should be 7 days.

11.3.3 Radiotherapy delay

If radiotherapy is delayed ≥ 1 week due to complications of chemotherapy or other reasons the local investigator should take contact with the resource group for discussion.

For radiobiological considerations, radiation techniques and specification see appendix D.

12. Safety determination

12.1 Adverse Event

Definition:

An adverse event (AE) is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does *not necessarily have a causal relationship* with this treatment. Thus, any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product are classified as AE.

In SSG XX, selected AEs judged to be of specific interest are documented on the case report forms (CRF), but also other AEs are to be reported if considered of importance by the investigator. All AEs are graded for severity according to Common Terminology Criteria for Adverse Event v. 3.0 (CTCAE)(Appendix I). AEs suspected to be related to the study medications are sometimes called “adverse reactions” (ARs).

12.2 Serious Adverse Event or Reaction

Definition:

A serious adverse event (SAE) or serious adverse reaction (SAR) is defined as any untoward medical occurrence or effect that:

- a) results in death regardless of its cause during protocol treatment and for 30 days after terminating protocol treatment
- b) is life-threatening
- c) requires hospitalization or prolongation of an existing hospitalization
- d) results in persistent or significant disability or incapacity
- e) is a congenital anomaly or birth defect
- f) is a secondary malignancy during protocol treatment and for 30 days after terminating protocol treatment.

In SSG XX, the following SAEs are to be reported, without unnecessary delay, on specific SAE forms:

- *All deaths* including death due to disease progression, intercurrent diseases or accidents during protocol treatment and for 30 days thereafter. Death due to progression of disease will thus not constitute a SAE if it occurs at least 30 days after the last protocol treatment.

- All life threatening events. The term "life-threatening" refers to an event where the patient is at IMMEDIATE risk of death at the time of the event (e.g. requires IMMEDIATE intensive

care treatment). It does not refer to an event which hypothetically might cause death if it were more severe.

- Unexpected hospitalization, defined as at least one unplanned overnight admission caused by adverse events that is not well known as a consequence of the treatment given, e.g. neutropenic fever. Thus, neutropenic fever is not to be reported if not regarded as life threatening.

- Disability defined as a substantial disruption in a person's ability to conduct normal life functions (e.g. blindness, deafness). Disability resulting from tumor surgery does not constitute an SAE.

- Secondary malignancies are also considered as SAEs and are reportable on an SAE form during protocol treatment and for 30 days after the last protocol treatment. Note that secondary malignancies occurring after this time point shall be reported on follow-up forms.

If in doubt whether a SAE must be reported or not, please contact the national coordinator for SSG XX.

All SAE reports must be sent to the SSG secretariat in Lund as soon as possible. If the outcome of the SAE is not clear by the time of reporting, a further report must be sent later.

All deaths, all life-threatening and all unexpected SAEs must, be reported *within 7 days*. All serious and unexpected SAE's must be reported *within 15 days*.

Unexpected SAEs are those of which the nature or severity is not consistent with information in the relevant source documents. Suspected unexpected serious adverse reactions (SUSARs) including deaths and life-threatening SAEs must according to laws and GCP rules be further reported to the Medical Product Agencies and Ethics committees in the participating countries, which will be done by the SSG secretariat.

12.3 Late toxicity in relation to chemotherapy

Late effects of chemotherapy, i.e. cardiac and renal toxicity, will be documented on follow-up forms.

12.4 Acute and late toxicity in relation to radiation toxicity

During treatment, radiation toxicity is reported on chemotherapy CRF. Toxicity is assessed according to the RTOG Acute Radiation Toxicity Scoring (Appendix D8). As the radiation treatment is given in an accelerated and hyperfractionated regime the acute radiation toxicity is expected after the course of radiotherapy is completed. Acute radiation toxicity is principally seen in rapidly proliferating tissues such as skin and mucosa in the irradiated volume. Acute toxicity is reported at the start of every chemotherapy cycle following the radiation treatment and until follow-up at 6 weeks after completion of the entire treatment. Thereafter radiation toxicity is scored according to RTOG/EORTC Late Radiation Morbidity Scoring Scheme (Appendix D9). Late toxicity is reported at every follow-up. If the event of severe radiation toxicity during treatment or observed during follow-up, i.e. grade 4–5, or if a secondary malignancy develops in the irradiated volume, this is reported on the follow-up form. If grade 4-5 radiation toxicity occurs during treatment or follow-up report also immediately to one of the resource persons for radiotherapy (see section 1).

12.5 Secondary malignancies

Secondary malignancies may occur as a consequence of both chemotherapy and radiotherapy and must be reported as SAE if occurring during treatment or within 30 days weeks after last protocol treatment. If occurring later, a secondary malignancy will be reported on Follow-up form.

13. Data management

The SSG SOP (Standard Operating Procedure) for Data Management will be followed if nothing else is specifically stated in this protocol.

13.1 Patient registration

A registration form must be filled in and sent by fax to the SSG secretariat *before* any chemotherapy may be given. At the secretariat, the registration form will be completed with a unique patient identification number and a returning fax will be sent to the participating institution. The local investigators will keep a confidential patient identification list connecting the patient identification numbers with the full patient name and ID codes.

13.2 Case Report Forms (CRF)

Data will be recorded on case report forms (CRFs). The local investigator is responsible for the completion of CRFs. The surgical case report form is completed by the investigator (oncologist) after information from the surgeon. The pathology CRFs, however, will be completed by the local sarcoma pathologist and the SSG morphology group, respectively.

CRFs must be filled out with ink. Corrections should be performed as follows: A single line should be drawn through the incorrect information, the correct information written next to it and dated and signed, if necessary giving reasons for the correction. Data fields that can not be completed because of lack of information should be commented, e.g. “NK” (Not Known), “NA” (Not Applicable) or “ND” (Not Done). A copy of every CRF shall be kept at the participating institution, and the original shall be sent to the SSG secretariat.

13.3 Data processing

At the SSG secretariat, data will be entered into an electronic database. Implausible or missing data detected during data entry, monitoring visit or data validation will be corrected or supplemented after contacting the local investigator through queries. Query resolutions will be stored together with the corresponding CRFs and a copy shall be kept at the participating institution. Before final analysis a decision must be taken on final closure of the database.

13.4 Data storage

The originals of all trial documents including e.g., the protocol, amendments, all CRFs and correspondence with ethical committees and regulatory agencies, will be stored at the SSG secretariat for the time period defined by national laws and regulations, at least 15 years after publication of the final analysis.

Local investigators will store all necessary study documents, as advised by the SSG secretariat, including copies of the protocol, amendments and CRFs, the patient identification list, signed informed consents, CVs and signature list of all local investigators etc. Local investigators will also store the patient’s hospital charts for as long as required by national laws and regulations, at least 15 years after publication of the final analysis.

14. Quality assurance

14.1 Monitoring

Following GCP rules, SSG XX will be monitored by site visits to all participating institutions. For details, see appendix E.

14.2 Validity checks

Validity checks will be performed at the SSG secretariat according to the principles of SSG Standard Operating Procedure (SOP) for Data Management.

15. Protocol ethics

15.1 Patient information

Before entry into the trial all eligible patients will receive a written patient information describing the rationale for and the outline of the suggested therapy, as well as probable and possible side effects and risks. Oral information from one of the investigators at the institution will also be given, and the patient must have the opportunity to ask any question, and to consider participation together with her/his relatives. The written information will be constructed by the respective national coordinator of the study to be accepted by the respective ethics committees and regulatory authorities.

15.2 Informed consent

Prior to trial entry the patient must give her/his written consent after being informed as described above, and this must be stored at the institution. It is the right of the patient to withdraw her/his consent anytime during the study.

16. Administrative matters

One common protocol will be used by all participating centers. The master protocol will be in English.

16.1 Sponsor

SSG is the sponsor of the study.

16.2 Necessary approvals

Each country must nominate a National Coordinator who is responsible for ensuring that investigators, Ethical committee/-s and competent authorities in his/her country are informed of all relevant information and for answering any national queries.

Before start of the study in any country, an ethical approval must be given according to the regulations in the country. The protocol must also be approved by the Medical Product Agency. Handling of research samples must be approved by the national health authorities in each country.

16.3 Protocol amendments

Any amendments to the protocol must be agreed upon by the members of the working group. Additions to the protocol to address local needs may be performed by the centers, provided they have no influence on the essential aims of the protocol. Any modifications which may have an impact on the conduct of the study, or may affect patient safety, including changes of study objectives, study design, patient population, sample size, study procedures, or significant administrative aspects will require a formal amendment to the protocol.

16.4 Financial matters

Each center will provide its own financing.

16.5 Publication principles

The principal investigator will be responsible for the manuscript(s) reporting the results of this study, with possible exceptions for e.g., experimental biological research projects within the study, where the person responsible for this research project also is responsible for the report. The responsible person will be first author. All active persons within the working group will also be authors according to “Uniform requirements for manuscripts submitted to Biomedical Journals” (www.icmje.org/). The working group will decide the order of authorship in consensus, and also decide upon co-authorship of persons not originally members of the working group.

17. References

(Including those only referred to in the appendices)

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APPENDIX

Appendix A. Pathology

A1. Guidelines for performing and handling of biopsies and surgical specimens

The diagnostic biopsy should be planned and performed by the surgeon who will be responsible for the definitive surgery. If needle biopsy is used, the skin has to be tattooed for inclusion in the operative specimen. See section 9.1.

Group A:

Group A will undergo surgery without any preoperative treatment. Preoperative diagnosis sufficient for decision of surgery in group A can be based on a fine-needle aspirate evaluated by an experienced cytologist. If there is not sufficient material for diagnosis a core needle biopsy should be performed. It is important to rule out any tumor needing preoperative treatment, such as the small round cell tumors, which are not included in this protocol. The precise type and grading of the tumor is made on the operative specimen.

Group B:

Group B includes patients who will receive preoperative chemo-radiation before surgery. It is especially important to have good material for diagnosis in these circumstances since the tumor can be necrotic after preoperative treatment. A core needle biopsy or an open surgical biopsy for a histological diagnosis should be performed to classify the type and grade of sarcoma. Fine needle aspiration biopsy is not accepted for diagnosis in group B.

Fresh specimens

Fresh (unfixed) surgical specimens should be submitted immediately after removal to the Department of Pathology. If it is not possible to send the tumor fresh, use formalin as a fixative. The specimen should be handled immediately upon arrival for deep-freezing of samples (at least -70°) for tissue banking and optimal fixation for electron microscopy when this analysis is wanted (see appendix H). It is recommended to send samples for molecular and cytogenetic analysis. When there is a reason to believe that the sarcoma may have a characteristic cytogenetic abnormality, samples should be processed for genetic analyses (FISH-analysis and/or RT-PCR). Imprints (touch preparations) of samples that are deep-frozen or saved for special analyses are recommended to certify that representative sarcoma tissue has been saved. Frozen sections can also be performed later for the same purpose. From tumors in group B a core needle biopsy could be used for deep-freezing.

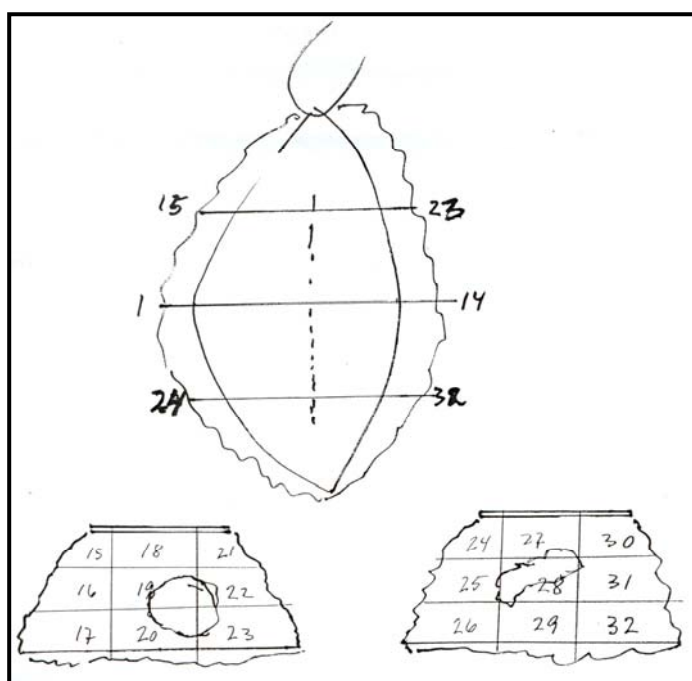
A2. Macroscopic examination

1. Ideally the surgeon and the pathologist should examine the specimen together or at least communicate about problems that may arise regarding orientation of the specimen and resection margins. The surgeon can help to identify the most critical resections margins by marking with sutures. Radiological studies are useful for orientation. Drawings should be submitted with the specimen whenever possible. Photographic documentation is advised.
2. The specimen and the tumor size are measured in three dimensions on the fixed specimen.
3. The type of surrounding tissues should be described.
4. The margin is assessed on the pathological specimens after fixation in formalin and ink dyeing of the specimen's surface. Acetic acid can fixate the ink better.
5. The specimen is sliced at maximum 1 cm increments and sections are made from areas of closest margin. The closest margin of resection should be measured and its type of tissue recorded. Cut also perpendicular to the first cut for better analysis of the margins.
6. The percentage of macroscopic necrosis (based on tumor volume) should be estimated.
7. The consistency, color and hemorrhage should be stated.
8. Examine vessels at amputation margins.

9. At least as many sections as the largest tumor dimension should be examined, e.g. at least 6 sections of a 6 cm tumor should be taken. A block guide is recommended. For very large tumors it is rarely necessary to take more than 10 blocks of the tumor itself.

Recommended sections:

- Sections of the tumor interface with surrounding tissues as well as macroscopically divergent areas including necrotic and hemorrhagic areas.
- Several sections from the peripheral part of the tumor. Vascular invasion is seen best in the tumor periphery.
- Sample skin to include biopsy tract.
- Any lymph nodes received should be sampled.
- Whole tumor or large and middle sized sections are excellent for the examination of tumor heterogeneity, relationship of the tumor to surrounding tissues (growth pattern with either pushing border or diffuse infiltration) and the presence of vascular invasion.



A3. Tumor depth

In resected specimens the tumor depth should be recorded both macroscopically and microscopically.

Cutaneous: The tumor originates in the skin and may extend into the subcutaneous tissue.

Subcutaneous: The tumor lies in the fatty tissue between the skin and the deep fascia. If it penetrates the deep fascia it should be categorized as deep.

Deep fascia: The fascia that divides the subcutaneous compartment from the muscular compartment.

Intramuscular/intraosseous: The tumor is located beneath the deep fascia, originates in a muscle/bone, and is surrounded by muscle fascia/periost, which is not engaged by the tumor.

Extramuscular/extraosseous: The tumor is located beneath the deep fascia, can lie between muscles or penetrates the boundaries of the muscle/bone.

Bone/periosteum involvement: Should be stated.

A4. Microscopic examination

Standardized histological diagnosis according to WHO should be used (Fletcher CD et al 2002).

“Second opinion” diagnoses are strongly recommended.

It is difficult to apply diagnostic recommendations on sarcomas *pretreated* with radiotherapy and/or chemotherapy. Histological typing, assessment of necrosis and grading are difficult in such cases. There will be no histological response evaluation in this protocol.

A4.1 Grading and mitotic count

There is no universally accepted grading system for sarcomas (Deyrup & Weiss 2006). The local pathologist should give a grade based on the Scandinavian system. The SSG morphology group will also classify the tumor according to the French grading system. Generally one should only grade untreated primary sarcomas and using good quality slides and representative tissue. Mitotic activity is counted in 10 high power fields (HPF) and a 40 x objective should be used.

A4.2 The four-tiered grading system

In the Scandinavian Sarcoma Group a four-tiered grading system is used. Grade I and II means low grade malignant and grade III and IV, high grade malignant. This system is primarily based on Broders grading of malignant tumors and considers the amount of necrosis, bleeding, mitotic count, cellularity, cell and nuclear polymorphism, and differentiation without given a score to the different parameters (Broders 1920).

For many soft tissue sarcomas the grade is understood or implicit in the diagnosis. For instance, atypical fibroxanthoma of skin, dermatofibrosarcoma protuberans and well differentiated liposarcoma are all grade I sarcomas. Typical examples of grade II sarcomas include myxoid liposarcoma and many subcutaneous myxofibrosarcomas. For high grade sarcomas (grades III and IV) the grade is partly based upon histogenetic diagnosis and partly upon the morphologic features. Examples of grade IV sarcomas include round cell liposarcoma and pleomorphic liposarcoma.

A4.3 The French grading system (FNCLCC)

This is based on tumor differentiation, mitotic count and the amount of tumor necrosis (Guillou et al. 1997, Rubin et al. 2006). The total score of these parameters gives the grade. See complete table, appendix A7 below.

A5. Definition of risk factors

A5.1 Tumor size

Tumor size is the maximum tumor diameter as measured on the surgical specimen fixed in formalaldehyde.

A5.2 Growth pattern

The peripheral tumor growth pattern is assessed microscopically. A pushing border has no signs of infiltrative growth. If there is any sign of infiltration into the surrounding tissue independent of amount, the growth pattern is classified as infiltrative. The amount of infiltration is not assessed (Engellau et al 2005).

A5.3 Vascular invasion

Vascular invasion can be seen within the tumor or in the adjacent tissues and is defined as the presence of tumor cells within any space having an obvious endothelial lining. Such tumor

either has to be adherent to the luminal aspect of the vessel wall or, if free-floating, has to be associated with adherent fibrin, red blood cells, or leucocytes. If an intact layer of endothelium covers the tumor, if the involved space has no discernible endothelial lining or if the tumor invades the vessel wall (but not the lumen), then this is not accepted within the definition of vascular invasion. In other words bulging of tumor cells into a vessel space is not considered as vascular invasion.

CD31 or another vascular marker can be used to confirm the endothelial differentiation.

A5.4 Necrosis

Microscopic tumor necrosis is defined as the presence of amorphous cellular debris, usually associated with a neutrophil polymorphonuclear cell response. Dead cells are generally arranged in sheets, often with ghost nuclear outlines. Individual cell death, apoptotic bodies, areas of hyalinosis or edema, areas of fibrinous exudate lacking tumor cells, and areas of acellular fibrosis are not accepted within the definition of necrosis. The amount should be stated in percent.

For macroscopic necrosis, see section A2.

A6. Definition of pathological and surgical resection margins

The most important margin is the poorest margin, i.e. the part of the specimen where the tissue coverage is poorest (qualitatively and quantitatively). In that area the pathologist should record the type of tissue (e.g. fat, connective tissue) and the thickness (mm) of tissues covering the tumor.

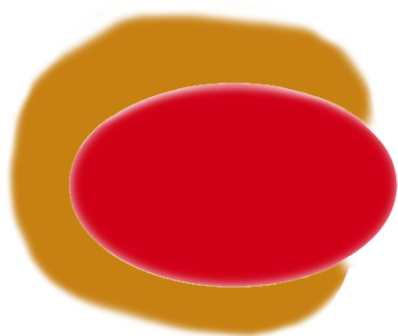
Two positive margins are defined:

Gross tumor left

The tumor is transected during the operation and macroscopic tumor tissue is left. This is reported by the surgeon.

Intralesional

Microscopic tumor tissue is seen at the resection border (reported by the pathologist) or leakage of fluid/tissue from the tumor into the wound occurs during surgery (reported by the surgeon).



Two negative margins are defined:

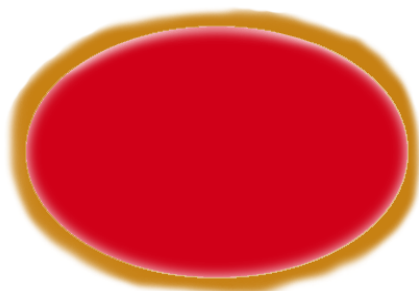
There are no tumor cells at the resection margin.

The local pathologist decides whether the margin is negative (tumor-free). In case of a negative margin the pathologist reports the shortest distance (mm) between tumor and resection border in fat, muscle or loose areole tissue.

The distinction between a *marginal* and *wide* margin is made by the surgeon and is based on the combined information from surgery and histopathologic examination. A fascia unengaged by the tumor is considered sufficient for a wide margin – irrespective of the distance between tumor and fascia. A total myectomy with the tumor completely surrounded by unengaged fascia needs no measurements and is by the surgeon classified as a wide margin.

Marginal

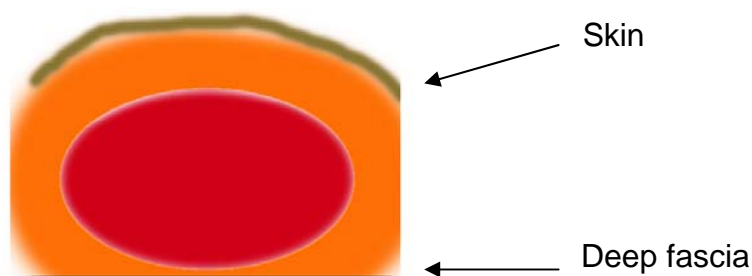
The closest margin is outside but near the tumor in one or more places (irrespective of how much healthy tissue is included elsewhere) or all around the tumor (shelling out). Microscopically the margin is negative all around the tumor (otherwise the margin is intralesional), but tumor cells may be only millimeters from the margin.



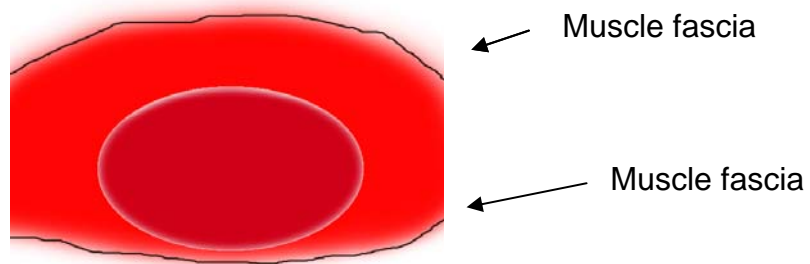
Wide

There is a cuff of healthy tissue all around the tumor. Unengaged fascia is considered a cuff regardless of the thickness of tissue between tumor and the fascia. A cuff of fatty or muscular or loose areole tissue must be minimum 10 mm thick as measured at the histopathologic examination to qualify for a wide margin.

Subcutaneous

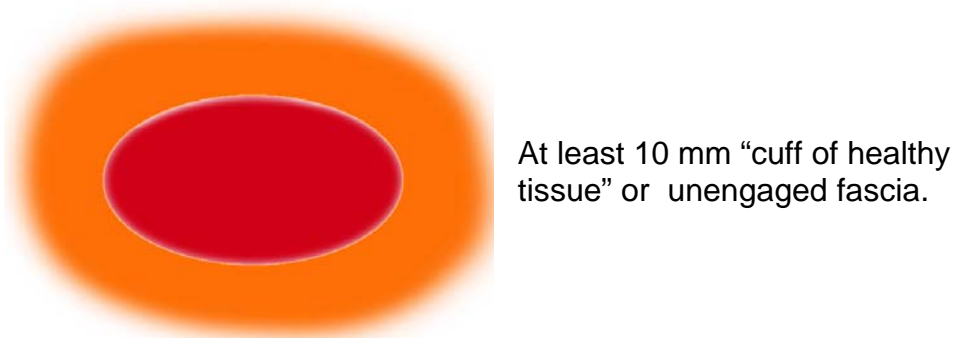


Intramuscular



A tumor within a muscle completely surrounded by an unengaged fascia is removed by total myectomy.

Deep extramuscular



A7. The French grading system

The French grade (FNCLCC grade) is only reported by the review pathologist.

Tumor differentiation:

Score 1:	sarcomas closely resembling normal adult mesenchymal tissue
Score 2:	sarcomas of certain histological type (e.g. myxoid liposarcoma, myxoid MFH)
Score 3:	Embryonal and undifferentiated sarcomas, sarcomas of doubtful type, synovial sarcoma, osteosarcoma, PNET

Tumor differentiation score of sarcomas in the French Federation of Cancer Centres Sarcoma Group System*

Diagnosis	Score
Well-differentiated liposarcoma	1
Myxoid liposarcoma	2
Round cell liposarcoma	3
Pleomorphic liposarcoma	3
Dedifferentiated liposarcoma	3
Fibrosarcoma	2
Myxofibrosarcoma (myxoid MFH)	2
Typical storiform MFH (sarcoma, NOS)	3
Pleomorphic MFH (patternless pleomorphic sarcoma)	3
Giant cell and inflammatory MFH (pleomorphic sarcoma, NOS with giant cells or inflammatory cells)	3
Well-differentiated leiomyosarcoma	1
Conventional leiomyosarcoma	2
Poorly diff./epithelioid/pleomorphic leiomyosarcoma	3
Synovial sarcoma (biphasic, monophasic and poorly differentiated)	3
Pleomorphic rhabdomyosarcoma	3
Mesenchymal chondrosarcoma	3
Extraskeletal osteosarcoma	3
Ewing's sarcoma/PNET	3
Malignant rhabdoid tumour	3
Undifferentiated sarcoma	3
PNET= primitive neuroectodermal tumor; MFH= malignant fibrous histiocytoma Note: Grading of malignant peripheral nerve sheath tumor, embryonal and alveolar rhabdomyosarcoma, angiosarcoma, extraskeletal myxoid chondrosarcoma, clear cell sarcoma and epithelioid sarcoma is not recommended.	

*Modified from Guillou et al. 1997 and Rubin et al. 2006.

Mitotic count:

Score 1: 0–9 mitoses per 10 HPF*

Score 2: 10–19 mitoses per 10 HPF

Score 3: ≥ 20 mitoses per 10 HPF

* A high power field (HPF) measures 0.1734 mm^2 .

Standardized HPF should be used.

Tumor necrosis:

Score 0: no necrosis

Score 1: $< 50\%$ tumor necrosis

Score 2: $\geq 50\%$ tumor necrosis

Histological grade (FNCLCC):

Grade 1: total score 2, 3

Grade 2: total score 4, 5

Grade 3: total score 6, 7, and 8

Appendix B. Radiology

B1. General guidelines for MR examination

MR should be done with an appropriate coil allowing sufficient field of view to depict the whole tumor area and sufficient detail resolution to evaluate anatomical details and tumor characteristics. It is recommended to include regional lymph nodes when relevant, especially for proximal lower extremity tumors. Intravenous contrast medium is mandatory. A recommended examination protocol can be found below, see B2.3.

The radiology report must include tumor size in three diameters and exact anatomical location (subcutaneous, subfascial/intramuscular, intermuscular). The relation with important anatomical structures (nerves, vessels, joints, fascia, adjoining soft tissues) should be recorded.

The presence or not of peritumoral edema, myxoid tumor, necrosis and skip lesions should be stated, as well as the pattern of contrast enhancement. Regional lymph nodes should be described if included in the field of view. As to evaluation of necrosis and peripheral growth pattern, see B4 below.

B2. Assessment of the tumor site

B2.1 MR of the primary tumor

Mandatory for all patients, unless contraindicated (e.g. pacemaker, intracranial magnetic clips). In these cases MR may be replaced by contrast enhanced CT.

Group A: The radiologist and the surgeon together decide if the quality of a previously performed MR exam (for example from a different institution) is sufficient for planning of surgery and evaluation of tumor characteristics. If the exam is considered insufficient, it must be repeated in accordance with guidelines below.

Group B: A previously performed MR exam from a different institution must be repeated at the tumor center where preoperative evaluation will be done if the quality is not optimal. In order to confidently evaluate therapy-induced changes, MR must be done with identical machine and examination protocol before and after preoperative chemo-radiation therapy. Any changes from the base line exam should be recorded.

B2.2 MR of the postoperative tumor site

MR should be performed about 6 weeks after chemotherapy completion for group A and group B. This exam serves as a baseline for further controls and should include the whole compartment on coronal images, with mandatory contrast enhancement on axial images.

MR of the postoperative tumor site at later follow-up is mandatory if recurrence is suspected clinically. With radiation-induced fibrosis and induration making clinical examination difficult, MR may give valuable information regarding recurrence. A simplified exam without contrast enhancement can be done if coronal STIR sequence shows no focal high signal (except explained by a postoperative seroma).

B 2.3 MR examination protocol

Coronal or sagittal STIR (depending on location)	mandatory	FOV large enough to cover the entire tumor compartment. Regional lymph nodes included when relevant.
Axial SE/TSE T1	mandatory	From healthy to healthy tissue. 3–5 mm slice thickness depending on size. FOV adjusted to the anatomical location.
Axial TSE T2	mandatory	As T1-sequence.
Axial SE/TSE T1 CE*	mandatory**	As T1-sequence. Fat suppression recommended.
Additional slice orientation and imaging options	optional	Optional.

The parameters in the different sequences cannot be given in detail because of varying capabilities of coils and equipment from different manufacturers.
The imaging protocol has to be adjusted to local feasibilities

*CE: contrast enhancement. May be omitted at follow-up if no focal high signal on STIR and T2.

**Mandatory for baseline, preoperative evaluation (group B) and 1st postoperative control.

B 2.4 Interpretation of MR**Primary tumor (Group A and B)**

Tumor location	subcutaneous/deep/intramuscular/intermuscular
Tumor size in centimeters	length x width x height
Peritumoral edema	yes/no
Myxoid tumor	yes/no/possible
Necrosis	yes/no/possible
Bleeding	yes/no/possible
Growth pattern	pushing/infiltrative/cannot be determined
Enhancement	peripheral, homogenous, heterogeneous
Regional lymph nodes	yes/no/not included

Preoperative examination after chemoradiotherapy (Group B)

Tumor size in centimeters	Change compared to exam 1
Peritumoral edema	Change compared to exam 1
Myxoid tumor	Change compared to exam 1
Regional lymph nodes	Change compared to exam 1
Enhancement	Change compared to exam 1
Necrosis or bleeding	Change compared to exam 1
Growth pattern	Change compared to exam 1

B3. Assessment of metastases

Chest X-ray (posteroanterior and lateral views): Mandatory for all patients, to evaluate possible lung metastases and serve as a baseline for later controls.

Chest CT scan: Mandatory for all patients included in both treatment arms. Contrast enhancement is optional for the evaluation of lung metastases. If additional information about mediastinal structures is required, contrast enhancement is recommended.

Evaluation of lung lesions: No radiological criterion for pulmonary tumor spread is 100% specific. The radiologist will give the interpretation of lung lesions, in discussion with the oncologist. Lung metastasis at diagnosis will exclude the patient from inclusion in the protocol. One pulmonary nodule of ≥ 1.0 cm, or 3 lesions ≥ 0.5 cm, is considered evidence of metastases, unless explained by other medical reasons. Smaller and fewer lesions are considered possible metastases, and inclusion in the protocol will be decided in each individual case.

B4. Distinction between high-risk and low-risk STS: pathologic-radiological correlation

The four prognostic factors used in the present protocol in order to discriminate between high risk and low risk STS are vascular invasion, tumor size ≥ 8.0 cm, infiltrative growth, and necrosis (section 4.2.1). Except for tumor size, this protocol does not include radiological evaluation of risk factors. MR cannot predict vascular invasion. The pathologist will determine the presence of macro- or microscopic necrosis. Contrast enhanced MR can identify necrosis in most cases, however, distinguishing between necrosis, bleeding and myxoid tumor components may be difficult or impossible.

Infiltrative versus pushing border: The peripheral tumor growth pattern as assessed microscopically by the pathologist is one of the prognostic factors discriminating between high risk and low risk STS. It remains to be proven if the diagnostic MR examination can be used to identify infiltrative or pushing peripheral growth pattern. So far no clear radiological criteria have been established to identify growth pattern in STS. Radiological assessment of growth pattern or pushing border does not form part of the SSG XX protocol, but a standard MR examination protocol will allow retrospective evaluation of pathologic-radiological correlation in a separate study.

The following preliminary criteria are recommended for radiological evaluation of infiltrative versus pushing growth: If tumor signal intensity extends into the adjacent muscle or fat, with a spiculated or nodular advancing margin and loss of interface, infiltrative growth pattern must be suspected. If the interface is preserved with a sharp border between the tumor and adjacent muscle or fat, this is compatible with pushing growth pattern. If one part of the tumor shows infiltrative pattern, this is considered representative of the tumor as a whole.

In general high grade STS have low signal intensity on T1-weighted images and intermediate to high signal intensity (lower than fat) on T2-weighted images. Subcutaneous tumors are surrounded by fat having high signal on both T1- and T2-weighted images. Deep subfascial / intramuscular tumors are surrounded by muscle and fascia having low signal on both T1- and T2-weighted images. In order to use identical criteria for evaluation of the border of both subcutaneous and deep tumors, a transverse T2-weighted sequence with thin slices and good signal-to-noise ratio is recommended. On contrast enhanced T1-weighted images tumor enhancement can be difficult to distinguish from peritumoral hyperemia. On STIR sequences, the distinction between tumor and edema may be difficult.

These radiological criteria must be validated or adjusted by comparing MR exams of pilot patients with microscopic examinations. If MR is shown to underestimate (or overestimate) infiltrative growth compared with pathology, the examination technique should be reassessed.

Appendix C. Chemotherapy

Handling of ifosfamide toxicity

C1. Bladder toxicity

Haemorrhagic cystitis with ifosfamide is rare if hydration and mesna are utilized appropriately. With macroscopic hematuria or microscopic hematuria (+++ or more) confirmed by microscopic examination (≥ 10 red blood cells/field) ifosfamide should be withheld, and NaCl 0.9% 1000 ml with mesna 1500 mg should be infused over 2 hours. Following the microscopic hematuria, the ifosfamide infusion should then be (re)started. After macroscopic hematuria ifosfamide will not be used in further cycles.

C2. CNS toxicity

CNS toxicity seldom occurs in the dose range of $< 6 \text{ g/m}^2$ and is more often seen by doses $> 10 \text{ g/m}^2$ ifosfamide treatment. It may be aggravated by metabolic acidosis, and the serum bicarbonate levels during ifosfamide infusions should be kept $> 21 \text{ mmol/l}$. If the patient develops symptoms of CNS toxicity (somnolence, cognitive disturbances, nightmares, hallucinations, convulsions) the infusion should be stopped and treatment instituted with methylene blue 50 mg i.v every 8 hours together with infusion of 2000 ml of glucose 5% with KCl 20 mmol + NaHCO_3 40 mmol over 8 hours. The symptoms generally disappear quickly, and 2–3 methylene infusions are usually sufficient. Usually, the ifosfamide course can be re-started. In subsequent cycles methylene blue must be given 50 mg i.v every 8 hours with start of ifosfamide infusion day 1 until end of day 3 (in total 9 times).

Appendix D. Radiotherapy

D1. Radiobiological considerations

There are experimental and clinical evidence that accelerated tumor cell proliferation during prolonged radiotherapy regimes or treatment breaks may result in a risk of decreased local tumor control. Data on rates of proliferation in surgically resected high-grade soft tissue sarcomas show a median of 20–30% of the tumor cells actively proliferating (Møller Nielsen 2001). There is no reason to assume that this proportion decreases following tumor resection with residual disease. Tissue activating factors involved in wound healing may well stimulate tumor regrowth too, although this has not been studied in mesenchymal tumors. Therefore, in general, radiotherapy treatment time should be as short as possible, without compromising wound healing, and within tissue tolerance for severe acute and late effects of radiotherapy.

In order to shorten the overall treatment period, the fractionation schedule in this phase-II study is 2 x 1.8 Gy/day, with an interval between the two daily fractions of at least 6 hours in order to allow for repair of sublethal damage in normal tissue, and five days of treatment per week. The rather slow component of repair reported for some late responding tissues (Ang et al 1992, Nyman and Turesson 1995), and thus the possibility of remaining incomplete repair, is taken into account by incorporating a dose per fraction modifying factor of 1.10 (cf SSG XIII) when establishing the total dose in the accelerated and hyperfractionated regime used in the current protocol. No such correction is deemed necessary for acute effects based on data for skin (Nyman and Turesson 1994, Nyman and Turesson 1995).

When radiotherapy is given in combination with chemotherapy - doxorubicin and ifosfamide being radiosensitizers; both increased acute and late effects are to be expected. The degree of this sensitization is not known, but dose modification needs to be taken into account when estimating the early and late effects when interpolating radiotherapy and doxorubicin containing chemotherapy regimes. In this protocol a chemotherapy modifying factor of 1.15 was assumed for both early and late effects. This dose modifying factor was used in the previous SSG XIII protocol in which radiotherapy and doxorubicin-ifosfamide was interpolated. In the SSG XIII both early and late effects were acceptable, with good local control for marginally resected tumors. For tumors in which an intralesional margin was obtained, an accelerated treatment to a total of 45 Gy in 13 patients given as a split course interpolated between chemotherapy-cycles resulted in local recurrences in three patients (unpublished data). In the current phase-II protocol, the total dose has been maintained for intralesional margins, but the treatment is given without a split course.

For estimates of early and late effects of the radiotherapy comparisons of Biologically Effective Dose (*BED*) were made for the conventional fractionation and the accelerated hyper-fractionated regime in the SSG XX protocol with dose modifying factors for late effects in the latter fractionation scheme.

$$BED = nd \left(1 + \frac{d}{\frac{\alpha}{\beta}} \right)$$

Late responding tissues ($\frac{\alpha}{\beta}=3$ Gy)

- Total dose 36 Gy in 20 fractions of 1.8 Gy per fraction, twice daily, five days per week; $BED=80$ Gy₃ with dose per fraction modifying factors of 1.10 and 1.15.
- Total dose 45 Gy in 25 fractions of 1.8 Gy per fraction, twice daily, five days per week; $BED=100$ Gy₃ with dose modifying factors of 1.10 and 1.15.

These schedules are equivalent to total doses of about 50 Gy ($BED=83$ Gy₃) and 60 Gy ($BED=100$ Gy₃), respectively, if administered with conventional fractionation, i.e. one fraction of 2 Gy per day.

Early responding tissues ($\frac{\alpha}{\beta}=10$ Gy)

- Total dose 36 Gy in 20 fractions of 1.8 Gy per fraction, twice daily, five days per week; $BED=50$ Gy₁₀ with a dose modifying factor of 1.15.
- Total dose 45 Gy in 25 fractions of 1.8 Gy per fraction, twice daily, five days per week; $BED=62$ Gy₁₀ with a dose modifying factor of 1.15.

If the difference in overall treatment time is neglected, these schedules are equivalent to total doses of about 40 Gy ($BED=48$ Gy₁₀) and 50 Gy ($BED=60$ Gy₁₀), respectively, if administered with conventional fractionation, i.e. one fraction of 2 Gy per day.

D2. Patient fixation

To reduce the set-up errors during treatment, care should be taken to ensure adequate use of fixation of the patient and affected body region to be treated. The position must be the same during planning, simulation and treatment. The type of fixation device/technique shall be reported in the RTQA-report (see below).

D3. Patient data acquisition

A CT study, and MR if applicable, shall be made in the treatment position on a flat table top with the patient, or the affected body region to be treated, in the fixation device. For group A, the scar should be marked with a lead thread. CT scanning shall be performed with a slice thickness of maximum 5 mm.

D4. Target volumes and organs-at-risk (OAR) volumes

The definitions of volumes follow the recommendations made by ICRU for photon and electron beam therapy (REF ICRU Report 50, 62, 71).

GROSS TUMOR VOLUME (GTV)

For preoperative therapy in treatment arm B, the macroscopic tumor volume is visualized on the CT-images for treatment planning, co-registered with MRI examination if possible. When CT images are not available the MR findings will be used to define the tumor extent.

CLINICAL TARGET VOLUME (CTV)

For tumors resected with a wide surgical margin, the CTV is defined as the preoperative tumor bed as defined on diagnostic MR and findings at surgery with a 2 cm margin in all directions. For tumors resected with a marginal margin, a 4 cm proximal-distal margin and a 2 cm lateral margin is added (where relevant and applicable) to the tumor bed. If underlying fascia borders are uninvolved, a 1 cm margin in this direction is regarded as adequate.

For preoperative radiotherapy, the *CTV* is defined by adding a 2 cm margin in all directions to the *GTV*.

If multiple primary tumor nodules or positive lymph nodes are present separate *CTV* volumes are defined, and labelled *CTV-T1*, *CTV-T2*, ... and *CTV-N1*, *CTV-N2*, ..., respectively (ICRU 71).

PLANNING TARGET VOLUME (PTV)

PTV is defined as the *CTV* with an additional margin of 1 cm in all directions.

ORGANS-AT-RISK (OAR)

For extremity localization, avoid circumferential irradiation to reduce the risk for subsequent distal lymphedema. Avoid inclusion of an entire joint space and full-dose irradiation of adjacent bone of weight bearing bones to reduce the risk of pathologic fractures.

For tumors in the dorsal aspect of the trunk wall, the spinal cord may be a dose limiting organ.

For the spinal cord, $\frac{\alpha}{\beta} = 2$ Gy is assumed, and the maximum dose should not exceed a *BED* of 96 Gy₂ including dose per fraction modifying factors of 1.10 and 1.15. This dose corresponds to 48 Gy in 24 daily fractions of 2 Gy.

D5. Radiation treatment technique

Three-dimensional conformal radiotherapy (3D-CRT, IMRT) should generally be delivered. For superficial tumors electron beams may be used.

It is left to each centre to decide upon the optimal technique (number of beams, beam weights, beam angles, wedges, beam shaping, bolus, etc.) as long as the dose-volume constraints are fulfilled.

D6. Dose specification and dose-volume constraints

Dose specification shall be in terms of the dose to the ICRU reference point. This point should be positioned centrally in the treated volume, if possible along the central rays of the beams. The prescribed target dose is the dose given to the ICRU reference point. All relative dose values are in percentage units in relation to the dose in this point.

The 95% isodose should cover at least 95% of the *PTV*. The maximum dose should not exceed 107%. For electron treatment the dose to the dose-maximum depth (D_{\max}) at a perpendicular angle to the surface is reported. The energy should be chosen for the *PTV* to be encompassed by the 90% isodose level.

D7. Quality assurance

DVH-data in ASCII-format, with doses and volumes in absolute numbers, for all delineated volumes including the volume defined by the patient external contour together with CRF for QA of the radiotherapy (Appendix D 10) should be sent to the SSG secretariat in Lund who will forward it to the QA-office in Lund. The DVH-data and the CRFs will be reviewed yearly by the SSG radiotherapists.

D8. RTOG Acute Radiation Toxicity Scoring

ORGAN TISSUE	0	1	2	3	4	5
Skin	No change	Follicular, faint erythema/ epilation/dry desquamation/ decreased sweating	Tender or bright erythema, patchy moist desquamation/ moderate edema	Confluent, moist desquamation other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis	DEATH AND FATAL COMPLICATIONS DIRECTLY RELATED TO THE RADIOTHERAPY
Mucous Membrane	No change	Injection/ may experience mild pain not requiring analgesic	Patchy mucositis, inflammatory serosanguinitis discharge/ may experience moderate pain requiring analgesia	Confluent fibrinous mucositis/ may include severe pain requiring narcotic	Ulceration, hemorrhage or necrosis	
Eye	No change	Mild conjunctivitis ± scleral injection/ increased tearing	Moderate conjunctivitis ± keratitis requiring steroids &/or antibiotics/ dry eye requiring artificial tears/ iritis with photophobia	Severe keratitis with corneal ulceration/ objective decrease in visual acuity or in visual fields/ acute glaucoma/ panophthalmitis	Loss of vision (unilateral or bilateral)	
Ear	No change	Mild external otitis with erythema, pruritis, secondary to desquamation not requiring medication. Audiogram ok	Moderate external otitis requiring topical medication/ serious otitis medius/ hypoacusis on testing only	Severe external otitis with discharge or moist desquamation/ symptomatic hypoacusis/tinnitus, not drug related	Deafness	
Salivary Gland	No change	Mild mouth dryness/ slightly thickened saliva/ may have slightly altered taste such as metallic taste	Moderate to complete dryness/ thick, sticky saliva/ markedly altered taste	-----	Acute salivary gland necrosis	
Pharynx & Esophagus	No change	Mild dysphagia or odynophagia/ may require topical anesthetic or non-narcotic analgesics/ may require soft diet	Moderate dysphagia or odynophagia/ may require narcotic analgesics/ may require puree or liquid diet	Severe dysphagia or odynophagia with dehydration or weight loss(>15% from pre-treatment baseline) requiring N-G feeding tube, I.V. fluids or hyper-alimentation	Complete obstruction, ulceration, perforation, fistula	
Larynx	No change	Mild or intermittent hoarseness/ cough not requiring antitussive/ erythema of mucosa	Persistent hoarseness but able to vocalize/ referred ear pain, sore throat, patchy fibrinous exudate or mild arytenoid edema not requiring narcotic/ cough requiring antitussive	Whispered speech, throat pain or referred ear pain requiring narcotic/ confluent fibrinous exudate, marked arytenoid edema	Marked dyspnea, stridor or hemoptysis with tracheostomy or intubation necessary	

ORGAN TISSUE	0	1	2	3	4	5
Upper G.I.	No change	Anorexia with <=5% weight/ nausea not requiring antiemetics/ abdominal discomfort not requiring parasympatholytic drugs or analgesics	Anorexia with <=15% weight loss/nausea &/ or vomiting requiring antiemetics/ abdominal pain requiring analgesics	Anorexia with >15% weight loss or requiring N-G tube or parenteral support. Nausea or vomiting requiring tube or parenteral support/abdominal pain, severe despite medication/hematemesis or melena/ abdominal distention (BÖS demonstrates distended bowel loops)	Ileus, subacute or acute obstruction, performance, GI bleeding requiring transfusion/abdominal pain requiring tube decompression or bowel diversion	DEATH AND FATAL COMPLICATIONS DIRECTLY RELATED TO THE RADIOTHERAPY
Lower G.I. including pelvis	No change	Increased frequency or change in quality of bowel habits not requiring medication/ rectal discomfort not requiring analgesics	Diarrhea requiring parasympatholytic drugs (e.g., Lomotil)/ mucous discharge not necessitating sanitary pads/ rectal or abdominal pain requiring analgesics	Diarrhea requiring parenteral support/ severe mucous or blood discharge necessitating sanitary pads/abdominal distention (BÖS demonstrates distended bowel loops)	Acute or subacute obstruction, fistula or perforation; GI bleeding requiring transfusion; abdominal pain or tenesmus requiring tube decompression or bowel diversion	
Lung	No change	Mild symptoms of dry cough or dyspnea on exertion	Persistent cough requiring narcotic, antitussive agents/ dyspnea with minimal effort but not at rest	Severe cough unresponsive to narcotic antitussive agent or dyspnea at rest/ evidence of acute pneumonitis/ O ₂ or steroids may be required	Severe respiratory insufficiency/ continuous O ₂ or assisted ventilation	
Genito Urinary	No change	Frequency of urination or nocturia twice pretreatment habit/ dysuria, urgency not requiring medication	Frequency of urination or nocturia which is less frequent than every hour. Dysuria, urgency, bladder spasm requiring local anesthetic (e.g., Pyridium)	Frequency with urgency and nocturia \geq hourly/ dysuria, pelvic pain or bladder spasm requiring narcotic/gross hematuria or clot passage	Hematuria requiring transfusion/ acute bladder obstruction not secondary to clot passage, ulceration or necrosis	
Heart	No change	Asymptomatic but objective evidence of EKG changes or pericardial abnormalities without other heart disease	Symptomatic with EKG changes and radiologic findings of congestive heart failure or pericardial disease/ no specific treatment required	Congestive heart failure, angina pectoris, pericardial disease responding to therapy	Unresponsive congestive heart failure, angina pectoris, pericardial disease, arrhythmias	
CNS	No change	Fully functional status (i.e., able to work) with minor neurologic findings, no medication needed	Neurologic findings present sufficient to require home care/ nursing assistance may be required/ medications including steroids/anti-seizure agents may be required	Neurologic findings requiring hospitalization for initial management	Serious neurologic impairment with paralysis, coma or seizures >3 per week despite medication/ hospitalization	

D9. RTOG/EORTC Late Radiation Morbidity Scoring Scheme

ORGAN TISSUE	0	Grade 1	Grade 2	Grade 3	Grade 4	5
Skin	None	Slight atrophy; Pigmentation change; Some hair loss	Patchy atrophy; Moderate telangiectasia; Total hair loss	Marked atrophy; Gross telangiectasia	Ulceration	DEATH AND FATAL COMPLICATIONS DIRECTLY RELATED TO THE RADIOTHERAPY
Subcutaneous tissue	None	Slight induration (fibrosis) and loss of subcutaneous fat	Moderate fibrosis but asymptomatic; Slight field contracture; <10% linear reduction	Severe induration and loss of subcutaneous tissue; Field contracture >10% linear measurement	Necrosis	
Mucous membrane	None	Slight atrophy and dryness	Moderate atrophy and telangiectasia; Little mucous	Marked atrophy with complete dryness; Severe telangiectasia	Ulceration	
Salivary glands	None	Slight dryness of mouth; Good response on stimulation	Moderate dryness of mouth; Poor response on stimulation	Complete dryness of mouth; No response on stimulation	Fibrosis	
Spinal cord	None	Mild L'Hermitte's syndrome	Severe L'Hermitte's syndrome	Objective neurological findings at or below cord level treated	Mono-, para-quadriplegia	
Brain	None	Mild headache; Slight lethargy	Moderate headache; Great lethargy	Severe headaches; Severe CNS dysfunction (partial loss of power or dyskinesia)	Seizures or paralysis; Coma	
Eye	None	Asymptomatic cataract; Minor corneal ulceration or keratitis	Symptomatic cataract; Moderate corneal ulceration; Minor retinopathy or glaucoma	Severe keratitis; Severe retinopathy or detachment Severe glaucoma	Panophthalmitis/Blindness	
Larynx	None	Hoarseness; Slight arytenoid edema	Moderate arytenoid edema; Chondritis	Severe edema; Severe chondritis	Necrosis	
Lung	None	Asymptomatic or mild symptoms (dry cough); Slight radiographic appearances	Moderate symptomatic fibrosis or pneumonitis (severe cough); Low grade fever; Patchy radiographic appearances	Severe symptomatic fibrosis or pneumonitis; Dense radiographic changes	Severe respiratory insufficiency/continuous O2/Assisted ventilation	
Heart	None	Asymptomatic or mild symptoms; Transient T wave inversion & ST Changes; Sinus tachycardia >110 (at rest)	Moderate angina on effort; Mild pericarditis; Normal heart size; Persistent abnormal T wave and ST changes ; Low ORS	Severe angina; Pericardial effusion; Constrictive pericarditis; Moderate heart failure; Cardiac enlargement; EKG abnormalities	Tamponade/ Severe heart failure/Severe constrictive pericarditis	

ORGAN TISSUE	0	Grade 1	Grade 2	Grade 3	Grade 4	5
Esophagus	None	Mild fibrosis; Slight difficulty in swallowing solids; No pain on swallowing	Unable to take solid food normally; Swallowing semi-solid food; Dilation may be indicated	Severe fibrosis; Able to swallow only liquids; May have pain on swallowing Dilation required	Necrosis/ Perforation Fistula	DEATH AND FATAL COMPLICATIONS DIRECTLY RELATED TO THE RADIOTHERAPY
Small and large intestine	None	Mild diarrhea; Mild cramping; Bowel movement 5 times daily Slight rectal discharge or bleeding	Moderate diarrhea and colic; Bowel movement >5 times daily; Excessive rectal mucus or intermittent bleeding	Obstruction or bleeding, requiring surgery	Necrosis/ Perforation Fistula	
Liver	None	Mild lassitude; Nausea, dyspepsia; Slightly abnormal liver function	Moderate symptoms; Some abnormal liver; function tests; Serum albumin normal	Disabling hepatic insufficiency; Liver function tests grossly abnormal; Low albumin; Edema or ascites	Necrosis/Hepatic coma or encephalopathy	
Kidney	None	Transient albuminuria; No hypertension; Mild impairment of renal function; Urea 25-35 mg%; Creatinine 1.5-2.0 mg%; Creatinine clearance >75%	Persistent moderate albuminuria (2+); Mild hypertension; No related anemia. Moderate impairment of renal function; Urea <36-60 mg%; Creatinine clearance 50-74%	Severe albuminuria; Severe hypertension; Persistent anemia (<10%); Severe renal failure; Urea >60 mg% Creatinine >4.0 mg% Creatinine clearance <50%	Malignant hypotension; Uremic coma/ Urea >100%	
Bladder	None	Slight epithelial atrophy; Minor telangiectasia (microscopic hematuria)	Moderate frequency; Generalized telangiectasia; Intermittent macroscopic hematuria	(<150 cc)	Necrosis/ Contracted bladder (capacity <100 cc); Severe hemorrhagic cystitis	
Bone	None	Asymptomatic; No growth retardation; Reduced bone Density	Moderate pain or tenderness; Growth retardation; Irregular bone sclerosis	Severe pain or tenderness; Complete arrest of bone growth; Dense bone sclerosis	Necrosis/ Spontaneous fracture	
Joint	None	Mild joint stiffness; Slight limitation of movement	Moderate stiffness; Intermittent or moderate joint pain; Moderate limitation of movement	Severe joint stiffness; Pain with severe limitation of movement	Necrosis/ Complete fixation	

D 10. QA-form¹ for SSG XX, radiotherapy

Patient data				
Patient initials:				
Date of birth (yyyy-mm-dd):			<input type="checkbox"/>	Group A, arm 1, 2 or 3
No in SSG XX:			<input type="checkbox"/>	Group B
Patient position and fixation				
<input type="checkbox"/>	Supine	<input type="checkbox"/>	Prone	
<input type="checkbox"/>	Body cast	<input type="checkbox"/>	Extremity fixation	<input type="checkbox"/> None <input type="checkbox"/> Other
Comment:				
Patient data acquisition and delineation of volumes				
CT slice thickness (mm):		<input type="checkbox"/> MR used for delineation		
Generation of tumor margins		<input type="checkbox"/> Auto	<input type="checkbox"/> Manual	<input type="checkbox"/> Both
Comment:				
Treatment technique				
<input type="checkbox"/>	3D-CRT	<input type="checkbox"/>	IMRT	<input type="checkbox"/> Opposing portals <input type="checkbox"/> Electrons
Comment:				
Treatment planning				
Photon beam quality (MV):		Total number of beams:		
Electron energy (MeV):				
Comment:				
Dose specification				
Dose in Gy to ICRU reference point (=100%):				
Comment:				
Fractionation				
Number of fractions:				
Date for first fraction (yyyy-mm-dd):				
Date for last fraction (yyyy-mm-dd):				
Total number of treatment days:				
Comment:				
Dose-volume data from DVHs				
CTV	Vol. (cm ³)	D _{max} (%)	D _{mean} (%)	D _{SD} (%)
PTV	V _{D=95%} (%)	D _{max} (%)	D _{mean} (%)	D _{SD} (%)
OAR		D _{max} (%)	D _{mean} (%)	D _{SD} (%)
Specify OAR:				
DVH for delineated structures			<input type="checkbox"/> Yes	<input type="checkbox"/> No
Entire circumference of affected extremity treated			<input type="checkbox"/> Yes	<input type="checkbox"/> No
Hospital: Date (yyyy-mm-dd):				
Planner:				
Physicist:				
Physician:				

¹ To be sent, within one month after end of treatment, to:
SSG secretariat, Regional Tumor Registry, Lund University Hospital, SE-221 85 Lund, Sweden

Appendix E. Monitoring

This clinical trial should be monitored in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with "Good Clinical Practice" including "International Conference on Harmonization Guidelines for Good Clinical Practice ICH-GCP" and applicable regulatory requirements.

Responsibilities

The monitoring activities will be performed by monitors from the following institutions in the respective Nordic countries.

- Sweden: Oncologic Centre at University Hospital in Lund. The monitoring will be done according to SOP 01–02 for monitoring, that exists at the department.
- Norway: Oncologic Centre at University Hospital in Lund or from Rikshospitalet-Radiumhospitalet HF, Montebello, 0310 Oslo

Extent of monitoring

Each site should be visited as early as possible after the first subject has entered active treatment in order to check protocol understanding and compliance. By failing routines it is necessary with early proposals/comments by the monitor to secure correct compliance at the local center. At a minimum a site must thereafter be visited at least once every 12 months. A closing visit will be done to each site at the end of the study.

Data to verify

At all study centres:

- Determining whether the investigator is maintaining the essential documents
- Determining whether the data required by the protocol are reported accurately on the CRFs and are complete, consistent with the source documents and ready for data entry
- Determining protocol compliance
- Determining whether storage of study documents are accurate
- Determining whether handling and storage of biological samples are accurate

Source data verification (SDV) for all patients:

- Verify all variables (100 % SDV) in at least one patient in all centres and one out of five if \geq five patients have been included (20 %)
- Choose patients from all parts of the inclusion period

Variables	Patient number (%) for source data verification
Patient identity (1)	100
Informed consent (2)	100
Eligibility-/exclusion criteria (3)	100
Visit dates	20
Study medication (compliance)	20
Reccurence (4)	100
Tests and examinations	20
AE	20
All SAE/SUSAR (5)	100

1. Verify that the patients initials and date of birth in the CRFs are consistent with the medical record.
2. Verify that written informed consent was obtained before each subjects participation in the trial and that this is documented in the medical record at the right time.
3. Verify that the patient is correctly included in the study, ie that all eligibility- and exclusion criteria are fulfilled and that relevant information about the patients participation in the trial (protocol name and purpose, patient number, treatment group (and if applicable treatment arm), study medication and dose level) are documented in the medical record.
4. Verify occurrence of 1:st metastasis or 1:st local recurrence or death during the follow-up periods.
5. Verify in the medical record, within the time periods required for documentation of SAE and SUSAR, that all SAE and SUSAR also are entered into the CRFs and are reported according to GCP, the protocol and the applicable regulatory requirements.

Reporting

The monitor should submit a written report to the national coordinator after each trial-site visit. The investigator at each study site will also receive a written report with comments of the findings and requests of changes/corrections if necessary. The national coordinator will submit a summary of the monitoring activities and results to the principal investigator.

Appendix F. Flow-sheet for group A

	Pretreatment	Before each cycle	Weekly	6 weeks after treatment	Follow-up*
Informed consent	X				
Performance status	X			X	
Height	X				
Weight		X			
Examination of tumor site				X	X
White blood counts	X	X	X	X	
Neutrophiles	X	X	X	X	
Thrombocytes	X	X	X	X	
Creatinine	X	X			
ALAT	X	X			
Bilirubin	X	X			
MR (CT) of involved tumor area	Before surgery			X	On suspicion of local recurrence
Chest x-ray	X	Before cycle 4		X	X
CT chest	X				On suspicion of lung metastases
LVEF	X	Before cycle 4		X	1,5 and 10 years after treatment
GFR	X	If creatinine is outside normal range		X	1,5 and 10 years after treatment
Adverse events		From start of cycle 1			
Late toxicity** in relation to chemotherapy					X
Scoring of acute RT effect (arm 2 and arm 3)		Before cycle 4, 5 and 6		X	
Scoring of late RT effect (arm 2 and arm 3)					X

*Years 1 (counted from follow-up at 6 weeks) - 2: every 3rd months

Years 3-5: every 6th months

Years 6-10: every year

** Cardiac and renal toxicity

Appendix G. Flow-sheet for group B

	Pre-treatment	Before each cycle	Before surgery	Weekly	6 weeks after treatment	Follow-up*
Informed consent	X					
Performance status	X				X	
Height	X					
Weight		X				
Examination of tumor site					X	X
White blood counts	X	X	X	X	X	
Neutrophiles	X	X	X	X	X	
Thrombocytes	X	X	X	X	X	
Creatinine	X	X	X			
ALAT	X	X	X			
Bilirubin	X	X	X			
MR (CT) of involved tumor area	X		X		X	On suspicion of local recurrence
Chest x-ray	X		X		X	X
CT chest	X					On suspicion of lung metastases
LVEF	X	Before cycle 4			X	1,5 and 10 years after treatment
GFR	X	If creatinine is outside normal range			X	1,5 and 10 years after treatment
Adverse events		From start of cycle 1				
Late toxicity** in relation to chemotherapy						X
Scoring of acute RT effect		Before cycle 3, 4, 5 and 6			X	
Scoring of late RT effect						X

*Years 1 (counted from follow-up at 6 weeks) - 2: every 3rd months

Years 3-5: every 6th months

Years 6-10: every year

** Cardiac and renal toxicity

Appendix H. Tumor biology studies

Inclusion criteria

Patients must meet the inclusion criteria for the main protocol and provide informed consent for participation in the translation research part of the study.

The research projects linked to the SSG XX trial consist of:

- A. Tumor biological studies with focus on profiling for genetic, expression-based or protein-based signatures (facilitated through SSG XX, but formally part of other research studies)*
- B. Pharmacogenetic studies with correlations between genetic variants and toxicity/prognosis*

The research projects linked to the SSG XX trial involve different approaches of profiling of tumor cells which represent areas of interest and expertise in several research groups in SSG. In addition, research areas where new information can improve treatment have been included e.g. pharmacogenetics and studies of specific genes or pathways of relevance for treatment response, toxicity and prognosis. The aim of the translational research part of the study is improved prognostic and treatment predictive factors.

A. Tumor biological studies with particular focus on profiling for genetic, expression-based or protein-based signatures

SSG aims at establishing tumor tissue banks at the different participating institutions, regulated under their respective ethical guidelines, but allowing joint analysis of tumor tissue from patients participating in the SSG XX trial. In order to ensure preservation of tissue from as many individuals as possible, the SSG XX includes patient information forms and informed consent forms for such collection. Thereby future joint studies of common interest are facilitated.

Tumor tissue collection is ongoing at most centers and the research studies include e.g. cytogenetic analyses, molecular characterization of fusion genes, genome-wide genetic profiling with array-platforms, and studies of specific target genes, establishment of xenograft models and studies hereon. Hence, the routines for how these samples should be preserved may vary between different laboratories and specific local requirements may apply. Guidelines for preservation of frozen tumor tissue have previously been established in the report “Analysis of genetic changes in musculoskeletal tumors” by the SSG tumor biology group in 2000 (www.ssg-org.net). Detailed descriptions on the requirements for the different methods have been described herein. As indicated in the SSG report referred to above, tissue samples from normal, surrounding tissue and blood samples may also be important as normal controls, e.g. regarding genetic alterations identified, comparison of expression levels or protein profiles. Availability of tissue material from metastases or local recurrences is also of great value for research purposes and in particular for determination of how tumor progression affects these phenomena.

B. Pharmacogenetic studies with correlations between genetic variants and toxicity/prognosis

Single nucleotide polymorphisms (SNPs) in a variety of biochemical pathways can alter/predispose cells for tumorigenesis and affect the response as well as the side effects of cancer treatment (Engle et al., 2006). Pharmacogenetics is defined as studies on the influence of genetic variations and changes in gene products on drug response and side-effects. An increasing number of genetic loci, mainly represented by SNPs within drug metabolizing enzymes have been identified and linked to interindividual differences in response and drug toxicity. These variations may be studied by analysis of these targets with different technologies, including DNA sequencing, DNA/RNA-based array techniques, and proteomic analysis. The variants were found in both coding, non-coding and promoter sequences. Now, more than 5 million SNPs are verified and published in different databases. However, a few SNPs have shown association between genotype and diseases or prognosis on effect of medical interventions (Sachidanandam et al. 2001). Analysis of SNPs can use various techniques, e.g. sequencing of a specified target or high-throughput analyses based on denaturing capillary gel electrophoresis (DGGE) or array-based techniques. The Norwegian Radium Hospital has a SNP facility based on DGGE, which allows analysis of SNPs in more than 90% of the genome, with good detection limit (0,1%) and high through put (96 samples/40 minutes) (Bjørheim et al. 2003a, and 2003b).

The overall benefit of improved pharmacogenomic profiling is to allow individualized treatment with dosing according to the individual patient's pharmacogenomic profile. Due to a low response rate (10-35%) aggressive chemotherapy regimens are used in soft tissue sarcoma treatment. Hence, patients selected for adjuvant therapy are burdened by side-effects, particularly haematologic toxicity (leucocytopenia, neutropenia, and thrombocytopenia) but also including renal and cardiac toxicity, alopecia, nausea, and CNS toxicity (Patel et al. 1997, Frustaci et al., 2001). Drug dosing does not yet take the interindividual variability in response and toxicity into account. Pharmacogenetics are increasingly being applied in clinical decision-making, but the knowledge about the different targets and their influence on treatment in soft tissue sarcomas is scarce (Berwick et al. 2004, Bion and Toffoli 2005, Choi et al. 2006).

The primary objective is to explore the relationship between genetic variants and side-effects of treatment in soft tissue sarcoma patients. Since novel genetic modifiers related to treatment are constantly detected and since the array-based and proteomic-based technologies applied cover the entire genome / transcriptome, a defined subset of markers to be studied can not be pre-defined. However, the studies will primarily focus on SNPs in drug target genes linked to toxicity and the following genetic variants are suggested in relation to doxorubicin and ifosfamide treatment:

Doxorubicin

The enzyme Carbonyl Reductase (CBR) is involved in the inactivation of doxorubicin. The functional SNP **CBR3*V244M** represents a common polymorphism that will be determined because of a possible impact on doxorubicin pharmacodynamics.

Ifosfamide

A large number of functional SNP's with potential modifying effect on ifosfamide treatment have been identified. The following will be profiled:

Glutathione S-transferase-p (GSTPI) **I105V** and **A114V**

CYP2B6*6 variants **Q172H**, **K266R** and **G516T** and the **CYP2B6** variants **R487C**, **M46V**, **G99E**, **K139E**, and **I391N**. These variants are potentially involved in the activity, hydroxylation and clearance of ifosfamide.

Study design

Patients enrolled in the SSG XX study will be asked to voluntarily enroll in the translational research on tumor biology of soft tissue sarcoma I) and III) and pharmacogenomics related to sarcoma treatment, doxorubicin and ifosfamide (II).

Participation in the translational research study includes collection of:

- I. **Fresh frozen tumor tissue** from the primary tumor (surgical specimen in arm A, core needle biopsy in arm B).

Tissue amount needed: tissue biopsy core or tumor piece 100-200 mg, approximately 0.5 cm (this amount will allow for DNA/RNA extraction at least once, if possible send duplicate samples). Tissue should be stored at -80° C.

Comparative **blood sample** (5 ml EDTA blood). Stored at -20° C

- II. **Blood samle** (5ml EDTA blood) obtained before chemotherapy. The vial can be stored at -20° C.

- III. **Fresh frozen tissue** from local recurrences/metastases (core needle biopsy or surgical specimen). Size as for primary tumor.

Tumor tissue collection can be performed at local laboratories, although registered with the SSG. When applicable the samples could also be sent to one of the national coordinators for the tumor biology (se below):

Tumor tissue (I or III) and blood samples (I) from the centres in Sweden:

- shipped on dried ice on mutually agreed date to: Attn. Mef Nilbert, Dept of Oncology, Lund University Hospital, SE-22185 Lund, Phone 46 46 177501, fax 46-46-147327
e-mail: mef.nilbert@med.lu.se.

Tumor tissue (I or III) and blood samples(I) from the centres in Norway:

- shipped on dried ice on mutually agreed date to: Attn. Ola Myklebost, Dept. of tumor biology, Institute for Cancer Research, Rikshospitalet-Radiumhospitalet Medical Centre, Montebello, NO-0310 Oslo. Phone +4722934299, fax +4722522421
e-mail: ola.myklebost@imbv.uio.no

Blood samples (II) for pharmacogenomic studies from the Swedish and the Norwegian centres:

-shipped on dry ice to the P.I of the study on mutually agreed date to: Attn. Kirsten Sundby Hall, Cancer Clinic, Rikshospitalet-Radiumhospitalet Medical Centre, Montebello NO-0310 Oslo, phone + 4722934000, fax +4722935599, e-mail: k.s.hall@klinmed.uio.no.

The analyses will be performed at Department of Immunology, Institute for Cancer Research, Rikshospitalet-Radiumhospitalet Medical Centre, Montebello, NO-0310 Oslo.