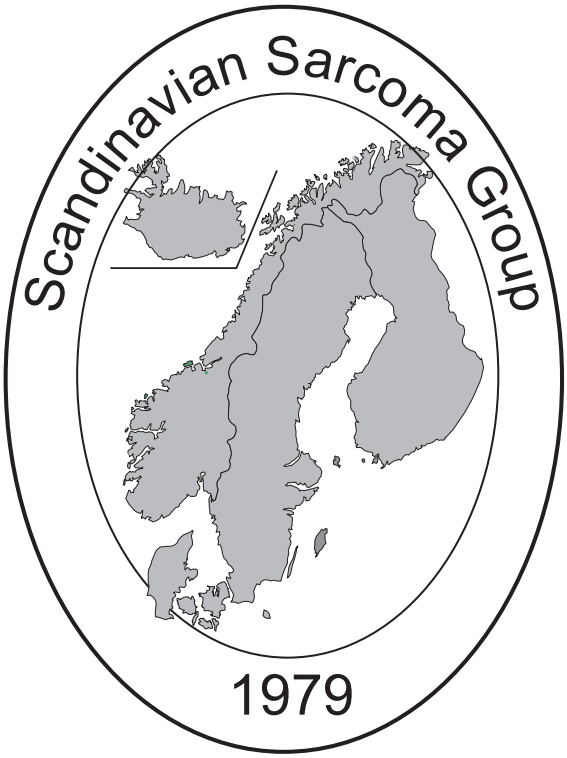
# The 31st Meeting of the Scandinavian Sarcoma Group

Reykjavik, Hotel Nordica

May 10-13, 2005




# Abstracts

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# 1. The SSG Registry of bone sarcomas

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1656 patients with bone sarcoma have been reported from Finland, Norway and Sweden to the SSG Registry since 1986. During 2004, the 2 major centers in Norway and 2 of 3 centers in Sweden have continuously reported new patients and follow-up. From these centers, the median follow-up of patients still alive was 7 years. In this report we focus on older patients with sarcoma of bone.

Overall referral of patients before open biopsy or surgery has improved from 73% before 1990 to 92% after 2000. The referral of chondrosarcoma patients improved from 71% to 93%, for chordoma from 65% to 90%, and for MFH of bone from 45% to 80%.

410 patients were operated for chondrosarcoma. Among 104 grade I lesions the local control rate at 5 years was .91 despite that 65% had an intralesional or marginal margin. The 5 years metastasis free survival was .98. Among the 306 patients with Grade II-IV lesions, a wide margin was achieved in 54%. The 5-year local control rate was .79 and the metastasis free survival was .72. Metastases were recorded in 14% of Grade II lesions, 26% of Grade III, and 48% of Grade IV.

Among 98 chordoma patients, 74 were operated. The 5-year local control rate was only .50.

121 patients had MFH or fibrosarcoma of bone, and there were 85 patients older than 40 years with osteosarcoma. The amputation rate was 35% and has only decreased marginally. The 5-year local control rate was .85 and the survival rate was .45.

In conclusion we have noted major improvement in referral of older patients with bone sarcomas. For all histological types there was a tendency for higher local control and survival rates for patients treated during the last 10 years. However, we need improved reporting of follow-up to conclude whether we are improving the outcome for these patients.

## 2. Radiological management of osteosarcoma

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Osteosarcomas comprise a family of malignant connective tissue tumors which characteristically produce osteoid or bone. Plain film radiography still provides high specificity and diagnostic information is seldom changed by other imaging modalities. However, in the evaluation of tumor extension CT and MRI are crucial as sometimes also for biopsy guidance. Bone scan is helpful in the detection of multiplicity. Osteosarcomas seldom pose diagnostic problem except for tumors exhibiting atypical radiographic appearance and/or atypical location. The cases posing difficulties also in the histopathological assessment are found in this radiologically atypical group.

Evaluation of tumor response during neo-adjuvant chemotherapy as assessed by CT or MRI has not proven reliable. In the literature PET has been reported to provide better measures of tumor response. The radio-pharmaceutical mostly used is FDG ($^{18}$F-fluor-2-deoxy-2-glukose) which is used in the cells sugar metabolism and thus is a relative measure of tumor activity. A draw back is that FDG also accumulates in inflammatory tissue. Other radio-pharmaceuticals used in PET are $^{11}$C-choline, which is incorporated in the cell-membrane and $^{18}$F-fluorothymidine used in the synthesis of DNA. The use of these pharmaceuticals in tumor evaluation still has to be examined. It remains to be seen if they can, in an individual case, provide reliable evaluation of tumor response and how early after start of anti-tumoral therapy then can be used as base for decision making.

## 3. Identification and possible targeting of pathways that are important in osteosarcoma development and progression

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By molecular cloning, genomic profiling, and functional studies, genes and regulatory pathways can be identified that may be useful as markers for clinically important tumor phenotypes. Some preliminary results from genomic profiling of osteosarcoma and chromosomal regions containing candidate tumor suppressor and oncogenes will be discussed. Examples will be presented of genes amplified in osteosarcoma and how they may affect tumor biology, and e.g. modulate the response to chemotherapy. An even more important aspect is the development of targeting strategies that may hit pathways that are critical for tumor survival, and that may be developed into biologically founded treatment regimens.

## 4. Can fine needle aspiration of osteosarcoma serve as a primary diagnostic modality?

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*Introduction* The role of fine needle aspiration biopsy (FNAB) in the examination of primary bone tumors is questioned by many pathologists and clinicians. We investigated the diagnostic accuracy of FNAB and reviewed diagnostic criteria and adjunctive methods, which can contribute to a confident diagnosis of osteosarcoma by FNAB.

*Patients and methods* We evaluated FNAB smears of osteosarcomas in 59 patients for details of their cytomorphology and occurrence of osteoid. In addition the usefulness of adjunctive methods was addressed and the correlation of FNAB findings to the clinical data and the histological features of excised tumors was analysed.

*Results* Reliable cytological criteria of malignancy were found in 49 smears of high-grade OS. These criteria, correlated with radiographic studies and complemented by ancillary techniques, allowed a diagnosis of OS or suspicion of OS in 44 cases. 21 of these patients received treatment without any histological examination. An additional 4 smears were diagnosed as sarcoma, 1 was erroneously diagnosed as being carcinoma metastasis and 10 were inconclusive for diagnosis (Table 1).

*Conclusion* FNAB is an efficient method in the diagnosis of high-grade osteosarcoma when correlated with radiological findings and appropriate clinical data. The main limitation of FNAB is the difficulty in obtaining diagnostic material from intraosseous, sclerotic and low-grade tumors.

Table 1. Diagnostic Accuracy of FNAB Examination of 59 Patients with Osteosarcomas

| **FNA** | **High-grade** | **Low-grade** |
|---|---|---|
| OS (47%) | 28 | - |
| Sarcoma, suspicion of OS (27%) | 16 | - |
| Sarcoma NOS (7%) | 4 | - |
| Carcinoma metastasis (2%) | 1 | - |
| Inconclusive (17%) | 8 | 2 |
| Summary (N=59) | 57 | 2 |

## 5. Prognostic factors in osteosarcoma

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*Background* In classical high-grade osteosarcoma (OS), a number of prognostic factors can be considered firmly established including serum alkaline phosphatase, tumor size and histological response to treatment.

*Methods* The last three OS-studies from the Scandinavian Sarcoma Group (SSGVIII, ISGSSG1 and in part SSGXIV; n=204) were combined for a new analysis of prognostic factors. Furthermore, the combined Italian and Scandinavian data of all patients included in ISGSSG1 (n=182) were analyzed separately including data on dose intensity and p glycoprotein (pGP).

*Results* The combined Scandinavian dataset confirms earlier reports of positive and independent prognostic impact for female sex (hazard ratio 2,3: 95% CI 1,3-4,2), normal baseline alkaline phosphatase (2,5: 1,4-4,6) and small tumor volume (p=0,01). The impact of sex is significant only in SSGVIII and the combined dataset. Alkaline phosphatase has to be sex and age corrected to show its full importance for survival. Independent impact for type of resection and histological response are lost in the multivariate analysis. The ISGSSG1 data show strong independent prognostic impact for alkaline phosphatase (2,1: 1,2-3,6) and a trend for tumor response (1,6: 0.95-2,8). Including pGP data (available for 96 patients) into the model makes this protein the single strongest predictor for survival (4,4: 1,8-11). Dose intensity is not prognostic in univariate analysis.

*Conclusion* In Scandinavia, female sex is favorably associated with outcome. For the first time prospectively, ISGSSG1 confirms the predictive value of pGP.

## 6. Surgical treatment of osteosarcoma. The SSG experience

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*Patients* 240 surgically treated patients who were reported to the SSG Register with high-grade, classical osteosarcoma since 1995 were studied. 4 chemo-therapy protocols were used during these years.

*Results* The rate of local excision was 0.85. A wide margin was obtained in 2/3 of all surgical procedures with a local control rate of 0.96 compared to 0.89 for marginal and 0.70 for intralesional margin. The overall local control rate was 0.94.

# 7. Osteosarcoma: Oncological treatment and clinical outcome

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*Introduction* Most osteosarcoma studies have included patients with classical disease characterized with a good prognosis. In this report we extend the analyses to include all patients with osteosarcoma reported to the SSG registry.

*Patients and methods* From 1982 SSG has conducted 4 clinical trials for classical osteosarcoma including a total of 334 patients. All chemotherapy regimens have utilized salvage therapy to poor histological responders. In the SSG register, from Jan 1986 to April 2005, 643 patients with the diagnosis of osteosarcoma are registered.

*Results* The 3-year event-free survival in SSG II (1982-89), SSG VIII (1990-1997), ISG-SSG 1 (1997-2000) and SSG XIV (2001- ) are 61%, 65%, 62% and 72% respectively. 3 treatment related deaths were recorded in SSG VIII (n=113), 1 in ISG/SSG-1(n=57) and by April 05, 3 in SSG XIV (n=63). The 5-year overall survival for patients with high-grade osteosarcoma (n=602) is 52% and for patients with non-extremity osteosarcoma (n=122) 30%, primary metastatic (n=98) 17% and patients aged > 40y (n=132) 27%.

*Conclusion* Outcome for patients with classical osteosarcoma seems to have reached a plateau. The prognosis for patients with non-classical osteosarcoma is still poor. In the upcoming Euramos-1 trial all patients with operable osteosarcoma are eligible and the effect of salvage therapy to poor responders will be tested in a randomized trial.

# 8. Ewing`s sarcoma. Diagnostic radiology

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Ewing sarcoma (ES) and Primitive Neuroectodermal Tumor (PNET) of the bone share the same radiological characteristics. I will show typical examples and most common differential diagnoses.

Origin: Usually in the medullary cavity. Occasionally in the periosteum or in the cortex or outside the skeleton.

Location: Equally common in short and flat bones as in long bones. When localized in a long tubular bone the lesion is metadiaphyseal in more than half of the cases, diaphyseal in the remaining cases. When localized in the vertebral column it is usually in the vertebral body.

Radiological findings: Ill defined intramedullary, permeative lytic lesion. Cortical destruction with thickening and sclerosis. Aggressive looking periosteal reaction (laminated, amorphus, sunburst). The bone lesion has usually (50-80 %) an extraskeletal soft tissue component which is often very large and almost always without calcifications. Compared to the smaller skeletal involvment, the soft tissue component can be extensive. Rarely, especially in flat bones, the bone lesion can be sclerotic, and mimic osteosarcoma.

Differential diagnoses: Osteomyelitis. Langerhans cell histiocytosis/eosinophilic granuloma. Lymphoma (older age group). Metastasis from neuroblastoma (younger age group).

Plain radiography is the method which bring us closest to the final diagnosis. CT can add information about the tumor type, and give additional information about the extent of the lesion. In adddition CT of the lungs can show pulmonary metastases.

MRI gives the best information about the extension and the viability of the tumor. However, there are still difficulties in defining the border between the tumor and peritumoral edema and hyperemia. Intravenous administration of MRI-contrast can to some degree aid in this differentiation, but this subject is still controversial. Skip metastases and distant metastases to the skeleton are also best evaluated using MRI. Scintigraphy may not detect ES metastases to the skeleton.

# 9. Cytogenetics and molecular genetics in Ewing sarcoma

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Ewing tumors are characterized by a highly specific translocation t(11;22)(q24;q12) and a chimeric fusion transcript of *EWSR1* (22q12) and *FLI1* (11q24). *FLI1* is a member of the ETS family of transcription factors. A minority of the Ewing tumors have variant translocations, in which *EWSR1* is fused with other members of the *ETS* gene family: *ERG* (21q22), *ETV1* (7p22), *ETV4* (17q21) and *FEV* (2q33). Fusion of *EWSR1* and *FLI1* or of *EWSR1* and *ERG* leads to a similar tumor phenotype and no correlations between different gene fusions and clinical features have been detected. Type 1 *EWS-FLI1* fusion transcripts have been associated with better outcome when compared to all other *EWS* fusion transcripts. From the additional changes, gain of 1q has been reported to be associated with adverse overall survival and event-free.

*Assays. Conventional cytogenetic analysis* is the basic method. Ewing translocations can be detected by *interphase-FISH* also from paraffin-embedded material. *Multicolor FISH* can be used for detailed analysis of all chromosomal changes, which greatly improves karyotype determination. Sensitive *PCR* can be used to detect the translocation-associated fusion genes and it is suitable for detection of minimal residual disease. *Comparative genomic hybridization* and *microarrays* enable study of DNA and gene copy number changes and gene expression.

*The future prospects* unfold through novel research lines for molecular classification of Ewing sarcoma. Gene copy number profiling combined with gene expression profiling can be used to identify a signature set of genes that will serve as an independent prognostic determinant of outcome. To achieve this goal, we need extensive collaboration and biobanks. It is necessary to ensure adequate availability of material for basic diagnostics as well as for research purposes. Our plain experience is that it is impossible to obtain enough material from needle biopsies for all diagnostic tests, let alone for research or storage in a biobank.

## 10. Prognosis and therapeutic targets in the Ewing family of tumors - sixth framework programme

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The project through collaborative studies will define prognostic markers and new therapeutic targets in the Ewing sarcoma family of tumors (ESFT) to provide rigorous scientific justifications for the development of clinical trials for this rare disease. By providing an organisational framework for collaboration the project will also allow multi-centre collection and analysis of cases as well as suitable collaborative research to allow genetic studies for the screening of high-risk patients and patients responding differently to chemotherapy. The main objective of this project is to evaluate the prognostic relevance of selected markers (EWS/FLI-1, secondary genetic alterations, CD99, IGF-IR, NOVH, erbB-2 and TTF1) and the effectiveness of therapeutic approaches targeting some of these molecules. Another major goal of the project is the construction of ESFT c-DNA microarrays and tissue arrays, which will be used for the analysis of different histological subtypes of ESFT, primary and metastatic tumors and poor and good responders to chemotherapy. This will lead to: 1) the definition of forthcoming risk-adapted strategies and targeted molecular treatments to be advantageously combined with established therapies; 2) improved quality of life and survival for ESFT patients; 3) prevention on risk in groups at risk.

# 11. Pathological diagnosis of Ewing family of tumors (EFT)

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EFT is a group of high-grade malignant sarcomas mostly affecting the bone marrow or soft tissue. EFT consists of Ewing's sarcoma and PNET (peripheral primitive neuroectodermal tumor) which shows a continuum of differentiation and the same genetic findings. EFT belongs to the group of morphological small round cell tumors and several differential diagnoses must be considered. The pathological diagnosis is based on a combination of morphology, histochemistry, immunohistochemistry, ultrastructural pathology, and genetics.

Microscopically these tumors show sheets of undifferentiated small, round, rather uniform "blue" cells with scant cytoplasm with round to oval prominent nuclei. There are many mitotic figures and apoptotic cells creating the typical cytological picture with light and dark cells. A diagnosis based only on the morphological picture is today not considered sufficient. The tumor stains positively to antibodies to vimentin in the cytoplasm, CD99 with membranous staining and FLI-1 in the nuclei. The cells can be positive for NSE, CD56, CD57, protein S-100, synoptophysin, chromogranin, and cytokeratin. PNET is often considered when two neuroectodermal markers are positive.

Genetic analysis can come from karyotyping, RT-PCR or FISH. The tumors in the Ewing family are characterised by translocations involving the *EWSR1* gene. The most common is t(11;22)(q24;q12) with a fusion of *FLI-1* to the *EWSR1* gene, but other gene fusions is also seen. It is important that one always provides tissue for ancillary genetic analysis and tumor bank.

## 12. Surgical treatment of Ewing´s sarcoma. The SSG experience

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*Patients* 139 patients who were reported to the SSG Register with Ewing´s sarcoma since 1995 were studied. Two chemotherapy protocols were used during these years.

*Results* Almost 2/3 were treated surgically but only 4 amputations were performed. A wide margin was obtained in 0.6 of all surgical procedures. The local control rate after surgery with or without radiotherapy was 0.99. In 34 pelvic ES, more than half were surgically treated with or without radiotherapy and the local control rate was 1.0. In 12 sacral ES, 2 were treated with surgery and the remaining 10 with radiotherapy (1 local recurrence).

## 13. Ewing sarcoma family tumors (ESFT): Oncological treatment and clinical outcome

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*Background* The ISG/SSG III and IV protocols were developed to improve outcome in standard and high risk ESFT using intensified chemotherapy for patients with a poor prognosis.

*Patients and methods* From June 1999 232 patients (ISG 195, SSG 37) has entered the ISG/SSG III study and 71 patients the ISG/SSG IV study (ISG 57, SSG 14).

*Results* Tumor location was extremity (51%), pelvis (18%) and other central location (25%) in study III and extremity (27%), pelvis (31%) and other central location (27%) in study IV. Local treatment was 59% surgery, 20% combined surgery and radiotherapy surgery and 21% radiotherapy in study III and 34%, 14% and 52 % in study IV. In study IV 78% of the patients have obtained remission upon primary therapy with 8 patients progressed before high-dose treatment.The 3-year projected event-free survival rates are 68% (III) and 44% (IV) and overall survival 80% (III) and 56% (IV). In study III, 18 patients have experienced a local recurrence; 9/121 after surgery, 1/41 after combined local treatment and after 8/44 after radiotherapy. In a univariate analyses outcome is better for patients treated with surgery (3y EFS 74%) compared to radiotherapy alone (3y EFS 52%). The outcome for poor and good responders are similar, 71% vs. 66% (log rank p=0,2). 4 treatment related deaths are recorded, 2 from acute myelogenous leukemaia.

*Conclusion* The protocols are feasible, toxicity is acceptable and the preliminary survival data encouraging. The strategy to boost poor responders with HDCT seems successful.

## 14. An overview of the EURO-E.W.I.N.G. 99 protocol

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In 1999, the **Euro**pean **E**wing tumor **W**orking **I**nitiative of **N**ational **G**roups came together in a treatment protocol for Ewing family tumors. It is a randomized, prospective, multi-centre, international study, linking UKCCSG, GPOH, SFCE (formerly SFOP), EORTC-STBSG and SIAK, in cooperation with EBMT regarding high dose therapy. In 2002, the Swedish paediatric solid tumor group, VSTB, decided to join the protocol. In 2003, COG joined the $R2_{pulm}$ arm.

The rationale behind the protocol and the randomization choices (R1, $R2_{loc}$ and $R2_{pulm}$), according to prognostic indicators (presence or absence of metastatic disease, site of metastases, local therapy options and histological response to chemotherapy and initial tumor volume), will be presented.

A study update including 1389 patients, based on data exchange February 2005, will be presented. Accrual according to study arms will be shown. 604 patients have been randomized so far. Swedish patients have been included.

No outcome data for the randomized arms have yet been released by the DMC. The numbers of randomized events in R1 and $R2_{loc}$ have recently triggered a second and first DMC-check, respectively. Preliminary data for the non-randomized R3 arm will be presented.

## 15. Surgical treatment of bone metastases in the pelvis and extremities. Overview of planned thesis. A SSG project

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*Introduction* The aim of surgical treatment of bone metastases, myeloma or lymphoma is to remove pain and maintain function for improved quality of life. In 1999 the Scandinavian Sarcoma Group (SSG) initiated the Skeletal Metastasis Register as a multi-centric, prospective study to provide a scientific basis for treatment recommendations of bone metastases in the pelvis and extremities.

*Patients and methods* More than 700 patients with skeletal metastases surgically treated at 9 Scandinavian Sarcoma Centres have been included in the study.

Studies to be included in a planned thesis:
***1. Survival after surgery for bone metastases in the pelvis and extremities.*** The study was presented at the SSG meeting in St. Petersburg May 2004 and published in Acta Orthop Scand Suppl April 2004.
***2. Functional outcome and pain after surgery for bone metastases in the pelvis and extremities***. The study was presented as a poster at the CTOS meeting in Montreal, Canada november 2004 and are presented at the SSG meeting in Reykjavik, Iceland May 2005.
***3. Complications after surgery for bone metastases in the pelvis and extremities.*** The data are collected and analyzed.
***4. Incidence of operative treated bone metastases.*** An epidemiology study in Aarhus Amt, Denmark. Data are collected.
**5. Patients treated surgically for bone metastases at Sarcoma Centres. Selected material?** A validity study. Planned to start 2006.

## 16. Biallelic somatic inactivation of the *NF1* gene through chromosomal translocations in a sporadic neurofibroma

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*Introduction* NF1 is caused by constitutional mutations in the *NF1* gene, located in chromosome band 17q11. Whereas the involvement of the *NF1* gene in neurofibroma development in NF1 patients has been fairly well characterized, the significance of inactivation of this gene in sporadic neurofibromas remains less well investigated. Inactivation of both copies of *NF1* has been described in a few neurofibromas from NF1-patients, and LOH at the same locus has been reported in additional cases. We report the cytogenetic and molecular cytogenetic findings in a sporadic neurofibroma that at G-banding analysis showed a translocation between one chromosome 2 and the long arms of both copies of chromosome 17.

*Method and results* FISH analysis using a set of three BAC clones covering the entire coding region of *NF1* revealed the complete loss of one allele and the deletion of the 5' portion of the second allele as a result of two translocation events.

*Conclusion* To the best of our knowledge, this is the first demonstration of a somatic biallelic inactivation of the *NF1* gene in neurofibroma, providing further evidence for the importance of *NF1* inactivation also in sporadic neurofibromas.

## 17. The myxoid/round cell liposarcoma (MLS/RCLS) fusion oncogene FUS-DDIT3 and the normal DDTI3 induce a liposarcoma phenotype in SCID mouse xenografted human fibrosarcoma cells

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*Introduction* MLS/RCLS is characterized by a specific fusion oncogene FUS-DDIT3. We investigated the role of this oncogene in development of liposarcoma.

*Materials and methods* FUS-DDIT3, the normal DDIT3 and the sequences encoding the first 180 amino acids of FUS was transfected to a human low differentiated sarcoma cell line. Severe combined immune deficient (SCID) mice were used as recipients of transfected and wild type HT1080 cells. Transfected and wild type HT1080 cell lines were treated in vitro with adipogenesis induction medium. Microarray based comparison of the HT 1080, the transfected cells and an MLS/RCLS derived cell line was performed.

*Results* FUS-DDIT3 and DDIT3 expressing cells grew as liposarcomas in SCID mice. The tumors developed a capillary network similar to the network of MLS/RCLS. DDIT3 transfected cells responded in vitro to adipogenic factors by accumulation of fat and developed a lipoblast like morphology. Microarray based comparison showed that the FUS-DDIT3 and DDIT3 transfected variants were more similar to the MLS/RCLS cell line.

*Conclusion* FUS-DDIT3 and the normal DDIT3 induce a liposarcoma phenotype in primitiv sarcoma cell line. MLS/RCLS may develop from cell types other than preadipocytes and may explain the preferential occurence of MLS/RCLS in non adipogenic tissues.

## 18. Irradiation of myxoid/round cell liposarcoma induces growth arrest and lipogenic maturation

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*Introduction* We investigated the clinical and morphological effects of radiotherapy of myxoid/round cell liposarcoma (MLS/RCLS).

*Patients and methods* 33 primary and metastatic MLS/RCLS in 15 patients were treated with radiation. 27 of the 33 tumors were surgically removed after preoperative radiation (34-46 Gy) while 6 tumors were given radiotherapy alone (44-60 Gy). The pre-treatment diagnosis was in all 15 patients based on fine needle aspirates or histologic findings. Tumor size was measured by CT or MRI before and after radiotherapy in 30 tumors. 13 tumors from 11 patients were genetically characterised before and/or after radiation therapy.

*Results* 24 of 30 irradiated tumors showed a median reduction in tumor volume of 43% and 6 lesions a median progression of 39%. All 27 surgically removed tumors revealed histological features of radiation response. The most striking morphological changes were lipogenic maturation, paucicellularity and hyalinization. 12 of 13 tumors analyzed before and / or after radiation therapy showed the FUS-CHOP translocation.

*Conclusions* Radiation therapy of MLS/RCLS induces growth arrest and lipogenic maturation and may facilitate resectability.

## 19. Rare variants of chondrosarcoma: Clear cell chondrosarcoma (CCC) and mesenchymal chondrosarcoma (MC), similarities and differences illustrated by 6 cases

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*Introduction:* CCC is considered low-grade malignant and consists histologically of lobular groups of bland clear cells in addition to cartilage of hyaline type. MC is highly malignant and shows a biphasic pattern with groups of small cells and presence of hyaline cartilage.

*Patients CCC:* 2 men and 1 woman, aged 41, 37, and 27, were admitted in 1973(Pt.1), 98(Pt.2), and 02(Pt.3). Pt.1 had a tumor removed from the femoral head with unknown margins. Between 1979 and 98 multiple bone and soft tissue metastases were resected. He got radiotherapy and radioactive isotopes, Metastron and Samarium, but died in 1999. Pt.2 had a tumor in the femoral head removed with negative margins. No evidence of disease since. Pt.3 had a tumor in L2, developed total pareses, decompressed, intralesional surgical margins. A local recurrence in the surgical field was removed by marginal margins followed by radiotherapy. No signs of disease since.

*MC* 3women, aged 39, 21 and 39, were admitted in 1986(Pt.4), 91(Pt.5) and 02 (Pt.6). Pt.4 had a tumur removed from her mamma, biopsy showed small cell sarcoma. Staging showed a tumor in the pelvis inside the acetabulum, and biopsy showed MC. Hemipelvectomi was done after neoadjuvant chemotherapy (T10 protocol. Between 1988 and 01 multiple bone and soft tissue metastases were removed. She died in 2003. Pt.5 had a tumor removed with intralesional margins from the left parietal/occipital region. Between 1994 and 05 she was operated for local recurrences 8 times, had external radiation, “gammaknife” treatment and radioactive isotope therapy with Boron and is alive with disease. Pt.6 had a MC in the thigh removed by a local surgeon, intralesional margins. Postoperative staging showed a tumor in the body of Th10. She was treated according to ISG/SSG III protocol, had the body of Th10 removed with free margins and good tumor response. The thigh was reexcised without signs of tumor and was radiated postoperatively. Mediastinal metastases were detected in Jan 05, she is now again treated by surgery, chemo- and radiotherapy.

*Conclusions* These diseases may have a long and protracted clinical course. CCC appears less malignant than MC. Long term follow up is mandatory.

## 20. Phase I-study: Exclusion of side-effects of the silver-coated tumor endoprosthesis in 20 patients with bone metastasis

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*Introduction* Deep infection of tumor endoprosthesis occurs in 10-20% and is a serious complication. Silver-coated tumor endoprostheses proved their effectiveness in a rabbit model (7% infection versus 47% in non-coated); toxicological side-effects could not be found (Gosheger et al., Biomaterials 2004).

*Patients and methods* A prospective study is conducted including 230 patients in 2 Phases: Phase I to exclude side-effects (20 patients with bone metastasis) and secondly prove the effectiveness. Phase I consists of patients undergoing resection of large metastasis with an impending (n=7) or a diagnosed fracture (n=11) or an infected osteosynthesis (n=2) with progressive metastatic disease. The age ranged from 32-82, the mean follow up was 16 months.

*Results* Preliminary results did not show any side-effects related to the silver-coating. Loosening, infection and material failure could not be observed. The laboratory parameters (GOT, GPT, creatinine, CK, etc.) did not show pathologic changes due to the silver-coated endoprosthesis. In two autopsies analysing the implant area no foreign body granuloma were found and the surface was stable.

*Conclusion* In summary, we have fond no side-effects of the silver-coated endoprosthesis up to now so it can be recommended in difficult patients suffering from infection, when postoperative radiotherapy will be given or in cases with problematic soft tissue coverage.

## 21. Pegylated interferon α2b as sole treatment of metastatic giant cell tumor of bone. Case report

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*Introduction* There is no consensus on the use of systemic treatment of inoperable metastases of giant cell tumors of bone. The main experience is with cytotoxic chemotherapy. Only a few reports on the use of antiangiogenic treatment with interferons are available. We report the response kinetics during treatment of advanced lung metastases from a giant cell tumor of the femoral neck with pegylated interferon in a 10-year-old girl.

*Patient and methods* The girl was 9 years and 10 months old in July 2002 when she had curettage and cementation of a lytic tumor of the right femoral neck. The morphological diagnosis was benign giant cell tumor. After 9 months she was admitted with right sided pleural fluid and multiple bilateral lung metastases with a maximum diameter of 6 cm. There was no local recurrence. Core needle biopsy of the largest metastasis indicated benign giant cell tumor. There were no malignant cells in the pleural fluid. Reevaluation of the morphological material did not indicate any signs of osteosarcoma or other malignancy. She was treated with pegylated interferon α2b at an initial dose of 0.5 µg/kg s.c. weekly. The dose was increased gradually and reached 1 µg/kg weekly in August 2003.

*Results* The only side effects were temporary minor flu-like symptoms and insomnia. After 5 months a gradual reduction of pleural fluid and volume reduction of lung metastases were observed. The latest CT-observation, 20 months after start of interferon treatment, showed complete regression of the pleural fluid and further regression of all metastases, some of the smallest lesions had disappeared completely. As there were no side effects and a continuous response we decided to continue interferon treatment with unchanged doses and postpone resection until maximum response.

*Conclusion* This case raises questions, e.g., of how long time the treatment should last, the optimum drug doses and optimum timing of eventual metastasectomies. Due to the rarity of the condition cooperative studies are necessary.

## 22. Current status of the quality of life and long- term morbidity project in survivors of extremity bone sarcoma

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*Introduction* This is a SSG study to evaluate function, long-term morbidity and quality of life (QoL) in survivors of extremity localized osteosarcoma or Ewing sarcoma minimum 5 years after treatment.

*Patients and methods* 57 patients (>15 years of age) from Norway and 24 patients from Sweden are included by Jan 05. The function was evaluated according to Enneking's system and TESS and QoL by SF 36 and HADS.

*Results* 81 pts have answered the questionnaires (42 men) and 70 pts have been examined. Median age at follow up was 29 years, and median time since surgery was 11 years. Limb-sparing surgery was done in 57. Median TESS score was 89% (43-100) and median Enneking score was 73% (17-100). The amputated patients had a significantly lower Enneking score compared to the limb sparing ones, but the TESS score was similar. Both men and women had a significantly lower score in the Physical health components of SF-36 compared to a comparison group. There was no significant difference in the SF-36 scores between the limb-sparing and the amputated patients.

16 patients had other diseases than cancer and 39 used some kind of medication. 26 pts had bilateral hearing loss. 7 women and 11 men had become a parent. 38 had higher education. 45 were working full time. 36% of the women and 17% of the men had increased anxiety score (HADS-A$\geq$8) and 12% of the men had increased depression score (HADS-D$\geq$8). 22 pts had once thought of suicide.

*Present status* The final analysis will be performed after examination of the remaining 11 patients.

## 23. SSG Registry, abdominal sarcomas

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## 24. Gastrointestinal stroma tumors (GISTs) and PET

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Intra-abdominal sarcomas and GISTs should be managed in a multidisciplinary unit to ensure the best outcome. The radiological evaluation of these tumors entails CT, MRI and endoscopic ultrasound. The radiological features are often complex.

For the treatment of GISTs a new drug has been developed, imatinib mesylate. The treatment response is myxoid degeneration, which often is accompanied by unchanged tumor size. In these cases the RECIST criteria for evaluating treatment response cannot be used. In the literature there are several reports of using positron emission tomography (PET) as a reliable mean of early evaluation of treatment response. Thus PET, although its relatively high cost and low accessibility, can be the method of choice in the evaluation of tumor response to imatinib mesylate.

## 25. Gastrointestinal stromal tumors with *KIT* exon 11 deletions are associated with poor prognosis

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*Introduction* Gain-of-function mutations in the *KIT* receptor tyrosine kinase gene and rare mutations in the platelet derived growth factor receptor alpha (*PDGFRA*) gene are early, important events in gastrointestinal stromal tumor (GIST) development. Different mutations are reportedly associated with distinctive phenotypes and potentially clinical behavior. We attempted to detect a correlation between mutation type, phenotype and clinical course in a population-based series of GIST with longterm follow-up.

*Materials and methods* Genomic DNA was extracted from archival tumors of 233 GIST patients and analyzed for *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12 and 18 mutations using denaturating high performance liquid chromatography (dHPLC) and bidirectional direct sequencing.

*Results KIT* exon 11 mutations were detected in GIST from 117/233 patients (69 deletions, 27 missense mutations, and 21 duplications); wild type (WT) *KIT* and *PDGFRA* were detected in 102; *KIT* exon 9 and exon 17 mutations in 7 and 1, respectively; and *PDGFRA* exons 12 and 18 mutations in 3 each. *KIT* exon 11 deletions were detected in a significantly higher proportion of high risk or overtly malignant groups and significantly associated with a higher risk score (>7) compared to those with WT. *KIT* exon 11 deletions adversely affected disease-free survival. *KIT* exon 11 duplications and exon 9 mutations were found exclusively in gastric and small intestinal GIST, respectively.

*Conclusions KIT* exon 11 deletion is an independent adverse prognostic factor in patients with GIST. Different *KIT* and *PDGFRA* mutations in GIST are associated with certain phenotypes.

## 26. Pathology of abdominal sarcomas

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Sarcomas in the abdominal region include a broad spectrum of histological entities. Liposarcoma and leiomyosarcoma constitutes two thirds of the sarcoma in the retroperitoneum. Other tumors in this region are solitary fibrous tumor, gastrointestinal stromal tumor (GIST), malignant peripheral nerve sheath tumor, malignant fibrous histiocytoma (MFH) and fibrosarcoma.

The most abundant subtypes in the *intraabdominal region* are GIST, but leiomyosarcoma. MFH, intra-abdominal desmoplastic small round cell tumor, rhabdomyosarcoma, and synovial sarcoma can also been seen.

*Gynecological sarcomas* are mostly leiomyosarcoma or endometrial stromal sarcoma. The majority of the *GISTs* are localised in the stomach followed by the small intestine and 10% at other sites (oesophagus, colon, rectum, mesentery and omentum). These tumors are thought to arise from mesenchymal stem cells, which also give rise to the interstitial cells of Cajal. Histology shows a cellular tumor with spindle and epithelioid cells. The GISTs have an immunoprofile which helps to separate them from leiomyomatous tumors. Generally there is a strong reaction with antibodies to CD117 and CD34, and negative findings for desmin and p S-100. GISTs have previously been overlooked and it is therefore recommended that all spindle cell tumors in the abdominal region should be analyzed with antibodies to CD34, CD117, p S-100, actin, desmin, SMA, AE1/AE3 and vimentin. C-kit mutations are found in most tumors. This gives a gain-of-function mutation leading to spontaneous tyrosine kinase activation. The size and the mitotic count/50 HPF are currently used for defining the risk of aggressive behaviour in GISTs. Patients with suspect abdominal sarcomas should be referred to a sarcoma centre without attempts to perform an open biopsy.

## 27. Surgery of retroperitoneal and gastrointestinal soft tissue sarcoma

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Surgery remains the cornerstone of the treatment of retroperitoneal and gastrointestinal soft tissue sarcoma (STS). The aim is to achieve complete resections with microscopically tumor-free margins (R0- resection) which requires en bloc resection of surrounding tissue and also most often adjacent organs. Careful preoperative evaluation is mandatory to reach best results. R0 resection seems to be of importance, both in the management of primary as well as in recurrent disease. However, adequate management at the time of primary presentation is likely the best way to improve the chance for long-term survival. Adjuvant therapy may in selected patients with high grade tumors enhance outcome.

The treatment of gastrointestinal stromal tumors (GIST) has changed tremendously with Imatinib. However, surgery is still the main treatment in primary GIST since it has become evident that many patients, who initially respond, develop resistance, sometimes in just one of several lesions. Many new questions have been raised as how to manage with neo-adjuvant/ adjuvant treatment, the role of surgery in poor and partial responders, patients who develop resistance and patients with metastatic disease or local recurrence.

A multidisciplinary approach including surgeons, medical oncologists, radiologists, and pathologists is crucial for the optimal management of these patients.

## 28. Oncological treatment and clinical outcome of GIST

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Imatinib is considered as the standard treatment of metastatic GIST. The starting dose is 400 mg once daily, taken with food. A higher dose (800 mg/d) may result in a longer progression-free survival than the standard dose of 400 mg/d, but with greater toxicity, cost, and no proven survival advantage. Approximately 65-70% of patients with metastatic GIST respond to imatinib, 20% have stabilized disease, and 10% show primary resistance to imatinib. The median duration of response is approximately 2 years, but the longest responses now approach 5 years. The likelihood of response is associated with the type of mutation in the *KIT* and *PDGFRA* genes, GISTs with wild-type *KIT* being least likely to respond. Resistance to imatinib is likely multifactorial, but second mutations in the ATP binding pocket of the kinase domain are an important cause for acquired resistance. SU11248 has shown most convincingly activity in the treatment of imatinib-resistant GIST, but several other compounds are being evaluated including PTK787, BMS354825, and AMG706. Adjuvant treatment of GIST with imatinib is regarded experimental. Randomized trials including the SSGXVIII/AIO trial that compares 12 vs. 36 months of adjuvant imatinib in the treatment of high-risk GIST are ongoing.

## 29. The SSG Registry of soft tissue sarcomas

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3 504 patients with soft tissue sarcoma have been reported from Finland, Norway and Sweden to the SSG Registry since 1986. Up to 2004, the 2 major centers in Norway and 3 of 5 centers in Sweden and 1 in Finland have continuously reported new patients and follow-up. Follow-up has been reported in more than 90% of the patients. Among those still alive the median follow-up was 5 years.

Overall referral of patients before open biopsy or surgery has improved from 61% before 1990 to 71% after 2000. The improvement was mostly achieved during the last years and for deep lesions. During 2000-2004, 85% of patients with deep lesions were referred to a sarcoma center before surgery.

2 702 patients without metastases at diagnosis were operated for primary tumor at a center. The amputation rate was 6%. A wide surgical margin was reported in 59% of the patients and has not changed over time – it was actually lowest after 1999, 55%. The use of adjuvant treatment has increased considerably. Before 1990 only 16% had radiotherapy compared 38% after 1999. Similarly, chemotherapy use has increased from 3% to 14%.

The overall 5-year local control rate was .85. For patients treated before 1990 it was 0.78 compared to .87 during 1995-1999. For those treated 2000-2004 the 3-year rate was .94. The metastasis-free survival has also improved slightly, from .70 before 1990, to .75 1995-1999.

The improved outcome was not only due to increased referral of patients with small, subcutaneous tumors. Hence, local control for deep seated lesions improved from .76 (1986-1989) to .86 (1995-1999). The metastasis free survival improved from .64 to .70.

During the almost 20 years of accrual to the SSG Registry, we found a continuous improvement in outcome in terms of local control and survival. It is difficult to pinpoint one factor as the most decisive for the progress. Rather, it appears to be a result of better management leading to better referral process and better multimodality treatment.

## 30. Imaging of soft tissue sarcoma

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The radiological diagnosis of soft tissue sarcoma is usually relatively easy, expecially if the lesion is subfascial, but there are lesions with unusual appearance that can be challenging for the radiologist. Also there are some benign lesions which can simulate sarcoma. MRI is the method of chocie for the imaging of soft tissue tumors. The imaging sequences necessary in the diagnostic work-up of soft tissue tumors will be discussed as well as the main diagnostic pitfalls. The value of PET and new MR imaging sequences for evaluation of adjuvant tumor therapy will also be addressed.

## 31. Mutations of *PIK3CA* in soft tissue sarcomas

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The somatic mutations in soft tissue sarcomas (STS) occur in two major contexts; as tumor-specific translocations that result in type-specific fusion proteins (e.g. in synovial sarcomas and myxoid liposarcomas) or as part of a complex karyotype with multiple acquired changes (e.g. in high-grade leiomyosarcomas and pleomorphic sarcomas).
Recently, somatic mutations in the *phosphatidylinositol 3-kinase* (*PIK3CA)* gene, which belongs to a family of lipid kinases that regulate cell growth and proliferation, has been demonstrated in several types of human carcinomas. Somatic genetic alterations of the p110α subunit of *PIK3CA* have been reported in e.g. breast cancer, colorectal cancer, and ovarian cancer.

Since the PI3K-AKT signaling pathway may be involved in STS, we evaluated *PIK3CA* mutations in a mixed series of 50 STS and identified missense mutations within exons 9 and 20 in a small subset of the tumors. Hence, also mesenchymal tumors may develop through mutations in the *PIK3CA* gene.

## 32. Synovial sarcoma: molecular, biological and clinical implications

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Doctoral Thesis

The rare soft tissue tumor synovial sarcoma (SS) is cytogenetically characterized by the recurrent and specific t(X,18)(p11.2;q11.2). This translocation results in fusion of the 5' part of the *SS18* gene, and the 3' part of either the *SSX1, SSX2* or *SSX4* gene. The fusion gene *SS18-SSX* constitutes a valuable molecular tool for diagnosis of SS.

*Study I* A previously *SS18-SSX* negative case was re-diagnosed as SS after method optimization. Molecular information of the oncogenic properties of *SS18-SSX* was provided, since an unusual breakpoint potentially encoded an altered fusion protein.

*Study II* A renal tumor was re-investigated after a highly aggressive behavior, usually not compatible with the originally diagnosis of hemangiopericytoma. After molecular confirmation of the presence of *SS18-SSX*, the new diagnosis was SS.

*Study III* Antisense strategy was used in combination with a cDNA microarray in order to investigate downstream events of *SS18-SSX*. The gene expression profiles of *SS18-SSX* inhibited cells and non-inhibited were compared, showing altered expression levels of genes involved in cancer-relevant functions.

*Study IV SS18-SSX* is able to stabilize expression of cyclin D1, and IGF-1R expression is associated with poor prognosis in SS. Here we suggest a proliferative role of *SS18-SSX* since its expression maintained cell cycling even without mitogenic signals. Inhibition of the IGF-1R caused massive cell death. A potential therapeutic strategy could use the continued cell cycle and kill SS cells by inhibition of IGF1-1R.

## 33. Prognostic factors in soft tissue sarcoma - tissue microarray for immunostaining, the importance of whole-tumor sections and time-dependence

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*Introduction* In adult soft tissue sarcoma (STS) of the extremities and trunk wall various prognostic factors are included in the many prognostic systems currently in use. Several immunohistochemical (IHC) expression of biological markers have been suggested to be prognostic. The time-dependence of prognostic factors in STS is unclear.

*Materials and methods* Based on the SSG register, and utilizing pathology peer-reviewed tumors, immunohistochemical expression of Ki-67 in 11 malignant fibrous histiocytomas (MFH) was analysed with tissue microarray technique (TMA), and with conventional staining methods for validation of TMA in STS. TMA was then used to study the IHC expression of multiple markers (Ki-67, p53, cyclin A, bcl-2, ß-catenin, CD44, and Pgp) in 218 MFH and in 140 mixed STS. In the mixed STS series whole-tumor sections were used for assessment of necrosis, vascular invasion and peripheral tumor growth pattern and TMA was performed in the peripheral tumor growth zone.

*Results* The use of TMA was validated for Ki-67 in STS. In the 218 MFH only tumor size and Ki-67 provided independent prognostic information. In the 140 mixed STS, vascular invasion, hazard ratio (HR) 3.5, tumor necrosis (HR 2.8), and an invasive tumor growth pattern (HR 3.2) were strong independent prognostic factors for metastasis, and growth pattern also for local recurrence (LR). Histological malignancy grade, tumor size, and depth were not of independent prognostic value. When TMA was performed from the peripheral tumor growth zone, the IHC expression of Ki-67 (HR 1.9), ß-catenin (HR 2.7), CD44 (HR 2.1) and Pgp (HR 2.4) were independent prognostic factors. Prognostic factors were found to be time-dependent, and lost their prognostic value after 2 years, whereas LR was a strong prognostic factor for metastasis whenever it occurred.

*Conclusions* TMA is applicable in STS for assessment of IHC staining patterns, but should probably be performed in the peripheral tumor growth zone. Prognostication of metastasis in STS can be improved by considering IHC expression of Ki-67, and ß-catenin, CD44 and Pgp are candidate markers for further prognostic evaluation. Whole-tumor sections are of value for assessment of morphological prognostic factors in STS. Vascular invasion, necrosis and peripheral tumor growth pattern are strong prognostic factors for metastasis, and

growth pattern also for LR. Tumor-related prognostic factors are time-dependent.

## 34. Treatment results of SSG XIII and a proposal for new protocol

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*Introduction* The SSG XIII protocol for high-risk soft-tissue sarcoma was opened for recruitment July 1998. We here report preliminary results.

*Patients and methods* Adult patients with high-grade STS of the trunk wall or extremities featuring two or more of the following risk factors; size $\geq$ 8 cm, necrosis and vascular invasion, are eligible. All patients are scheduled for 6 adjuvant cources of a doxorubicin/ifosfamide combination and radiotherapy is delivered by a hyperfractionated/accelerated regimen according to margins and tumor depth.

*Results* By March 05, 101 patients have been recruited. 11 pts are ineligible leaving 90 patients for this analysis. Radiotherapy has been given to 80% of the patients and 92% have received all 6 cources of chemotherapy. With a median follow-up of 42 months the projected 5-year metastases-free survival is 64% (+/- 12%). 11 patients have experienced a local recurrence. 2 treatment related deaths are reported, from pulmonary embolism and nephrotoxicity.

*Conclusion* The regimen is well tolerated with most patients receiving the planned therapy with acceptable acute toxicity. The survival data is promising compared to historical controls. A proposal for a new protocol based on the current protocol, SSG experience and international literature will be presented.