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Abstract: Background: Doxorubicin is an effective drug against rhabdomyosarcoma (RMS), but its role in combination with an established multidrug regimen remains controversial. The RMS2005 trial evaluated the possible benefit of early dose intensification with doxorubicin in patients with non-metastatic RMS.
Methods: For this multicentre trial we recruited patients aged 6 months-21 years with a previously untreated RMS from 108 hospitals in 14 countries. Patients with embryonal RMS incompletely resected and localized at unfavourable sites with or without nodal involvement or with alveolar RMS without nodal involvement were considered at high risk of relapse and were randomly assigned (1:1) to either 9 x 21-day cycles of IVA (ifosfamide 3g/m2 days 1 and 2, vincristine 1*5 mg/m2 day 1, actinomycin-D 1*5 mg/m2 day 1) or 4 cycles of IVA with doxorubicin 30 mg/m2 on days 1 and 2 (IVADo) followed by 5 cycles of IVA. The primary endpoint was 3-year event free survival (EFS) in the intention to treat population. The trial is registered with EudraCT- Nr: 2005-000217-35.
Findings: Between October 1, 2005 to December 17, 2013 242 patients were randomized in each arm. Median follow-up was 63*9 months (IQR 44*6 - 78*9). The 3-yr EFS was 67*5% (95% CI 61*2 - 73*1) in the IVADo arm and 63*3% (95% CI 56*8 - 69*0) in the IVA arm (HR 0*87, 95%CI 0*65-1*16; p=0*3352). The 3-yr OS was 78*3% (95%CI 72*4 - 83*0) and 80*6% (95% CI 74*9 - 85*1) in the IVADo vs. IVA arm (HR 1*17, 95%CI 0*82-1*67; p=0*3736). Grade 3-4 leukopenia, anaemia, thrombocytopenia, number of infections and gastrointestinal toxicity were significantly more common with IVADo.
Interpretations: The addition of dose intensified doxorubicin to standard chemotherapy failed to show a significant improvement in the outcome of

patients with high-risk non metastatic RMS. Therefore the IVA regimen remains the standard of care for patients with localised RMS in Europe.
Funding: Fondazione Città della Speranza, Italy. Association Léon Berard Enfant Cancéreux, France.

The role of doxorubicin in the treatment of rhabdomyosarcoma: the European paediatric Soft tissue sarcoma Study Group RMS2005 randomized controlled phase 3 trial

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ABSTRACT

Background: Doxorubicin is an effective drug against rhabdomyosarcoma (RMS), but its role in combination with an established multidrug regimen remains controversial. The RMS2005 trial evaluated the possible benefit of early dose intensification with doxorubicin in patients with non-metastatic RMS.

Methods: For this multicentre trial we recruited patients aged 6 months-21 years with a previously untreated RMS from 108 hospitals in 14 countries. Patients with embryonal RMS incompletely resected and localized at unfavourable sites with or without nodal involvement or with alveolar RMS without nodal involvement were considered at high risk of relapse and were randomly assigned (1:1) to either 9 x 21-day cycles of IVA (ifosfamide 3g/m^2 days 1 and 2, vincristine 1·5 mg/m^2 day 1, actinomycin-D 1·5 mg/m^2 day 1) or 4 cycles of IVA with doxorubicin 30 mg/m^2 on days 1 and 2 (IVADo) followed by 5 cycles of IVA. The primary endpoint was 3-year event free survival (EFS) in the intention to treat population. The trial is registered with EudraCT- Nr: 2005-000217-35.

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Interpretations: The addition of dose intensified doxorubicin to standard chemotherapy failed to show a significant improvement in the outcome of patients with high-risk non metastatic RMS. Therefore the IVA regimen remains the standard of care for patients with localised RMS in Europe.

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INTRODUCTION

Rhabdomyosarcoma (RMS) is an aggressive tumour that can develop in almost any part of the body and thought to arise from primitive mesenchymal cells. It is the commonest form of soft tissue sarcoma in children and young adults and accounts for approximately 4-5% of all childhood malignancy with an annual incidence of 4·5 per million children under the age of 20. The peak incidence is seen early in childhood with a median age at diagnosis of about 5 years.[1]

Two main forms of RMS have been identified on histological appearance: the embryonal subtype accounts for approximately 80% of all RMS and carries a better prognosis and the alveolar subtype (15-20% of RMS) characterized by the fusion of the FOXO transcription factor gene to either the PAX3 or PAX 7 transcription factor genes and associated with poorer outcomes.

Although RMS is an aggressive tumour, survival rates for patients with non-metastatic disease have improved over the last 30 years owing to the application of a multimodality approach that includes chemotherapy, coordinated with surgery and, in the majority of cases, radiotherapy.

This strategy has been promoted by several cooperative Groups, the largest being the Children Oncology Group (COG) in the USA and the more recently founded European paediatric Soft tissue sarcoma Study Group (EpSSG) in Europe. A series of studies have established that a chemotherapy regimen including an alkylating agent, cyclophosphamide or ifosfamide, combined with vincristine and actinomycin D (VAC or IVA) represents the standard combination for patients with RMS. [2,3]

Different attempts to improve cure rates by adding other drugs to this combination have been made for patients with unfavourable prognostic factors such as alveolar histology or a primary tumour arising in unfavourable sites but failure free survival remained around 55-70%. [4,5] However to date no randomised trial has shown a survival advantage compared to standard VAC or IVA.[3]

Doxorubicin has often been used in the treatment of patients at high risk of relapse or those with metastatic disease because the response rate to doxorubicin, used as single drug in the up front window setting, is one of the highest among chemotherapeutic agents. However its contribution when combined with an established multidrug regimen remains controversial .[6,7]

The E*p*SSG RMS2005 study incorporated a trial with two consecutive independent randomisations to investigate the benefit of early dose intensification with doxorubicin and the value of a maintenance treatment after standard therapy in patients with high risk localized RMS. We report here the results regarding the doxorubicin dose intensification question.

METHODS

Study Design and participants

This prospective phase III randomised clinical trial was conducted at 108 Hospitals in 14 Countries (Argentina, Belgium, Brazil, Czech Republic, France, Israel, Italy, Norway, Slovakia, Slovenia, Spain, The Netherlands, United Kingdom, and Ireland). All participating centres were required to obtain written approval from their local authorities and ethics committees and written informed consent from the patient and/or from their parents or legal guardians. The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines (European Union Drug Regulating Authorities Clinical Trials EUDRACT No. 2005-000217-35).

The eligibility criteria were: age > 6 months to < 21 years; pathologically proven diagnosis of RMS; no evidence of distant metastatic lesions; previously untreated except for primary surgery; no pre-existing illness preventing treatment; no previous malignant tumours and an interval between diagnostic surgery and systemic treatment less than 8 weeks. Histopathological material had to be available for central diagnostic review even though risk group and randomisation were assigned on the basis of the local assessment. Molecular confirmation of the presence of a FKHR translocation was not mandatory to classify a tumour as alveolar.

Each patient was assigned to a specific risk group according to six prognostic factors identified in a common retrospective analysis of European protocols: pathology (embryonal vs. alveolar), IRS Group, tumour primary site, nodal involvement, tumour size and patient age (Figure 1). Patients eligible for the RMS 2005 trial who were assigned to the High Risk group were eligible for the IVA vs. IVADo randomisation. Patients with alveolar paratesticular RMS were excluded in recognition of the better prognosis of this group of patients.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to receive the standard or the investigational chemotherapy. The randomisation was performed using a web based system and was stratified, in block sizes of four, by enrolling country (France, Italy, UK and Eire, Brazil and Argentina, other countries) and risk subgroup (E, F and G). Neither investigators nor patients were blinded to treatment allocation.

Procedures

The diagnostic work up included CT and/or MRI of the primary tumour, chest CT scan, radionuclide bone scan, bone marrow aspirate and biopsy. FDG-PET was optional and a baseline echocardiogram was also required.

Primary tumour resection was recommended only for patients where a complete resection was considered feasible without harming the patient, otherwise a biopsy was requested to establish the diagnosis.

Standard chemotherapy was a combination of ifosfamide 3 g/m^2 on day 1 and 2, vincristine 1·5 mg/m^2 (weekly during the first 7 weeks then only on day 1 of each cycle) and actinomycin D 1·5 mg/m^2 on day 1 (IVA). This same regimen with the addition of doxorubicin 30 mg/m^2 on day 1 and 2 comprised the investigational arm (IVADo). Four cycles of chemotherapy had to be administered in the initial part of treatment before local control measures were implemented. Subsequently both arms received 5 cycles of IVA (Figure 2). In children aged 6 to 12 months or less than 10 kg body weight drug doses were calculated according to body weight: vincristine 0·05 mg/kg/dose, actinomycin D 0·05 mg/kg/dose, ifosfamide 100 mg/kg/dose and doxorubicin 1 mg/kg/dose.

Tumour dimensions were measured at diagnosis using the 3 maximum diameters (a= length, b = width, c = thickness) and tumour volume estimated with the following formula: π/6 x a x b x c. Response assessment in patients with macroscopic residual disease after initial surgery (IRS group III) was evaluated at week 9 choosing, as far as possible, the diameters selected at diagnosis. and at the end of the treatment (with further assessments at the clinicians' discretion) and defined as follow: complete response (CR), clinically or histologically confirmed complete disappearance of disease; very good partial response (VGPR): tumour volume reduction more than 90%; partial response (PR): tumour volume reduction more than 66% but less than 90%; minor response (MR), reduction greater than 33% less than 66%; no response or stable disease (SD): less than 33% reduction in tumour volume; progressive disease (PD), any increase in tumour size of any measurable lesion or appearance of new lesions. When all the 3 diameters were not available two dimensions were used to establish the tumour response with corresponding 2D cut offs. All responses had to last at least 4 weeks without evidence of tumour progression or relapse

After the initial 3 cycles of chemotherapy (week 9) a full clinical and radiological assessment of the tumour was performed. Patients in complete remission or with evidence of tumour volume reduction > 33% continued the allocated treatment. In case of SD or PD patients were considered off study and the protocol recommended to switch to different chemotherapy regimens, including doxorubicin if initially allocated to the IVA arm, in the attempt to obtain a better tumour response.

The ‘local treatment’ of the tumour was planned after the tumour response assessment and implemented at week 13. Where a residual mass was present, surgical resection was encouraged if

free margins without organ or function impairment were anticipated. Marginal resection in sites where complete resection was not deemed possible was accepted, provided that it was always followed by radiotherapy.

Radiotherapy represented the only local treatment possible for patients that could not undergo secondary surgery due to the tumour location (e.g. parameningeal RMS). Radiotherapy doses were delivered according to histology, chemotherapy response and surgical results: 41·4 Gy were given to patients with alveolar RMS in IRS Group I or II, for patients in IRS Group III who achieved a complete remission after secondary surgery, and to patients with embryonal RMS that achieved a complete remission with initial chemotherapy; 50·4 Gy for cases of incomplete or unfeasible secondary resection. A boost of 5·4 Gy in 3 fractions to the residual tumour was allowed for large tumours with poor response to chemotherapy. Radiotherapy to the involved lymph node sites was recommended at a dose of 41·4 Gy independently of histology and surgical resection. Treatment was delivered with megavoltage photons, one fraction per day, five days per week, with conventional fraction sizes of 1·8 Gy per day. In patients with large abdominal or cranio-spinal fields, or in patients less than 3 years old smaller fractions were allowed (e.g. 1·5 Gy).

The clinical target volume (CTV) was defined as the initial gross tumour volume (GTV) + 1 cm in all directions, except for limb tumours where the longitudinal GTV to CTV expansion was 2 cm. The CTV to planning target volume margin was typically 1 cm. In patients receiving 50·4 Gy, the PTV was reduced by 1 cm after 41·4 Gy. In patients with orbital tumours, the initial radiation of the whole orbit was reduced to a PTV of the GTV + 1 cm after 36 Gy. At the start of the trial in 2005, 3D conformal radiotherapy plans were most commonly used, but the as the trial covered a period of increasing availability of more sophisticated radiotherapy planning and treatment delivery, advanced photon techniques such as intensity modulated radiotherapy became more commonly used. Alternative techniques such as brachytherapy, electrons, and proton beam therapy were permitted when clinically appropriate. The protocol mandated doxorubicin therapy to be completed before starting radiotherapy. Actinomycin D was omitted during radiotherapy.

Further assessment of the tumour was performed after the 9th chemotherapy cycle. Patients with high risk RMS (included in the first randomisation or excluded for whatever reason) in complete remission were eligible for the second randomisation: to stop treatment or to continue with the administration of weekly vinorelbine and low dose continuous oral cyclophosphamide for 6 months. This randomisation was closed in December 2016 and results will be reported separately.

Supportive care was according to institutional guidelines. Primary prophylaxis with granulocyte colony-stimulating factor (G-CSF) was not mandated, however it was recommended during IVADo treatment for life-threatening neutropenic infection, or treatment delay > 1 week due to neutropenia

in a previous cycle.

Outcome

The primary endpoint was event-free survival (EFS), defined as the time from random assignment to the time of the first event defined as: death for all reasons, progression of disease (in cases where complete tumour remission was never achieved), relapse following previous complete remission, appearance of a new tumour and switch to second line chemotherapy in patients with unsatisfactory chemotherapy response (SD or PD) or time of the latest follow-up. Secondary end points were: overall survival (OS), measured as time from date of first randomisation up to death for all reasons or time of the latest follow-up; progression-free survival (PFS) measured as time from date of randomisation to tumour progression, relapse or time of the latest follow-up; response rate to initial treatment (9^{th} week) and toxicity according to NCI-CTC version 3.

Statistical analysis

The trial was originally projected to enroll 600 patients with high risk disease to detect an increase of 10% in the 3-year EFS with IVADo treatment, assuming a baseline 3-year EFS of 50% in the IVA arm, equivalent to a hazard ratio (HR) of 0·74. Overall, 343 events were needed to ensure a power of 80%, with a two-sided α level of 5%. Two interim analyses were planned after 1/3 and 2/3 of events, using an O'Brien-Fleming boundary for the efficacy boundary and the Harrington-Fleming-O'Brien process of repeated testing of the alternative hypothesis at an α level of 0·005 for futility monitoring.

An independent data-monitoring committee (IDMC) periodically monitored safety and efficacy during the study. The patients recruitment was slower than expected so in December 2011, the IDMC recommended a sample size re-estimation with a reduction in the HR to 0·65, maintaining the power of the study and extending the enrolment period. With these assumptions, a new sample size of 500 patients and 169 events and one interim analysis after observing 50% of events was planned.

At the time of the planned interim analysis, the IDMC recommended to continue the randomisation as planned, asking for a second interim analysis in December 2013. This analysis was performed when 79% of the expected information was available. The estimate of the HR was 1·024, IVADo vs IVA, with a p-value of 0·89. Repeated testing of the alternative hypothesis has been performed to assess futility (log relative risk estimate= 0·02414, standard error = 0·17288, log relative risk β = -0·4308, T=2·631) obtaining a p-value of 0·004, suggesting the study could be stopped for futility.

The randomisation IVADo vs. IVA was closed on the 17th December 2013.

Survival probabilities were estimated according to the intention to treat principle, i.e. including patients in the group to which they were assigned, whether or not they received the allocated treatment, using the Kaplan-Meier method and heterogeneity among strata of selected variables was assessed using the log-rank test. A sensitivity efficacy analysis for the per protocol population, i.e. eligible patients that received the allocated treatment, was performed. The 3-year and 5-year EFS, OS and PFS were reported with 95% confidence intervals (CIs), calculated according to the Greenwood's method. HRs with the 95% CI, calculated according to the Wald method, and p values for the interaction between treatment effect and any subgroup variable were estimated from the Cox regression model for event free survival and overall survival in relevant clinical subgroups of patients. For the primary end point analysis HR was adjusted for the stratification factors at randomization.

The description of treatment exposure was summarized using descriptive statistics (median, min, max). Since the dose intensity of doxorubicin was a critical factor in our study, we compared the time interval between the start of treatment and the administration of the fourth cycle in the two arms and the cumulative dose of doxorubicin administered having as target 240 mg/m^2. We included in this intention to treat analysis also those patients that did not complete the 4 cycles of chemotherapy or did not receive the complete dosage of doxorubicin.

Toxicities were analysed according to the actual treatment received. Comparison of distribution was performed with the χ^2 test.

Data collected as of June 16, 2017 was analysed using SAS software 9·4 (SAS Institute, Cary, NC).

Role of funding source

EpSSG designed and coordinated the trial. The funders had no role in the design of the study, data collection and analysis or writing the report. The corresponding author had the final responsibility for the decision to submit for publication on behalf of the EpSSG Board members.

RESULTS

Between 1 November 2005 and 16 December 2013, 645 patients with high risk characteristics were assessed for eligibility and 161 (25·0%) of them were excluded. Overall 484 patients were randomized: 242 in each arm (Figure 3). Thirty-three patients were found not to fulfil the eligibility criteria after the randomisation (20 assigned to IVA and 13 to IVADo), mainly due to incorrect staging or change of histological diagnosis. One patient, randomized to IVA regimen, rapidly progressed and was treated according to IVADo regimen. All these patients were included in the

analysis according to the intention to treat principle.

An incorrect staging for metastatic lesions or nodal involvement were found in 10 and 5 patients, respectively, while the size of tumour was incorrectly recorded in 2; one patient was affected by a genetic syndrome with cardiovascular anomalies preventing the administration of anthracyclines. The diagnosis of RMS was not confirmed in 8 patients and the subtypes was changed in 7 (5 embryonal to alveolar and 2 alveolar to embryonal). Six cases received a rapid review soon after the diagnosis and did not start the treatment while in 9 the diagnosis was changed when they had already received the treatment. Overall 410 (84·7 %) cases were submitted for central pathology review at national and/or international level. Fourteen patients allocated to the IVA arm did not start the allocated treatment after randomisation but none in the IVADo group. This is explained by the fact that EpSSG protocols recommended IVADo treatment for patients in the very high risk or metastatic group so patients in the IVADo arm that were upstaged after diagnosis or staging review simply continued the treatment as allocated while those in the IVA arm changed to IVADo.

Patients and disease characteristics were well balanced between the 2 arms (Table 1). Patients were also balanced considering the treatment received after the 9 cycle of chemotherapy: 87 patients in the IVA arm and 89 in the IVADo arm received maintenance because included in the second randomized trial or by physician choice.

The median time between the start of treatment and the administration of the fourth cycle was similar in the 2 arms: 9·4 weeks (range 8·4-16) for IVADo and 9·3 weeks (5·9-15·9) for IVA. The doxorubicin median cumulative dose administered was 237·2 mg/m^2 (range 60·0-262·7). The tumour response rate (CR, VGPR, PR and MR) evaluated after initial chemotherapy was 88·9% vs. 92·3% for IVA vs. IVADo, respectively (p =0·24). Radiotherapy treatment was administered in 210 (86·8%) patients randomized to IVA and in 202 (83·5%) randomized to IVADo regimen. Two-hundred and thirty-four patients underwent at least one secondary surgery (118 in IVA and 116 in IVADo arm) and complete tumour resection was achieved in 149 of them (71 IVA and 78 IVADo).

The median survival follow-up for alive patients was 63·9 months (IQR 44·6–78·9): 63·2 months (IQR 45·2-77·7) in the control arm and 64·3 months (IQR 41·4-79·4) in the experimental arm. The 3-yr EFS was 67·5% (95% CI 61·2 – 73·1) in the IVADo arm and 63·3% (95% CI 56·8 – 69·0) in the IVA arm (HR 0·87, 95%CI 0·65-1·16; p=0·3352). The 3-yr OS was 78·3% (95%CI 72·4 – 83·0) and 80·6% (95% CI 74·9 – 85·1) in the IVADo vs. IVA arm (HR 1·17, 95%CI 0·82-1·67; p=0·3736) (Figure 4).

Overall 181 patients experienced an event. The type of event distribution was similar in the two arms, however a number of patients in the IVA arm switched to second line treatment for insufficient tumour response (Table 2). This switch was considered an event for the EFS calculation

but not for PFS that was 67·6 (95% CI 61·1-73·3) in the IVA arm at 3-yr and 68·1 (95% CI 61·7-73·6) in the IVADO arm, demonstrating that this phenomenon did not affect significantly on the overall trial results (p=0·97).

450 patients met the criteria for the per-protocol analysis: the 3-yr EFS was 68·8% (95% CI 62·3 – 74·4) in the IVADo arm and 63·1% (95% CI 56·4 – 69·1) in the IVA arm (HR 0·82, 95%CI 0·60-1·10; p=0·1924). The 3-yr OS was 79·2% (95% CI 73·3 – 84·0) and 81·1% (95% CI 75·2 – 85·7) in the IVADo vs. IVA arm (HR 1·13, 95%CI 0·78-1·65, p= 0.5101).

An analysis taking into account the most relevant clinical variables including age at diagnosis, gender, histological subtype, nodal involvement, primary tumour invasiveness, size, and site, did not show any difference among the two arms in any subgroup of patients (Figure 5).

Complete toxicity data for the initial chemotherapy phase (cycle 1 to 4) were available for 476 patients (Table 3). Considering grade 3-4 together, children treated in the IVADo arm experienced significantly more myelotoxicity with leukopenia (p=0·0061), anaemia (p<0·0001) and thrombocytopenia (p<0·0001). The higher rate of myelotoxicity in the IVADo arm prompted the investigators to a more frequent use of G-CSF that was administered in the 37·7% of cycles in the IVADo arm vs 22·5% in the IVA arm. For non-haematological toxicity, patients included in the experimental arm experienced a higher number of infections (p < 0·0001). Approximately one third of children in the IVADo arm experienced grade 3-4 gastrointestinal toxicity (p < 0·0001) and this was mainly due to mucositis likely caused by the concomitant administration of doxorubicin and actinomycin D. The same combination was expected to increase the risk of veno-occlusive disease but only 3 patients in the IVADo arm experienced this type of toxicity vs. 5 patients in the IVA arm. No difference in acute cardiotoxicity were noted: 5 grade 3-4 events occurred in each arm). The number of patients with grade 3-4 thrombocytopenia was higher in the IVADo arm even in the following phase of treatment (5 cycles IVA in both arms) (p=0·0372), while no differences were observed for other toxicities.

Discussion

Doxorubicin is a very active drug against RMS as demonstrated by early studies performed initially in relapsed RMS[8] and more recently in patients with newly diagnosed metastatic RMS.[6] A phase II window study in children with newly diagnosed metastatic RMS demonstrated the activity of ifosfamide and doxorubicin with a 63% response rate after 12 weeks of treatment.[9] This is very similar to the 65% response rate obtained by the administration as initial treatment of 2 cycles of single agent doxorubicin 60 mg/m² over 2 days.[6]

However the role of doxorubicin role as part of a multidrug regimen is controversial. It is not clear whether its addition to an established regimen such as VAC or IVA improves the survival of patients with RMS. A possible benefit of doxorubicin addition must be carefully considered as the toxicity profile of the drug may worsen immunosuppression and gut toxicity in the short term and cause cardiotoxicity in the long term.

Different randomized trials performed by the Intergroup Rhabdomyosarcoma Study (IRS) Group have not shown a substantial difference in survival and progression free survival for patients with RMS treated with VAC or VAC plus anthracyclines. In the IRS-I protocol, the addition of 5 VadrC (vincristine, adriamycin, cyclophosphamide) courses to VAC did not improve the outcomes in patients with gross residual disease after surgery or metastatic disease at diagnosis (IRS clinical groups III and IV).[10] In the IRS-II study a similar comparison was performed, however doxorubicin was given repetitively in pulse combination with vincristine and cyclophosphamide (pulse VAdrC). This regimen was given alternating with VAC cycles and was compared with repeated VAC as the standard arm. Additionally the cumulative dose of doxorubicin (480 mg/m^2) was higher than in the IRS-I protocol. Despite this intensification the two arms showed similar results (EFS 75% vs. 70% p 0·84) and the authors concluded that doxorubicin did not offer any survival advantage and was more toxic.[11] The role of doxorubicin was further investigated in the IRS-III study showing conflicting results.[12] A randomised comparison showed a significant benefit from the addition of doxorubicin in patients in clinical IRS-group II (microscopic post surgical disease). This advantage however disappeared when the historical control from IRS-II protocol were considered in the analysis. Other patients' subgroups showed better results in the IRS-III study in comparison with those obtained in the IRS-II study but doxorubicin was included along with other chemotherapy agents making its contribution hard to determine. Overall, the investigators of the IRS-II and IRS-III studies concluded that that the precise role of doxorubicin in newly diagnosed patients required further study.[7]

It should be noted that in IRS Group studies the treatment schemes were based on the alternating administration of cycles VAC and VadrC, consequently the intervals between doxorubicin containing courses were wide, reducing the anthracycline dose-intensity.

In Europe a trial conducted by the Malignant Mesenchymal Tumour (MMT) Group of the International Society of Pediatric Oncology (SIOP) compared the IVA regimen against a six drug regimen containing anthracycline (epirubicin). No difference in survival was found between the two arms. Once again the anthracyclines dose-intensity was low because epirubicin was included in only 3 out of a total of 9 cycles.[5]

The possibility that increasing anthracylines dose-intensity may be of benefit for the patients is supported by a meta-analysis of several trials performed in patients with bone sarcomas. This analysis demonstrated that an induction treatment including doxorubicin in every course was better than a schema alternating doxorubicin with actinomycin D.[13] To explore this strategy we added doxorubicin to the IVA regimen avoiding to alternate courses with and without anthracyclines as has been done in previous studies. We also hypothesized that the use of IVADo in the initial part of treatment could have induced a higher number of tumour responses allowing a better local control with surgery and radiotherapy. The IVADo combination had previously been tested in a pilot study and was shown to be feasible.[14]

The RMS2005 trial confirms on a larger scale that the IVADo regimen is manageable. The doxorubicin dose intensity was maintained as demonstrated by the median interval between the initial and last IVADo and the median cumulative dose of doxorubicin administered. As expected there was significantly more toxicity in the patients receiving IVADo compared to the IVA group, particularly in terms of myelosuppression and mucositis.

Despite IVADo feasibility and the toxicity suffered by patients, our study clearly shows that the addition of doxorubicin did not add any meaningful benefit to patient's survival.

It is interesting that this result is in line with evidence progressively collected by other studies dedicated to paediatric tumours. Recent publications have demonstrated that doxorubicin can be omitted from the treatment plan of patients with standard hepatoblastoma or favourable histology stage II and III Wilms tumour without jeopardizing outcome.[15,16] Therefore the role of anthracyclines in the first line treatment has to be rethought in a growing number of pediatric tumours.

Since the seventies a series of randomized clinical trials have been performed with the aim of improving the treatment of children with high risk RMS. None of the trials performed so far has been able to identify a chemotherapy regimen more effective than the standard VAC or IVA.

Despite these "negative" results, the survival of children with RMS has progressively increased over the years. The same has happened with this trial: we were not able to demonstrate that the "new" IVADo was more effective than IVA but the observed 3-year EFS for the whole population was substantially better than anticipated. This can been explained by a general improvement of care with better imaging, surgery and radiotherapy planning but one major reason may rely on the higher number of patients that received radiotherapy during first line treatment (85·1%) in comparison with previous European studies (approximately 60%) (5).

In conclusion, we believe that this study represents good evidence that the addition of doxorubicin has no benefit in standard first line chemotherapy for patients with localized RMS. This will save a

substantial number of children and adolescents with localized RMS significant acute toxicity and potential late morbidity. The IVA regimen remains the standard of care for patients with localised RMS in Europe.

Contributors

All authors contributed to study design, data collection and interpretation, managemente of the clinical trial, writing and review of the paper and approval of the final version. GB act as principal investigator. GB, MJ, CB, SG, AF, OO, MC, MS. AK, MNG, HM, GLDS wrote the protocol and organized data collection. AK coordinate the central pathology review, MNG and HM coordinate the radiotherapy and surgical review, respectively. GLDS coordinated the data centre and performed the statistical analysis with ADP.

Declaration of interest

Authors do not have competing interest.

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References

1. Bisogno G, Ferrari A. Soft Tissue Sarcoma in Cancer in Children. Clinical management, pp 274-292. Eds MCG Stevens, HN Caron, A Biondi. Oxford University press 6th edition, 2012.

2. Sultan I, Ferrari A. Selecting multimodal therapy for rhabdomyosarcoma. Expert Rev Anticancer Ther. 2010;10:1285-301.

3. Hawkins D, Gupta AA, Rudzinski ER. What is new in the biology and treatment of pediatric rhabdomyosarcoma? Curr Opin Pediatr. 2014;26:50-6.

4 Arndt CA, Stoner JA, Hawkins DS, et al. Vincristine, actinomycin, and cyclophosphamide compared with vincristine, actinomycin, and cyclophosphamide alternating with vincristine, topotecan, and cyclophosphamide for intermediate-risk rhabdomyosarcoma: children's oncology group study D9803. J Clin Oncol. 2009;27:5182-8.

5. Oberlin O, Rey A, Sanchez de Toledo J, et al. Randomized comparison of intensified six-drug versus standard three-drug chemotherapy for high-risk nonmetastatic rhabdomyosarcoma and other chemotherapy-sensitive childhood soft tissue sarcomas: long-term results from the International Society of Pediatric Oncology MMT95 study. J Clin Oncol. 2012 ;30:2457-65.

6. Bergeron C, Thiesse P, Rey A, et al. Revisiting the role of doxorubicin in the treatment of rhabdomyosarcoma: an up-front window study in newly diagnosed children with high-risk metastatic disease. Eur J Cancer. 2008;44:427-31.

7. Arndt CA. Role of Doxorubicin in rhabdomyosarcoma: is the answer knowable? Am Soc Clin Oncol Educ Book. 2012:621-3

8. Bonadonna G, Monfardini S, De Lena M, Fossati-Bellani F, Beretta G. Phase I and preliminary phase II evaluation of adriamycin (NSC 123127). Cancer Res 1970;30:2572–82.

9. Sandler E, Lyden E, Ruymann F, et al. Efficacy of ifosfamide and doxorubicin given as a phase II ‘‘window’’ in children with newly diagnosed metastatic rhabdomyosarcoma: a report from the Intergroup Rhabdomyosarcoma Study Group. Med Pediatr Oncol 2001;37:442–8

10 Maurer HM, Beltangady M, Gehan EA, et al. The Intergroup Rhabdomyosarcoma Study-I. A final report. Cancer. 1988; 61:209-20.

11 Maurer HM, Gehan EA, Beltangady M, et al. The Intergroup Rhabdomyosarcoma Study-II. Cancer. 1993;71:1904-22.

12. Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. J Clin Oncol. 1995;13:610-30.

13. Smith MA, Ungerleider RS, Horowitz ME, Simon R. Influence of doxorubicin dose intensity on response and outcome for patients with osteogenic sarcoma and Ewing's

sarcoma. J Natl Cancer Inst 1991;83:1460-70.

14. Bisogno G, Ferrari A, Bergeron C, et al. The IVADo regimen--a pilot study with ifosfamide, vincristine, actinomycin D, and doxorubicin in children with metastatic soft tissue sarcoma: a pilot study on behalf of the European pediatric Soft tissue sarcoma Study Group. Cancer. 2005;103:1719-24.

15. Perilongo G, Maibach R, Shafford E, et al. Cisplatin versus cisplatin plus doxorubicin for standard-risk hepatoblastoma. N Engl J Med. 2009;361:1662-70.

16. Pritchard-Jones K, Bergeron C, de Camargo B, et al. SIOP Renal Tumours Study Group. Omission of doxorubicin from the treatment of stage II-III, intermediate-risk Wilms' tumour (SIOP WT 2001): an open-label, non-inferiority, randomised controlled trial. Lancet. 2015;386:1156-64.

Research in context

Evidence before this study

We searched pubmed for all randomized trials in patients with rhabdomyosarcoma. We also searched for published papers discussing the use of doxorubicin in patients with rhabdomyosarcoma using the following terms: rhabdomyosarcoma and randomiz(s)ed trial, rhabdomyosarcoma and doxorubicin. We found 4 randomized trials investigating the role of anthracyclines containing regimen (4 doxorubicin, 1 epirubicin) and 1 meta-analyses. Regimens containing doxorubicin have been shown to improve survival in selected subgroups of rhabdomyosarcoma patients in a trial but overall not convincing evidence of doxorubicin benefit was found.

Added value of this study

In our trial doxorubicin was added to the standard chemotherapy regimen in the initial part of the treatment and administered in a more intensive way in comparison with previous studies.
However, we did not find any survival benefit and toxicity was more severe in the experimental arm.

Implications of all the available evidence

Doxorubicin can be omitted from the first line chemotherapy of patients with localized rhabdomyosarcoma sparing them from significant acute toxicity and late morbidity.

The role of doxorubicin in the treatment of rhabdomyosarcoma: the European paediatric Soft tissue sarcoma Study Group RMS2005 randomized controlled phase 3 trial

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ABSTRACT

Background: Doxorubicin is an effective drug against rhabdomyosarcoma (RMS), but its role in combination with an established multidrug regimen remains controversial. The RMS2005 trial evaluated the possible benefit of early dose intensification with doxorubicin in patients with non-metastatic RMS.

Methods: For this multicentre trial we recruited patients aged 6 months-21 years with a previously untreated RMS from 108 hospitals in 14 countries. Patients with embryonal RMS incompletely resected and localized at unfavourable sites with or without nodal involvement or with alveolar RMS without nodal involvement were considered at high risk of relapse and were randomly assigned (1:1) to either 9 x 21-day cycles of IVA (ifosfamide 3g/m^2 days 1 and 2, vincristine 1·5 mg/m^2 day 1, actinomycin-D 1·5 mg/m^2 day 1) or 4 cycles of IVA with doxorubicin 30 mg/m^2 on days 1 and 2 (IVADo) followed by 5 cycles of IVA. The primary endpoint was 3-year event free survival (EFS) in the intention to treat population. The trial is registered with EudraCT- Nr: 2005-000217-35.

Findings: Between October 1, 2005 to December 17, 2013 242 patients were randomized in each arm. Median follow-up was 63·9 months (IQR 44·6 – 78·9). The 3-yr EFS was 67·5% (95% CI 61·2 – 73·1) in the IVADo arm and 63·3% (95% CI 56·8 – 69·0) in the IVA arm (HR 0·87, 95%CI 0·65-1·16; p=0·3352). The 3-yr OS was 78·3% (95%CI 72·4 – 83·0) and 80·6% (95% CI 74·9 – 85·1) in the IVADo vs. IVA arm (HR 1·17, 95%CI 0·82-1·67; p=0·3736). Grade 3-4 leukopenia, anaemia, thrombocytopenia, number of infections and gastrointestinal toxicity were significantly more common with IVADo.

Interpretations: The addition of dose intensified doxorubicin to standard chemotherapy failed to show a significant improvement in the outcome of patients with high-risk non metastatic RMS. Therefore the IVA regimen remains the standard of care for patients with localised RMS in Europe.

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INTRODUCTION

Rhabdomyosarcoma (RMS) is an aggressive tumour that can develop in almost any part of the body and thought to arise from primitive mesenchymal cells. It is the commonest form of soft tissue sarcoma in children and young adults and accounts for approximately 4-5% of all childhood malignancy with an annual incidence of 4·5 per million children under the age of 20. The peak incidence is seen early in childhood with a median age at diagnosis of about 5 years.[1]

Two main forms of RMS have been identified on histological appearance: the embryonal subtype accounts for approximately 80% of all RMS and carries a better prognosis and the alveolar subtype (15-20% of RMS) characterized by the fusion of the FOXO transcription factor gene to either the PAX3 or PAX 7 transcription factor genes and associated with poorer outcomes.

Although RMS is an aggressive tumour, survival rates for patients with non-metastatic disease have improved over the last 30 years owing to the application of a multimodality approach that includes chemotherapy, coordinated with surgery and, in the majority of cases, radiotherapy.

This strategy has been promoted by several cooperative Groups, the largest being the Children Oncology Group (COG) in the USA and the more recently founded European paediatric Soft tissue sarcoma Study Group (EpSSG) in Europe. A series of studies have established that a chemotherapy regimen including an alkylating agent, cyclophosphamide or ifosfamide, combined with vincristine and actinomycin D (VAC or IVA) represents the standard combination for patients with RMS. [2,3]

Different attempts to improve cure rates by adding other drugs to this combination have been made for patients with unfavourable prognostic factors such as alveolar histology or a primary tumour arising in unfavourable sites but failure free survival remained around 55-~~65~~ 70%. [4,5] However to date no randomised trial has shown a survival advantage compared to standard VAC or IVA.[3]

Doxorubicin has often been used in the treatment of patients at high risk of relapse or those with metastatic disease because the response rate to doxorubicin, used as single drug in the up front window setting, is one of the highest among chemotherapeutic agents. However its contribution when combined with an established multidrug regimen remains controversial .[6,7]

The E*p*SSG RMS2005 study incorporated a trial with two consecutive independent randomisations to investigate the benefit of early dose intensification with doxorubicin and the value of a maintenance treatment after standard therapy in patients with high risk localized RMS. We report here the results regarding the doxorubicin dose intensification question.

METHODS

Study Design and participants

This prospective phase III randomised clinical trial was conducted at 108 Hospitals in 14 Countries (Argentina, Belgium, Brazil, Czech Republic, France, Israel, Italy, Norway, Slovakia, Slovenia, Spain, The Netherlands, United Kingdom, and Ireland). All participating centres were required to obtain written approval from their local authorities and ethics committees and written informed consent from the patient and/or from their parents or legal guardians. The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines (European Union Drug Regulating Authorities Clinical Trials EUDRACT No. 2005-000217-35).

The eligibility criteria were: age > 6 months to < 21 years; pathologically proven diagnosis of RMS; no evidence of distant metastatic lesions; previously untreated except for primary surgery; no pre-existing illness preventing treatment; no previous malignant tumours and an interval between diagnostic surgery and systemic treatment less than 8 weeks. Histopathological material had to be available for central diagnostic review even though risk group and randomisation were assigned on the basis of the local assessment. Molecular confirmation of the presence of a FKHR translocation was not mandatory to classify a tumour as alveolar.

Each patient was assigned to a specific risk group according to six prognostic factors identified in a common retrospective analysis of European protocols: pathology (embryonal vs. alveolar), IRS Group, tumour primary site, nodal involvement, tumour size and patient age (Figure 1). Patients eligible for the RMS 2005 trial who were assigned to the High Risk group were eligible for the IVA vs. IVADo randomisation. Patients with alveolar paratesticular RMS were excluded in recognition of the better prognosis of this group of patients.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to receive the standard or the investigational chemotherapy. The randomisation was performed using a web based system and was stratified, in block sizes of four, by enrolling country (France, Italy, UK and Eire, Brazil and Argentina, other countries) and risk subgroup (E, F and G). Neither investigators nor patients were blinded to treatment allocation.

Procedures

The diagnostic work up included CT and/or MRI of the primary tumour, chest CT scan, radionuclide bone scan, bone marrow aspirate and biopsy. FDG-PET was optional and a baseline echocardiogram was also required.

Primary tumour resection was recommended only for patients where a complete resection was considered feasible without harming the patient, otherwise a biopsy was requested to establish the diagnosis.

Standard chemotherapy was a combination of ifosfamide 3 g/m^2 on day 1 and 2, vincristine 1·5 mg/m^2 (weekly during the first 7 weeks then only on day 1 of each cycle) and actinomycin D 1·5 mg/m^2 on day 1 (IVA). This same regimen with the addition of doxorubicin 30 mg/m^2 on day 1 and 2 comprised the investigational arm (IVADo). Four cycles of chemotherapy had to be administered in the initial part of treatment before local control measures were implemented. Subsequently both arms received 5 cycles of IVA (Figure 2). In children aged 6 to 12 months or less than 10 kg body weight drug doses were calculated according to body weight: vincristine 0·05 mg/kg/dose, actinomycin D 0·05 mg/kg/dose, ifosfamide 100 mg/kg/dose and doxorubicin 1 mg/kg/dose.

Tumour dimensions were measured at diagnosis using the 3 maximum diameters (a= length, b = width, c = thickness) and tumour volume estimated with the following formula: π/6 x a x b x c. Response assessment in patients with macroscopic residual disease after initial surgery (IRS group III) was evaluated at week 9 choosing, as far as possible, the diameters selected at diagnosis. and at the end of the treatment (with further assessments at the clinicians' discretion) and defined as follow: complete response (CR), clinically or histologically confirmed complete disappearance of disease; very good partial response (VGPR): tumour volume reduction more than 90%; partial response (PR): tumour volume reduction more than 66% but less than 90%; minor response (MR), reduction greater than 33% less than 66%; no response or stable disease (SD): less than 33% reduction in tumour volume; progressive disease (PD), any increase in tumour size of any measurable lesion or appearance of new lesions. When all the 3 diameters were not available two dimensions were used to establish the tumour response with corresponding 2D cut offs. All responses had to last at least 4 weeks without evidence of tumour progression or relapse

After the initial 3 cycles of chemotherapy (week 9) a full clinical and radiological assessment of the tumour was performed. Patients in complete remission or with evidence of tumour volume reduction > 33% continued the allocated treatment. In case of SD or PD patients were considered off study and the protocol recommended to switch to different chemotherapy regimens, including doxorubicin if initially allocated to the IVA arm, in the attempt to obtain a better tumour response.

The ‘local treatment’ of the tumour was planned after the tumour response assessment and implemented at week 13. Where a residual mass was present, surgical resection was encouraged if

free margins without organ or function impairment were anticipated. Marginal resection in sites where complete resection was not deemed possible was accepted, provided that it was always followed by radiotherapy.

Radiotherapy represented the only local treatment possible for patients that could not undergo secondary surgery due to the tumour location (e.g. parameningeal RMS). Radiotherapy doses were delivered according to histology, chemotherapy response and surgical results: 41·4 Gy were given to patients with alveolar RMS in IRS Group I or II, for patients in IRS Group III who achieved a complete remission after secondary surgery, and to patients with embryonal RMS that achieved a complete remission with initial chemotherapy; 50·4 ~~51·4~~ Gy for cases of incomplete or unfeasible secondary resection ~~or when secondary surgery was not feasible~~. A boost of 5·4 Gy in 3 fractions to the residual tumour was allowed for large tumours with poor response to chemotherapy. Radiotherapy to the involved lymph node sites was recommended at a dose of 41·4 Gy independently of histology and surgical resection. Treatment was delivered with megavoltage photons, one fraction per day, five days per week, with conventional fraction sizes of 1·8 Gy per day. In patients with large abdominal or cranio-spinal fields, or in patients less than 3 years old smaller fractions were allowed (e.g. 1·5 Gy).

The clinical target volume (CTV) was defined as the initial gross tumour volume (GTV) + 1 cm in all directions, except for limb tumours where the longitudinal GTV to CTV expansion was 2 cm. The CTV to planning target volume margin was typically 1 cm. In patients receiving 50·4 Gy, the PTV was reduced by 1 cm after 41·4 Gy. In patients with orbital tumours, the initial radiation of the whole orbit was reduced to a PTV of the GTV + 1 cm after 36 Gy. At the start of the trial in 2005, 3D conformal radiotherapy plans were most commonly used, but the as the trial covered a period of increasing availability of more sophisticated radiotherapy planning and treatment delivery, advanced photon techniques such as intensity modulated radiotherapy became more commonly used. Alternative techniques such as brachytherapy, electrons, and proton beam therapy were permitted when clinically appropriate. The protocol mandated doxorubicin therapy to be completed before starting radiotherapy. Actinomycin D was omitted during radiotherapy.

Further assessment of the tumour was performed after the 9th chemotherapy cycle. Patients with high risk RMS (included in the first randomisation or excluded for whatever reason) in complete remission were eligible for the second randomisation: to stop treatment or to continue with the administration of weekly vinorelbine and low dose continuous oral cyclophosphamide for 6 months. This randomisation was closed in December 2016 and results will be reported separately.

Supportive care was according to institutional guidelines. Primary prophylaxis with granulocyte colony-stimulating factor (G-CSF) was not mandated, however it was recommended during IVADo

treatment for life-threatening neutropenic infection, or treatment delay > 1 week due to neutropenia in a previous cycle.

Outcome

The primary endpoint was event-free survival (EFS), defined as the time from random assignment to the time of the first event defined as: death for all reasons, progression of disease (in cases where complete tumour remission was never achieved) ~~a residual tumour~~, relapse following previous complete remission, appearance of a new tumour and switch to second line chemotherapy in patients with unsatisfactory chemotherapy response (SD or PD) or time of the latest follow-up. Secondary end points were: overall survival (OS), measured as time from date of first randomisation up to death for all reasons or time of the latest follow-up; progression-free survival (PFS) measured as time from date of randomisation to tumour progression, relapse or time of the latest follow-up; response rate to initial treatment (9^{th} week) and toxicity according to NCI-CTC version 3.

Statistical analysis

The trial was originally projected to enroll 600 patients with high risk disease to detect an increase of 10% in the 3-year EFS with IVADo treatment, assuming a baseline 3-year EFS of 50% in the IVA arm, equivalent to a hazard ratio (HR) of 0·74. Overall, 343 events were needed to ensure a power of 80%, with a two-sided α level of 5%. Two interim analyses were planned after 1/3 and 2/3 of events, using an O'Brien-Fleming boundary for the efficacy boundary and the Harrington-Fleming-O'Brien process of repeated testing of the alternative hypothesis at an α level of 0·005 for futility monitoring.

An independent data-monitoring committee (IDMC) periodically monitored safety and efficacy during the study. The patients recruitment was slower than expected so in December 2011, the IDMC recommended a sample size re-estimation with a reduction in the HR to 0·65, maintaining the power of the study and extending the enrolment period. With these assumptions, a new sample size of 500 patients and 169 events and one interim analysis after observing 50% of events was planned.

At the time of the planned interim analysis, the IDMC recommended to continue the randomisation as planned, asking for a second interim analysis in December 2013. This analysis was performed when 79% of the expected information was available. The estimate of the HR was 1·024~~:1·00~~, IVADo vs IVA, with a p-value of 0·89. Repeated testing of the alternative hypothesis has been

performed to assess futility (log relative risk estimate= 0·02414, standard error = 0·17288, log relative risk β = -0·4308, T=2·631) obtaining a p-value of 0·004, ~~was observed~~ suggesting the study could be stopped for futility ~~(p-value < 0·005).~~

The randomisation IVADo vs. IVA was closed on the 17th December 2013.

Survival probabilities were estimated according to the intention to treat principle, i.e. including patients in the group to which they were assigned, whether or not they received the allocated treatment, using the Kaplan-Meier method and heterogeneity among strata of selected variables was assessed using the log-rank test. A sensitivity efficacy analysis for the per protocol population, i.e. eligible patients that received the allocated treatment, was performed. The 3-year and 5-year EFS, OS and PFS were reported with 95% confidence intervals (CIs), calculated according to the Greenwood's method. HRs with the 95% CI, calculated according to the Wald method, and p values for the interaction between treatment effect and any subgroup variable were estimated from the Cox regression model for event free survival and overall survival in relevant clinical subgroups of patients. For the primary end point analysis HR was adjusted for the stratification factors at randomization. ~~HR with the 95% CI, calculated according to the Wald method, was reported for significant variables.~~

The description of treatment exposure was summarized using descriptive statistics (median, min, max). Since the dose intensity of doxorubicin was a critical factor in our study, we compared the time interval between the start of treatment and the administration of the fourth cycle in the two arms and the cumulative dose of doxorubicin administered having as target 240 mg/m^2. We included in this intention to treat analysis also those patients that did not complete the 4 cycles of chemotherapy or did not receive the complete dosage of doxorubicin.

Toxicities were analysed according to the actual treatment received. Comparison of distribution was performed with the χ^2 test.

Data collected as of June 16, 2017 was analysed using SAS software 9·4 (SAS Institute, Cary, NC).

Role of funding source

EpSSG designed and coordinated the trial. The funders had no role in the design of the study, data collection and analysis or writing the report. The corresponding author had the final responsibility for the decision to submit for publication on behalf of the EpSSG Board members.

RESULTS

Between 1 November 2005 and 16 December 2013, 645 patients with high risk characteristics were assessed for eligibility and 161 (25·0%) of them were excluded. Overall 484 patients were

randomized: 242 in each arm (Figure 3). Thirty-three patients were found not to fulfil the eligibility criteria after the randomisation (20 assigned to IVA and 13 to IVADo), mainly due to incorrect staging or change of histological diagnosis. One patient, randomized to IVA regimen, rapidly progressed and was treated according to IVADo regimen. All these patients were included in the analysis according to the intention to treat principle.

An incorrect staging for metastatic lesions or nodal involvement were found in 10 and 5 patients, respectively, while the size of tumour was incorrectly recorded in 2; one patient was affected by a genetic syndrome with cardiovascular anomalies preventing the administration of anthracyclines. The diagnosis of RMS was not confirmed in 8 patients and the subtypes was changed in 7 (5 embryonal to alveolar and 2 alveolar to embryonal). Six cases received a rapid review soon after the diagnosis and did not start the treatment while in 9 the diagnosis was changed when they had already received the treatment. Overall 410 (84·7 %) cases were submitted for central pathology review at national and/or international level. Fourteen patients allocated to the IVA arm did not start the allocated treatment after randomisation but none in the IVADo group. This is explained by the fact that EpSSG protocols recommended IVADo treatment for patients in the very high risk or metastatic group so patients in the IVADo arm that were upstaged after diagnosis or staging review simply continued the treatment as allocated while those in the IVA arm changed to IVADo.

Patients and disease characteristics were well balanced between the 2 arms (Table 1). Patients were also balanced considering the treatment received after the 9 cycle of chemotherapy: 87 patients in the IVA arm and 89 in the IVADo arm received maintenance because included in the second randomized trial or by physician choice.

The median time between the start of treatment and the administration of the fourth cycle was similar in the 2 arms: 9·4 weeks (range 8·4-16) for IVADo and 9·3 weeks (5·9-15·9) for IVA. The doxorubicin median cumulative dose administered was 237·2 mg/m^2 (range 60·0-262·7). The tumour response rate (CR, VGPR, PR and MR) evaluated after initial chemotherapy was 88·9% vs. 92·3% for IVA vs. IVADo, respectively (p =0·24). Radiotherapy treatment was administered in 210 (86·8%) patients randomized to IVA and in 202 (83·5%) randomized to IVADo regimen. Two-hundred and thirty-four patients underwent at least one secondary surgery (118 in IVA and 116 in IVADo arm) and complete tumour resection was achieved in 149 of them (71 IVA and 78 IVADo).

The median survival follow-up for alive patients was 63·9 months (IQR 44·6–78·9): 63·2 months (IQR 45·2-77·7) in the control arm and 64·3 months (IQR 41·4-79·4) in the experimental arm.

The 3-yr EFS was 67·5% (95% CI 61·2 – 73·1) in the IVADo arm and 63·3% (95% CI 56·8 – 69·0) in the IVA arm (HR 0·87, 95%CI 0·65-1·16; p=0·3352). The 3-yr OS was 78·3% (95%CI

72·4 – 83·0) and 80·6% (95% CI 74·9 – 85·1) in the IVADo vs. IVA arm (HR 1·17, 95%CI 0·82-1·67; p=0·3736) (Figure 4).

Overall 181 patients experienced an event. The type of event distribution was similar in the two arms, however a number of patients in the IVA arm switched to second line treatment for insufficient tumour response (Table 2). This switch was considered an event for the EFS calculation but not for PFS that was 67·6 (95% CI 61·1-73·3) in the IVA arm at 3-yr and 68·1 (95% CI 61·7-73·6) in the IVADO arm, demonstrating that this phenomenon did not affect significantly on the overall trial results (p=0·97).

450 patients met the criteria for the per-protocol analysis: the 3-yr EFS was 68·8% (95% CI 62·3 – 74·4) in the IVADo arm and 63·1% (95% CI 56·4 – 69·1) in the IVA arm (HR 0·82, 95%CI 0·60-1·10; p=0·1924). The 3-yr OS was 79·2% (95% CI 73·3 – 84·0) and 81·1% (95% CI 75·2 – 85·7) in the IVADo vs. IVA arm (HR 1·13, 95%CI 0·78-1·65, p= 0.5101).

An analysis taking into account the most relevant clinical variables including age at diagnosis, gender, histological subtype, nodal involvement, primary tumour invasiveness, size, and site, did not show any difference among the two arms in any subgroup of patients (Figure 5).

Complete toxicity data for the initial chemotherapy phase (cycle 1 to 4) were available for 476 patients (Table 3). Considering grade 3-4 together, children treated in the IVADo arm experienced significantly more myelotoxicity with leukopenia (p=0·0061), anaemia (p<0·0001) and thrombocytopenia (p<0·0001). The higher rate of myelotoxicity in the IVADo arm prompted the investigators to a more frequent use of G-CSF that was administered in the 37·7% of cycles in the IVADo arm vs 22·5% in the IVA arm. For non-haematological toxicity, patients included in the experimental arm experienced a higher number of infections (p < 0·0001). Approximately one third of children in the IVADo arm experienced grade 3-4 gastrointestinal toxicity (p < 0·0001) and this was mainly due to mucositis likely caused by the concomitant administration of doxorubicin and actinomycin D. The same combination was expected to increase the risk of veno-occlusive disease ~~(VOD)~~ but only 3 patients in the IVADo arm experienced this type of toxicity vs. 5 patients in the IVA arm. No difference in acute cardiotoxicity were noted: 5 grade 3-4 events occurred in each arm). The number of patients with grade 3-4 thrombocytopenia was higher in the IVADo arm even in the following phase of treatment (5 cycles IVA in both arms) (p=0·0372), while no differences were observed for other toxicities.

Discussion

Doxorubicin is a very active drug against RMS as demonstrated by early studies performed initially in relapsed RMS[8] and more recently in patients with newly diagnosed metastatic RMS.[6] A phase II

window study in children with newly diagnosed metastatic RMS demonstrated the activity of ifosfamide and doxorubicin with a 63% response rate after 12 weeks of treatment.[9] This is very similar to the 65% response rate obtained by the administration as initial treatment of 2 cycles of single agent doxorubicin 60 mg/m² over 2 days.[6]

However the role of doxorubicin role as part of a multidrug regimen is controversial. It is not clear whether its addition to an established regimen such as VAC or IVA improves the survival of patients with RMS. A possible benefit of doxorubicin addition must be carefully considered as the toxicity profile of the drug may worsen immunosuppression and gut toxicity in the short term and cause cardiotoxicity in the long term.

Different randomized trials performed by the Intergroup Rhabdomyosarcoma Study (IRS) Group have not shown a substantial difference in survival and progression free survival for patients with RMS treated with VAC or VAC plus anthracyclines. In the IRS-I protocol, the addition of 5 VadrC (vincristine, adriamycin, cyclophosphamide) courses to VAC did not improve the outcomes in patients with gross residual disease after surgery or metastatic disease at diagnosis (IRS clinical groups III and IV).[10] In the IRS-II study a similar comparison was performed, however doxorubicin was given repetitively in pulse combination with vincristine and cyclophosphamide (pulse VAdrC). This regimen was given alternating with VAC cycles and was compared with repeated VAC as the standard arm. Additionally the cumulative dose of doxorubicin (480 mg/m^2) was higher than in the IRS-I protocol. Despite this intensification the two arms showed similar results (EFS 75% vs. 70% p 0·84) and the authors concluded that doxorubicin did not offer any survival advantage and was more toxic.[11] The role of doxorubicin was further investigated in the IRS-III study showing conflicting results.[12] A randomised comparison showed a significant benefit from the addition of doxorubicin in patients in clinical IRS-group II (microscopic post surgical disease). This advantage however disappeared when the historical control from IRS-II protocol were considered in the analysis. Other patients' subgroups showed better results in the IRS-III study in comparison with those obtained in the IRS-II study but doxorubicin was included along with other chemotherapy agents making its contribution hard to determine. Overall, the investigators of the IRS-II and IRS-III studies concluded that that the precise role of doxorubicin in newly diagnosed patients required further study.[7]

It should be noted that in IRS Group studies the treatment schemes were based on the alternating administration of cycles VAC and VadrC, consequently the intervals between doxorubicin containing courses were wide, reducing the anthracycline dose-intensity.

In Europe a trial conducted by the Malignant Mesenchymal Tumour (MMT) Group of the International Society of Pediatric Oncology (SIOP) compared the IVA regimen against a six drug

regimen containing anthracycline (epirubicin). No difference in survival was found between the two arms. Once again the anthracyclines dose-intensity was low because epirubicin was included in only 3 out of a total of 9 cycles.[5]

The possibility that increasing anthracylines dose-intensity may be of benefit for the patients is supported by a meta-analysis of several trials performed in patients with bone sarcomas. This analysis demonstrated that an induction treatment including doxorubicin in every course was better than a schema alternating doxorubicin with actinomycin D.[13] To explore this strategy we added doxorubicin to the IVA regimen avoiding to alternate courses with and without anthracyclines as has been done in previous studies. We also hypothesized that the use of IVADo in the initial part of treatment could have induced a higher number of tumour responses allowing a better local control with surgery and radiotherapy. The IVADo combination had previously been tested in a pilot study and was shown to be feasible.[14]

The RMS2005 trial confirms on a larger scale that the IVADo regimen is manageable. The doxorubicin dose intensity was maintained as demonstrated by the median interval between the initial and last IVADo and the median cumulative dose of doxorubicin administered. As expected there was significantly more toxicity in the patients receiving IVADo compared to the IVA group, particularly in terms of myelosuppression and mucositis.

Despite IVADo feasibility and the toxicity suffered by patients, our study clearly shows that the addition of doxorubicin did not add any meaningful benefit to patient's survival.

It is interesting that this result is in line with evidence progressively collected by other studies dedicated to paediatric tumours. Recent publications have demonstrated that doxorubicin can be omitted from the treatment plan of patients with standard hepatoblastoma or favourable histology stage II and III Wilms tumour without jeopardizing outcome.[15,16] Therefore the role of anthracyclines in the first line treatment has to be rethought in a growing number of pediatric tumours.

Since the seventies a series of randomized clinical trials have been performed with the aim of improving the treatment of children with high risk RMS. None of the trials performed so far has been able to identify a chemotherapy regimen more effective than the standard VAC or IVA. Despite these "negative" results, the survival of children with RMS has progressively increased over the years. The same has happened with this trial: we were not able to demonstrate that the "new" IVADo was more effective than IVA but the observed 3-year EFS for the whole population was substantially better than anticipated. This can been explained by a general improvement of care with better imaging, surgery and radiotherapy planning but one major reason may rely on the higher

number of patients that received radiotherapy during first line treatment (85·1%) in comparison with previous European studies (approximately 60%) (5).

In conclusion, we believe that this study represents good evidence that the addition of doxorubicin has no benefit in standard first line chemotherapy for patients with localized RMS. This will save a substantial number of children and adolescents with localized RMS significant acute toxicity and potential late morbidity. The IVA regimen remains the standard of care for patients with localised RMS in Europe.

Contributors

All authors contributed to study design, data collection and interpretation, managemente of the clinical trial, writing and review of the paper and approval of the final version. GB act as principal investigator. GB, MJ, CB, SG, AF, OO, MC, MS. AK, MNG, HM, GLDS wrote the protocol and organized data collection. AK coordinate the central pathology review, MNG and HM coordinate the radiotherapy and surgical review, respectively. GLDS coordinated the data centre and performed the statistical analysis with ADP.

Declaration of interest

Authors do not have competing interest.

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We would like to acknowledge the contribution of Ilaria Zanetti for her valuable data management activities and Paola dal Bianco for her advices on the statistical design and analysis.

We would like to thank all the Investigators that worked at the EpSSG centres and patients/families that consented to participate in this trial contributing to its success. In particular we would like to acknowledge the work of national coordinators: Adriana Rose (Argentina), Christine Devalck (Belgium), Sima Ferman (Brasil), Peter Mudry (Czech Republik), Christophe Bergeron (France) Myriam Weyl Ben Arush (Israel), Gianni Bisogno (Italy), Heidi Glosli (Norway), Daniela Sejnova (Slovakia), Maja Cesen (Slovenia), Soledad Gallego (Spain), J. Hans Merks (The Netherlands) Meriel Jenney (United Kingdom and Ireland).

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References

1. Bisogno G, Ferrari A. Soft Tissue Sarcoma in Cancer in Children. Clinical management, pp 274-292. Eds MCG Stevens, HN Caron, A Biondi. Oxford University press 6th edition, 2012.

2. Sultan I, Ferrari A. Selecting multimodal therapy for rhabdomyosarcoma. Expert Rev Anticancer Ther. 2010;10:1285-301.

3. Hawkins D, Gupta AA, Rudzinski ER. What is new in the biology and treatment of pediatric rhabdomyosarcoma? Curr Opin Pediatr. 2014;26:50-6.

4 Arndt CA, Stoner JA, Hawkins DS, et al. Vincristine, actinomycin, and cyclophosphamide compared with vincristine, actinomycin, and cyclophosphamide alternating with vincristine, topotecan, and cyclophosphamide for intermediate-risk rhabdomyosarcoma: children's oncology group study D9803. J Clin Oncol. 2009;27:5182-8.

5. Oberlin O, Rey A, Sanchez de Toledo J, et al. Randomized comparison of intensified six-drug versus standard three-drug chemotherapy for high-risk nonmetastatic rhabdomyosarcoma and other chemotherapy-sensitive childhood soft tissue sarcomas: long-term results from the International Society of Pediatric Oncology MMT95 study. J Clin Oncol. 2012 ;30:2457-65.

6. Bergeron C, Thiesse P, Rey A, et al. Revisiting the role of doxorubicin in the treatment of rhabdomyosarcoma: an up-front window study in newly diagnosed children with high-risk metastatic disease. Eur J Cancer. 2008;44:427-31.

7. Arndt CA. Role of Doxorubicin in rhabdomyosarcoma: is the answer knowable? Am Soc Clin Oncol Educ Book. 2012:621-3

8. Bonadonna G, Monfardini S, De Lena M, Fossati-Bellani F, Beretta G. Phase I and preliminary phase II evaluation of adriamycin (NSC 123127). Cancer Res 1970;30:2572–82.

9. Sandler E, Lyden E, Ruymann F, et al. Efficacy of ifosfamide and doxorubicin given as a phase II ‘‘window’’ in children with newly diagnosed metastatic rhabdomyosarcoma: a report from the Intergroup Rhabdomyosarcoma Study Group. Med Pediatr Oncol 2001;37:442–8

10 Maurer HM, Beltangady M, Gehan EA, et al. The Intergroup Rhabdomyosarcoma Study-I. A final report. Cancer. 1988; 61:209-20.

11 Maurer HM, Gehan EA, Beltangady M, et al. The Intergroup Rhabdomyosarcoma Study-II. Cancer. 1993;71:1904-22.

12. Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. J Clin Oncol. 1995;13:610-30.

13. Smith MA, Ungerleider RS, Horowitz ME, Simon R. Influence of doxorubicin dose intensity on response and outcome for patients with osteogenic sarcoma and Ewing's

sarcoma. J Natl Cancer Inst 1991;83:1460-70.

14. Bisogno G, Ferrari A, Bergeron C, et al. The IVADo regimen--a pilot study with ifosfamide, vincristine, actinomycin D, and doxorubicin in children with metastatic soft tissue sarcoma: a pilot study on behalf of the European pediatric Soft tissue sarcoma Study Group. Cancer. 2005;103:1719-24.

15. Perilongo G, Maibach R, Shafford E, et al. Cisplatin versus cisplatin plus doxorubicin for standard-risk hepatoblastoma. N Engl J Med. 2009;361:1662-70.

16. Pritchard-Jones K, Bergeron C, de Camargo B, et al. SIOP Renal Tumours Study Group. Omission of doxorubicin from the treatment of stage II-III, intermediate-risk Wilms' tumour (SIOP WT 2001): an open-label, non-inferiority, randomised controlled trial. Lancet. 2015;386:1156-64.

Research in context

Evidence before this study

We searched pubmed for all randomized trials in patients with rhabdomyosarcoma. We also searched for published papers discussing the use of doxorubicin in patients with rhabdomyosarcoma using the following terms: rhabdomyosarcoma and randomiz(s)ed trial, rhabdomyosarcoma and doxorubicin. We found 4 randomized trials investigating the role of anthracyclines containing regimen (4 doxorubicin, 1 epirubicin) and 1 meta-analyses. Regimens containing doxorubicin have been shown to improve survival in selected subgroups of rhabdomyosarcoma patients in a trial but overall not convincing evidence of doxorubicin benefit was found.

~~The doxorubicin dose intensity was low in the published trials.~~

Added value of this study

In our trial doxorubicin was added to the standard chemotherapy regimen in the initial part of the treatment and administered in a more intensive way in comparison with previous studies.

However, we did not find any survival benefit and toxicity was more severe in the experimental arm.

Implications of all the available evidence ~~This result allows the omission of~~

Doxorubicin can be omitted from the first line chemotherapy of ~~for~~ patients with localized rhabdomyosarcoma sparing them from significant acute toxicity and late morbidity.

RMS 2005

a protocol for non metastatic rhabdomyosarcoma

VERSION 1.3 INTERNATIONAL
MAY 2012

Contents

1. Protocol Sponsor

It is responsibility of each participating national Group or Institution to arrange sponsorship in line with the requirements of the European Union directive on Good Clinical Practice in Clinical Trials.

2. Protocol Co-ordination

This protocol is co-ordinated by the European paediatric Soft tissue sarcoma Study Group (in its abbreviated form E*p*SSG). This new collaborative structure has been founded by

- **The Co-operative Weichteilsarkom Studie (CWS)**
- **The AIEOP Soft Tissue Sarcoma Committee (AIEOP STSC)**
  (former ICG: Italian Cooperative Group for paediatric soft tissue sarcoma)
- **The SIOP Malignant Mesenchymal Tumour Committee (SIOP MMT)**

These Groups decided to join forces to design and implement a portfolio of pan-European studies addressed at children and adolescents affected by soft tissue sarcoma.

The three cooperative Groups act on behalf of the following Societies:
AIEOP - Associazione Italiana di Ematologia e Oncologia Pediatrica
BSPHO - Belgian Society of Paediatric Haematology Oncology
GPOH - *Germany, Austria***:** Gesellschaft für pädiatrische Onkologie und Hämatologie
NOPHO *Denmark, Norway, Sweden* **-** Nordic Organisation of Paediatric Haematology and Oncology
SEOP - Sociedad Española de Oncologìa Pediátrica
SFCE - Société Française de lutte contre les Cancers de l'Enfant et de l'adolescent
UKCCSG - United Kingdom Children's Cancer Study Group

This study will not introduce or try to license chemotherapeutic agents for treatment of paediatric sarcoma. Treatment will rely on already licensed and introduced chemotherapeutic drugs. Therefore, chemotherapeutic agents and other therapeutic substances needed for treatment in E*p*SSG RMS 2005 will not be paid for by the study nor will these substances be provided by pharmaceutical companies.

Important note:
It is emphasised that no legal responsibility for possible consequences resulting from the application of recommendations from this protocol will be taken by the members of the E*p*SSG. Treatment and follow-up of patients with soft tissue sarcoma requires a high degree of medical competence and humane presence existing only in hospitals with adequate infrastructure. A state of emergency due to complications from the underlying disease or from its treatment can develop in every patient at any time. An experienced team with multidisciplinary competences should thus treat children with STS.

3. E*p*SSG Structure

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Surgical Panel

Prof. Helene Martelli (France)

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Dr. Gian Luca De Salvo (Italy)

Other collaborators:

Dr. Andrea Ferrari: maintenance treatment
Dr. Alberto Donfrancesco: second line treatment

4. Protocol E*p*SSG 2005 – Administrative organisation

The protocol is co-ordinated by a Trial Monitoring Committee under the supervision of E*p*SSG Board.
The Committee will meet at least twice a year to monitor the progress of the study.
Protocol Panels will normally meet at the same time as the protocol Committee.
The structure of EpSSG is described in the document: "Structure and standard for EpSSG members".
The member of the different EpSSG Committee and Panels is reported in the document: "EpSSG Structure & Membership".

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Partecipating Centres

A list of participating centres breakdown by countries is provided in the *"EpSSG Structure & Membership list"* document.

4.1 SAFETY DESK

To deal with the safety issue throughout the EpSSG RMS 2005 trial a safety desk has been instituted. A guide denominated "*Standard Operative Procedures for managing Serious Adverse Events (SAEs) and Suspect Unexpected Serious Adverse Reactions (SUSARs) throughout the EpSSG RMS 2005 Trial"* has been distributed to the EpSSG investigators.
In brief The EpSSG Safety Desk is responsible for the ongoing safety evaluation of the investigational medicinal products. The Safety Desk will monitor all communication regarding any findings that may adversely affect the health of subjects, have an impact on the conduct of the trial or induce the competent authority to withdraw authorisation to continue the trial in accordance with Directive 2001/20/EC.

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5. Abbreviations

ACT-D	Actinomycin D
Adria	Adriamycin (doxorubicin)
aRMS	Alveolar Rhabdomyosarcoma
BM	Bone Marrow
Carbo	Carboplatin
CAV	Cyclophosphamide, Adriamycin, Vincristine
CEVAIE	Carboplatin, Epirubicin, Vincristine, Actinomycin, Ifosfamide, Etoposide
CPM	Cyclophosphamide
CR	Complete Remission
CT	Chemotherapy
CTC	Common Toxicity Criteria
CWS	Cooperative Weichteilsarkom Studie
DFS	Disease Free survival
Doxo	Doxorubicin
EFS	Event Free Survival
eRMS	Embryonal Rhabdomyosarcoma
EFS	Event Free Survival
E*p*SSG	the European paediatric Soft tissue Sarcoma Study Group
GCP	Good Clinical Practice
GU BP	Genito Urinary Bladder Prostate
GU non BP	Genito Urinary non Bladder Prostate
HN non PM	Head and Neck non Parameningeal
HN PM	Head and Neck Parameningeal
IDMC	International Data Monitoring Committee
IFO	Ifosfamide
IRS	Intergroup Rhabdomyosarcoma Study
IRSG	Intergroup Rhabdomyosarcoma Study Group
IVA	Ifosfamide, Vincristine, Actinomycin,

IVADo	Ifosfamide, Vincristine, Actinomycin, Doxorubicin
MMT	Malignant Mesenchymal Tumours
NOS	Not Otherwise Specified
OS	Overall Survival
PFS	Progression free Survival
PNET	Peripheral Primitive NeuroEctodermal Tumour
PD	Progressive Disease
PR	Partial Response
RDE	Remote Data Entry
RMS	Rhabdomyosarcoma
RT	Radiotherapy
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SIOP	Société Internationale d'Oncologie Pédiatrique
SD	Stable Disease
STS	Soft Tissue Sarcoma
STSC	Italian Soft Tissue Sarcoma Committee
Topo	Topotecan
TNM	Tumour-Node-Metastasis
VA	Vincristine, Actinomycin
VAC	Vincristine, Actinomycin, Cyclophosphamide
VAIA	Vincristine, Actinomycin, Ifosfamide, Adriamycin (doxorubicin)
VAdrC	Vincristine, Adriamycin (doxorubicin) Cyclophosphamide,
VCR	Vincristine
VNL	Vinorelbine
VOD	Veno-Occlusive Disease

6. Summary

Three Cooperative Groups have been working in Europe on paediatric soft tissue sarcoma for the last twenty years: the SIOP MMT Committee, the CWS and the AIEOP STSC (former ICG). Cooperation has intensified over the last few years and has led to the foundation of the European paediatric Soft tissue sarcoma Study Group (E*p*SSG). This is the first E*p*SSG protocol and it addresses the treatment of children and young people presenting with non-metastatic rhabdomyosarcoma.
The protocol contains a randomised trial for "high risk patients" and observational studies for patients categorized in other risk groups.

Patients with metastatic rhabdomyosarcoma or other soft tissue sarcoma (non rhabdomyosarcoma) will be treated according to different protocols elaborated within the framework of E*p*SSG. These patients must be therefore registered in these protocols.

Objectives:
To give a homogenous local and systemic treatment Europe-wide according to the risk of local and metastatic relapse in patients categorized in Low, Standard and Very High Risk Groups (observational study)
To investigate the role of doxorubicin dose intensity and maintenance chemotherapy in patients included in the High Risk Group (randomised trial)

PATIENTS ELIGIBILITY

A) To the observational study
- Patients with pathologically confirmed rhabdomyosarcoma
- No evidence of metastatic disease
- Age 0 - < 21 years
- Previously untreated except for primary surgery
- No pre-existing illness preventing treatment
- No previous malignant tumours.
- Interval between diagnostic surgery and start of chemotherapy no longer than 8 weeks
- Diagnostic material available for pathology review
- Available for long term follow up through the treatment centre
- Written informed consent for treatment available

B) To the investigational study (randomised trial)
- Eligible to the protocol
- Included in the High Risk Group
- Age > 6 months (younger children are eligible for the protocol study treatment but they will not enter in the randomised trial)
- Informed consent given for the randomised study

Adults with RMS (> 21 years) may be eligible for registration and treatment on study (according to institutional preference) but not for randomisation.

PATIENTS STRATIFICATION

Patients are subdivided according to the risk factors that have emerged from the analyses of previous European studies. A new stratification has been developed taking into account histology (alveolar vs. non alveolar RMS), post surgical stage (according to IRS grouping), tumour site and size, node involvement and patient age.
According to their risk profile four Groups have been identified: Low Risk, Standard Risk, High Risk and Very High Risk (see Table 1). Different objectives and treatment plans have been elaborated for each group.

1) Low Risk Group:

Stratification: favourable histology (non alveolar), IRS Group I, any site, N0, favourable age (< 10 years) and favourable tumour size (≤ 5 cm) - SUBGROUP A (see Table 1).
Objective: to further investigate whether low risk patients can be treated with Vincristine and Actinomycin D alone.
Surgery: no further surgery after initial complete resection.
Chemotherapy: Vincristine + Actinomycin D (VA) over 8 blocks (22 weeks).
Radiotherapy: not indicated in these patients.

2) Standard Risk Group

Stratification: this risk Group comprises 3 different sets of patients. All patients must have favourable histology and no evidence of nodal involvement. SUBGROUP B*:* IRS Group I, and unfavourable size or age; SUBGROUP C: IRS II or III and favourable site; SUBGROUP D*:* IRS II or III, unfavourable site but favourable size and age (see Table 1).
Objective: to evaluate whether a) the addition of a limited dose of ifosfamide may improve the results in SUBGROUP B; b) chemotherapy intensity may be reduced decreasing the cumulative dose of the alkylating agent ifosfamide (SUBGROUP C) or avoiding anthracycline (SUBGROUP D)
Surgery: no further surgery after initial resection in IRS groups I and II (but a primary re-excision should be considered in group II patients). In IRS group III patients delayed surgery should be considered after initial chemotherapy, if feasible. Delayed surgery in orbital RMS is not encouraged, however.
Chemotherapy: Ifosfamide + Vincristine + Actinomycin D (IVA) over 9 blocks. However ifosfamide will be withheld in Subgroup B and C after the initial 4 blocks.
Radiotherapy: Irradiation will be avoided when the tumour has been completely removed at diagnosis (Subgroup B). All other patients are to receive radiotherapy according to the radiotherapy guidelines with doses ranging between 36 Gy and 50.4 Gy depending on resection margins and response. Exceptions can be made in very young patients or in patients with tumours in particularly sensitive sites.

3) High Risk Group

Stratification: patients in IRS group II or III, with favourable pathology but unfavourable site and size or age (SUBGROUP E); patients in IRS Group I, II or III with favourable pathology, site, size and age but with nodal involvement (SUBGROUP F); all patients with unfavourable histology (SUBGROUP G) except alveolar N1 (see Table 1)
Objective: to improve the EFS of the whole group evaluating through a double randomisation 1) the value of adding doxorubicin in the initial part of the treatment and 2) the role of low dose maintenance chemotherapy.
Surgery: no further surgery after initial resection in IRS groups I and II (but a primary re-excision should be considered in group II patients). In group III patients delayed surgery should be considered after initial chemotherapy, if feasible.

Chemotherapy: IVA vs. IVADo (IVA + Doxorubicin) over the initial 4 blocks followed by 5 IVA blocks. All patients in complete remission will then be randomised to stop treatment or to continue with low dose maintenance therapy with a combination of cyclophosphamide and vinorelbine.
Radiotherapy: All patients are to receive radiotherapy according to the radiotherapy guidelines with doses ranging between 36 Gy and 50.4 Gy depending on histology, resection margins and response.

4) Very High Risk Group
Stratification: unfavourable histology (alveolar) and node involvement (N1), regardless of the other risk factors (see Table 1)
Objective: to improve the EFS by adding doxorubicin in the initial part of the treatment and low dose maintenance chemotherapy.
Surgery: no further surgery after initial resection in IRS groups I and II (but a primary re-excision should be considered in group II patients). In group III patients delayed surgery should be considered after initial chemotherapy, if feasible.
Chemotherapy: IVADo over the initial 4 blocks followed by 5 IVA blocks and low dose cyclophosphamide and vinorelbine.
Radiotherapy: patients are to receive radiotherapy according to the radiotherapy guidelines with doses ranging between 41.4 Gy and 50.4 Gy depending on resection margins and response.

PATHOLOGY AND BIOLOGY

The diagnosis of the patients registered into the protocol will be reviewed by the E*p*SSG Pathology Panel to confirm the diagnosis and the RMS subtype, as this is essential in the patient's management. Different pathology studies will be implemented to analyse the prognostic meaning of several features including cellular anaplasia and post chemotherapy maturation.
Tumour samples will be also analysed, using the RT-PCR technique, to specifically detect transcripts that can be used for the identification of paediatric sarcomas. Among others, PAX-FKHR transcripts that characterize alveolar rhabdomyosarcoma; EWS-FLI1 and EWS-ERG that are expressed in the Ewing's family of tumours; ETV6-NTRK3 in congenital infantile fibrosarcoma; EWS-WT1 in desmoplastic sarcoma, and SYT-SSX1 and SYT-SSX2 in synovial sarcoma.
Other transcripts such as MyoD1 and Myogenin will be used in the study of minimal bone-marrow infiltration. New molecular markers may be identified in the future that could have clinical applications

STATISTICAL CONSIDERATIONS

This study is a prospective phase III international, multi-institutional, non-blinded double-randomised clinical trial.
Aims of the trial are to evaluate the addition of doxorubicin to the standard therapy with ifosfamide, vincristine and actinomycin (IVA) in paediatric patients with rhabdomyosarcoma in high risk group – *intensification question,* and the role of a maintenance therapy with vinorelbine and cyclophosphamide in the same category of patients who have achieved a complete remission with first line treatment – *maintenance question.*
The estimated number of patients to be included in the randomised trial is 600 and the expected accrual period of the trial is 5 years followed by a minimum follow up period of 3 years.

ORGANIZATION OF THE STUDY

The E*p*SSG is an inter-group structure, which is based on the already existing national and international organisations built with the efforts of the participants to CWS, STSC and SIOP MMT studies over many years.

The existing national coordinating centres, will continue their work ensuring pathology review, clinical advice and data quality control.
All clinical centres previously part of the SIOP, CWS or STSC Co-operative Group are expected to participate in the E*p*SSG study. New clinical centres, whose national group does not take part as a whole, who wish to participate must demonstrate their ability to participate in the study.
The E*p*SSG Co-ordinating Centre will supervise the data collection and data quality and will be responsible for the statistical analysis within the trial at given time periods in collaboration with the panel of statisticians from individual groups.

DATA MANAGEMENT AND ANALYSIS

The E*p*SSG RMS trial will be managed via a web-based system provided by CINECA (Casalecchio, Italy).
Standard Operative Procedures for the electronic data management will be agreed on and followed by the Co-ordinating Centres.
Reports on the study progress will be prepared twice yearly, describing accrual of the patients, group allocations, local therapy modalities and toxicity of the treatments given. This report will be circulated to the Principal Investigators.
The international study committee shall meet as appropriate to consider patient accrual, eligibility, treatment allocation and outcome and ensure a smooth conduct of the study.
Results of the interim analysis shall be reported to the International Data Monitoring Committee (IDMC) as scheduled by the protocol. The IDMC may recommend early stopping, continuation or extension of the study to the Protocol Committee.

ETHICAL CONSIDERATIONS

The protocol will be submitted, before patients enrolment, to the Ethics Committee of each participating Centre for review and approval according to law in force.
The patient's and/or parent's written consent to participate in the study must be obtained after a full explanation has been given of the treatment options including the conventional and generally accepted methods of treatment and the manner of treatment allocation.
Consent for participation for data management and biology material handling will be also obtained.
All patients and/or their parents must give written consent to inclusion into the trial, data processing and – if applicable – to sending diagnostic material to reference institutions, which in all participating countries has to conform to the national data protection legislation.
The investigator agrees, by accepting the protocol, to adhere to the principles of Good Clinical Practice.

Table 1 - Risk Stratification for EpSSG non metastatic RMS study

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
Low Risk	**A**	Favourable	I	Any	N0	Favourable
Standard Risk	***B***	Favourable	I	Any	N0	Unfavourable
	C	Favourable	II, III	Favourable	N0	Any
	D	Favourable	II, III	Unfavourable	N0	Favourable
High Risk	***E***	Favourable	II, III	Unfavourable	N0	Unfavourable
	F	Favourable	II, III	Any	N1	Any
	G	Unfavourable	I, II, III	Any	N0	Any
Very High Risk	***H***	Unfavourable	I, II, III	Any	N1	Any

- **Pathology:**

Favourable = all embryonal, spindle cells, botryoid RMS
Unfavourable = all alveolar RMS (including the solid-alveolar variant)

- **Post surgical stage** (according to the IRS grouping, see appendix A.2):

Group I = primary complete resection (R0);
Group II = microscopic residual (R1) or primary complete resection but N1;
Group III = macroscopic residual (R2);

- **Site:**

Favourable = orbit, GU non bladder prostate (i.e. paratesticular and vagina/uterus) and non PM head & neck
Unfavourable = all other sites (parameningeal, extremities, GU bladder-prostate and "other site")

- **Node stage** (According to the TNM classification, see appendix A1 and A.5):

N0 = no clinical or pathological node involvement
N1 = clinical or pathological nodal involvement

- **Size & Age:**

Favourable = Tumour size (maximum dimension) ≤5cm ***and*** Age <10 years
Unfavourable = all others (i.e. Size >5 cm ***or*** Age ≥10 years)

Notes:
- *for paratesticular alveolar RMS see paragraph 8.4.4.*
- *for patients with RMS N.O.S, Undifferentiated STS and Ectomesenchymoma see paragraph 29.4*
- *Children with ascites/pleural effusion or CSF positive for malignant cells should be enrolled in the protocol for metastatic RMS*

6.1 SUMMARY FOR ELIGIBILITY

Diagnosis of Rhabdomyosarcoma or other malignant mesenchymal tumours*

ELIGIBLE FOR REGISTRATION

A pathologically proven diagnosis of RMS
Age < 21 years
Previously untreated except initial surgery
No pre-existing illness preventing treatment
No previous malignant tumours

Diagnosed ≤ 8 weeks
Pathology available for central review
Available for follow up
Written consent for treatment available

YES

NO

Registration only

Evidence of Distant Metastases

NO

YES

Treat in EpSSG Stage IV Study

ELIGIBLE FOR RMS 2005 PROTOCOL

Low Risk Group	**Standard Risk Group**	**High Risk Group**	**Very High Risk Group**
Subgroup A: ♦ VA x8	*Subgroup B:* ♦ IVA + VA *Subgroup C:* ♦ IVA ±VA *Subgroup D:* ♦ IVA	*Subgroup E* *Subgroup F* *Subgroup G* *if* -Age > 6 months -Informed consent given **Randomised trial No. 1** *(IVA vs. IVADo)* *if* -In CR or with minimal anomalies at the end of treatment **Randomised trial No. 2** (stop treatment vs. maintenance)	*Subgroup H* ♦ IVADo + maintenance

** undifferentiated soft tissue sarcoma and ectomesenchymoma are included in this protocol*

6.2 TREATMENT SUMMARY: LOW RISK GROUP

Low Risk Group	**Localised non alveolar RMS, microscopically completely resected (IRS Group I), at all sites,** *and* **nodes negative** *and* **tumour size ≤ 5 cm** *and* **age < 10 years**

	V	V	V	V			V	V	V	V			V	V	V	V			V	V	V	V
Surgery	A			A			A			A			A			A			A			A
Weeks	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Cycle no.	1			2			3			4			5			6			7			8

V = Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection.
A = Actinomycin D 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection.

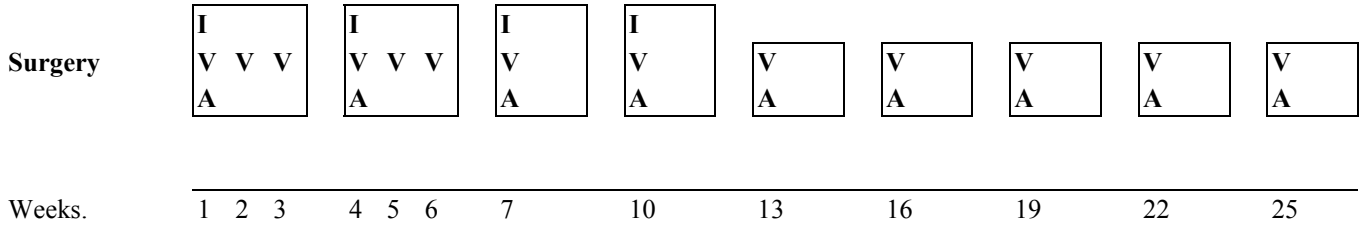
Cycles should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.
Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.

For children ≤ 1 year (or ≤ 10 kg body weight) see first cycle doses will be calculated by body weight and increased in the following cycles if tolerated, see chapter 24.4.1.

For Low Risk Group treatment details: see chapter 13
For chemotherapy guidelines and dose modifications: see chapter 24.

6.3 TREATMENT SUMMARY: STANDARD RISK GROUP - SUBGROUP B

SUBGROUP B	**Localised non alveolar RMS, microscopically completely resected (IRS Group I), at all sites,** *and* **nodes negative** *and* **tumour size > 5 cm *or* age ≥ 10 years**



I Ifosfamide 3 g/m^2 is given as a 3 hour i.v. infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) is given as a single i.v. injection on day 1 of each course and weekly, for total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/ m^2 (maximum single dose 2 mg) as a single i.v. injection on day 1 of each course.

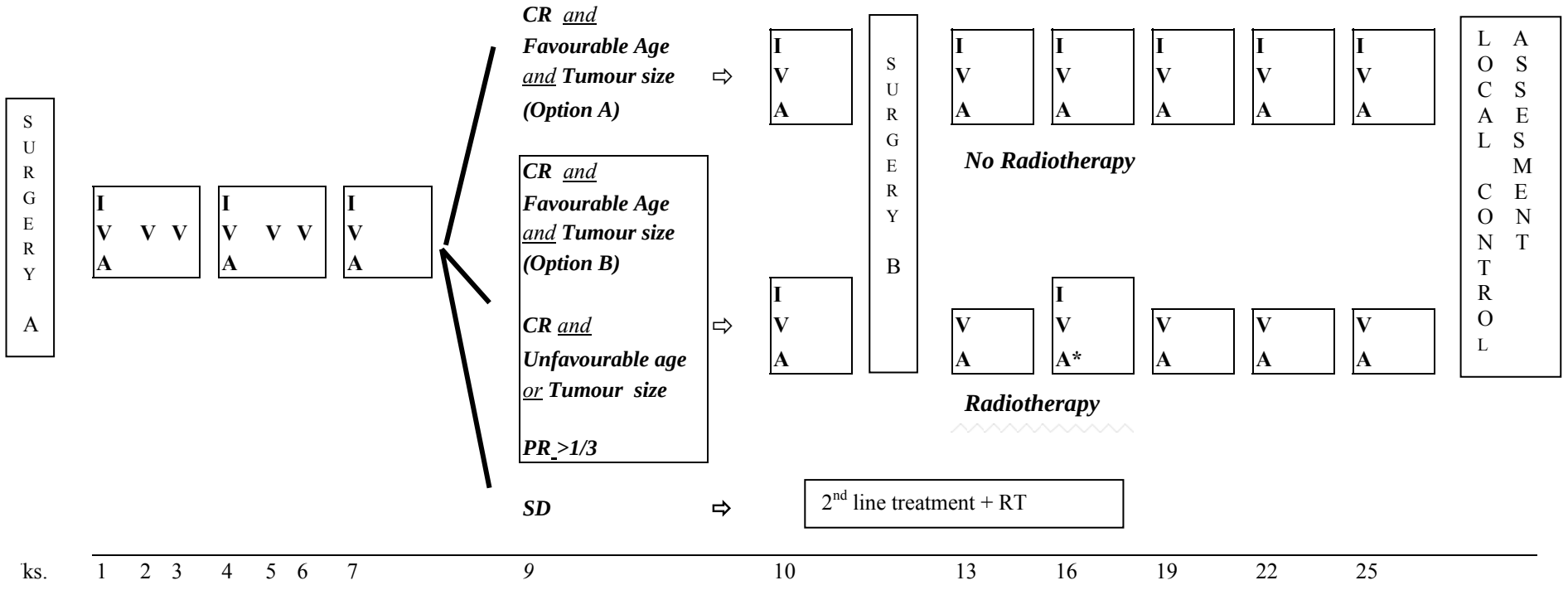
Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.
Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.

For children ≤ 1 month VA only should be administered in the 1st cycle. For children ≤ 1 year (or ≤ 10 kg) specific precautions (doses calculated by body weight, reduced ifosfamide dose if age < 3 mos, …) must be applied, see chapter 24.4.1.

For *Standard Risk Group-Subgroup B* treatment details see chapter 6.3.
For chemotherapy guidelines and dose modifications: see chapter 24.

6.4 TREATMENT SUMMARY: STANDARD RISK GROUP – SUBGROUP C

SUBGROUP C	**non alveolar RMS, IRS Group II or III, localised in orbit, head and neck non PM or GU non bladder-prostate,** *and* **nodes negative** *and* **any size *or* age**



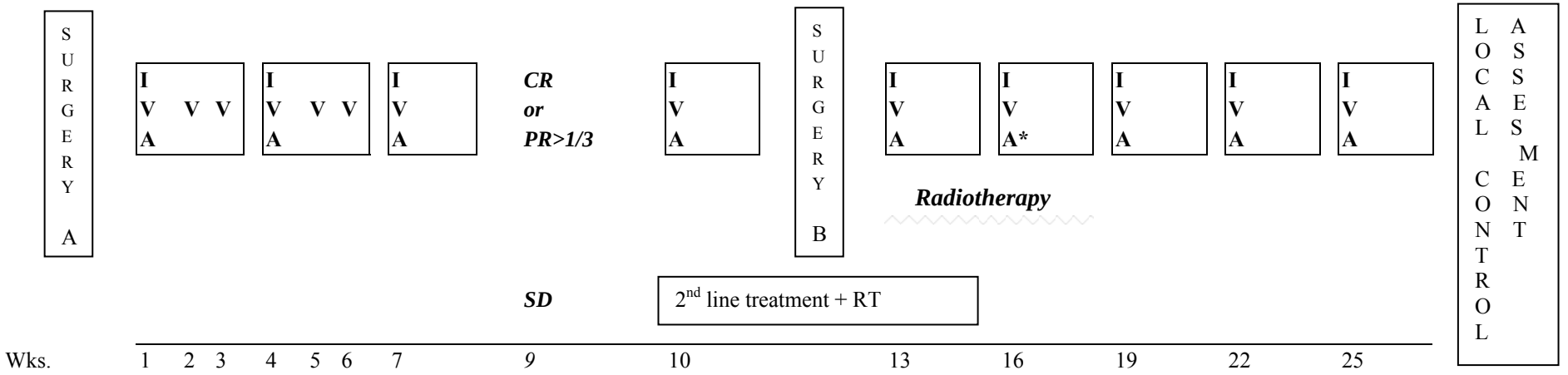
I Ifosfamide 3 g/m^2 is given as a 3 hour i.v. infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO/course = 6 g/m^2).
V Vincristine 1.5 mg/m^2 (max. single dose 2 mg) is given as a single i.v. injection on day 1 of each course and weekly, for a total of 7 consecutive doses, from week 1 to 7.
A Actinomycin D 1.5 mg/ m^2 (maximum single dose 2 mg) as a single i.v. injection on day 1 of each course.
** Actinomycin may be given at the very beginning of RT (week 13) but is omitted during RT (week 16), see chapter23.11.*

Note: Patients with favourable age (< 10 years) and tumour ≤ 5 cm at diagnosis, who achieve the complete remission after the initial treatment (3 courses of IVA + surgery) have two options:

- **Option A:** patients will receive 6 courses of IVA without radiotherapy.
- **Option B:** patients will receive 6 courses of IVA without radiotherapy only if the CR has been obtained through a secondary operation (histologically CR). Otherwise they will be treated as patients in CR with unfavourable features. *NOTE: The German (CWS), the Italian (STSC) and the Spanish Group do recommend option B.*

6.5 TREATMENT SUMMARY: STANDARD RISK GROUP – SUBGROUP D

SUBGROUP D	**non alveolar RMS, IRS Group II or III, localised in parameningeal, extremities, GU bladder-prostate or "other sites"** *and* **nodes negative** *and* **tumour size ≤ 5 cm and age < 10 years**



I Ifosfamide 3 g/m^2 is given as a 3 hour i.v. infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

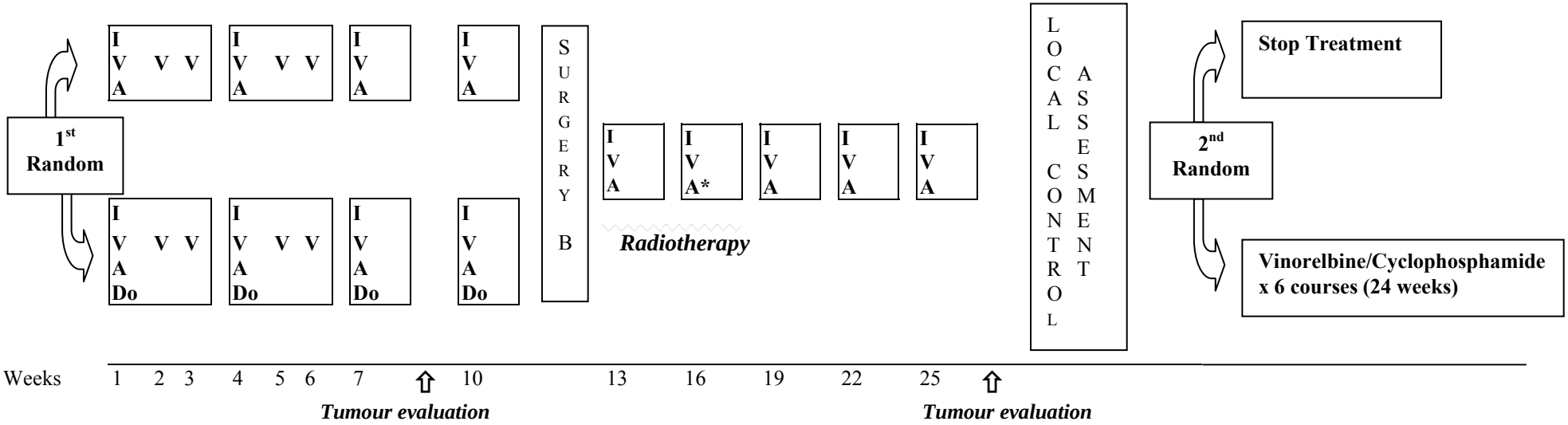
V Vincristine 1.5 mg/m^2 (max. single dose 2 mg) is given as a single i.v. injection on day 1 of each course and weekly, for a total of 7 consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/ m^2 (maximum single dose 2 mg) as a single i.v. injection on day 1 of each course.
** Actinomycin may be given at the very beginning of RT (week 13) but is omitted during RT (week 16), see chapter 23.11.*

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l/µl neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.
Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.
For children ≤ 3 months VA only should be administered in the 1st cycle. For children ≤ 1 year (or ≤ 10 kg) specific precautions (doses calculated by body weight, reduced ifosfamide dose if age < 3 mos, …) must be applied, see chapter 24.4.1.
For *Standard Risk Group - Subgroup D* treatment details see chapter 14.5.
See chapter 24 for chemotherapy guidelines and dose modifications.

6.6 TREATMENT SUMMARY: HIGH RISK GROUP

SUBGROUP E	**non alveolar RMS, IRS Group II or III, localised in parameningeal, extremities, GU bladder-prostate or "other sites"** ***and*** **nodes negative,** ***and*** **tumour size > 5 cm** ***or*** **unfavourable age ≥ 10 year**
SUBGROUP F	**non alveolar RMS, IRS Group I or II or III, any site** ***and*** **nodes positive,** ***and*** **any tumour size or age**
SUBGROUP G	**alveolar RMS, and any IRS Group I or II or III, and any site** ***and*** **nodes negative,** ***and*** **any tumour size or age**

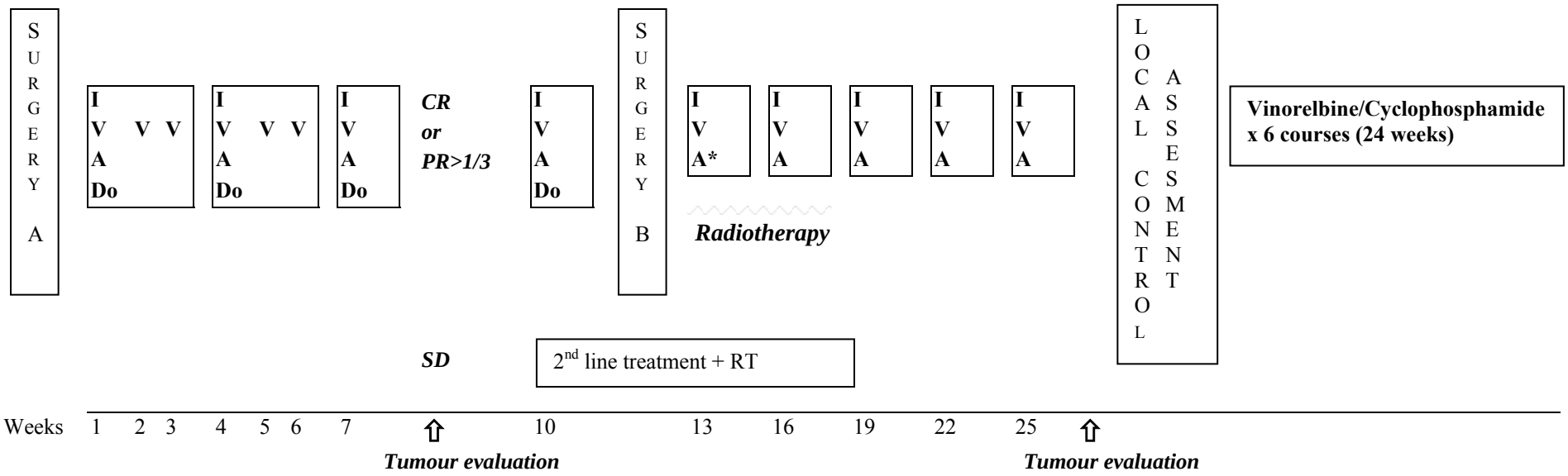


I Ifosfamide 3 g/m^2 is given as a 3 hour i.v. infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO/course = 6 g/m^2).
V Vincristine 1.5 mg/m^2 (max. single dose=2 mg) is given as a single i.v. injection on day 1 of each course and weekly for a total of 7 consecutive doses, from week 1 to 7.
A Actinomycin D 1.5 mg/m^2 (maximum single dose = 2 mg) as a single i.v. injection on day 1 of each course of treatment.
** Actinomycin may be given at the very beginning of RT (week 13) but is omitted during RT (week 16), see chapter23.11.*
Do Doxorubicin 30 mg/m^2 given as a 4-hour i.v. infusion daily on days 1 & 2 for courses 1-4 of treatment (total dose per course = 60 mg/m^2).

First Randomisation: eligible patients must be randomised before chemotherapy treatment is started using the RDE system. *If the randomization is refused or not applicable for whatever reason patients should be treated in Arm A (IVA).*
Second Randomisation: eligible patients should be randomised within 6 weeks following the administration of the 9th course of chemotherapy. *If the randomization is refused or not applicable for whatever reason the standard treatment strategy is to stop treatment.*
Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction. Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.
For *High Risk Group* treatment details: *s*ee chapter 15. For chemotherapy guidelines and dose modifications: see chapter 24.

6.7 TREATMENT SUMMARY: VERY HIGH RISK GROUP

SUBGROUP H	**alveolar RMS and nodes positive** (independently from any other variable such as tumour histology, site, size or patient age)



I Ifosfamide 3 g/m^2 is given as a 3 hour i.v. infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (maximum single dose = 2 mg) is given as a single i.v. injection on day 1 of each course of IVA and weekly for a total of seven consecutive doses, until week 7. Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.

A Actinomycin D 1.5 mg/m^2 (maximum single dose = 2 mg) as a single i.v. injection on day 1 of each course of IVA.
** Actinomycin may be given at the very beginning of RT (week 13) but is omitted during RT (week 16), see chapter 23.11.*

Do Doxorubicin 30 mg/m^2 given as a 4-hour i.v. infusion daily on days 1 & 2 for courses 1-4 of treatment (total dose per course = 60 mg/m^2).

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/lWBC (or 1 x10^9/l neutrophils) + 80x10^9/l platelets + absence of any relevant organ dysfunction.
For *High Risk Group* treatment details: *s*ee chapter 16. For chemotherapy guidelines and dose modifications: see chapter 24

7. Background

The prognosis of children with localized rhabdomyosarcoma has improved dramatically since the introduction of co-ordinated multimodality treatment. Cure rates have improved from 25% in the early seventies, when combination chemotherapy was first implemented, to approximately 70% in more recent years.
A major role in developing new strategies has been carried by Cooperative Groups working in Europe and North America. They have optimised the treatment for children with RMS matching the complexity of treatment against known prognostic factors such as site, stage and pathological subtype.
In fact the role of radiotherapy, surgery and chemotherapy regimen in different risk groups has been explored in a series of multicentre clinical trials on both sides of the Atlantic.
This protocol has been derived from the evolving cooperation of European Groups, namely the SIOP Malignant Mesenchymal Tumours (MMT) Committee, the AIEOP Soft Tissue Sarcoma Committee (AIEOP STSC) (former ICG: Italian Cooperative Group for paediatric soft tissue sarcoma) and the German Co-operative Soft Tissues Sarcoma Group (CWS).
This collaboration will allow the recruitment of patients from all over Europe to the same protocol and thus be able to answer more rapidly some still unanswered questions regarding the treatment of children with soft tissue sarcomas.

7.1 RHABDOMYOSARCOMA

Rhabdomyosarcoma (RMS) is thought to arise from primitive mesenchymal cells committed to develop into striated muscles. It can be found virtually anywhere in the body, including those sites where striated muscles are not normally found. It is the commonest form of soft tissue sarcoma in children and young adults and accounts for approximately 4 - 5 % of all childhood malignancy with an annual incidence of 5.3 per million children under the age of 15. The peak incidence is seen early in childhood with a median age at diagnosis of about 5 years. Males are reported to be more frequently affected than females.
The aetiology is unknown. Genetic factors may play an important role as demonstrated by an association between RMS and familial cancer syndrome (Li Fraumeni), congenital anomalies (involving the genitourinary and central nervous system) and other genetic conditions, including neurofibromatosis type 1.

Depending on histological appearance two main forms of RMS have been distinguished: the embryonal (which accounts for approximately 80% of all RMS) and the alveolar subtypes (15 - 20% of RMS).
However it has been shown that some subtypes have an impact on survival. In 1995 pathologists from the different Cooperative Groups agreed a new classification which identified prognostically significant and reproducible subtypes [1]. Three main classes have been identified:
1) superior prognosis: including botryoid RMS and spindle cell or leiomyomatous RMS;
2) intermediate prognosis: represented by embryonal RMS;
3) poor prognosis: including alveolar RMS and its variant solid alveolar.
This classification system does not include the pleomorphic category, as this is very rarely observed in children.

Molecular biology studies have identified two characteristic chromosomal alterations in RMS: reciprocal chromosomal translocations t(2;13)(q 35; q14) or t(1;13)(p36;q14) in alveolar RMS [2] whilst genetic loss on chromosome 11p15.5 has been shown in embryonal RMS [3]
Different staging systems have been elaborated to classify RMS into categories from which treatment can be planned and prognosis predicted. The most widely used are the pre-treatment TNM staging and the postoperative IRS Grouping system (see appendix A.2). However with the evolution of treatment and trial results new, more complex, categorization has been used to better tailor the treatment to the risk of relapse.
Modern risk grouping attempts to take into account all the factors shown to be prognostically important. The most important are *Stage, Site and Histology.* They are also interdependent with, for instance, orbital tumours being almost exclusively of the embryonal subtype and limb tumours over represented amongst those with alveolar histology
The size of the tumour has a prognostic impact similar to that of other soft tissue sarcomas. More recently the patient's age at diagnosis has been recognised as a predictor of survival, with the older children ($\geq$ 10 years old) having the worse outcome [4].

7.2 TREATMENT STRATEGIES

A multimodality approach involving surgery, chemotherapy and radiotherapy is necessary in the treatment of children with RMS. The optimal timing and intensity of these three treatment modalities must be planned with regard to the prognostic factors and considering the late effects of treatment.
Local control is necessary to cure children with localized RMS and this may be achieved with surgery and/or radiotherapy. A conservative approach is recommended and tumour resection or irradiation is usually performed taking into account the activity of chemotherapy in reducing the tumour volume.
Different drug combinations proved to be effective against RMS. The most widely used regimens are: VAC (vincristine, actinomycin D, cyclophosphamide), VACA (VAC plus adriamycin alternating with actinomycin D), IVA (as VAC, but with ifosfamide replacing cyclophosphamide) and VAIA (IVA with adriamycin alternating with actinomycin D).
The multimodality approach according to different strategies and different chemotherapy regimens has been tested in several clinical trials run by the Cooperative Groups already named. Their results constitute the evidence for this protocol.

7.3 SIOP MMT STUDIES

The philosophy behind the SIOP studies has explored the use of more intensive primary chemotherapy in an attempt to reduce, where possible, the systematic use of definitive local therapy (surgery or radiotherapy). The objective has been to reduce the risk of important late functional or cosmetic sequelae, whilst maintaining satisfactory overall survival.

SIOP 75 and MMT 84
SIOP 75 was performed between 1975 and 1984 and compared treatment with a VAC based regimen given before or after definitive local therapy. Although there was no difference between the 2 arms (overall survival = 52%), the patients who received initial chemotherapy followed by local therapy achieved a similar survival with less aggressive local treatment and, predictably less important sequelae [5] .

MMT 84 followed this by using the strategy of intensified initial chemotherapy (IVA, IFO 6 g/m^2/course, VCR and ACT-D) to try and reduce or avoid local therapy for patients who achieved complete remission (CR) with chemotherapy with or without conservative surgery. Patients achieving CR with chemotherapy +/- surgery did not receive radiotherapy or further extensive surgery. Those remaining in partial remission (PR) required definitive local therapy, or if not feasible, a trial of second line chemotherapy. Only patients over the age of 5 years with parameningeal tumours, and those aged >12 years with tumours at any site, received systematic radiotherapy.
The overall results of MMT 84 demonstrated a high CR rate (91%) in patients with localised disease. CR was achieved with chemotherapy alone in 48% patients. Overall survival at 5 years was 68% with an event free survival of 53% [6]. Only 34% patients received intensive local therapy.

MMT 89
The overall objectives of MMT 89 were to improve treatment outcome for children with non-metastatic RMS and to continue to reduce the systematic use of local therapy to reduce, where possible, the consequences of local therapy.
For standard and high risk patients, specific aims were a) to improve outcome by evaluating early tumour response and modifying chemotherapy in poor responders and b) to explore the value of an increased dose intensity of IFO (9 g/m^2/course compared to 6 g/m^2/course in MMT 84). Intensified chemotherapy using the multiagent (6 drug) combination was used for patients with high risk (stage III) disease and for young patients with parameningeal disease. Systematic radiotherapy again was avoided in patients who achieved CR with chemotherapy with or without surgery, except in children $\geq$3 years with parameningeal tumours.
In patients with a very good prognosis (completely resected disease at favourable sites) an attempt was made to further reduce the sequelae of treatment by avoiding the use of alkylating agents.
Complete remission was achieved in 93% of patients. Five-year overall and event free survival were 71% and 57%, respectively. Overall survival was not significantly better than that achieved in the previous MMT 84 study but 49% of survivors (33% of all patients) were cured with limited local therapy.
Other key findings were:

- In low stage disease (pT1) it was confirmed that duration and intensity of therapy can be reduced as there was no reduction in overall survival in patients treated with two drugs (VCR and ACT-D) for two cycles compared to historical controls treated in MMT 84 with three drug (additional IFO) over 6 cycles. However EFS was less satisfactory (67% vs. 85%).
- There was an improvement in survival for patients with regional lymph node (SIOP Stage III) disease treated with 6 drugs (including anthracyclines) in MMT 89, compared to those treated with IVA in MMT 84 (5 year OS 60% compared to 42%).

For younger patients ($\leq$3 years) with parameningeal disease, the results of MMT 89 demonstrate that the survival in these patients in whom radiotherapy was deferred was not significantly worse than others receiving systematic radiotherapy. However almost all those who survived ultimately received radiotherapy (only 3/27 patients were cured without radiotherapy). The question of whether a delay in the administration of radiotherapy is of long term benefit remains unanswered.

Issues of local control
It was to be expected that the strategy of determining local therapy based on initial chemotherapy response (as in MMT 84 and MMT 89) would result in higher local relapse rates compared to other treatment strategies. However, a secondary objective of the SIOP studies has been to determine whether patients initially treated with chemotherapy without local definitive therapy could be salvaged by local treatment and further chemotherapy at the time of relapse. Although certain subsets of patients appear to benefit from this strategy (e.g. those with orbital [7] or bladder prostate

tumours [8] this has not been true for all. It has become clear that following the analysis of mature data from MMT 89 when compared to equivalent data from the IRS III and IV studies, the modification of local treatment strategy for some groups of patients was necessary. Systematic radiotherapy is now recommended for all patients ≥3 years with alveolar tumours (excluding paratesticular), and, regardless of pathology, those with non-parameningeal head and neck and those over 10 years with limb primaries.

In summary IVA remains the therapy for standard and high risk patients within the MMT studies. The strategy of withholding systematic local therapy has been of benefit to certain subsets of patients, minimising the late effects of therapy whilst others clearly require more aggressive local treatment.

7.4 CWS STUDIES

The first multi-centre German STS study (CWS-81) was conducted under the auspices of the German Society of Paediatric Oncology (GPOH) between 1981 and 1986, the second CWS-86 between 1986-1990. The results of these studies have already been reported [9, 10]. The CWS-91 was conducted between 1991-1996 and CWS-96 between 1996-2002.

Chemotherapy
In the CWS-81 and -86 Studies, all patients received a four drug chemotherapy regimen comprising VCR, AMD, Doxo, and alkylating agent: CPM in the CWS-81 Study (VACA cycle) or IFO in the CWS-86 Study (VAIA cycle). This decision was based on data showing that IFO appeared to be a more effective agent in the treatment of some paediatric tumours. The replacement of CPM by IFO improved the response in patients with macroscopic residual tumour by increasing the proportion of patients with 2/3 or more tumour volume reduction. However, no clear benefit for the event-free and overall survival was seen.
Due to the lack of result improvement and a relatively high incidence of nephrotoxicity, a decision was made to reintroduce CPM in place of IFO in the CWS-91 study for better prognostic groups of patients. In the CWS-91 Study the chemotherapy was also intensified for poor prognostic patients by adding VP16 to VAIA combination (EVAIA cycle). The results did not show a definitive survival advantage, in particular there was no change in the local relapse rate.
The intensification of chemotherapy did not reduce the number of patients who required radiotherapy: the proportion of irradiated patients was similar in the three studies, CWS-81: 77%, CWS-86: 79% and CWS-91: 85%.

Local treatment
In the CWS-81 Study radiation was stratified according to the results of second look surgery at week 16-20, given only to patients who still had microscopic (40 Gy) or macroscopic (50 Gy) residual disease. In the CWS-86 Study radiation was given prior to second look surgery after one cycle of chemotherapy (7-10 weeks). The cumulative dose was stratified according to the degree of tumour volume reduction (32 Gy and 54.4 Gy) and given simultaneous to chemotherapy. In the CWS-91 Study radiation was stratified by tumour invasiveness (T) characteristic, the degree of tumour volume reduction and the results of second look surgery at week 10-13. Since 1986 the German STS studies (CWS) recommend an accelerated hyperfractionated irradiation (2x1,6 Gy daily). The prognosis improved dramatically in the CWS-86 and -91 Study in the group of patients who responded to chemotherapy and had been irradiated mainly prior to secondary surgery in comparison to the CWS-81 Study (EFS 69% vs.67% vs. 41%) .

It is noteworthy that 130 patients in the CWS-86 and -91 studies were irradiated with 32 Gy, the local control rate in this group was 73% and 77% respectively [11]. The comparable dose of 40 Gy conventionally fractionated was given to 25 children in the CWS 81 Study (local tumour control rate 48%).

It has been concluded that: 1) Tumour-volume reduction after preoperative chemotherapy combined with primary tumour size in patients with residual tumour can be used as a basis for risk adapted radiation. 2) Early (10-13 weeks), hyperfractionated, accelerated radiation given simultaneously to chemotherapy improved local tumour control in patients with a good response after preoperative chemotherapy. 3) The dose of 32 Gy when accelerated and hyperfractionated, given simultaneously to chemotherapy is adequate for local tumour control in patients showing a good response to preoperative chemotherapy. Whether the same principle can be applied to each histological entity cannot be answered on the basis of the CWS-Studies.

7.5 AIEOP STSC STUDIES

The Italian studies tried to identify patients with low risk characteristics for whom treatment could be reduced and those patients who needed a more intensive treatment.
Despite the variation in chemotherapy regimens between protocols, the treatment philosophy which dictated the therapeutic decisions was quite similar in the first (RMS 79) and second (RMS 88) Italian protocols. It was based on a) conservative surgery or biopsy at diagnosis; b) initial chemotherapy according to different regimen adopted; c) disease evaluation after an initial 3 to 4 courses of chemotherapy; d) second look surgery in case of residual disease, e) adjuvant chemotherapy following initial or delayed radical surgery, and f) radiotherapy in patients with persistent disease.
In RMS 79 protocol patients classified in Group I received 12 courses of alternating CAV (CPM, Adria, VCR) and VAC (VCR, ACT, CPM) over 11 courses. Group II and III patients received alternating CAV and VAC for a total of 12 courses. Patients with alveolar histology or primary tumour located in the extremities received 18 alternating courses of CAV/VAC. RT was avoided in Group I but delivered to a total dose of 40-45 Gy to Group II and III patients.
In the RMS 88 protocol chemotherapy was reduced to 22 weeks VCR and ACT-D in patients with embryonal histology in IRS group I.
In patients staged in IRS Group II or III chemotherapy intensity was increased in RMS 88 protocol compared to RMS 79 replacing cyclophosphamide with ifosfamide, increasing the ACT-D dose and using the VCR more intensively in the first part of treatment. The regimens used were VAIA and IVA. Radiotherapy doses did not vary substantially but it was administered according to the hyperfractionated and accelerated techniques in RMS 88 study.
In RMS 88 study the 5 years PFS resulted 82%, 72%, and 59% in patients in Group I , II, and III respectively. The overall 5-year PFS and OS were 65.6% and 74% respectively. This represents an improvement from RMS 79 (5 yrs PFS 53.5 and OS 64%). The patients who benefited more were those with the following characteristics: embryonal histology, parameningeal or other primary site, large and invasive tumours (size > 5 cm and T2), node negative [12].
More detailed analyses for subset of patients were carried out. A joint Italian/German study on paratesticular RMS confirmed the good outcome of patients with localized disease (5 years survival 94.6%). Major prognostic factors were tumour invasiveness, size, resectability as well as nodal involvement and age. This allowed the identification of subset of patients at low risk that could be treated with VA. Alveolar histology did not have an adverse impact on the patients outcome (5 year survival 93.3% vs. 88.1% in non alveolar RMS) [13].

In conclusion the Italian experience showed that it is possible to avoid the administration of anthracyclines and alkylating agents in patients with favourable characteristics and chemotherapy intensification improved the results in some subsets of high risk patients. Due to the improved results in RMS 88 an IFO-based regimen became the reference regimen in the Italian studies.

7.6 IRSG STUDIES

The IRS Group has concluded 4 consecutive studies (from IRS-I to IV) from 1972 to 1997. The IRS-V study is currently ongoing.
The 5-year survival improved significantly from 55% on the IRS I protocol, to 63% on the IRS-II and to more than 70% on the IRS-III and IV protocols. [14].
The initial studies used the IRS grouping system to stratify patients and treatment.
Early IRS trials showed that for patients in Group I VCR and ACT-D are enough and radiotherapy is not necessary [15]. More recent analysis showed a role of irradiation for patients with alveolar histology [16].
In Group II patients the VA regimen (VCR, ACT-D) with radiotherapy have been considered the standard treatment for non alveolar non extremity RMS. The benefit of the addition of other drugs such as doxorubicin and cyclophosphamide is not clear due to the contradictory results noted in IRS-III trial [17].
In Group III patients the intensification of treatment increasing the cumulative drug dose and moving from standard VAC to pulsed VAC has improved the survival from 52% in IRS-I to 74% in IRS-III [15]. No clear benefit was evident with the addition of doxorubicin.

In more recent IRS trials other prognostic factors have been recognised and used to decide the treatment, in particular histology, tumour site and size.
In IRS-IV the 3 years survival was 86%. In this study patients were randomised to receive chemotherapy with VAC or VAI or VIE. No significant difference in outcome was noted and the VAC was elected as gold standard by the American investigators due to the lower cost and nephrotoxicity of cyclophosphamide [4].

7.7 RESULTS OF CWS/RMS 96 AND MMT 95 STUDIES

These studies represent the basis for the ongoing European collaboration. In fact a common stratification has been used (Table 2) and a similar randomised study has been run by the three Cooperative Groups. With the goal to explore the value of more intensive chemotherapy for RMS, the regimen used in the European Intergroup Stage IV Protocol (CEVAIE) was randomised against the standard treatment, i.e. VAIA in the German/Italian CWS/RMS 96 or IVA in the MMT 95 study. Differences in local treatment philosophy at that time precluded the possibility of planning a common study.

Table 2 - CWS/RMS 96 & MMT 95 Common Stratification

N-Status	Histology	Group	Site	pT-Status	Risk Group
N0	ERMS/RMS nos	I	Any	pT1	***LOW***
		I	Any	pT2	***STANDARD***
		II+III	ORB, HN, NBP	pT3a/b/c	
		II+III	PM, UG-BP, EXT, OTH	pT3a/b/c	***HIGH***
	ARMS, EES/PNET	Any	Any	Any	
N1	All				

In the CWS/STSC experience Low, Standard and High Risk Group showed good preliminary results: EFS 88%, 77% and 62% respectively and OS 97%, 95% and 78% respectively (see Figure 1 and 2).

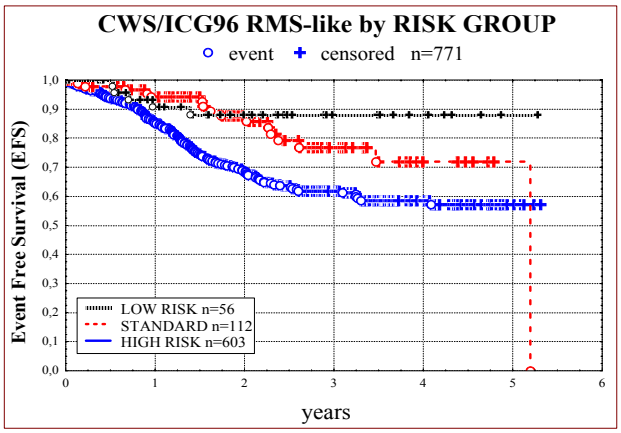


Figure 1: Event free survival according to risk group

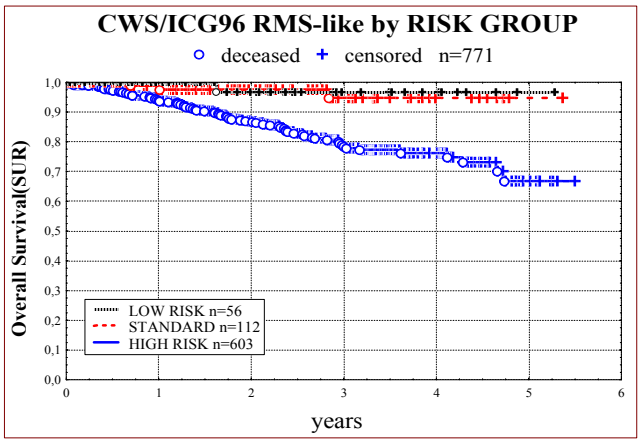


Figure 2: Overall survival according to risk group

Low Risk: patients were treated both in the SIOP MMT 95 and the CWS/RMS 96 study with Vincristine and Actinomycin D only. This treatment approach followed the Italian experience of the RMS 88 study in which 4 blocks of VA was used for the first time. The good results achieved with this low toxic regimen has led to its adoption in this protocol [13 18].

Standard risk: These patients have been treated with IVA (9 blocks over 25 weeks) both in MMT 95 and CWS/RM S96. This represented a treatment reduction for the CWS group that used anthracyclines in the previous protocol. The total length of therapy has also been reduced from 35 (CWS-81 and RMS 88) to 25 weeks.
In the CWS/STSC experience events in this group were mainly local. The main reason for the greater number of relapses has been attributed to the cautious administration of RT. Of the irradiated group within the standard risk group only 7% relapsed vs. 15% in the non-irradiated group even though the irradiated group has been negatively selected (prognostic risk factors poorer than in non-irradiated group).

High risk: In the CWS/RMS 96 protocol these patients were enrolled in a randomised trial with the aim to compare a 6-drug regimen (CEVAIE) with the "standard" 4-drug regimen VAIA. In the MMT 95 study CEVAIE was randomised against IVA.
Both studies failed to show a superior outcome for patients treated with CEVAIE (unpublished data).

7.8 CONCLUSIONS

The treatment of patients with RMS undergoes continuing evolution and should be adapted constantly as new evidence emerges from clinical trials. It is therefore not possible to define a true "standard treatment" for these patients. This evolving process has lead to the improved survival seen over the last decades and should continue in the future.

- A more accurate prognostic assessment at diagnosis is needed to ensure that those patients with a good prognosis are not over treated and to identify those with a poorer prognosis who require a more aggressive approach. Histology, staging (IRS grouping), node involvement tumour site and size and patient's age have been presently identified as major prognostic factors.

- A better selection of children that can be treated with less intensive treatment (VA alone $\pm$ radiotherapy) should be attempted to avoid acute and late sequelae of alkylating agents and anthracyclines.

- Chemotherapy regimens based on the VAC or IVA combinations appear equally effective and may be considered the "reference regimen" for most children with RMS. However a substantial proportion of children are not cured with such regimens and the search for new combinations must continue. The value of the addition of other drugs should be investigated in randomised trials.

- Local treatment is a fundamental part of RMS but the advantages and disadvantages of aggressive surgery and/or radiotherapy should be balanced against the late effects for young children.

- Conservative surgery is recommended, and experience should be gathered to select those children for whom surgery may be the only necessary local treatment.

- Although it is possible to cure about 30% of patients without radiotherapy, only a subgroup of them (i.e. embryonal tumour completely resected at diagnosis) can confidently be identified at diagnosis. Further efforts should be made to better define a favourable population in whom irradiation and its late effects can be avoided.

Increasing international collaboration should improve the treatment stratification and explore through well designed, randomised studies better treatment strategies for children with RMS.

8. Rationale for the E*p*SSG RMS 2005 protocol

8.1 RATIONALE FOR THE NEW E*p*SSG STRATIFICATION

An analysis carried out by the CWS group using the CWS/RMS 96 preliminary data and validated using the data of studies with longer follow up: the SIOP MMT 84 and 89, the German CWS-81 and 91 and the Italian RMS 79 and RMS 88 studies (see Table 4) identified as significant prognostic factors for localized RMS the following:

- HISTOLOGY (aRMS *vs.* eRMS)
- POST SURGICAL STATUS (AS DEFINED BY IRS GROUPING SYSTEM),
- TUMOUR SITE,
- NODE INVOLVEMENT (N0 ABSENT, N1 PRESENT),
- TUMOUR SIZE (> OR ≤ 5 CM)
- PATIENT'S AGE (UNFAVOURABLE IF ≥10 YEARS)

Combining these factors 8 subgroups of patients have been identified (see Table 3).

Table 3 – Patient Subgroups

Subgroup	**Pathology**	**IRS Group**	**Site**	**Node Stage**	**Tumour Size & Age**
A	eRMS	I	Any	N0	≤5 cm and <10 yr
B	eRMS	I	Any	N0	>5 cm or ≥10 yr
C	eRMS	II, III	Orbit; Head & Neck non PM GU non Bladder-Prostate	N0	Any
D	eRMS	II, III	Extremity; Parameningeal; Bladder-Prostate; Other sites	N0	≤5 cm and <10 yr)
E	eRMS	II, III	Extremity; Parameningeal; Bladder-Prostate; Other sites	N0	>5 cm or ≥10 yr
F	eRMS	I, II, III	Any	N1	Any
G	aRMS	I, II, III	Any	N0	Any
H	aRMS	I, II, III	Any	N1	Any

Table 4 - Results for each subgroup in the different European studies

	CWS/RMS 96		MMT 84 & 89		CWS81 & 91		RMS 79 &88	
Subgroups	**% of patients**	**3 yrs EFS**	**% of patients**	**5 yrs EFS**	**% of patients**	**5 yrs EFS**	**% of patients**	**5 yrs EFS**
A	7	93%	6	93%	8	88%	6	94%
B	6	73%	8	69%	27	72%	6	78%
C	18	81%	21	61%			18	72%
D	11	77%	10	61%			9	83%
E	27	59%	29	52%	57	59%	27	55%
F	10	43%	10	55%			8	51%
G	15	64%	12	28%			20	52%
H	6	25%	4	31%	7	36%	6	39%

Taking into consideration these results and their implications for treatment, 4 Risk Groups have been identified. (see Table 5)

Table 5 - Risk Group and predicted EFS and OS

Risk Group	Subgroup	Estimated % of patients	Estimated 3 yrs EFS
Low Risk	***A***	***6-8%***	***90%***
Standard Risk	***B***	***25-35%***	***70-80%***
	C		
	D		
High Risk	***E***	***55-60%***	***50-55%***
	F		
	G		
Very High Risk	***H***	***4-7%***	***30-40%***

8.2 RATIONALE FOR LOW RISK PATIENTS TREATMENT

This represents a very selected group of patients, accounting for 6 to 8% of the whole population of localized RMS, with an excellent outcome. Most of these patients are represented by children with paratesticular RMS.
Reducing the toxicity without jeopardizing the results is therefore the goal in this group of patients.
The VA chemotherapy adopted in the previous protocols RMS 88, CWS/RMS 96 and SIOP MMT 95 showed good results with event-free and overall survival above 80 and 90%, respectively. [13].
The results achieved in MMT 89 with 12 of 41 stage I patients relapsing after only 2 blocks of VA suggest caution in further reducing the treatment in this subset of patients [18].
In conclusion VA x 22 weeks (8 VA blocks) represents a low-toxic, effective regimen for this group of patients and will be adopted in this protocol.

8.3 RATIONALE FOR STANDARD RISK PATIENTS TREATMENT

This group includes patients with a satisfactory prognosis for whom the goal is to reduce the treatment without compromising survival.
Three Subgroups of patients have been identified with similar outcome. However because their characteristics are quite different it has not been possible to design an identical treatment. Three treatment arms have been proposed, maintaining IVA as the regimen of reference.

8.3.1 Subgroup B: Treatment Arm SR-B

These patients are similar to the ones included in the Low Risk Group but tumour size or age are unfavourable. Most of these patients are represented by children with paratesticular RMS older than 10 years and/or with large tumour (> 5 cm).
There is increasing evidence from the European and USA experience that older children ($\geq$ 10 years) with low risk characteristics fare worse than their younger counterparts [13, 18]. In the IRS studies an increased risk of nodal relapse have been seen in Group I patients with paratesticular tumour and age $\geq$ 10 years. This prompted the IRSG colleagues to return to a surgical staging for older patients [4].
The European experience reported a lower rate of nodal involvement as laparotomy with nodal exploration is avoided, but caution has been recommended in reducing the treatment in such patients**.**
The Subgroup B has been created to upstage these patients and treat them with a limited dose of alkylating agents with the aim of reduce the risk of relapse and avoiding important toxicity.

8.3.2 Subgroup C: Treatment Arm SR-C

This group is mainly represented by orbital and head and neck non parameningeal RMS.
The Italian, German and North American experience is in favour of the use of systematic irradiation of these patients. The MMT studies have demonstrated, however, that some children can be treated with chemotherapy alone and eventually salvaged after relapse with irradiation [7].
In the more recent IRS IV study patients with orbital RMS in IRS Group I or II have been treated with VA and irradiation with an excellent outcome [4]. The same strategy is currently used for all orbital RMS in the ongoing IRS V study.
Therefore it seems possible in this subgroup *a)* to reduce the cumulative dose of alkylating agents compared with previous European protocols using radiotherapy and *b)* to try to select prospectively patients with favourable features that can avoid irradiation. These patients will be selected

according to chemotherapy response (CR after the initial 3 blocks of IVA) and favourable tumour size and age.

8.3.2 Subgroup D: Treatment Arm SR-D

Patients with embryonal RMS, N0, favourable age and tumour size are included in this category. They are mainly represented by young children with small tumour arising in the extremities, parameningeal, bladder-prostate or other site areas.
An analysis of patients included in the high risk category according to CWS/RMS 96 and MMT 95 stratification showed that children with embryonal RMS, N0, favourable age and tumour size (see Table 4) have a prognosis comparable to patients treated in the standard risk group of CWS/RMS.
Consequently these patients have been included in the Subgroup D in this protocol and down staged to receive the treatment planned for the standard risk group.
These patients will continue to receive the IVA regimen as in the MMT 95 study but this represents a treatment reduction in comparison with the CWS/RMS 96 protocol where the VAIA regimen was used.

8.4 RATIONALE FOR HIGH RISK PATIENTS TREATMENT

Patients with large embryonal RMS localized in unfavourable sites, alveolar RMS, and N1 are included in this Group.
The different Subgroups included in this category share the same unsatisfactory prognosis and therefore the need for a more effective strategy.
This protocol will try to improve the outcome of these patients by implementing two novel strategies:

1) the intensification of initial chemotherapy adding anthracyclines to the standard IVA regimen
2) the adoption of a low dose maintenance treatment after 1st line chemotherapy.

8.4.1 Doxorubicin in RMS treatment

Doxorubicin (Doxo) is an effective drug in the treatment of RMS. However its role as part of a multidrug regimen is controversial. It is not clear whether adding Doxo to an established regimen such as VAC or IVA improves the survival of patients. This must be carefully considered as the toxicity profile of the drug may worsen the immunosuppression in the short term and cause cardiotoxicity in the long term.
An IRS phase II window in children with newly diagnosed metastatic rhabdomyosarcoma demonstrated the efficacy of IFO and Doxo with a 63% CR+PR rate at 12 weeks [19]. Furthermore the preliminary results of the window study with Doxo in high risk RMS in the SFCE experience (65% CR+PR) support the value of Doxo as an efficient drug in RMS (Bergeron C, unpublished data). Doxo is also considered an important drug in the treatment of other paediatric sarcomas such as osseous Ewing's and PNET [20]. Moreover a meta-analysis of several trials demonstrated that an induction treatment including Doxo in every course was better than a schema alternating Doxo with ACT-D [21].
Doxo is also one of the most effective drugs in the treatment of soft tissue sarcoma in adult patients [22].
Unfortunately different randomised trials performed by the IRS Group did not show a substantial difference in survival and progression free survival for patients with RMS treated with VAC or VAC plus anthracyclines. In IRS-I the addition of 5 VadrC course to VAC did not improve the results [23]. In IRS-II a similar comparison, but with higher cumulative doses of Doxo (480 mg/m^2) showed no improvement [24]. In IRS III further randomised comparison did not yield to different

results. However it was noted that a more complex therapy including administration of Doxo and cisplatin appeared to have caused a significative improvement in some subgroups of patients i.e. IRS group I/II alveolar histology and special pelvic sites [17]
It should be noted that in IRSG studies the treatment scheme was based on the alternating administration of VAC and VadrC, consequently the intervals between Doxo containing courses were wide, reducing the anthracycline dose-intensity.
In conclusion Doxo seems a very effective drug against RMS, however its role as part of a multi-drug regimen remains to be established.

8.4.2 The IVADo Regimen

This regimen combines the Doxo with the standard combination IVA. This allows the intensification of the chemotherapy avoiding the need to alternate courses with and without the anthracyclines as has been done up to now. This combination has been tested in a pilot study conducted by the STSC in which 29 patients with metastatic STS have been treated with the IVADo regimen (G. Bisogno et al, Cancer in press). Toxicity was mainly haematological with grade 4 neutropenia encountered in 67% of evaluable cycles and 17 patients and 8 patients receiving blood and platelets, respectively. Major toxicity occurred in two patients: VOD and seizures. Grade 3-4 organ toxicity were constipation (9.7% of cycles), mucositis (6.5%) and peripheral neuropathy (6.5%). The median interval between courses was 23 days (range 19-51). Clinical complete response after three IVADo was evident in 5 patients, PR in 17, minor PR in 2, mixed response in 2. Stable tumour was evident in 2 children with desmoplastic small round cell tumour, whereas tumour progression was evident in a patient with malignant schwannoma.
These data are also supported by preliminary data from a window study for metastatic RMS run by the SFCE group in France where no unexpected toxicities were observed in the first 7 patients enrolled.
In conclusion the IVADo regimen has proved to be active against soft tissue sarcomas but, more importantly, it is feasible because no unacceptable toxicities have been reported.

8.4.3 Maintenance treatment in RMS

Chemotherapy regimens have been progressively intensified [15] improving the survival of patients with localised disease. However patients with unfavourable characteristics, such as unfavourable site or alveolar subtype, did not show major improvements [25] and any attempt to further increase the drug dose in metastatic RMS has not significantly changed the poor prognosis of these patients [26]. When complete remission has been achieved, minimal residual disease, resistant to high dose short-term treatment, remains an obstacle to major increases in cure rate.
It is, therefore, important to identify new approaches to improve the outcome for high-risk patients.
Low dose continuous chemotherapy has been used with some success [27] and new hypotheses on antitumour mechanism have been advanced [28]. This approach is also attractive if we consider the reduced toxicity of low dose treatment.
Although there is little experience in the treatment of soft tissue sarcoma promising results have been reported by the CWS group. They used standard chemotherapy in children with metastatic soft tissue sarcoma followed by high dose chemotherapy (thiotepa + cyclophosphamide and melphalan + etoposide) or an oral treatment with trofosfamide + idarubicine. The results in 62 patients are very promising with 3-year EFS above 50% for patients taking oral treatment (and EFS 20% after high dose). Since the comparison was not randomised a risk bias between the two groups must be taken into consideration. It seems though that oral maintenance therapy has a greater benefit for group IV patients than does high dose chemotherapy.

The duration of treatment should also be addressed. This has been progressively decreased over years without apparently impairing the results. In IRS-I chemotherapy was administered for 2 years. In the latest North American protocols most patients received one year of treatment.
In the SIOP studies the treatment duration for the majority of patient was 27 weeks.
In Italian protocols the treatment duration has been progressively reduced from 52-78 weeks in the first study to 22-37 weeks in the second and 25 in the third one. Also in the CWS studies the treatment duration have been reduced for most patients from 35 weeks in the early studies to 25 in the latest ones.

The drug doses administered in each cycle have been increased progressively in the most modern protocols and this may have hindered the benefit of a longer treatment. Up to now no studies have been performed to establish which is the optimal duration of treatment for RMS.

In this protocol we propose to investigate the role of low dose chemotherapy in patients with RMS.

On the basis of previous experience with RMS cyclophosphamide appears an interesting drug for the following reasons:
a) is active against RMS
b) has been successfully used at low dose (2.5 mg/kg/day for up to 2 years) in the initial IRS studies [23, 24].
c) it may be easily included in the current European protocols where different drugs are used during the initial intensive treatment.

The activity of vinorelbine in the treatment of heavily pre-treated patients with soft tissue sarcoma has recently been published [29]. A dose finding study has been performed by the STSC [30].
Therefore the combination of these two drugs is proposed to investigate the role of low dose chemotherapy in patients with rhabdomyosarcoma. Patients in complete remission at the end of standard treatment will be randomised to stop the therapy or to continue for 6 more months with the vinorelbine-cyclo regimen.

8.4.4 Alveolar Paratesticular tumours

Despite unfavourable pathology this very small group of patients showed a good outcome in previous European studies. In the CWS/STSC experience they represented 8% of all paratesticular RMS and the 5 year survival rate was 93% after IVA ± doxorubicin chemotherapy [31]. However 4 relapses occurred. Similar data come out from the SIOP experience.
According to these data patients with paratesticular alveolar RMS will be kept in the high risk group, according to the histology factor, however in consideration of the better outcome they will not be included in the randomised trial and will be treated with IVAx9 (avoiding anthracyclines).
Patients with Alveolar N1 tumor will be treated according to the very high risk arm.

8.5 RATIONALE FOR VERY HIGH RISK PATIENTS TREATMENT

In an attempt to better define patients at high risk of relapse, an analysis of the High risk arm of the CWS/RMS 96 has been made. The group of patients with alveolar RMS and nodal involvement had the poorest outcome, comparable to that of group IV patients. In CWS/RMS 96 the 3 years EFS was 28% and OS 29%.
Results in the SIOP experience were only partially better with 5-year EFS of 39%.
These patients consequently will be treated with the more intensive strategy outlined in this protocol comprising the IVADo regimen and the maintenance chemotherapy to improve the results in comparison with historical controls.

9. References

1. Newton WA, Jr., Gehan EA, Webber BL, et al. Classification of rhabdomyosarcomas and related sarcomas. Pathologic aspects and proposal for a new classification--an Intergroup Rhabdomyosarcoma Study. *Cancer* 1995;76:1073-1085.

2. Barr FG. Molecular genetics and pathogenesis of rhabdomyosarcoma. *J Pediatr Hematol Oncol* 1997;19:483-491.

3. Scrable H, Witte D, Shimada H, et al. Molecular differential pathology of rhabdomyosarcoma. *Genes Chromosomes Cancer* 1989;1:23-35.

4. Crist WM, Anderson JR, Meza JL, et al. Intergroup rhabdomyosarcoma study-IV: results for patients with nonmetastatic disease. *J Clin Oncol* 2001;19:3091-3102.

5. Flamant F, Rodary C, Voute PA, Otten J. Primary chemotherapy in the treatment of rhabdomyosarcoma in children: trial of the International Society of Pediatric Oncology (SIOP) preliminary results. *Radiother Oncol* 1985;3:227-236.

6. Flamant F, Rodary C, Rey A, et al. Treatment of non-metastatic rhabdomyosarcomas in childhood and adolescence. Results of the second study of the International Society of Paediatric Oncology MMT84. *Eur J Cancer* 1998;34:1050-1056.

7. Oberlin O, Rey A, Anderson J, et al. Treatment of orbital rhabdomyosarcoma: survival and late effects of treatment--results of an international workshop. *J Clin Oncol* 2001;19:197-204.

8. Anderson J, Raney RB, Carli M, al. e. International study of characteristics and outcome of patients with primary rhabdomyosarcoma of the bladder / prostate. *Med Pediatr Oncol* 2001;37:181.

9. Koscielniak E, Jurgens H, Winkler K, et al. Treatment of soft tissue sarcoma in childhood and adolescence. A report of the German Cooperative Soft Tissue Sarcoma Study. *Cancer* 1992;15:2557-2567.

10. Koscielniak E, Harms D, Henze G, et al. Results of treatment for soft tissue sarcoma in childhood and adolescence: a final report of the German Cooperative Soft Tissue Sarcoma Study CWS-86. *J Clin Oncol* 1999;17:3706-3719.

11. Koscielniak E, Schmidt B, Knietig R, al. e. Effectiviy of a 32 Gy radiation dose in children with rhabdomyosarcoma: Report of the German Cooperative Soft Tissue Sarcoma Studies (CWS). *Med Pediatr Oncol* 2001;37:186.

12. Carli M, Bisogno G, Cecchetto G, al. e. Childhood Rhabdomyosarcoma: Results of the Italian Cooperative study RMS88. *Medical and Pediatric Oncology* 1998;31:262.

13. Ferrari A, Bisogno G, Casanova M, et al. Paratesticular rhabdomyosarcoma: report from the Italian and German Cooperative Group. *J Clin Oncol* 2002;20:449-455.

14. Raney RB, Anderson JR, Barr FG, et al. Rhabdomyosarcoma and Undifferentiated Sarcoma in the First Two Decades of Life: A Selective Review of Intergroup Rhabdomyosarcoma Study Group Experience and Rationale for Intergroup Rhabdomyosarcoma Study V. *Am J Pediatr Hematol Oncol* 2001;23:215-220.

15. Pappo AS, Shapiro DN, Crist WM, Maurer HM. Biology and therapy of pediatric rhabdomyosarcoma. *J Clin Oncol* 1995;13:2123-2139.

16. Wolden SL, Anderson JR, Crist WM, et al. Indications for radiotherapy and chemotherapy after complete resection in rhabdomyosarcoma: A report from the Intergroup Rhabdomyosarcoma Studies I to III. *J Clin Oncol* 1999;17:3468-3475.

17. Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. *J Clin Oncol* 1995;13:610-630.

18. Stewart RJ, Martelli H, Oberlin O, et al. Treatment of children with nonmetastatic paratesticular rhabdomyosarcoma: results of the Malignant Mesenchymal Tumours studies (MMT 84 and MMT 89) of the International Society of Pediatric Oncology. *J Clin Oncol* 2003;21:793-798.

19. Sandler E, Lyden E, Ruymann F, et al. Efficacy of ifosfamide and doxorubicin given as a phase II "window" in children with newly diagnosed metastatic rhabdomyosarcoma: a report from the Intergroup Rhabdomyosarcoma Study Group. *Med Pediatr Oncol* 2001;37:442-448.

20. Rodriguez-Galindo C, Spunt SL, Pappo AS. Treatment of Ewing sarcoma family of tumors: current status and outlook for the future. *Med Pediatr Oncol* 2003;40:276-287.

21. Smith MA, Ungerleider RS, Horowitz ME, Simon R. Influence of doxorubicin dose intensity on response and outcome for patients with osteogenic sarcoma and Ewing's sarcoma. *J Natl Cancer Inst* 1991;83:1460-1470.

22. Spira AI, Ettinger DS. The use of chemotherapy in soft-tissue sarcomas. *Oncologist* 2002;7:348-359.

23. Maurer HM, Beltangady M, Gehan EA, et al. The Intergroup Rhabdomyosarcoma Study-I. A final report. *Cancer* 1988;61:209-220.

24. Maurer HM, Gehan EA, Beltangady M, et al. The Intergroup Rhabdomyosarcoma Study-II. *Cancer* 1993;71:1904-1922.

25. Baker KS, Anderson JR, Link MP, et al. Benefit of intensified therapy for patients with local or regional embryonal rhabdomyosarcoma: results from the Intergroup Rhabdomyosarcoma Study IV. *J Clin Oncol* 2000;18:2427-2434.

26. Carli M, Colombatti R, Oberlin O, et al. High-dose melphalan with autologous stem-cell rescue in metastatic rhabdomyosarcoma. *J Clin Oncol* 1999;17:2796-2803.

27. Kushner BH, Kramer K, Cheung NK. Oral etoposide for refractory and relapsed neuroblastoma. *J Clin Oncol* 1999;17:3221-3225.

28. Kamen BA, Rubin E, Aisner J, Glatstein E. High-time chemotherapy or high time for low dose. *J Clin Oncol* 2000;18:2935-2937.

29. Casanova M, Ferrari A, Spreafico F, et al. Vinorelbine in previously treated advanced childhood sarcomas: evidence of activity in rhabdomyosarcoma. *Cancer* 2002;94:3263-3268.

30. Casanova M, Ferrari A, Bisogno G, Merks JH, De Salvo GL, Meazza C, Tettoni, K, Provenzi M, Mazzarino I, Carli M. Vinorelbine and low-dose cyclophosphamide in the treatment of pediatric sarcomas: pilot study for the upcoming European Rhabdomyosarcoma Protocol. *Cancer.* 2004; 101:1664-71.

31. Ferrari A, Bisogno G, Casanova M, et al. Is alveolar histotype a prognostic factor in paratesticular rhabdomyosarcoma? The experience of Italian and German Soft Tissue Sarcoma Cooperative Group. *Pediatr Blood Cancer* 2004;42:134-138.

10. Study structure

This protocol includes:

- an observation study
for patients in the low, standard and very high risk groups.

- an investigational study (randomised trial)
for children within the high risk group

10.1 STRATIFICATION AND RISK GROUPS

Patients have been stratified in 8 Subgroups (A through H) that are subsequently grouped in 4 Risk Groups: low, standard, high and very high.

The prognostic factors considered are:

- **Pathology:**

Favourable = all embryonal, spindle cells, botryoid RMS
Unfavourable = all alveolar tumours (including the solid-alveolar variant)

- **Post surgical stage:**

according to the IRS grouping. Briefly
Group I = primary complete resection (equivalent to SIOP pT1);
Group II = microscopic residual (equivalent to SIOP pT3a) or primary complete resection but node involvement (N1);
Group III = macroscopic residual (equivalent to SIOP pT3b).
For more details on IRS grouping system see also appendix A.2.

- **Site:**

Favourable = orbit, GU non bladder prostate (i.e. paratesticular and vagina/uterus) and head & neck non PM
Unfavourable = all other sites (parameningeal, extremities, GU bladder-prostate and "other site")

- **Node stage**

According to the TNM classification
N0 = no clinical or pathological node involvement
N1 = clinical or pathological nodal involvement

- **Size & Age:**

Favourable = Tumour size (maximum dimension) ≤5 cm ***and*** Age < 10 years
Unfavourable = all others (i.e. Size >5 cm ***or*** Age ≥ 10 years)

Note: patients with malignant effusion (i.e. tumour cell in peritoneal or pleural fluid) or cells in the spinal fluid should be treated according to the protocol for metastatic RMS

Table 6 - Risk Stratification for EpSSG non metastatic RMS study

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
Low Risk	**A**	Favourable	I	Any	N0	Favourable
Standard Risk	***B***	Favourable	I	Any	N0	Unfavourable
	C	Favourable	II, III	Favourable	N0	Any
	D	Favourable	II, III	Un favourable	N0	Favourable
High Risk	***E***	Favourable	II, III	Unfavourable	N0	Unfavourable
	F	Favourable	II, III	Any	N1	Any
	G	Unfavourable*	I, II, III	Any	N0	Any
Very High Risk	***H***	Unfavourable	I, II, III	Any	N1	Any

** Note: for paratesticular alveolar RMS see paragraph 8.4.4..*

11. Patient eligibility

Participating centres are expected to register all patients with Rhabdomyosarcoma and other Soft tissue sarcomas.

There are two levels of eligibility for this protocol: according to the risk group patients may be eligible to be treated according to the observation study or to entry (high risk group) into the randomised trial (see summary chart chapter 11.3).
Centres are not allowed to enrol patients only in the trial or in the observation study.

11.1 Eligibility to the Protocol (study + trial)

Patients with the following criteria are eligible for EpSSG RMS 2005 protocol:

- A pathologically proven diagnosis of Rhabdomyosarcoma.
- No evidence of metastatic lesions.
- Age less than 21 years (20 years and 364 days) of age.
- Previously untreated except for primary surgery.
- No pre-existing illness preventing treatment, in particular renal function must be equivalent to grade 0-1 nephrotoxicity, no prior history of cardiac disease and normal shortening fraction (> 28%) and ejection fraction (> 47%).
- No previous malignant tumours.
- Interval between diagnostic surgery and chemotherapy no longer than 8 weeks.
- Diagnostic material available for pathology review.
- Available for long term follow up through the treatment centre.
- Written informed consent for treatment available.

Patients with a diagnosis of RMS not satisfying the above criteria will be registered, but not evaluated for the purpose of this study.

Patients with ***RMS N.O.S, Undifferentiated STS*** *and* ***Ectomesenchymoma*** *are eligible to RMS 2005 protocol: see paragraph 29.4*

Notes
- *patients with malignant effusion* (i.e. tumour cell in peritoneal or pleural fluid) or malignant cells in the spinal fluid should be treated according to the protocol for metastatic RMS.

- *Adults with RMS* (> 21 years) may be eligible for registration and treatment on study (according to institutional preference) but not for randomisation.

11.2 Eligibility to the Randomised trials

Patients eligible for the EpSSG RMS 2005 Protocol are also eligible for the randomised trials when the following criteria are satisfied:

11.2.1 FIRST RANDOMISATION

This randomisation will take place after the diagnostic surgery, before chemotherapy treatment is started using the Remote Data Entry (RDE) system (see randomisation procedure chapter 33.5).

Patients with the following criteria are eligible for EpSSG RMS 2005 randomised:

- stratification according to the High Risk Arm
- age > 6 months (and < 21 years)
- informed consent given for the randomised study

11.2.2 SECOND RANDOMISATION

- stratification and treatment according to the High Risk Arm
- age > 6 months at the moment of randomisation (some infants, not eligible for the first randomisation, may be randomised here) and < 21 years at diagnosis (patients older than 21 years at the moment of second randomisation are eligible too)
- in complete remission or with minimal abnormalities* on imaging studies at the end of "standard " treatment (9 courses of chemotherapy ± surgery ± radiotherapy).

** It is intended that minimal radiological anomalies describe imaging studies in which there may be residual abnormalities, compatible with fibrosis and the responsible clinician would be ready to stop the treatment.*

Patients must be randomised within 8 weeks after the end of treatment.
The end of treatment is defined as the last day of the 9th chemotherapy cycle. However:
- if surgery is performed after the 9th chemotherapy cycle, the date of surgery will be considered;
- if radiotherapy is administered after 9 cycles of chemotherapy, the date of the end of RT will be considered. Since maintenance CT should be started within 8 weeks from the last day of the 9th CT cycle, it would be better to start the maintenance CT during irradiation.(See randomisation procedure chapter 33.5).

Note: the RDE system will guide the clinician to check the eligibility criteria and assign the risk group.

11.3 SUMMARY FOR ELIGIBILITY

Diagnosis of Rhabdomyosarcoma or other malignant mesenchymal tumours

ELIGIBLE FOR REGISTRATION

A pathologically proven diagnosis of RMS
Age < 21 years
Previously untreated except initial surgery
No pre-existing illness preventing treatment
No previous malignant tumours

Diagnosed ≤ 8 weeks
Pathology available for central review
Available for follow up
Written consent for treatment available

YES

NO

Registration only

Evidence of Distant Metastases

NO

YES

Treat in EpSSG Stage IV Study

ELIGIBLE FOR RMS 2005 PROTOCOL

Low Risk Group	**Standard Risk Group**	**High RiskGroup**	**Very High Risk Group**
Subgroup A: ♦ VA x8	*Subgroup B:* ♦ IVA + VA *Subgroup C:* ♦ IVA ±VA *Subgroup D:* ♦ IVA	*Subgroup E* *Subgroup F* *Subgroup G* *if* -Age > 6 months -Informed consent given **Randomised trial No. 1** *(IVA vs IVADo)* if -In CR or with minimal anomalies at the end of treatment **Randomised trial No. 2** (stop treatment vs. maintenance)	*Subgroup H* ♦ IVADo + maintenance

12. Pre Treatment Investigations

With the pre-treatment investigations a patient will be tested for eligibility and staging criteria. The pre-treatment investigations must be performed no more than 4 weeks before the beginning of chemotherapy.

12.1 DIAGNOSIS

This must be established pathologically. Open surgical biopsy is the preferred approach as this maximises the tissue available for diagnostic procedures, biological studies and central pathology review. Open biopsy is essential if initial needle biopsy is non diagnostic or equivocal. On rare occasions diagnosis may be achieved by cytology of a malignant effusion or bone marrow aspirate.
(See Surgical Guidelines about initial biopsy techniques and Pathology Guidelines for details about tissue handling and diagnostic pathology techniques)

12.2 CLINICAL ASSESSMENT

- Weight, Height and Body Surface Area
- Blood pressure, pulse
- Site and clinical extent of the tumour. *For site definition see Appendix A.4.*
- Regional lymph node involvement should be assessed and recorded in all cases, including biopsy if involvement is suspected but is clinically/radiologically uncertain - under these circumstances needle biopsy or fine needle aspirate cytology may be sufficient to confirm tumour infiltration.

12.3 LABORATORY INVESTIGATIONS

- Blood Full Blood Count, Differential WBC and Platelet Count, Creatinine (and formal GFR measurement if possible), Na, K, Ca, Mg, PO_4, Cl and HCO_3 or Total CO_2, LDH, Liver function including ALT / AST, Bilirubin and Alkaline Phosphatase
- Early Morning Urine sample for Phosphate, Creatinine, Osmolarity and routine urinalysis (included as baseline for Ifosfamide nephrotoxicity evaluation)
- Bone Marrow: at least one bone marrow aspirate and trephine should be performed. Patients with evidence of node or distant metastases and all those with alveolar primaries should have bilateral aspirates and trephines.
- CSF Examination for cytospin and cell count is required only for parameningeal tumours

- **Echocardiogram:** baseline assessment is required in all patients included in High and Very High risk groups.

Optional investigations:

- Pulmonary function test
- Hormonal status in patients with tumours close to endocrine organs (thyroid gland, adrenal gland, hypophysis etc).

Note: Semen storage should be considered in post-pubertal boys before commencing chemotherapy.

12.4 RADIOLOGICAL GUIDELINES

First locoregional evaluation should be made with MRI. The choice between CT and MR depends also on local availability.
MRI is preferable for most locations, other than the chest, including head and neck tumours with possible skull base invasion [1-5].
MRI is mandatory for genito-urinary primaries and paraspinal tumours.
CT is occasionally useful for assessing subtle bone destruction but MRI is sufficient for most head and neck lesions.

Pre-treatment re-evaluation must be performed after excision biopsy since this can significantly modify initial tumour volume.

All imaging data should be stored in DICOM format for further review (on CDROM if PACS is not locally available)

See also Appendix A.6 for MRI and CT scanning technical recommendations.

- **CT scan or MRI** of the primary site (+ initial ultrasound if follow-up with ultrasound is possible).

CT or MRI examination should be carried out with the use of contrast.
The investigation will need to be performed (again) after surgical excision biopsy if significant volume has been resected

Imaging of the primary site should include tumour volume measurement and examination of regional lymph nodes especially if not evaluable clinically or if clinically suspicious.

- **Chest CT scan:** the presence of lung metastases must be evaluated in all patients at diagnosis by CT scan *and* Postero-Anterior and Lateral **Chest X-Ray.**

Intravenous-contrast enhancement is mandatory for limb or abdominal primaries (and ideally for other primaries)

- **Abdomen-pelvic CT scan** (during same acquisition as chest CT)

For abdominal, pelvic primaries if MRI has not been performed. To assess the presence of abdominal lymphadenopathy in case of paratesticular or lower limb primaries.
Intravenous-contrast enhancement is mandatory.

- **Abdomen US**

If abdominal CT is equivocal regarding lymphadenopathy or liver metastases

- **Radionuclide Bone Scan** (with plain X rays and / or MRI of any isolated abnormal site).

Mandatory in all patients at diagnosis

- **Craniospinal MRI**

If intraspinal extension or suspected meningeal involvement
Optional investigation:

- **PET-CT:** According to local availability and local protocols

Special Notes

Paratesticular tumours must have evaluation of regional (para aortic) lymph nodes by CT/MRI and Ultrasound.
Limb tumours
- Lower limb tumours must have evaluation of pelvic lymph nodes by CT or MRI even if femoral nodes are clinically/radiologically (including ultrasound) normal.
- Upper and lower limb tumours must have surgical evaluation of axillary or inguinal nodes, respectively, even if nodes are clinically/radiologically normal. This applies for both alveolar and embryonal RMS.

Tumour dimensions should be recorded in 3 diameters choosing, as far as possible, the 3 maximum diameters (sagittal, coronal and axial)

The tumour volume will be calculated according to the following:

Tumour volume (V) calculation:

a= length (in cm)
b= width (in cm) $\mathbf{V = \pi/6 \times a \times b \times c = 0.52 \times a \times b \times c}$ in cm^3
c= thickness (in cm)

12.4.1 Evaluation of lung lesions

Chest CT scan at diagnosis is mandatory in all patients. Defining pulmonary spread of tumour is critical to staging, although differentiation between metastatic or benign nodules (i.e. granulomatous disease, hamartoma, intrapulmonary lymph nodes, bronchiolitis…) can be impossible [6-8]. Several criteria are commonly used to diagnose metastastic lesions: number, size, morphology (non-calcified, round and well-defined) and location (inferior lobes, subpleural spaces, vessels-branching). Actually, no radiological criterion has a 100% specificity.
For EpSSG studies it is the radiologist, expert in such problems, that gives the interpretation of lung lesions, in discussion with the oncologist. Similarly to what is recommended for other solid tumours (i.e. Ewing sarcoma), one pulmonary/pleural nodule of 1 cm, or lesions > 0.5 cm in more than one site, are considered evidence of pulmonary metastasis, as long as there is no other clear medical explanation for these lesions. For EpSSG studies, the following patterns will be considered as metastatic pulmunary disease (assuming there is no other clear medical explanation for the these lesions):
- one or more pulmonary nodules of 10 mm or more diameter;
- or: two or more well-defined nodules of 5 to 10 mm diameter;
- or: 5 or more well-defined nodules smaller than 5 mm;

Hence, 4 or less small nodules (<5mm) at diagnosis will not be considered as pulmonary metastatic disease and should be classified only as "non-specific pulmonary lesions".
In such cases a biopsy may be performed but it is not recommended. In fact, these lesions may be considered as "evident micrometastasis" and because micrometastasis are probably present in every case of localized RMS the patients will be eligible for the protocol for localised RMS.
The same lung window settings should be used when pulmonary nodules are being measured at diagnosis and follow-up.
Please note there is a specific item in the RDE system to collect data on these particular patients.

12.4.2 Evaluations of Lymph nodes

Defining lymph nodal spread of tumour is critical to staging [9], although accurately evaluating pathological lymph node (LN) extension of tumour can be problematic.

- Oval shaped nodes (with a preserved hilum at sonography) and a short axis diameter of less than 1cm are considered normal nodes.
- Locoregional nodes which show only peripheral enhancement on CT or MRI (probable necrotic centres) are likely to be involved by tumour also, even if less than 1 cm axis.
- Mildly enlarged locoregional nodes pose a diagnostic challenge but when round in shape, over 1.5-2 cm in short axis with a heterogenous appearance are likely invaded by tumour.
- All suspicious lymph nodes merit biopsy or another form of nodal sampling.
- Sampling of loco-regional nodes is mandatory for all limb primaries (regardless of imaging findings).

Regional lymph nodes are defined as those appropriate to the site of the primary tumour: see appendix A.5.

Evidence of nodal involvement beyond the regional lymph nodes must be interpreted as distant metastasis and the patient must be treated according to the protocol for metastatic RMS.
Examples:

- perineal tumour with nodes above the pelvis
- thigh tumour with iliac or periaortic nodes
- intrathoracic tumour with subdiaphragmatic nodes
- unilateral tumour with controlateral involved lymph nodes (except in the head and neck).

12.5 REFERENCES – RADIOLOGY

1. McHugh K, Boothroyd AE. The role of radiology in childhood rhabdomyosarcoma. *Clin Radiol* 1999;54:2-10.

2. De Schepper AM, De Beuckeleer L, Vandevenne J, Somville J. Magnetic resonance imaging of soft tissue tumors. Eur Radiol 2000;10:213-223.

3. Kransdorf MJ, Murphey MD. Radiologic evaluation of soft-tissue masses: a current perspective. AJR Am J Roentgenol 2000;175:575-587.

4. Berquist TH, Dalinka MK, Alazraki N, et al. Soft tissue masses. American College of Radiology. ACR Appropriateness Criteria. Radiology 2000;215 Suppl:255-259.

5. Tomura N, Hirano H, Sashi R, et al. Comparison of MR imaging and CT in discriminating tumor infiltration of bone and bone marrow in the skull base. Comput Med Imaging Graph 1998;22:41-51

6. Picci P, Vanel D, Briccoli A, et al. Computed tomography of pulmonary metastases from osteosarcoma: the less poor technique. A study of 51 patients with histological correlation. Ann Oncol 2001;12:1601-1604

7. Seo JB, Im JG, Goo JM, Chung MJ, Kim MY. Atypical pulmonary metastases: spectrum of radiologic findings. Radiographics 2001;21:403-417

8. Grampp S, Bankier AA, Zoubek A, et al. Spiral CT of the lung in children with malignant extra-thoracic tumors: distribution of benign vs malignant pulmonary nodules. Eur Radiol 2000;10:1318-1322.

9. Lawrence W, Jr., Hays DM, Heyn R, et al. Lymphatic metastases with childhood rhabdomyosarcoma. A report from the Intergroup Rhabdomyosarcoma Study. Cancer 1987;60:910-915.

13. Low Risk Group Study

13.1 OBJECTIVE

To further improve the outcome, trying to better select patients that can be treated with VA alone.

13.2 PATIENTS AND TREATMENT

All patients eligible for the protocol (see chapter 11) with the following characteristics:

SUBGROUP A:

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
Low Risk	**A**	Favourable	I	Any	N0	Favourable

Localised non alveolar RMS, microscopically completely resected (IRS Group I), at all sites,
and
nodes negative
and
tumour size ≤ 5 cm
and
age < 10 years

This group of patients must be selected with great accuracy as they receive limited chemotherapy. It is necessary, therefore, to be very careful about the adequacy of resection margins and to ensure that the case is discussed in detail with the surgeon and pathologist before agreeing allocation to Low Risk treatment.

Primary re-excision is justified if this can be done without important functional or cosmetic sequelae, and if there is a realistic prospect of achieving complete microscopic resection (see paragraph 22.4). If the primary re-excision confirms clear margins, whether or not there is residual tumour in the resected specimen, the patient will be classified in the Low Risk Group and treated accordingly. If there is any doubt whatsoever about the completeness of resection, the patient should be allocated and treated in the Standard Risk Group (Subgroup C or D).

Note: *patients with paratesticular disease in whom the initial surgical approach has been through the scrotum should receive hemiscrotectomy, otherwise they cannot be treated in this group and will be upstaged to standard risk – Subgroup B (see Surgical guidelines Chapter 22)*

Urgent pathology review is required for any patient eligible for Low Risk Group strategy in which a diagnosis of Embryonal RMS is made by the local pathologist.
Previous experience has shown a high concordance between centre diagnosis and central review in cases of alveolar RMS. The agreement is lower when the centre reaches a diagnosis of embryonal RMS.

13.3 LOW RISK GROUP TREATMENT DETAILS

The treatment consists of 8 courses of Vincristine and Actinomycin D (VA) separated by a 3-week rest period. Weekly vincristine will be administered between cycle 1 and 2, 3 and 4, 5 and 6, 7 and 8. The total duration of chemotherapy is 22 weeks.

	V	V	V	V			V	V	V	V			V	V	V	V			V	V	V	V
	A			A			A			A			A			A			A			A
Weeks	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Cycle no.	1			2			3			4			5			6			7			8

V = Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection.
A = Actinomycin D 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection.

VA cycles should not be started unless all these condition are present: 2 x10^9/l WBC or 1 x10^9/l neutrophils + 80 x10^9/l platelets + absence of any relevant organ dysfunction.
Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good conditions.

See chapter 24 for chemotherapy guidelines and dose modifications
For children $\leq$ 1 year (or $\leq$ 10 kg body weight) see chapter 24.4.1.

No further local treatment procedures are needed after the initial complete resection (except for primary re-excision when indicated, see chapter 22.4)

14. Standard Risk Group Study

14.1 OBJECTIVES

Subgroup B: to evaluate whether the outcome for older patients with favourable features may be improved /maintained by administering a treatment with limited intensity.

Subgroup C: to evaluate whether chemotherapy intensity for standard risk patients can be reduced, lowering the cumulative dose of Ifosfamide from 54 g/m² to 36 g/m².

Subgroup D: to evaluate whether the treatment can be reduced in a subgroup of patients with RMS arising in *unfavourable* site (parameningeal, other site) but with *favourable* size and age.

14.2 PATIENTS AND TREATMENT

Patients included in the subgroups B, C and D are part of the Standard Risk Group. The treatment varies in the different Subgroups.

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
Standard Risk	***B***	Favourable	I	Any	N0	Unfavourable
	C	Favourable	II, III	Favourable	N0	Any
	D	Favourable	II, III	Unfavourable	N0	Favourable

Urgent pathology review is required for any patient eligible for Standard Risk Group strategy in which a diagnosis of Embryonal RMS is made by the local pathologist.
Previous experience has shown a high concordance between centre diagnosis and central review in cases of alveolar RMS. The agreement is lower when the centre reaches a diagnosis of embryonal RMS.

14.3 SUBGROUP B

All patients eligible to the protocol (see chapter 11) with the following characteristics:

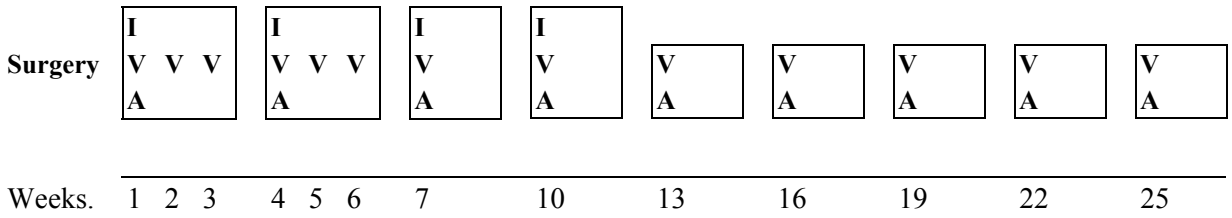
Localised non alveolar RMS, microscopically completely resected (IRS Group I), at all sites,
and
nodes negative
and
tumour size > 5 cm *or* age ≥ 10 years

Note: *patients with paratesticular RMS in whom the initial surgical approach was through the scrotum should be treated in this group if primary re-excision with hemiscrotectomy has not been performed, even if they have favourable characteristics.*

14.3.1 Subgroup B Treatment (ARM SR-B)

The treatment comprises of 4 cycles of Ifosfamide, Vincristine and Actinomycin D (IVA) followed by 5 courses of Vincristine and Actinomycin D (VA). The total duration of chemotherapy is 25 weeks.

These patients are in complete remission after initial surgery therefore they will not receive further local treatment (no RT or second look surgery).



I Ifosfamide 3 g/m^2 is given as a 3 hour intravenous infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) is given as a single intravenous injection on day 1 of each course and weekly, for a total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/ m^2 (maximum single dose 2 mg) as a single intravenous injection on day 1 of each course of treatment.

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.

See chapter 24 for chemotherapy guidelines and dose modifications
For children $\leq$ 1 year (or $\leq$ 10 kg body weight) see chapter 24.4.1.

Growth factors may be used at the physicians' discretion. It is suggested to use them in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay $\geq$ 1 week due to toxicity after previous cycles. For the use of growth factors see also chapter 27.2.

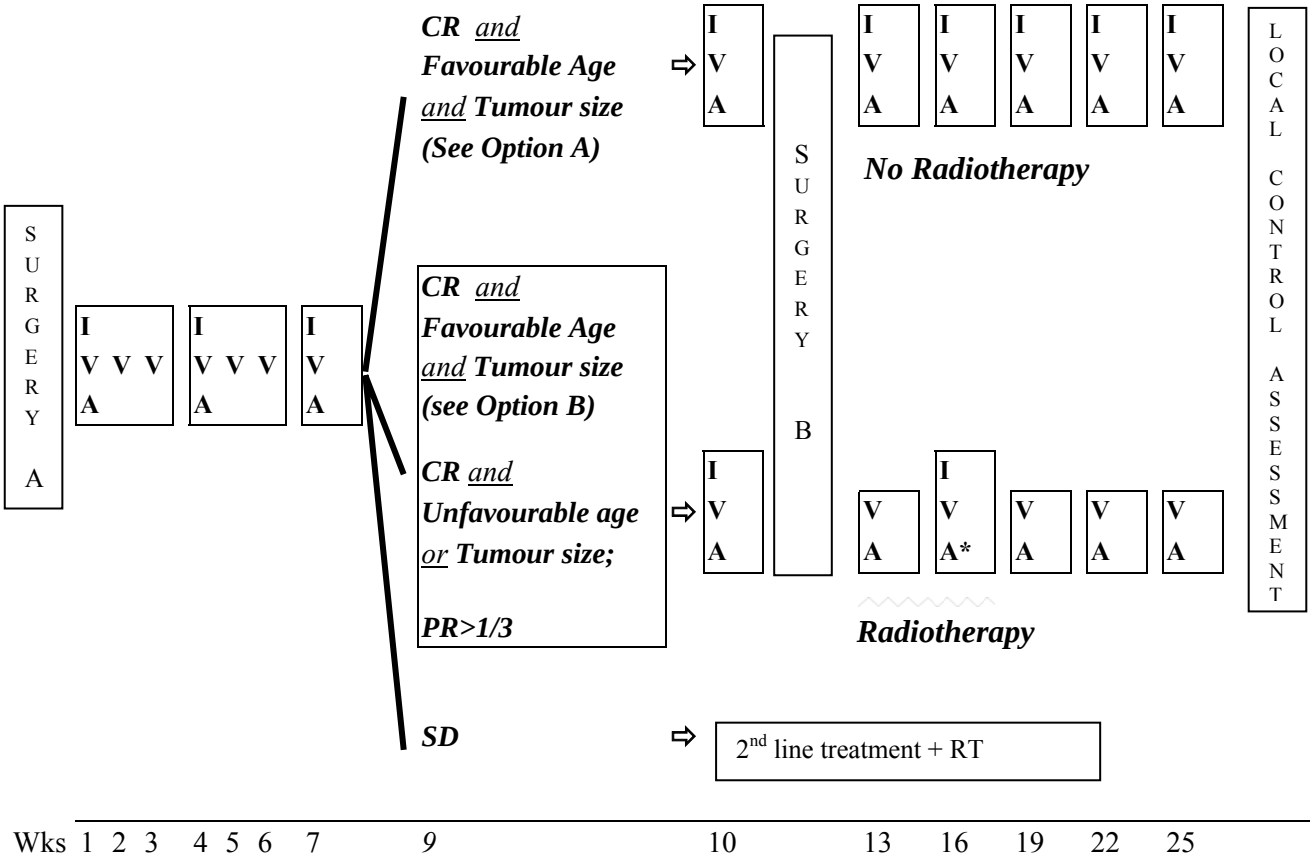
14.4 SUBGROUP C

All patients eligible for the protocol (see chapter 11) with the following characteristics:

non alveolar RMS, IRS Group II or III,
localised in orbit, head and neck non PM or GU non bladder-prostate,
and
nodes negative
and
any size *or* age

14.4.1 Subgroup C treatment (ARM SR-C)

The treatment comprises of 5 courses of Ifosfamide, Vincristine and Actinomycin (IVA) and 4 courses of Vincristine and Actinomycin (VA) ± Ifosfamide.
Local treatment will be administered at week 13.



I Ifosfamide 3 g/m^2 is given as a 3 hour intravenous infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (max. single dose 2 mg) is given as a single intravenous injection on day 1 of each course and weekly, for a total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection on day 1 of each course of treatment.

** Actinomycin should be given at the very beginning of RT (week 13) but may be omitted during RT (week 16). Caution is needed in the administration of week 19 ACT-D. For more details see chapter 23.11.*

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.

See chapter 24 for chemotherapy guidelines and dose modifications
For children ≤ 1 year (or ≤ 10 kg body weight) see chapter 24.4.1.

Growth factors may be used at the physicians' discretion. It is suggested to use them in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay ≥ 1 week due to toxicity after previous cycles. For the use of Growth factors see also chapter 27.2.

14.4.2 Assessment of tumour response and treatment decisions

- *1st assessment*: after the initial 3 cycles of chemotherapy (week 9) a full clinical and radiological assessment of the tumour response will be evaluated.

➲ At this time local control modality must be decided

a) Patients with favourable age (< 10 years) and tumour ≤ 5 cm at diagnosis, who achieve the complete remission after the initial treatment (3 courses of IVA ± surgery):
Please note that complete remission must be confirmed by central review
Two options are contemplated in this protocol:
- Option A: patients will receive 9 courses of IVA without radiotherapy.
- Option B: patients will receive 9 courses of IVA without radiotherapy if the CR has been obtained through a secondary operation (histological CR). Otherwise they will be treated as patients in CR with unfavourable features (radiotherapy plus VA chemotherapy).

NOTE: The German (CWS), the Italian (STSC) and the Spanish Group do recommend option B.

b) Patients in CR with unfavourable features (age ≥ 10 years and/or tumour size > 5 cm) or tumour volume reduction > 1/3 will continue the treatment they have been allocated at diagnosis.

c) Patients with stable disease (SD: tumour volume reduction < 1/3), will be eligible for 2nd line treatment (see chapter 20)

After the tumour response assessment one more chemotherapy cycle will be administered and in the meantime the appropriate local control modality will be planned and implemented at week 13.

Surgery: where residual masses are demonstrated or in cases of doubt, surgical resection should be done (surgery B). However resection in this Subgroup may be difficult because of the anatomical sites.
Surgery for tumours localised in the head and neck may not be feasible and the final decision in these cases is left to the discretion of the individual Surgeon.
In orbital RMS a delayed surgery is discouraged and radiotherapy should be the preferred local treatment.

Secondary operations are not indicated if clinically and radiologically (CT and/or MRI) there is no visible tumour (see chapter 22.5)
Secondary operations should, as a rule, be conservative but demolitive operations may be appropriate in certain circumstances. "Debulking" is not recommended. Particular care must be taken to ascertain completeness of resection.
Week 13 chemotherapy (5th cycle) and radiotherapy should begin after recovery from surgery B.
Surgery may be appropriate at the end of treatment in order to assess the or to achieve the local control after chemotherapy + radiotherapy. Mutilating surgery ("salvage surgery") could be considered in some cases.

Radiotherapy Patients in IRS Group II and III must be irradiated (unless in CR after the inital 3 cycles of chemotherapy and with favourable age and tumour size). Different doses will be delivered according to chemotherapy response and delayed surgery results (see Chapter 23 for details). Radiotherapy must be performed concomitantly with the 5th cycle (week 13).
If Surgery B is not possible and radiotherapy is decided this must be delivered beginning at week 13. Guidelines for patients less than 3 years of age are given in chapter 23.12.

Adjustments to the chemotherapy schedule are necessary during radiotherapy in particular for the administration of actinomycin (see paragraph 23.11).

- *2nd assessment* a second assessment of tumour response may be undertaken after 6-7 courses of chemotherapy (week 18)
  Any patient with progressive disease must proceed to 2nd line treatment.

- *3rd assessment:* a third assessment must be performed after 9 courses of chemotherapy (end of standard treatment).
  At this point surgery should be reconsidered (Local control assessment) in case of residual tumour.

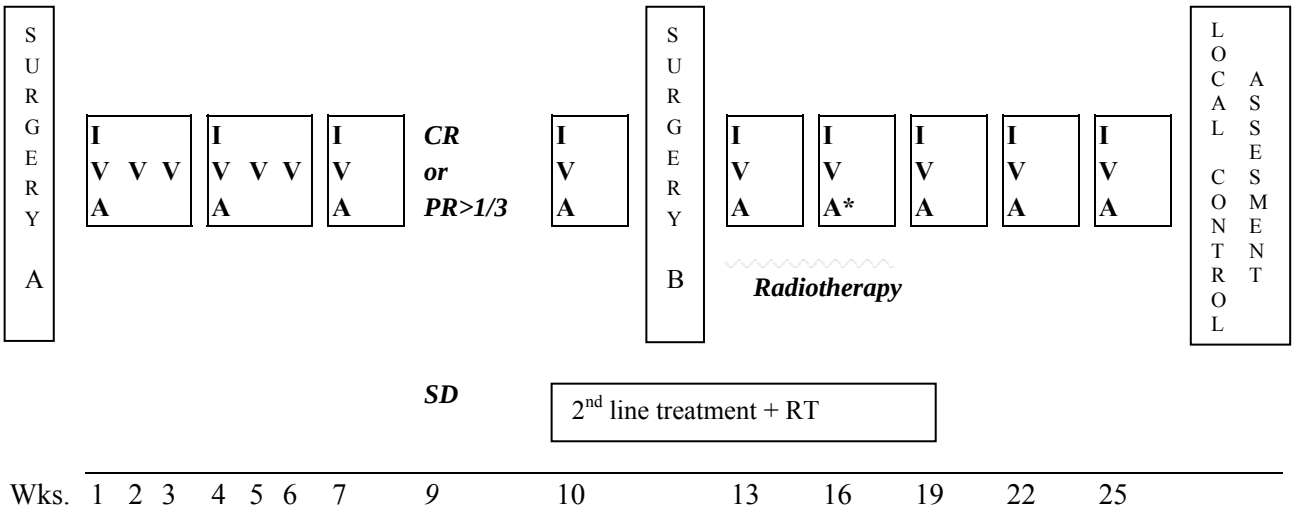
14.5 SUBGROUP D

All patients eligible to the protocol (see chapter 11) with the following characteristics:

Localised non alveolar RMS, IRS Group II or III,
arising in parameningeal, extremities, GU bladder-prostate or "other sites"
and
nodes negative
and
tumour size ≤ 5 cm and age < 10 years

14.5.1 Subgroup D Treatment (ARM SR-D)

The treatment comprises of 9 courses of Ifosfamide, Vincristine and Actinomycin (IVA).
Local treatment (radiotherapy ± surgery) will be administered at week 13.



I Ifosfamide 3 g/m2 is given as a 3 hour intravenous infusion daily, with Mesna (3 g/m2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m2).

V Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) is given as a single intravenous injection on day 1 of each course and weekly, for a total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/ m2 (maximum single dose 2 mg) as a single intravenous injection on day 1 of each course of treatment.

** Actinomycin should be given at the very beginning of RT (week 13) but may be omitted during RT (week 16). Caution is needed in the administration of week 19 ACT-D.*
For more details see chapter 23.11)

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.
Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.

See chapter 24 for chemotherapy guidelines and dose modifications
For children ≤ 1 year (or ≤ 10 kg body weight) see chapter 24.4.1.

Growth factors may be used at the physicians' discretion. It is suggested to use them in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay ≥ 1 week due to toxicity after previous cycles. For the use of growth factors see also chapter 27.2.

14.5.2 Assessment of Tumour response and treatment continuation

- *1st assessment*: after the initial 3 blocks of chemotherapy (week 9) a full clinical and radiological assessment of the tumour response will be evaluated.

➲ *At this time local control modality must be decided*

Patients in CR or tumour volume reduction > 1/3 will continue the treatment as detailed above.
Patients with stable disease (SD: tumour volume reduction ≤ 1/3), will be eligible for 2nd line treatment (see chapter 20)

After the tumour response assessment one more chemotherapy cycle will be administered and in the meantime the appropriate local control modality will be planned and implemented at week 13.

Surgery Secondary operations are not indicated if clinically and radiologically (CT and/or MRI) there is no visible tumour (see chapter 22.5). Where residual masses are demonstrated, or in cases of doubt, surgical verification is recommended.

Secondary operations (Surgery B) should, as a rule, be conservative but demolitive operations may be appropriate in certain circumstances. "Debulking" is not recommended. Particular care must be taken to ascertain completeness of resection.
Surgery may be appropriate at the end of treatment in order to assess the or to achieve the local control after chemotherapy + radiotherapy. Mutilating surgery ("salvage surgery") could be considered in some cases.

Radiotherapy Patients in IRS II and III must be irradiated. Different doses will be delivered according to chemotherapy response and delayed surgery results (see Chapter 23 for details). *Radiotherapy must be performed beginning at week 13.*
The local treatment modality in patients with parameningeal RMS must be radiotherapy.

Guidelines for patients less than 3 years of age are given in chapter 23.12.

Adjustments to the chemotherapy schedule are necessary during radiotherapy in particular for the administration of actinomycin (see paragraph 23.11).

- *2nd assessment* A second assessment of tumour response may be undertaken after 6-7 courses of chemotherapy (week 18)
  Any patient with progressive disease must proceed to 2nd line treatment.

- *3rd assessment:* A third assessment must be performed after 9 courses of chemotherapy (at the end of treatment).

15. High Risk Group Trial

15.1 OBJECTIVES

To improve the outcome of this group of patients investigating in a randomised way:

1. the value of early intensification with Doxorubicin comparing in the initial part of the treatment, a standard chemotherapy regimen IVA (Ifosfamide, Vincristine, Actinomycin D) with a novel combination with additional doxorubicin (IVADo)
2. the role of low dose "maintenance" chemotherapy with 6 months of cyclophosphamide and vinorelbine in the experimental arm

End points for both randomisations are:
a) primary: 3-year EFS
b) secondary: response to initial treatment (9^{th} week) and 5 yrs OS

15.2 PATIENTS AND TREATMENT

The following patients are eligible for EpSSG RMS 2005 randomised trial:

- eligibility to E*p*SSG RMS2005 protocol (see paragraph 11.1)
- stratification according to the High Risk Arm
- age > 6 months (and < 21 years)
- not paratesticular alveolar RMS
- informed consent given for the randomised study

The High risk group includes patients with different characteristics, however the treatment will be the same for the different Subgroups.

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
High Risk	***E***	Favourable	II, III	Unfavourable	N0	Unfavourable
	F	Favourable	I, II, III	Any	N1	Any
	G	Unfavourable*	I, II, III	Any	N0	Any

Note: For patients < 6 months see chapter 24.4.1 for chemotherapy modifications according to age.

SUBGROUP E

non alveolar RMS, IRS Group II or III, localised in parameningeal, extremities, GU bladder-prostate or "other sites"
and
nodes negative
and
tumour size > 5 cm *or* unfavourable age (≥ 10 year)

SUBGROUP F

non alveolar RMS, IRS Group I or II or III, any site
and
nodes positive
and
any tumour size or age

SUBGROUP G

alveolar RMS,
and
any IRS Group I or II or III, and any site
and
nodes negative
and
any tumour size or age

Notes*:*
The following groups of patients are included in the High Risk Group but they are not eligible to the randomised trial and should be treated according to Arm A, with 9 cycles of IVA
a) alveolar RMS: See also chapter 8.4.4
b) patients with Undifferentiated soft tissue sarcoma or ectomesenchymoma. See chapter 29.4.

15.3 RANDOMISED TRIAL NO. 1 – *THE INTENSIFICATION QUESTION*

After the diagnosis of RMS has been established and written informed consent obtained eligible patients will be randomised to receive:

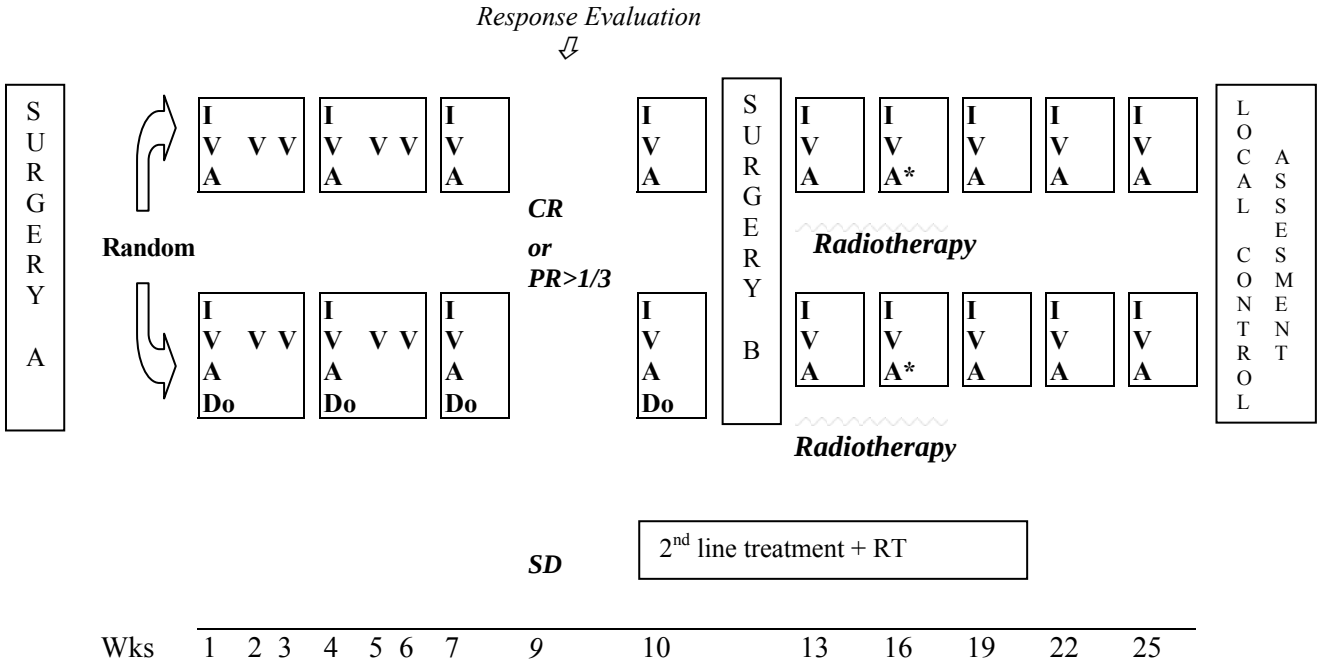
- **Arm A:** 4 courses of Ifosfamide, Vincristine and Actinomycin D (IVA)

or

- **Arm B:** 4 courses of IVA+ Doxorubicin (IVADo).

Eligible patients must be randomised before chemotherapy treatment is started using the RDE system.
For randomisation procedure see chapter 33.5.
If the randomization is refused or not applicable for whatever reason patients should be treated in Arm A.

After the diagnostic surgery primary re-operation can be considered, before chemotherapy start, in selected cases (see paragraph 22.3).



I Ifosfamide 3 g/m2 is given as a 3 hour intravenous infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) is given as a single intravenous injection on day 1 of each course and weekly, for a total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection on day 1 of each course of treatment.

Do Doxorubicin 30 mg/m2 given as a 4-hour intravenous infusion daily on days 1 & 2 for courses 1-4 of treatment (total dose per course = 60 mg/m^2).

** Actinomycin should be given at the very beginning of RT (week 13) but may be omitted during RT (week 16). Caution is needed in the administration of week 19 ACT-D. See chapter 23.11)*

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC or 1 x10^9/l neutrophils + 80 x10^9/l platelets + absence of any relevant organ dysfunction.

Weekly vincristine should be administered irrespective of pancytopenia providing the child is in good condition.

See chapter 24 for chemotherapy guidelines and dose modifications
For children ≤ 1 year (or ≤ 10 kg body weight) see chapter 24.4.1.

Growth factors may be used at the physicians' discretion. It is suggested to use them in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay ≥ 1 week due to toxicity after previous cycles. For the use of growth factors see also chapter 27.2.

15.3.1 Assessment of tumour response and treatment decisions

- *1st assessment*: after the initial 3 cycles of chemotherapy (week 9) a full clinical and radiological assessment of the tumour response will be evaluated.

➲ At this time local control modality must be decided

Patients in CR or tumour volume reduction > 1/3 will continue the treatment they have been allocated at diagnosis.
Patients with stable disease (SD: tumour volume reduction ≤ 1/3), will be eligible for 2nd line treatment (see chapter 20)

After the tumour response assessment, one more chemotherapy cycle (IVA or IVADo according to the treatment arm) will be administered and in the meantime the appropriate local control modality will be planned and implemented at week 13.

Surgery Where residual masses are demonstrated or in case of doubt, surgical resection should be done (surgery B), although there may be certain anatomical sites, particularly in the head and neck, where this may not be feasible and the final decision in these cases is left to the discretion of the individual Surgeon. Secondary operations are not indicated if clinically and radiologically (CT and/or MRI) there is no visible tumour (see chapter 22.5).
Secondary operations should, as a rule, be conservative but demolitive operations may be appropriate in certain circumstances. "Debulking" is not recommended.
Week 13 chemotherapy (5th cycle) should begin after recovery from surgery B, and radiotherapy should start with the fifth chemotherapy cycle
Surgery may be appropriate at the end of treatment in order to assess the or to achieve the local control after chemotherapy + radiotherapy. Mutilating surgery ("salvage surgery") could be considered in some cases.

Radiotherapy Patients in IRS Group II and III must be irradiated. Different doses will be delivered according to chemotherapy response and delayed surgery results (see Chapter 23 for details). Radiotherapy must be performed concomitantly with the 5th cycle (week 13).
If Surgery B is not possible and radiotherapy is decided this must be delivered beginning at week 13, after the administration of the fourth cycle.
Guidelines for patients less than 3 years of age are given in chapter 23.12.

Adjustments to the chemotherapy schedule are necessary during radiotherapy in particular for the administration of actinomycin (see paragraph 23.11).

- *2nd assessment* a second assessment of tumour response may be undertaken after 6-7 courses of chemotherapy (week 18)
  Any patient with progressive disease must proceed to 2nd line treatment.

- *3rd assessment:* a third assessment must be performed after 9 courses of chemotherapy (end of standard treatment).
  Patients in CR or with evidence of minimal radiological anomalies are eligible for the second randomization.
  At this point surgery should be reconsidered (Local control assessment) in case of residual tumour. Patients who achieve a CR after surgery are eligible for the second randomisation

15.4 RANDOMISED TRIAL NO. 2 - *THE MAINTENANCE QUESTION*

The following patients are eligible for second randomisation:

- eligibility to E*p*SSG RMS2005 protocol (see paragraph 11.1)
- stratification and treatment according to the High Risk Arm
- age > 6 months at the moment of randomisation (some infants, not eligible for the first randomisation, may be randomised here) and < 21 years at diagnosis (some patients may be older than 21 years at the moment of second randomisation)
- in complete remission or with minimal abnormalities* on imaging studies at the end of "standard " treatment (9 courses of chemotherapy ± surgery ± radiotherapy)
- absence of severe vincristine neuropathy (requiring discontinuation of vincristine treatment)

After the end of standard therapy, patients have received 9 blocks of chemotherapy similar to that administered in previous European protocols. Clinicians must be prepared to end the patient's treatment because the patient is in CR or minimal radiological anomalies are evident and they are reasonably suspected not to be disease (i.e. fibrosis). This frequently occurs in parameningeal RMS and the standard strategy is to stop treatment.
Following the 9th block of chemotherapy, surgery or a biopsy of what appears to be a possible residual tumour, may be performed. Patients are not eligible for the second randomisation if viable tumour is found and the clinician thinks that more chemotherapy would be appropriate.
If the clinician wants to give more chemotherapy after the initial 9 blocks this should NOT be vinorelbine/cyclophosphamide and these patients will not be eligible for randomisation.

Patients must be randomised within 8 weeks after the end of treatment.
The end of treatment is defined as the last day of the 9th chemotherapy cycle. However:
- if surgery is performed after the 9th chemotherapy cycle, the date of surgery will be considered;
- if radiotherapy is administered after 9 cycles of chemotherapy, the date of the end of RT will be considered. Since maintenance CT should be started within 8 weeks from the last day of the 9th CT cycle, it would be better to start the maintenance CT during irradiation.

Patients in CR will be randomised to:
- **Arm C:** stop treatment

or
- **Arm D:** 6 courses Vinorelbine + Cyclophospamide

► Randomization should be performed and (if allocated) treatment started within 8 weeks following the end of treatment.

N.B. If the first randomization has been refused or not done for whatever reason patients are still eligible for the second randomization if they satisfy the eligibility criteria

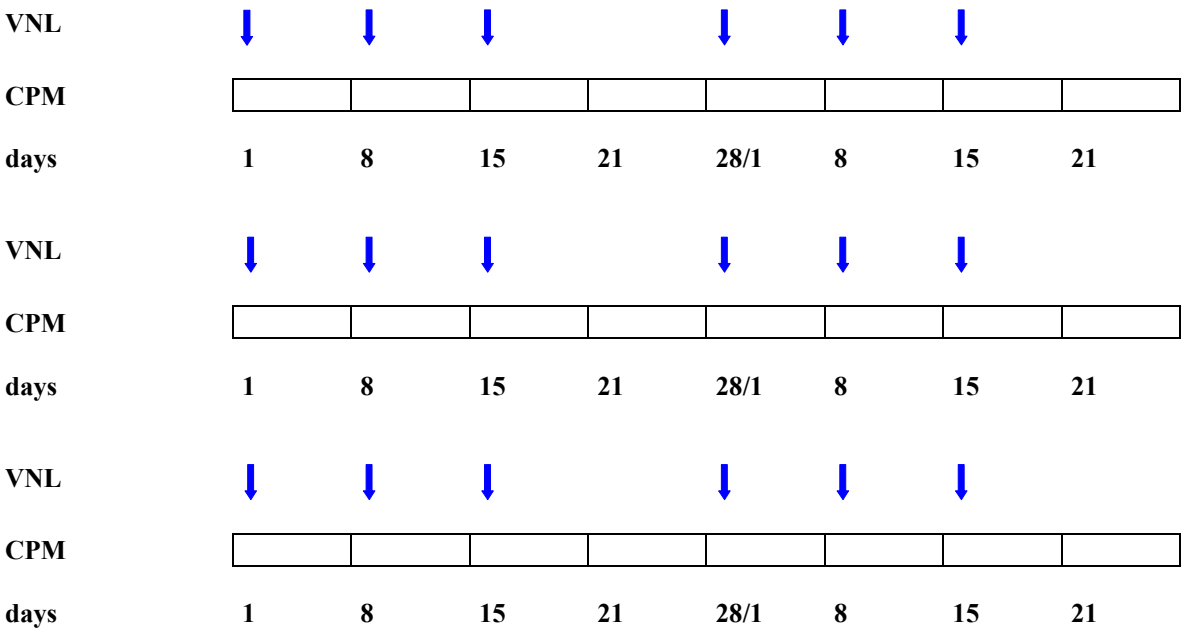
Stop treatment

9th CT block ⇦ **decision & randomisation within 8 weeks** ⇨ **Vinorelbine/Cyclophosphamide x 6 courses (24 weeks)**

Very important:

The standard treatment strategy is to stop treatment because the benefit of vinorelbine/cyclophospamide maintenance is not proven.
Consequently when randomization is refused or thought by the responsible clinician not to be appropriate for the patient, no further treatment after the initial 9 blocks should be administered.

15.4.1 Vinorelbine / cyclophosphamide Maintenance schema



VNL: Vinorelbine 25 mg/m^2 i.v. over 5-10 minutes day 1,8,15 of each cycle
CPM: Cyclophosphamide 25 mg/m^2 per os every day (no rest between cycles)

This treatment is usually given on an outpatient basis.

N.B. Cyclophosphamide is only available in capsules of 50 mg, which cannot be cut in smaller capsules so the doses should be divided over more days. Capsules should be administered early in the day and followed by adequate fluid intake to minimize bladder toxicity.
For drug administration details see also paragraph 24.2 and 24.3.

16. Very High risk Group Study

16.1 OBJECTIVE

To improve the results in this poor prognosis group of patients administering the more intensive treatment IVADo plus maintenance chemotherapy.

16.2 PATIENTS AND TREATMENT

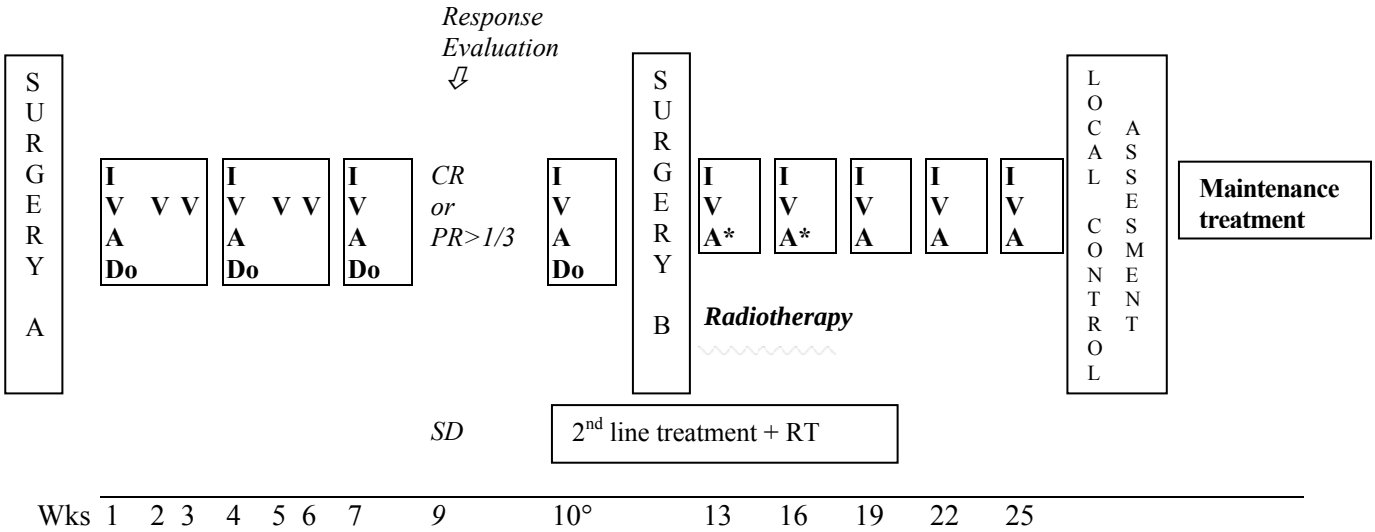
All patients eligible to the protocol (see paragraph 11.1) with the following characteristics:

Localised alveolar RMS
and
nodes positive

(independently from any other variable such as tumour site, size or patient age)

After the diagnostic surgery primary re-operation can be considered, before chemotherapy start, in selected cases (see paragraph 22.4).

16.2.1 Very high risk patients: Intensive Treatment



I Ifosfamide 3 g/m^2 is given as a 3 hour intravenous infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) is given as a single intravenous injection on day 1 of each course and weekly, for a total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection on day 1 of each course of treatment.

Do Doxorubicin 30 mg/m^2 given as a 4-hour intravenous infusion daily on days 1 & 2 for courses 1-4 of treatment (total dose per course = 60 mg/m^2).

** Actinomycin should be given at the very beginning of RT (week 13) but may be omitted during RT (week 16). Caution is needed in the administration of week 19 ACT-D.*
For more details see chapter 23.11)

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.

For children ≤ 1 month VA only should be administered in the 1st cycle. For children ≤ 1 year (or ≤ 10 kg body weight) first cycle doses will be calculated by body weight and increased in the following cycles if tolerated. See chapter 24.4.1.

Growth factors may be used at the physicians' discretion. It is suggested to use them in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay ≥ 1 week due to toxicity after previous cycles.
For the use of growth factors see also chapter 27.2.

16.2.1.1 ASSESSMENT OF TUMOUR RESPONSE AND TREATMENT DECISIONS

- *1st assessment*: after the initial 3 cycles of chemotherapy (week 9) a full clinical and radiological assessment of the tumour response will be evaluated.

➲ At this time local control modality must be decided

Patients in CR or tumour volume reduction > 1/3 will continue the treatment they have been allocated at diagnosis.
Patients with stable disease (SD: tumour volume reduction ≤ 1/3), will be eligible for 2nd line treatment (see chapter 20).

After the tumour response assessment, one more chemotherapy cycle will be administered and in the meantime the appropriate local control modality will be planned and implemented at week 13.

Surgery Where residual masses are demonstrated or in case of doubt, surgical resection should be done (surgery B), although there may be certain anatomical sites, particularly in the head and neck, where this may not be feasible and the final decision in these cases is left to the discretion of the individual Surgeon. Secondary operations are not indicated if clinically and radiologically (CT and/or MRI) there is no visible tumour (see chapter 22.5).
Secondary operations should, as a rule, be conservative but demolitive operations may be appropriate in certain circumstances. "Debulking" is not recommended. Particular care must be taken to ascertain completeness of resection.
Radical lymph node dissections are not indicated and involved lymph nodes should be irradiated, whether resected or not. There are rare occasions when, if radiotherapy is contraindicated (e.g. age ≤ 3 years), a lymph node dissection may be considered as definitive local treatment.
Week 13 chemotherapy (5th cycle) should begin after recovery from surgery B, and radiotherapy should start with the fifth chemotherapy cycle.

Radiotherapy Patients in IRS Group II and III must have the primary tumour irradiated. Different doses will be delivered according to chemotherapy response and delayed surgery results (see Chapter 23 for details). Radiotherapy must be performed concomitantly with the 5th cycle (week 13).
If Surgery B is not possible and radiotherapy is decided this must be delivered beginning at week 13, after the administration of the fourth cycle.
Radiotherapy to the involved lymph node sites is performed independently of histology and surgical resection. (see paragraph 23.5)
Guidelines for irradiation of patients less than 3 years of age are given in paragraph 23.12.
Adjustments to the chemotherapy schedule are necessary during radiotherapy in particular for the administration of doxorubicin and actinomycin (see paragraph 23.11).

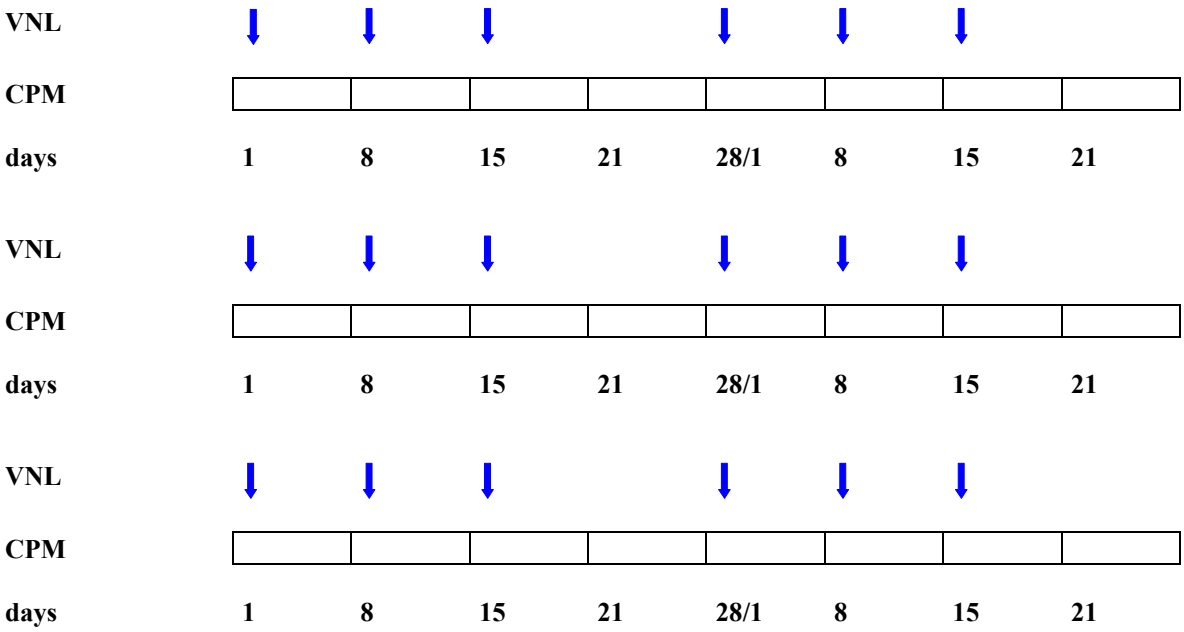
- *2nd assessment* a second assessment of tumour response may be undertaken after 6-7 courses of chemotherapy (week 18)
  Any patient with progressive disease must proceed to 2nd line treatment.

- *3rd assessment:* a third assessment must be performed after 9 courses of chemotherapy (end of standard treatment).

At this point surgery should be reconsidered (Local control assessment) in case of residual tumour.

16.2.2 Very High risk patients: Maintenance Treatment

Following the 9th block of chemotherapy, surgery or a biopsy of what appears to be a possible residual tumour may be performed. Patients may not continue with the maintenance treatment if viable tumour is found and the clinician thinks that more intensive chemotherapy would be appropriate. However in presence of limited quantity of viable tumour maintenance treatment should be adopted.



VNL: Vinorelbine 25 mg/m^2 i.v. over 5-10 minutes day 1,8,15 of each cycle
CPM: Cyclophosphamide 25 mg/m^2 per os every day (no rest between cycles)

This treatment is given on an outpatient basis.

N.B. Cyclophosphamide is only available in capsules of 50 mg, which cannot be cut in smaller capsules so the doses should be divided over more days. Capsules should be administered early in the day and followed by adequate fluid intake to minimize bladder toxicity.
For drug administration details see also paragraph 24.2 and 24.3.

17. Specific Site treatment Information

17.1 PARAMENINGEAL SITE

Unfavourable site
MRI is recommended as radiological investigation.
CSF examination at diagnosis has to be performed.
Complete surgical resection is difficult and generally not possible.
An initial resection will not be accepted if permanent severe uncorrectable functional dysfunction or mutilation results. In all cases where resectability is uncertain a resection should not be attempted and only a biopsy taken. Neck dissections should not be performed initially.
Radiotherapy is always necessary in patients over 3 year of age and should be given at week 13 regardless of response to initial chemotherapy.
Only after radiotherapy is a secondary resection acceptable. Secondary resections in PM site should only be performed in centres with experience in this field.

17.2 CSF POSITIVE

Patients with malignant cells in the spinal fluid will be treated in the protocol for metastatic tumours.

17.3 ORBIT

Favourable site if not bone involvement and not parameningeal site involvement. In case the orbital bone is perforated the tumour has to be classified as PM and the appropriate guidelines have to be applied.
Initial surgery should almost always include a biopsy only. Complete resection of an orbital tumour is rarely possible and most of the time associated with a loss or impairment of vision.
Local treatment: after 4 blocks of chemotherapy most orbital tumours will receive radiotherapy independently of resection status. The doses and target volume definitions will follow the general guidelines. The radiation of the entire orbit is not mandatory and is dependent on the initial tumour size and location. The decision for or against radiotherapy in patients with favourable characteristics (included in Subgroup C) and clinical complete remission following chemotherapy is made according to the recommendations described in chapter 14.4.2.
After chemotherapy + radiotherapy a secondary resection or a biopsy may be indicated in patients with residual tumour. Enucleation or exenteration are very rarely indicated in the course of first line treatment.

17.4 HEAD AND NECK

Favourable site
MRI is recommended as radiological investigation.
An initial complete resection may be achieved but in all cases where resectability is uncertain only a biopsy should be taken. Revision of suspicious lymph nodes should be performed but neck dissections are not indicated.
In some circumstances a major tumour resection with reconstruction may be considered after neoadjuvant chemotherapy. In Non-Responders or tumours that are locally persistent a mutilating approach may be indicated.

17.5 BLADDER/PROSTATE

Unfavourable site

MRI is recommended as radiological investigation

Cystoscopy should be done at diagnosis and during follow up.

Initial resection (rather than a simple biopsy) should be done only in the case of very small tumours arising in the fundus of the bladder, far from the trigone.

In all other cases a biopsy has to be performed and secondary surgery will be planned after chemotherapy (see chapter 19). A conservative surgery of Bladder-Prostate RMS may be considered (partial cystectomy and/or partial prostatectomy) in conjunction with brachytherapy. If conservative treatment is not feasible, the choice of treatment is between radiotherapy and total cystectomy and/or total prostatectomy. The doses and target volume definitions will follow the general guidelines. Gonads should be positioned out of the treatment volume if possible (in girls oophoropexy must be discussed). Individual planning and discussion with the respective reference centre is advised.

17.6 VAGINA / UTERUS

Favourable site

Examination under general anaesthesia may be required to define the local extent of tumour. Complete tumour resection at diagnosis is not recommended and only a biopsy should be performed. RMS of the vagina with favourable histology (embryonal RMS) will not receive local treatment (surgery or radiotherapy) if in clinical complete remission after chemotherapy. In case of residual tumour partial vaginectomy may be feasible, but brachytherapy is often preferable.

Patients with unfavourable histology (alveolar RMS) need to be treated with radiotherapy. Depending on the extent and infiltration of the disease these patients may be treated with afterloading techniques/brachytherapy. Individual planning and discussion with the respective reference centre is advised. Oophoropexy has to be considered in order to avoid irradiation of the ovary in all girls treated for pelvic tumours.

17.7 PARATESTICULAR

Favourable site

Paratesticular tumours should have scrotal ultrasound and must have evaluation of regional (paraaortic) lymph nodes by CT scan and ultrasound.

Complete tumour resection at diagnosis is possible but should be performed according to the recommendations (see chapter 22.3). Retroperitoneal lymphadenectomy or node sampling at diagnosis is not recommended unless there is uncertainty on imaging.

If the initial surgical approach was through the scrotum a primary re-operation should be done according to the recommendations (see chapter 22.4). When there is a doubt about a scrotal dissemination, hemiscrotectomy should be performed, if not the tumour will be upstaged.

Incompletely resected paratesticular RMS need radiotherapy (see chapter 23.4.11).

Paratesticular alveolar RMS will be kept in the high risk group, according to the histology, however in consideration of the better outcome they will not be included in the randomised trial and will be treated with IVAx9 (avoiding anthracyclines). Radiotherapy should not be performed if group I.

17.8 LIMBS

Unfavourable site

Particular attention is recommended to the initial evaluation of regional lymph nodes. Lower Limb tumours must have evaluation of pelvic lymph nodes by CT or MRI even if inguinal nodes are

clinically/sonographically normal. Even if the inguinal nodes are clinically/sonographically normal a surgical picking will be performed.
When the primary tumour can be completely resected, regional lymph nodes should be systematically biopsied during the same procedure, even if they are not clinically suspect.
At secondary operation, formal compartmental resection may be appropriate for some tumours but less "anatomical" resections may be better providing an adequate margin of normal tissue.
According to the protocol extremity tumours should be irradiated (see chapter 23). Tissue contaminated during surgery must be included in the irradiated field. After surgical procedures, all scars and drainage sites should be irradiated with a safety margin of 1-2 cm.

17.9 PATIENTS WITH PLEURAL EFFUSION OR ASCITES

In case of important effusion, examination of the fluid is mandatory (biological studies may be of help).
If malignant cells are found on morphology the patients will be treated according to the metastatic protocol.
If peritoneal or pleural nodules are evident on imaging the tumour will be considered as metastatic and treated accordingly.
In case of a small amount of fluid this may be „reactive" and sampling is not necessary, i.e. a tumour located below the diaphragm with limited ipsilateral pleural effusion, the patient will be treated in the E*p*SSG RMS 2005 protocol according to his risk profile.

18. Investigation during and at the end of treatment

18.1 EXAMINATIONS DURING THERAPY

18.1.2 Physical Examination

A thorough physical examination should be performed prior to every block of chemotherapy.

18.1.3 Laboratory Investigations

- Full blood count (including differential white cell count and platelets) before each course of chemotherapy (neutrophils > 1 $x10^9$/l and platelets > 80 $x10^9$/l is required before the start of each course of chemotherapy).
- Serum creatinine, electrolytes and liver function tests: before each block of chemotherapy

- *Ifosfamide Nephrotoxicity Monitoring:* in standard and high risk patients, ifosfamide nephrotoxicity needs to be monitored periodically. Monitoring must include:
  - Blood for Na, K, Ca, Mg, PO_4, Cl, Total CO_2/HCO_3 and AP
  - Early morning urine sample for PO_4, Creatinine and Osmolarity
  - GFR
  - Renal Tubular Threshold for Phosphate (Tm_p/GFR)

18.1.4 Tumour Reassessment

Evaluation during treatment should be performed when possible **with the same techniques** as initially used.
If no signs of progression are present a formal tumour revaluation is advised

- *low risk patients:* at the end of treatment
- *standard risk patients:* after the initial 3 blocks of chemotherapy (with tumour response evaluation, see chapter 19) and at the end of treatment
- *high risk patients:* after the initial 3 blocks of chemotherapy (with tumour response evaluation, see chapter 19) and after 9 blocks of chemotherapy (at the time of randomisation decision, see chapter 19). During maintenance treatment: after 3 and 6 months.
- *very high risk patients:* after the initial 3 blocks of chemotherapy (with tumour response evaluation, see chapter 19), after 9 blocks of chemotherapy. During maintenance treatment: after 3 and 6 months.

MRI or CT remains necessary prior to surgery.

18.2 INVESTIGATIONS BEFORE RANDOMISATION

For the first randomisation, the investigations at diagnosis are sufficient.
For the second randomisation, CR should be achieved before randomisation, therefore a thorough tumour evaluation should be performed 2-3 weeks after the administration of the 9^{th} block of chemotherapy.
If eligible, the child should be randomised within 8 weeks after the end of treatment.

18.3 INVESTIGATIONS AT THE END OF TREATMENT

Investigations required at this point are:

- Thorough physical and neurological examination (weight, height, pubertal status)
- Blood: Full Blood Count, liver enzymes, K, Na, Ca, PO_4, Cl, Mg, Glucose, AP, H_2CO_3, creatinine.
- Urine: Na, Ca, Glucose, PO_4, Creatinine, pH, Total Protein; 24 h urine: Calculate GFR, 24 h Ca, PO_4 and Glucose loss, max. PO_4 reabsorption/GFR.
- MRI/CT/ultrasound of primary tumour site, Chest x-ray, abdominal ultrasound.
- Echocardiogram if doxorubicin has been administered
- Other investigations if previously abnormal (CSF, hormonal status, ECG, PET) may be indicated but are not generally recommended.

19. Tumour response evaluation

A choice has been made for this study to rely on volume measurements for tumour response assessment. Tumours do not necessarily grow or shrink in a rounded fashion and 3D evaluation may be more accurate than uni or bidimensional criteria.
It is planned to also measure the maximum unidimensional measurement as suggested by the RECIST guidelines and later compare the volume with unidimensional measurements in terms of tumour response. The maximum lesion diameter in any plane should be recorded as the longest tumour diameter, and measurements may be taken from CT or MRI (contrary to the formal RECIST guidance) but the maximum tumour measurement must always be in the same plane (axial, coronal or sagittal).
The presence or absence of a post-therapeutic residue should be stated in the radiology report.
Very good partial response and minor partial response criteria are not recognised international criteria but have been added for this protocol.

A clinical assessment of tumour response should be made at each visit in order to detect tumour progression at any point during treatment. This should be supplemented by radiological examination as appropriate.

For the patients in standard and high risk group with evidence of macroscopical residues after initial surgery a formal reassessment of Tumour Response is undertaken *at week 9, after the initial 3 cycles of chemotherapy.*

Assessment must include a detailed clinical examination with external tumour measurements where relevant and radiology using comparable techniques to those used at diagnosis (MRI and/or CT scan).
Tumour dimensions should be recorded in three diameters and can be compared choosing, as far as possible, the diameters selected at diagnosis.

Tumour volume (V) calculation:

a= length (in cm)
b= width (in cm)
c= thickness (in cm)

$$\mathbf{V = \pi/6 \times a \times b \times c = 0.52 \times a \times b \times c} \text{ in } cm^3$$

19.1 RESPONSE EVALUATION CRITERIA

Response in patients with macroscopic residual disease after initial surgery (IRS group III) will be evaluated as follow:

Complete Response ***(CR)***	Complete disappearance of all visible disease
Very Good Partial Response ***(VGPR)***	Tumour volume reduction ≥ 90% but < 100%
Partial Response ***(PR≥2/3)***	Tumour volume reduction ≥ 66% but < 89%
Minor Partial Response ***(PR<2/3)***	Tumour volume reduction > 33% but < 66%
Stable disease ***(SD)***	No criteria for PR or PD (< 33% tumour volume reduction)
Progressive Disease ***(PD)***	Any increase of more than 40% in volume (or > 25% in area) of any measurable lesion, or appearance of new lesions.

All response must last at least 4 weeks without evidence of tumour progression or relapse

Residual disease should be defined as macroscopic measurable residue. Residual ill-defined areas of high density on CT-scan, or residual signal abnormalities on MR such as low intensity on T1WI, high intensity on T2WI and ill-defined margins of enhancement areas are commonly observed after chemotherapy. If no measurable mass, these may be regarded as post-therapeutic residue, and should not exclude the classification as CR.

20. Second line therapy

A poor response to initial chemotherapy appears correlated with a poor prognosis in RMS patients. Data from the CWS-81 and CWS-86 studies demonstrated a significantly worse prognosis for children with a poor response after the initial three blocks of chemotherapy.
Therefore the current management of patients with evidence of poor response after the initial chemotherapy phase includes the administration of drugs not previously administered and the implementation of local treatment measures (surgery and/or radiotherapy).
In this protocol, we suggest to treat such patients with alternative chemotherapy combinations along with surgery and radiotherapy. Chemotherapy regimen should be chosen taking into account chemotherapy previously administered and patient tolerance. We suggest different chemotherapy regimens that could be used by the responsible clinicians.

Local treatment must be considered at any time when an unsatisfying response to initial chemotherapy is evident.

When more chemotherapy treatment is thought appropriate by the responsible clinician before local control measures (surgery and/or radiotherapy) chemotherapy response evaluation will be possible.
A proper phase II study is not part of this protocol, however we ask centres to record the response to the regimen administered to collect more information.
Patients eligible to second line chemotherapy response evaluation may be for instance:
- young patients for whom local treatment is thought to be excessively toxic or not possible
- patients in good condition with stable tumour for whom a second chemotherapy test is retained appropriate
- patients for whom surgery or irradiation is not possible in a short time (i.e. within 6-8 wks)

20.1 SECOND LINE CHEMOTHERAPY

Drugs not administered during first line therapy should be used.
- Topotecan has been demonstrated to be active in paediatric malignancies including RMS. Carboplatin has been part of previously used regimens (CEVAIE) that proved to be effective against RMS. It has also been used alone in a window study conducted by the UKCCSG (unpublished data). A phase II trial has been performed at the Bambino Gesù Hospital in Rome showing the feasibility of the proposed regimen. The Topo-Carbo combination is also used as window treatment in the current CWS protocol for metastatic RMS.

- Doxorubicin may be used instead of Topotecan in patients if they have not received anthracyclines in the initial treatment.

After 2 cycles there will be a tumour response evaluation and decisions will be taken accordingly:

a) **Good response** (including CR, VGPR and PR): the initial chemotherapy will continue: *see second line treatment schema.*

b) **No response** (stable or progressive disease): local treatment must be evaluated and a new chemotherapy regimen may be considered.

20.1.1 SECOND LINE TREATMENT SCHEMA

Topo - Carbo Regimen

	Topo Carbo	Topo Carbo	*Good Response*	Topo Cyclo	VP16 Carbo	Topo Cyclo	VP16 Carbo
weeks	1	4	⇧	7	10	13	16

Tumour response evaluation

Topotecan: 2 mg/m^2/day on day 1 to 3 (total dose 6 mg/m^2/course) in 30 minutes.
Carboplatin: 250 mg/m^2/day in 1 hour on day 4 and 5 when given with topotecan, on day 1 and 2 when given with VP16 (total dose 500 mg/m^2 course).
Cyclophosphamide: 1500 mg/m^2 /day on day 1 and 2 (total dose 3000 mg/m^2 course) in 4 hours.
VP16: 100 mg/m^2/day on day 1 to 3 (total dose 300 mg/m^2 course) in 1 hour.

Doxo – Carbo Regimen

	Doxo Carbo	Doxo Carbo	*Good Response*	Doxo Cyclo	Doxo Carbo	Doxo Cyclo	Doxo Carbo
weeks	1	4	⇧	7	10	13	16

Tumour response evaluation

Doxorubicin: 60 mg/m^2/day on day 1 (total dose 60 mg/m^2/course) 1 to 6 hours according institutional policies.
Carboplatin: 250 mg/m^2/day on day 1 and 2 (total dose 500 mg/m^2 course) in 1 hour.
Cyclophosphamide: 1500 mg/m^2 /day on day 1 and 2 (total dose 3000 mg/m^2 course) in 4 hours.

Chemotherapy modulation
The interval between courses should be 21 days and the following chemotherapy course should not be started unless all these conditions are present:

- 2 x10^9/l WBC, or 1 x10^9/l neutrophils
- 80 x10^9/l platelets are reached.
- absence of any relevant organ dysfunction

Response assessment

This should be done according to the same recommendations and criteria adopted for the first line treatment (see paragraph 19.1). However the tumour volume after the initial two courses of second line chemotherapy must be compared to the tumour evaluated at week 9th and not at diagnosis.

Important note: **please remember that patients in CR after second line chemotherapy are still eligible to second randomisation**

21. Adequate Local Therapy Diagram

(for details see surgical and radiotherapy guidelines)

Upfront local therapy

Initial surgery is only recommended when not mutilating and when macroscopic and microscopic complete resection is possible; evaluate complete non-mutilating reexcision in Group II and III pts

In extremity sites : biopsy of axillary or inguinal lymph nodes

Initial chemotherapy according to risk groups

Restaging at week 9

Radiotherapy planning in pts. with alveolar RMS IRS group I and in all patients with IRS group II

Start of radiotherapy at week 13.
In high risk patients, the application of doxorubicin has to be completed.

Decision about further local therapy in pts. with IRS group III

Non-mutilating appropriate oncologic resection with negative margins possible ?

Yes
Resection should be performed*

No

POSTOPERATIVE RTX ACCORDING TO THE MARGIN STATUS	
R0	36 Gy in eRMS and CR or PR (>2/3) following induction chemotherapy 41.4 Gy in eRMS and less than PR(>2/3) 41.4 Gy in aRMS
R1	50.4 Gy
R2	50.4 Gy

PLANNING OF RTX STARTING AT WEEK 13
CR and eRMS: .....
PR, SD, PD, aRMS: .....
(exception: orbit, see radiotherapy guidelines)

*preoperative RT in selected patients
who will receive reconstructive surgery

22. SURGICAL GUIDELINES

Local treatment is essential in non-metastatic RMS. It can be achieved by surgery, radiotherapy (external beam RT and/or brachytherapy) or both.
The aim of local treatment is to cure the patient with no, or minimal, long term sequelae. The choice of local treatment will depend on the site and the size of the primary tumour, the age of the patient and the response to neoadjuvant chemotherapy. Surgical planning should include all reconstructive procedures with optimal timing of possible additional radiotherapy.

22.1 DEFINITIONS

The quality of the resection is defined by its worst margin and is usually classified as follows for extremity tumours but definitions can be extended to other sites whenever possible.

R0 resection (= microscopically complete resection = *radical* resection)

- *Wide*
It is an en-bloc resection through normal tissue, beyond the reactive zone, with the removal of the tumour with its pseudocapsule and a margin of normal tissue; a resection could be defined as "wide" when the tumour is covered at every point by healthy tissue (muscle, subcutaneous tissue, thick fascia or intermuscular septum) according to the growth pattern of the tumour.
When the tumour involves more than one anatomical compartment, the *wide* resection may include adjacent muscle compartment, bone, blood vessels or nerves and should be immediately followed by reconstructive surgery.

- *Compartmental surgery*
When the tumour is removed en-bloc with the entire muscular or anatomical compartment and is covered by intact deep fascia. This surgery is feasible when tumour is entirely anatomically confined.

R1 resection *(= microscopically incomplete resection = marginal resection)*
When the tumour surface emerges macroscopically at the resection surface (e.g. surgical plane through the reactive zone or pseudo-capsule), or when microscopic tumour extension is present at the margin of resection, but without evidence of macroscopic disease residue.

- *Contaminated*: When accidental rupture of the tumour pseudocapsule with spillage of material into the operating field occurs, and also when the pseudocapsule has simply emerged at the margin of resection. In these cases spillage of material must be controlled by all means, and then the operating field must be rapidly washed and the resection margins widened. The contamination must be reported in the description of the surgical procedure and will be followed by complementary radiotherapy.

R2 resection *(= macroscopically incomplete resection = intralesional resection)*
When macroscopic tumour residue is left *in situ*.

22.2 BIOPSY

Aim: to provide enough material for histology, immunochemistry, cytogenetics, central pathology review and spare tissues for biological studies and frozen storage.

Biopsy should be the initial surgical procedure in all patients except when primary excision with adequate margins is possible (rare except for paratesticular tumours).
Open biopsy is recommended and should be **incisional** although US or CT scan guided core needle (Tru-cut) biopsies may be appropriate in difficult or inaccessible sites. Fine needle aspiration biopsy is not recommended. Endoscopic biopsies are appropriate for bladder, prostate or vaginal tumours.

In planning the surgical approach for biopsy it must be kept in mind that:

- **Incisional biopsy:**
  - The scar and the biopsy track must be included en bloc in the subsequent definitive surgical procedure (this also applies to needle biopsy)
  - In case of sarcoma of the extremities, the incision must always be longitudinal to the limb (transverse and inappropriately placed incisions that traverse multiple tissue compartments must be avoided, because they interfere with the further delayed surgery)
  - Very careful hemostasis must be ensured, to avoid post-surgical haematoma. If drains are used (not recommended), the tract of the drain must be in-line with the skin incision and as close as possible from it.

- **Tru-cut biopsy:**
  - 18 or 16 Gauge needles (1.2 or 1.6 mm) should be used with 4 to 6 cores performed.
  - The biopsy track must always go directly to the tumour, through the muscle fibres with minimal use of retractors
  - The biopsy track must contaminate only the anatomical compartment in which the tumour is situated, avoiding major neurovascular structures.

Tissue should always be sent fresh to the laboratory if possible. If fixative has to be used it should be formalin based (see Pathology Guidelines).

IMAGING-GUIDED BIOPSY

- Surgical open biopsy is recommended, but, according to local procedures, US or CT scan-guided core needle biopsies may be appropriate, especially in difficult or inaccessible sites, whereas endoscopic biopsies are appropriate for bladder, prostate or vaginal tumours.
- - 18 or 16 Gauge (1.2 – 1.6 mm) needles may be used depending of local procedures. Fine needle aspiration (22 Gauge – 0.7 mm) only is not recommended, but additional FNA may provide additional cellular material which can be used for genetical examinations (i.e. DNA ploidy and chromosomal analysis) [2].
- For limb primaries in particular the biopsy tract must contaminate only the anatomical compartment in which the tumour is situated, avoiding major neurovascular structures. Useful anatomical landmarks may be found in the following reference [3].
- For limb or superficial primaries it is recommended the biopsy tract is marked e.g. with ink (tattooing), at the time of biopsy to allow later surgical excision of the tract.
- Local arrangements with the histopathology department should be in place regarding fast transport of fresh tumour biopsy specimens.

- Direct fixation must be avoided since no cytogenetic studies are possible when a specimen is placed in formaldehyde, but RPMI medium (Roswel Park Memorial Institute 1640) may be used for specimen transport without jeopardizing genetic studies.

22.3 PRIMARY RESECTION (SURGERY A)

Aim: to achieve complete resection (R0: microscopically complete resection), without danger or mutilation.

Primary resection is indicated

1. if there is no clear clinical evidence of lymph node or metastatic disease
2. if the tumour can be excised with adequate margins and without danger or mutilation.

A layer of healthy tissue between tumour and resection margins should exist. This layer of healthy tissue is defined as a **safety distance**. The metric definition of the safety distance (2-5 cm) cannot be used in paediatric tumour surgery. The kind of tumour growth has to be settled as well defined with pseudocapsule or locally infiltrating, and should be documented. This information is important to characterize the biological behaviour of the tumour, and thus contribute to the evaluation of further local therapeutical measures.

In order to ensure the evaluation concerning complete resection, the risk stratification, and therefore further treatment, a close **cooperation between surgeon and pathologist** is necessary. The surgeon should perform an exact drawing of the tumour, including resection margins being important for the evaluation of safety distance (also marked at the tumour). It should be possible for the pathologist to reconstruct the tumour and biopsies taken from the resection margins according to the surgeon's drawing and information. An agreement between surgeon and pathologist concerning TNM-status should be achieved. It will be important for the pathologist to examine the specimen with the surgeon so that correct orientation is ensured for accurate evaluation of the margins. The surgeon must help the pathologist to identify the most critical resection margin and likewise must ensure that points where the tumour emerges only due to muscle retraction following surgical removal are not identified as critical margins.

In practice, the only common situation when primary resection is appropriate is a paratesticular tumour.

"Debulking" procedures are not recommended.

Extensive, "mutilating" operations should never be considered at primary resection. "Mutilating" is defined as: leading to significant long term anatomical, functional or cosmetic impairment. Mutilating surgery are considered:

- orbital exenteration,
- major resection of the face
- pneumonectomy
- pelvic exenteration with definitive intestinal or urinary diversion
- total cystectomy
- total prostatectomy
- hysterectomy
- extremity amputation and major muscular resections leading to important functional impairment

22.4 PRIMARY RE-OPERATION (SURGERY A)

Aim: To achieve complete resection (R0) in patients with microscopic (certain or doubtful) residue after primary operation, before other therapies, if this can be done without danger or mutilation.

If a primary marginal excision or excisional biopsy (not recommended) has already been done, or where histological evaluation is inadequate, then primary re-excision should be considered [1,2]. This applies particularly to trunk, limb and paratesticular tumours. The interval between initial surgical approach and chemotherapy, including primary reexcision should be as short as possible and should never exceed 8 weeks. In case of adequate margins (or no tumour) on specimen from primary re-excision, patient should be classified as IRS Group I only if the description of first surgery allows to be confident that no tumour spill and contamination has occurred.

22.5 SECONDARY OPERATION (SURGERY B)

Aim : to achieve complete resection of a residual mass after neoadjuvant chemotherapy (R0).

Secondary operations and even multiple biopsies for verification of local control are not indicated if clinically, endoscopically and on CT or MRI scanning there is no visible tumour[3].
Where a residual mass is demonstrated or in doubtful cases, surgical resection should be done, although there may be certain anatomical sites, particularly in the head and neck, where this may not be feasible and the final indication in these cases is left to the decision of the individual surgeon. It should however be remembered that negative biopsies of the residual mass, even if multiple, may be unrepresentative. Marginal resection (R1 resections) in sites where R0 resection is not possible may also be acceptable, provided that they are always followed by radiotherapy.
If residual mass is not completely resected, radiotherapy should be given.

Secondary operations should, as a rule, be conservative, anticipating local radiotherapy for residual disease, but “mutilating” operations may be appropriate after unsuccessful neo-adjuvant chemotherapy or radiotherapy or in patients under 3 years for whom external beam radiotherapy is not indicated.

“Debulking” procedures are not recommended.

22.6 LOCAL CONTROL ASSESSMENT

Surgery may be discussed at the end of treatment in order to assess or achieve the tumour local control after chemotherapy + radiotherapy. Mutilating surgery ("salvage surgery") could be appropriate in some cases.

22.7 RECONSTRUCTIVE SURGERY AND LOCAL CONTROL

Reconstructive procedures have to be included early enough in the planning of the resection. It is desirable to have the histological evaluation before reconstructive surgery. In cases however where reconstructive vascular surgery or microvascular surgery is involved, this is mostly not possible. Therefore in some cases resection and reconstructive surgery have to be performed at the same time without histological confirmation of the status of the resection.

Pre or post operative irradiation has to be considered depending on the necessary reconstructive measures :

- Bone reconstruction (e.g. microvascular transfers of fibula or iliac bone) is incompatible with post-operative irradiation
- Free flaps for soft tissue replacement can help lymphatic reconstruction only if they are not irradiated (proximal part of arm or thigh tumours).
- The integration of metal implants in general for joint replacement may be disturbed by radiation.

22.8 SURGERY OF THE LYMPH NODES

Aim : to confirm nodal involvement with nodal sampling avoiding radical lymph node dissection.

Clinically or radiologically suspicious regional lymph nodes should be sampled on initial presentation and at relapse. Cytology or true-cut biopsy may be useful to confirm nodal involvement but only if a conventional biopsy of the primary tumour has been obtained for diagnostic purposes.

In extremity sites, systematic biopsy of regional nodes (see definition in appendix A5) should be performed even if nodes are not palpable or enlarged on imaging. New techniques of sentinel node mapping (with blue dye and/or radioactive tracer) are recommended whenever feasible [4].

Radical lymph node dissections are generally not indicated and involved lymph nodes should be irradiated, as should enlarged nodes at relapse, whether resected or not. It should be remembered that the combination of radiation therapy and radical lymph node dissection should be absolutely avoided as it can induce severe lymph oedema.
There are rare occasions when, if radiotherapy is contraindicated (e.g. age < 3 years), a lymph node dissection may be considered as definitive local treatment.

22.9 SPECIFIC SITES

22.9.1 Parameningeal site

Complete surgical resection is difficult and generally not possible. Radiotherapy is always necessary in patients over 3 years and should be given at week 13 regardless of response to initial chemotherapy.
An initial resection will not be accepted if permanent severe uncorrectable functional dysfunction or mutilation results. In all cases where resectability is uncertain a resection should not be attempted and only a biopsy taken. Neck dissections should not be performed initially.
Only after radiotherapy a secondary resection is acceptable. Secondary resections in PM site should only be performed in centres with experience in this field.

22.9.2 Orbit

Biopsy is usually the only surgical procedure required for orbital tumours.
Secondary resections are not recommended. Enucleation or exenteration are very rarely indicated [5].
Depending on the age of the child microsurgical reconstruction with a free flap or forearm flap in combination with an appropriate prosthetic device are recommended after exenteration of the orbit.

22.9.3 Head and Neck

Complete surgical excision is difficult but major resections with reconstruction may be appropriate in some circumstances, after neoadjuvant chemotherapy. Such operations should only be realised in centres with an interdisciplinary surgical team and with experience in microsurgical free flap reconstruction.
A combination of surgery and brachytherapy ("Amore" technique) is practised in some Centres [6].

22.9.4 Bladder/Prostate

Cystoscopy should be done at diagnosis and during follow up.
Initial resection (rather than biopsy alone) should only be done in the case of very small tumours arising in the dome of the bladder, far from the trigone.

Secondary operations:
Conservative surgery of bladder /prostate tumours could be done where feasible (partial cystectomy and/or partial prostatectomy) in conjunction with brachytherapy particularly in very young boys [7,8] or external beam radiotherapy.
Partial prostatectomy, without radiotherapy, carries a high risk of local relapse[9].
Where conservative treatment is not feasible, the treatment will include total cystectomy and/or total prostatectomy with or without post-operative radiotherapy.

22.9.5 Vagina

Partial vaginectomy may be feasible after chemotherapy but brachytherapy is often preferable after ovarian transposition [10].

22.9.6 Paratesticular

These should be excised via an inguinal incision, first ligating the cord at the internal inguinal ring. Orchidectomy is essential. In rare cases, if the tumour is very large and delivery into the groin would be difficult or traumatic, it is better to make a scrotal incision (keeping the tunica vaginalis intact) and deliver the testis and cord via this.
Retroperitoneal lymphadenectomy or nodal sampling at diagnosis is not recommended unless there is uncertainty on imaging [11,12].

If the initial operation before referral was scrotal then primary re-operation should be done to excise the cord at the internal ring. Complementary hemiscrotectomy is not necessary [13,14] if the patient is upstaged, being treated according to the Subgroup B strategy (i.e. if in the Low Risk group the child will be upstaged). When there is a doubt about scrotal contamination, hemiscrotectomy should be performed.

22.9.7 Extremities

Particular attention is recommended initially in evaluating the regional lymph nodes. Upper and lower limb tumours must have surgical evaluation of axillary or inguinal nodes, respectively, even if nodes are clinically/radiologically normal. New techniques of sentinel node mapping (with blue dye and/or radioactive tracer) are recommended whenever feasible [4].

At secondary operation, formal compartmental resection (en bloc resection of the tumour and the entire compartment of origin, where tumour was entirely anatomically confined) may be appropriate for some tumours but less “anatomical” wide resections (en bloc resection through normal tissue, beyond the reactive zone, with the removal of the tumour with its pseudocapsule and a margin of normal tissue) is usually sufficient, providing an adequate margin of normal tissue.

A wide cutaneous incision will be made along traditional lines (along the major axis of the tumour-bearing anatomical compartment), and must include en bloc the scar and the holes-track of previous biopsies or surgery. Once the skin-fat flaps have been prepared the tumour will be isolated within the tumour-bearing structure, with prompt recognition and careful dissection of the main vascular structures and motor nerves (femoral, sciatic, sciatic-popliteal, external/internal, median, ulnar and radial). These structures must not show tumour infiltration. Should doubt arise about a possible oedema or suspect thickening of the delimiting fascia (vascular external tunica, perineurium), it will be prudent to perform frozen section biopsy.

Care must be taken to avoid contamination of the surgical field, which can also occur if the tumour is allowed to emerge on the surface of resection. When minimal contamination has occurred, the patient will be classified as IRS group II, and complementary radiation therapy will have to be planned in any case. Once the malignancy has been isolated, it must be removed en bloc with the surrounding soft tissue, covered at every point by healthy tissue.

Compartmental operations will be performed only if made necessary by the site and dimensions of the tumour. If the lesion is near structures such as the vascular-nervous fascia or bone, it must be cautiously prepared by also removing the fascia covering these structures (vascular external tunica, perineurium or periostium). If these structures are also found to be infiltrated, they must be resected en bloc with the tumour, assessing the possibility of performing vascular, neurological or bone reconstruction as an alternative to mutilating procedures.

Specific problems that can arise from the combination with the irradiation should be considered already at the operation planning. These are:
- disturbance of growth because of irradiation of growth plates
- pathological fractures after marginal bone resection
- lymph oedema after regional lymph node dissection and nevertheless necessary irradiation, especially in the region of the shoulder and groin
- scarred contracture.

When considering radiotherapy, it should be remembered that amputation may be preferable in young children, bearing in mind the serious effects of radiation on growth and function.

22.9.8 Abdomen/Pelvis

If radiotherapy is anticipated for pelvic tumour the surgeon should consider exclusion of the ovaries from the radiotherapy field by transposition and could consider exclusion of small bowel from the pelvis by insertion of a tissue expander or absorbable mesh.

22.10 SURGERY FOR RELAPSE

This depends on the treatment used during primary treatment, but "mutilating" operations may be justified, particularly if radiotherapy options have already been exhausted.

22.11 MARKER CLIPS

If it is considered necessary to mark the tumour bed for postoperative radiotherapy, titanium rather than stainless steel clips should be used so as not to interfere with CT or MRI scans.

22.12 HISTOLOGY

Whenever possible, the case should be discussed with the Pathologist pre-operatively and the tissue sent fresh from the operating theatre to the laboratory. Marker sutures should be inserted to help in orientation and show crucial resection margins. If the tissue has to be sent fixed rather than fresh, a formalin based fixative is preferred.

22.11 REFERENCES – SURGERY

1. Hays DM, Lawrence W Jr, Wharam M, Newton W Jr, Ruymann FB, Beltangady M, Maurer HM. Primary reexcision for patients with 'microscopic residual' tumor following initial excision of sarcomas of trunk and extremity sites. J Pediatr Surg. 1989 Jan;24(1):5-10.
2. Cecchetto G, Guglielmi M, Inserra A, Zanetti I, Dall'Igna P, Gigante C, Carli M; Italian Cooperative Group on Soft-tissue Sarcomas. Primary re-excision: the Italian experience in patients with localized soft-tissue sarcomas. Pediatr Surg Int 2001 Sep;17(7):532-534
3. Godzinski J, Flamant F, Rey A, Praquin MT, Martelli H. Value of postchemotherapy bioptical verification of complete clinical remission in previously incompletely resected (stage I and II pT3) malignant mesenchymal tumors in children: International Society of Pediatric Oncology 1984 Malignant Mesenchymal Tumors Study. Med Pediatr Oncol. 1994;22(1):22-26.
4. McMulkin HM, Yanchar NL, Fernandez CV, Giacomantonio C. Sentinel lymph node mapping and biopsy : a potentially valuable tool in the management of childhood extremity rhabdomyosarcoma. Pediatr Surg Int. 2003, Aug;19(6):453-6
5. Oberlin O, Rey A, Anderson J, Carli M, Raney RB, Treuner J, Stevens MC. Treatment of orbital rhabdomyosarcoma: survival and late effects of treatment -- results of an international workshop. J Clin Oncol. 2001 Jan 1;19(1):197-204.
6. Buwalda J, Schouwenburg PF, Blank LE, Merkx JH, Copper MP, Strackee SD, , Voute TA. Caron HN. A novel local treatment strategy for advanced stage head and neck rhabdomyosarcomas in children: results of the AMORE protocol. Eur J Cancer. 2003;39(11):1594-602
7. Haie-Meder C, Breton-Callu C, Oberlin O, Martelli H, Audry G, Valayer J, Habrand JL, Terrier-Lacombe MJ, Gerbaulet A. Brachytherapy in the treatment of vesicoprostatic rhabdomyosarcomas in children. Cancer Radiother. 2000 Nov;4 Suppl 1:145s-149s.
8. Martelli H, Haie-Meder C, Oberlin O. Conservative surgery + brachytherapy treatment in very young boys with bladder-prostate rhabdomyosarcoma: a single team experience. Med Pediatr Oncol, 2003, 41, 260
9. Audry G, Oberlin O, Capelli C, Martelli H, Godzinski J, Spicer R, Gruner M, Praquin MT, Corkeri J, Ransley PG, Van Unnick A, Quintana E, Stevens MCG. The role of conservative surgery in bladder-prostate rhabdomyosarcoma – an update of the SIOP experience. Med Pediatr Oncol. 1998;31(4):198 (abstract).
10. Martelli H, Oberlin O, Rey A, Godzinski J, Spicer RD, Bouvet N, Haie-Meder C, Terrier-Lacombe MJ, Sanchez de Toledo J, Spooner D, Sommelet D, Flamant F, Stevens MC. Conservative treatment for girls with nonmetastatic rhabdomyosarcoma of the genital tract: A report from the Study Committee of the International Society of Pediatric Oncology. J Clin Oncol. 1999 Jul;17(7):2117-2122.
11. Olive D, Flamant F, Zucker JM, Voute P, Brunat-Mentigny M, Otten J, Dutou L. Paraaortic lymphadenectomy is not necessary in the treatment of localized paratesticular rhabdomyosarcoma. Cancer. 1984 Oct 1;54(7):1283-1287.
12. Ferrari A, Bisogno G, Casanova M, Meazza C, Piva L, Cecchetto G, Zanetti I, Pilz T, Mattke A, Treuner J, Carli M. Paratesticular rhabdomyosarcoma: report from the Italian and German Cooperative Group. J Clin Oncol. 2002 Jan 15;20(2):449-455.
13. Dall'Igna P., Bisogno G., Ferrari A., Treuner J., Carli M., Zanetti I., Guglielmi M., Cecchetto G. Primary transcrotal excision for paratesticular RMS. Is hemiscrotectomy really mandatory? Cancer, 2003, 97, 1981-1984
14. Stewart RJ, Martelli H, Oberlin O, Rey A, Bouvet N, Spicer RD, Godzinski J, Stevens MC. Treatment of Children With Nonmetastatic Paratesticular Rhabdomyosarcoma: Results of the Malignant Mesenchymal Tumors Studies (MMT 84 and MMT 89) of the International Society of Pediatric Oncology. J Clin Oncol. 2003 Mar 1;21(5):793-798.

23. RADIOTHERAPY GUIDELINES

23.1 ROLE OF RADIOTHERAPY

Radiotherapy is an essential treatment for selected patients with rhabdomyosarcoma. This chapter gives guidelines about indications for radiotherapy, doses and target volume definitions. Here are some of the underlying data and the rationale for the recommendations shown.

IRS group I *(initial complete resection, no microscopic or macroscopic residual tumour, no lymph node involvement):*
Data from the IRS trials I, II and III have been published about the use of radiotherapy in patients with IRS group I tumours [1]**.** In the IRS-I trial, the use of radiotherapy was randomised, in IRS-II, no radiotherapy was recommended and in IRS-III, radiotherapy was indicated for patients with alveolar histology only. In the analysis of all 3 trials, there was a trend for increased failure free survival (not statistically significant) for patients with favourable histology who received radiotherapy, but the overall survival with or without radiotherapy was identical (about 95 % after 10 years). Failure free survival in the IRS trials I-III was significantly improved for patients with alveolar RMS who received radiotherapy. In IRS I and II, the overall survival for patients with alveolar RMS was also statistically significantly improved with radiotherapy (82 % vs. 52 % after 5 years). There was also a trend for improved overall survival in IRS-III (95 % vs. 86 %; p=0.23). The conclusion is that patients with alveolar RMS IRS group I benefit from radiotherapy, but not patients with favourable histology. This is also the policy in the current E*p*SSG radiotherapy guidelines.

IRS group II *(grossly resected tumour with microscopic residual disease or evidence of regional lymph node involvement):*
An analysis of radiotherapy in patients with IRS group II RMS and RMS-like tumours has been performed for patients treated in the CWS trials 81, 86, 91 and 96 [2]. Indications for radiotherapy differed amongst the trials, but there were favourable subgroups of patients that did not receive radiotherapy. Radiation doses ranged between 32 Gy and 54 Gy. There was a statistically significant difference in local control and event free survival in favour of patients treated with radiotherapy despite selection bias. Local control after 5 years was 83 % with and 65 % without radiotherapy ($p<0.004$), event free survival was 76 % with and 58 % without radiotherapy ($p<0.005$). There was a trend for improved survival in the radiation group (84 % vs. 77 %, n.s.). The improvement in local control and event free survival was independent of histology (favourable vs. unfavourable), tumour size, tumour site and age of the patient. Even patients with favourable histology and small primary tumours ($\leq$ 5 cm) benefited from the use of radiotherapy. When the patients of each single trial (CWS 81, 86, 91 or 96) were analyzed separately, the difference in local control and event free survival was not statistically significant any more. The difference in overall survival for the whole study population, although better in all analyzed subgroups who received radiotherapy, was statistically significant only for patients with unfavourable histology (80 % vs. 56 % after 10 years).
In order to avoid a high local failure rate, the use of radiotherapy in patients with IRS group II is therefore recommended. This is compulsory for the patients treated in the high risk group. Because there is no statistically significant difference in overall survival for standard risk patients with

favourable histology, radiotherapy can be omitted if considering the tumour site and age of the patient, radiotherapy is too toxic. The risk of a higher local relapse rate must then be discussed.

IRS group III *(initial incomplete resection with gross residual disease):*

Radiotherapy is the only available local therapy in patients who cannot receive a secondary complete resection. Patients with vaginal tumours and favourable histology are usually very young and local control is acceptable without radiotherapy in patients in complete remission after chemotherapy [3, 4]. In patients with IRS group III disease at other sites with clinical complete remission without the option of second surgery and favourable histology, radiation doses of 32 Gy using accelerated hyperfractionation have resulted in satisfactory local control in the CWS trials [5,6]; with conventional fractionation, doses of 40 Gy or more have been reported to be sufficient to obtain local control [7]. For patients with alveolar RMS, a higher radiation dose has usually been given.
In the IRS IV trial, radiotherapy doses of 50.4 Gy in conventional fractionation were randomised against 59.4 Gy using hyperfractionation in patients with group III tumours [8]. The results with higher radiation doses were not improved, therefore 50 Gy is considered as sufficient for alveolar RMS independent of remission status and for embryonal RMS with residual disease following induction chemotherapy without an option for second surgery.
If delayed second surgery is possible and complete resection is achieved, patients still benefit from additional radiotherapy. In an analysis of the trials CWS 81, 86, 91 and 96, patients with RMS and RMS-like tumours who had IRS group III tumours with secondary complete resection (n=132) were evaluated. Indications for radiotherapy differed amongst the trials but radiotherapy was usually omitted in low risk patients. The calculated local control was 85 % for patients who did and 67 % for those who did not receive radiotherapy ($p<0.01$). EFS after 5 years was 77 % with and 58 % without radiotherapy ($p<0.02$). OS after 5 years with and without radiotherapy was 84 % and 79 % (n.s.). There was no difference in the incidence of systemic failures between the two groups. Patients with small as well as with large initial tumours profited from radiotherapy. The advantage for irradiated patients was seen in patients with favourable and unfavourable histology. The 5 year local control rate in patients without tumour cells in the resected specimen and no radiotherapy was 50 % compared with 89 % in those who did receive radiotherapy ($p<0.01$). Concerning patients with favourable histology and favourable site, overall survival is good following complete secondary resection even when postoperative radiotherapy is omitted, particularly in uro-genital non-bladder-prostate tumors. [3,4] Radiotherapy following second surgery is therefore usually indicated in this trial except for patients with favourable site and favourable histology (subgroup C). Moderate radiation doses are recommended (36 Gy or 41.4 Gy depending on histology). This is compulsary for the patients treated in the high risk group. Because there is no statistically significant difference in overall survival for standard risk patients with favorable histology, radiotherapy can be omitted if considering the tumour site and age of the patient, radiotherapy is too toxic. The risk of a higher local relapse rate must then be discussed.

23.2 EQUIPMENT

23.2.1 Megavoltage equipment

All patients will be treated with megavoltage equipment (4-20 MV linear accelerator preferably). For extremity tumours photons of 4 to 6 MV are recommended. Care must be taken to ensure an

adequate skin dose in high risk areas when high energy photons are used. For tumours of the trunk, photons of 6 to 20 MV energy are recommended.

23.2.2 Electrons

Electrons are allowed for superficial and moderately infiltrating tumours (to a maximum depth of 5 cm) either as an electron field matching on, or as boost to, linear accelerator planned fields. The use of electron fields alone should be avoided because of the late effects.

23.2.3 Brachytherapy

Brachytherapy may be used in cases of incompletely resected tumours of vagina, perineum, bladder, prostate and orbit. It may be used as boost technique before or after external beam irradiation or may in some cases replace external beam irradiation. This must be discussed with the reference centre for each individual patient. The dose for brachytherapy and external beam radiotherapy must take into account radiation-tolerance of adjacent tissue and should be calculated individually in each case.

23.3 TREATMENT PLANNING

3-D-conformal radiotherapy planning is recommended when critical structures lie in or nearby the target volume. The dose is prescribed according to ICRU 50.

23.4 RADIATION DOSE FOR THE PRIMARY TUMOUR

The radiation dose is prescribed according to histology of the tumour, response and the IRS group (extent of initial resection). The doses are summarized in table 1. This section relates to children aged 3 years and older.

- IRS group I (initial complete resection, no microscopic or macroscopic residual tumour, no lymph node involvement):

Radiotherapy is only performed in patients with alveolar RMS. The dose is 41.4 Gy in 23 fractions.
Exceptions: see below

- IRS group IIa (grossly resected tumour with microscopic residual disease, no evidence of regional lymph node involvement), IIb and c (with regional lymph node involvement):

All patients receive radiotherapy independently of histology. The dose is 41.4 Gy in 23 fractions.

- IRS group III (initial incomplete resection with gross residual disease):

In all patients with gross residual disease and residual disease following initial chemotherapy, a secondary complete resection is recommended. Second surgery should only be anticipated when a macroscopically and microscopically complete resection is possible. In case of second surgery, radiotherapy is usually given following second surgery. In patients with reconstructive second surgery, radiotherapy before this procedure may be recommendable.

- *Favourable (embryonal) histology:*
  Patients in subgroup C with complete secondary resection may not receive postoperative radiotherapy (see option A).
  In all other patients, a dose of 36 Gy in 20 fractions is given following complete secondary resection and good clinical response at restaging following initial chemotherapy.

  A dose of 41.4 Gy in 23 fractions is given following complete secondary resection and poor clinical response at restaging following initial chemotherapy.

  In patients who receive radiotherapy *before* (expected) complete second surgery, the same doses according to response are applied.

  The dose is 41.4 Gy in 23 fractions when there is complete clinical remission following initial chemotherapy and no second surgery is performed.

  A dose of 50.4 Gy in 28 fractions is given following incomplete second surgery.

  A dose of 50.4 Gy in 28 fractions is given in patients with residual tumour following initial chemotherapy (partial remission, progressive disease) when no second surgery is performed.

  A boost of 5.4 Gy in 3 fractions may be given in large tumours with poor response to chemotherapy.

- *Unfavourable (alveolar) histology:*
  A dose of 41.4 Gy in 23 fractions is given following complete secondary resection.

In patients who receive radiotherapy *before* (expected) complete second surgery, the same dose is applied.

A dose of 50.4 Gy in 28 fractions is given following incomplete second surgery.

The dose is 50.4 Gy in 28 fractions when there is complete clinical remission following initial chemotherapy (no second surgery) and in patients with residual tumour following initial chemotherapy (partial remission, progressive disease) when no second surgery is performed. A boost of 5.4 Gy in 3 fractions may be given in large tumours with poor response to chemotherapy.

Radiotherapy of lymph nodes: see following chapter.

Exceptions:
a. *Vaginal tumour* site and embryonal histology: no radiotherapy is performed if a complete remission is achieved after the completion of chemotherapy. In patients without complete remission, brachytherapy can be considered.
b. *Orbital tumour* site: The decision for or against radiotherapy in patients with group II and group III embryonal RMS is made individually following full informed consent. (see chapter treatment guidelines for special sites:orbit). Patients with partial remission (more than 66 % tumor shrinkage) receive 45 Gy instead of 50.4 Gy.
c. *Patients* $\leq$ *3 years* of age: see paragraph 23.12.

Important comment: The radiotherapy guidelines have to be followed strictly in all high risk patients. Furthermore they should be followed for patients treated in the standard risk group. As stated in the introduction of the radiotherapy chapter, event free survival is improved in patients with the use of radiotherapy in IRS groups II and III even when they had complete second surgery or are in complete clinical remission after initial chemotherapy. For patients in this situation presenting with favourable histology, despite differences in event free survival, there is no statistical difference in overall survival because of effective (but also aggressive) salvage treatment. Therefore, because of concerns of radiation-associated side effects, particularly in very young patients and/or vulnerable tumour sites, omission of radiotherapy may be justified in single patients who present with favourable histology and achieve clinical complete remission with chemotherapy and second surgery despite the higher risk of relapse. *This situation must be discussed with the reference centre and the patient/parents must be informed about the increased risk of local relapse.*

Table 7: **Radiation doses for the <u>primary tumour</u> according to histology and IRS - group for children age 3 years or older (RT: radiotherapy; F: fractions).**

IRS Group	**embryonal RMS**	**alveolar RMS**
I	no RT	41.4 Gy; 23 F
IIa, b and c	41.4 Gy; 23 F	41.4 Gy; 23 F
III followed by:		
- secondary complete resection	36 Gy; 20 F *(partial response)* 41.4 Gy; 23 *F (minor partial response, SD)* *Subgroup C: option A (no RT) or B (36 Gy)*	41.4 Gy; 23 F
- second look surgery but incomplete secondary resection	50.4 Gy; 28 F	50.4 Gy; 28 F
- clinical complete remission, no second look surgery	41.4 Gy; 23 F	50.4 Gy; 28 F
- partial remission, minor PR, SD, progressive disease, no second surgery	50.4 Gy; 28 F (+ Boost of 5.4 Gy; 3 F) orbit and PR (>2/3) 45 Gy; 25 F	50.4 Gy; 28 F (+ Boost of 5.4 Gy; 3 F)

23.4.1 Radiation in patients with stable or progressive disease at restaging

Patients who have stable or progressive disease at restaging at week 9 receive second line therapy. Patients in whom a secondary complete resection is possible will be treated with postoperative radiotherapy with 41.4 Gy, 23 F independently of histology. Patients with inoperable tumours or with incomplete second surgery will be treated with 50.4 Gy in 28 fractions and a boost of 5.4 Gy in 3 fractions at the discretion of the treating radiation oncologist.

23.5 RADIATION DOSE FOR INVOLVED REGIONAL LYMPH NODES

Radiotherapy to regional lymph nodes is only performed when there is clinical or pathological evidence of lymph node involvement. Radiotherapy is not performed when there is no evidence of lymph node involvement at diagnosis, either clinically or histologically. The risk of lymph node involvement in patients with embryonal RMS is very low, it is higher in patients with alveolar RMS. In the CWS trials 81-96, there were 184 patients with alveolar RMS without clinically involved lymph nodes at diagnosis. The incidence of loco-regional lymph node failure was 9 % overall. Analyzed according to tumour site, it was highest for extremity tumours (14 %;11 of 78 pts.). There was no difference in the incidence according to IRS group or according to age. Of the 17 lymph node relapses, only 7 were isolated relapses. Radiotherapy of clinically uninvolved regional lymph nodes seems therefore not justified.
Radiotherapy to the involved lymph node sites is performed independently of histology. In patients with clinical or pathological evidence of lymph node involvement, a radiation dose of 41.4 Gy is given when there are no enlarged lymph nodes following initial chemotherapy before the onset of radiotherapy. This dose is given also when a lymph node excision was performed initially. In patients with enlarged lymph nodes at the onset of radiotherapy, an additional boost of 9 Gy is applied.

Table 8: **Radiation dose for regional lymph node areas (RT: radiotherapy; F:fractions)**

Situation	**embryonal/alveolar RMS**
no clinical or pathological involvement of regional lymph nodes	no RT
clinically or pathologically positive lymph nodes; excised or in complete remission before RT	41.4 Gy; 23 F
positive lymph nodes, macroscopical residual disease before RT	41.4 Gy; 23 F + 9 Gy boost; 5 F

23.6 FRACTIONATION

Treatment is applied in conventional fractionation with 1.8 Gy per day. In patients with large abdominal or cranio-spinal fields, smaller fractions are used. In patients $\leq$ 3 years of age, smaller fractions may be used as well (1.6 Gy). The radiation dose is prescribed according to ICRU 50.

23.7 COMPENSATION FOR TREATMENT BREAKS

Standard fractionation is 5 days per week. If there is a treatment interruption, 2 fractions with an interval of at least 6 hours between fractions should be given to enable completion of treatment within the same overall time if feasible from the irradiated volume.

23.8 TARGET VOLUME DEFINITION FOR PRIMARY TUMOUR

1. The target volume is chosen according to the initial tumour volume (gross tumour volume; GTV). The pretherapeutic T1 MR image with contrast is usually the optimal imaging study. Exceptions: intrathoracic or pelvic tumour bulk (see paragraph 23.14)
2. The clinical target volume (CTV) is defined as the GTV + 1 cm (exception limbs: 2 cm in longitudinal direction).
3. Additionally, scars of the biopsy, of the initial surgery, of the second look surgery and of drain sites have to be included in the CTV. Furthermore all tissues that were potentially tumour-contaminated during surgery need to be included in the CTV.
4. The planning target volume (PTV) is defined as the CTV + 1 cm (exception chest wall: 2 cm). orbit: whole orbit included in the PTV up to 36 Gy).
5. In patients receiving 50.4 Gy, the CTV and hence the PTV is reduced by 1 cm after 41.4 Gy. In patients with orbital tumors, the initial radiation of the whole orbit is reduced to the initial tumor extent + 1 cm after 36 Gy.
6. In patients receiving a boost after 50.4 Gy, the PTV for the boost is the residual tumour at the start of radiotherapy plus a margin of 1-2 cm.
7. In growing patients, a radiation dose gradient through the epiphyseal growth plates should be avoided because of the risk of asymmetric growth. The growth plates should either be included in or, if feasible from the tumour extension, be excluded from the radiation fields. The same should be observed for vertebral bodies in order to avoid scoliosis.

Summary: The PTV consists of the initial tumour volume + 2 cm except for limb and chest wall tumours (+ 3 cm) for 41.4 Gy and except for orbit (entire orbit for 36 Gy). Areas contaminated during surgery including scars and drainage sites must be included in the PTV. If 50.4 Gy need to be applied, the PTV is reduced by 1 cm after 41.4 Gy (orbit: initial tumor size + 1 cm after 36 Gy).

23.9 TARGET VOLUME DEFINITION FOR LYMPH NODES

The dose of 41.4 Gy is applied to the entire lymph node site (axilla, groin, paraaortic lymph nodes etc.). When that approach results in very large radiation fields, this extent can be reduced to the involved lymph nodes plus a PTV margin of 3 cm at the discretion of the treating radiation oncologist. The boost is used for the enlarged lymph node(s) as it is defined in the CT or ultrasound examination before the onset of radiotherapy. An additional margin of 2 cm is to be used for the PTV of the boost.
If possible the draining lymphatic vessels between the primary tumour and the involved lymph node site should be irradiated. However, in some cases this would result in unacceptable large radiation fields. In these patients, two separate radiation fields have to be used to treat the primary tumour and the lymph node site excluding draining lymphatic vessels.

23.10 TIMING OF RADIOTHERAPY

In patients with IRS group III (macroscopical residual disease), the option for second surgery must be checked before the onset of radiotherapy. In patients receiving no second surgery, radiotherapy is performed at week 13. In high risk patients, the full dose of doxorubicin must have been given before the onset of radiotherapy.

After second surgery, postoperative radiotherapy should be started within 21 days except when there are postoperative complications.
In patients who receive reconstructive surgery, radiotherapy before second look surgery may be beneficial. This must be discussed with the study centre. The interval between the end of radiotherapy and second surgery should be approximately 5 weeks. Surgery immediately following radiotherapy can result in higher operative morbidity.

23.11 SYNCHRONOUS CHEMOTHERAPY AND RADIOTHERAPY

Synchronous application of radiotherapy and chemotherapy with doxorubicin and actinomycin D should in general be avoided.
However irradiation will take from 5 to 6 weeks and it is important not to reduce excessively the cumulative dose of the drugs administered.
According to the protocol the whole dose of doxorubicin will be administered before start of radiotherapy.

Parallel application of radiotherapy and actinomycin D should be given:
- when extremity tumours are treated
- mucosae are not included in the irradiation field.
- at the very beginning of RT (week 13)

Actinomycin-D should be omitted at week 16 when the treatment fields include the trunk, abdomen, or the head and neck

Caution is needed in the administration of Actinomycin-D at week 19: in general if 2 weeks have passed from the end of irradiation Actinomycin-D should be given. In case of a shorter interval Actinomycin-D may be re administered when no toxicity is anticipated (in case of doubt reduce Actinomycin dose to 50%)

The omitted doses of actinomycin will not be administered later.

23.12 AGE ADAPTATION

23.12.1 Age > 1 and ≤ 3 years at the time of radiotherapy

Embryonal RMS: Radiotherapy will only be performed if there is residual disease at the end of chemotherapy.
Exception: parameningeal tumours will always receive radiotherapy even when in complete clinical remission after chemotherapy. The radiation dose should be given according to older patients. Depending on tumour size and site, this can result in unacceptable toxicity. In these special cases, a dose reduction can be performed. This should be discussed with the reference center.

Alveolar RMS:	Group I:	no radiotherapy
	Group II and III:	radiotherapy according to older patients (no dose reduction; exceptions as above)

Smaller fraction sizes can be used (1.5 or 1.6 Gy).

23.12.2 Age ≤ 1 year

An individual decision for or against radiotherapy must be made depending on tumour histology, tumour site, response to chemotherapy, extent of previous resections and options for second surgery. This should be discussed with the study centre.

23.13 NORMAL TISSUE TOLERANCE GUIDELINES

	Conventional fractionation (F:fraction)
heart	**30.6** Gy; 17 F
whole liver	**19.8** Gy; 11 F
whole kidney	**14.4** Gy; 8 F
spinal cord (part)	**41.4** Gy; 23 F
spinal cord in pts. with residual spinal tumour (on MRI)	**50** Gy; 28 F
optic nerve/optic chiasm	**45** Gy; 25 F

23.14 TREATMENT GUIDELINES FOR SPECIAL SITES

23.14.1 Parameningeal tumours

Surgery in parameningeal tumours is usually incomplete. Therefore second surgery should not be performed. Radiotherapy must be applied at week 13.

23.14.2 No skull base erosion/no cranial nerve palsy

The brain/meninges are NOT routinely irradiated. The CNS volume irradiated will be that included within the fields required to cover the primary volume, (e.g. nasopharynx/paraspinal situations) according to the general guidelines.

23.14.3 Skull base erosion/cranial nerve palsy/no intracerebral component

RMS with skull base erosion/cranial nerve palsy but no intracerebral components will be irradiated as follows:
The PTV will be that required to treat the primary tumour (initial tumour volume + 2 cm). Radiation fields must adequately cover the initial skull base erosion but there is no routine whole brain irradiation.

23.14.4 Skull base erosion/cranial nerve palsy/with intracranial component

The PTV for the intracranial extent of the tumour is defined according to the residual intracranial component at restaging before the onset of radiotherapy with an additional safety margin of 2 cm. It is not necessary to consider the full initial intracranial tumour extent. The amount of skull base included in the PTV is as defined above.

23.14.5 Disseminated meningeal disease or CSF positive cytology

These patients are treated in the protocol for metastatic disease.

23.14.6 Target volume definition in parameningeal RMS with positive lymph nodes

The PTV is according to the treatment guidelines for parameningeal site and to the treatment guidelines for nodal involvement.

23.14.7 Head and neck non-parameningeal

Radiotherapy is given according to the general radiation guidelines described above. Patients in subgroup C (favourable histology) may not receive radiotherapy when a secondary complete resection was performed.

23.14.8 Orbit

The decision for or against radiotherapy in patients with group II and group III embryonal RMS and clinical complete remission following induction chemotherapy is made individually following full informed consent. Patients in this treatment situation who receive radiotherapy have a lower risk of local relapse, an improved event free survival but experience radiation associated side effects. Patients in this treatment situation who do not receive radiotherapy have a higher risk of local relapse, less good event free survival but no radiation associated side effects in case there is no local relapse and increased toxicity due to salvage treatment including radiotherapy if a relapse occurs. Overall survival in both approaches is equivalent. This is due to effective salvage treatment[9]. The decision for or against radiotherapy is therefore a question of priorities of the treating physician and of the patient/parents. Two options are given in this protocol (see chapter 14.4)
When given, radiation of the entire orbit is performed up to 36 Gy, then the PTV is reduced to the initial tumor size and an additional margin of 1 cm, if possible sparing the lacrimal gland. Patients with favourable histology and clinical complete remission following induction chemotherapy receive 41.4 Gy, patients with partial response (>2/3) 45 Gy, patients with minor partial response, SD or PD receive 50.4 Gy.

23.14.9 Extremities

Extremity tumours should be treated according to the general guidelines described above. Tissue contaminated during surgery must be included in the CTV. After surgical procedures, all scars and drainage sites should be irradiated with a safety margin of 1 - 2 cm. Circumferential radiotherapy must be avoided because of the danger of constrictive fibrosis and lymphoedema. In growing patients, a radiation dose gradient through the epiphyseal growth plates should be avoided because of the risk of asymmetric growth. The growth plates should either be included in or, if feasible from the tumour extension, be excluded from the radiation fields.
For lymph node positive extremity RMS see paragraphs 2.4 and 2.8.

23.14.10 Urogenital Bladder/Prostate Site

The doses and target volume definitions follow the general guidelines. Gonads should be positioned out of the treatment volume if possible (in girls oophoropexy must be discussed). Depending on the extent and infiltration of the disease, patients with bladder/prostate tumours may be treated with afterloading techniques/brachytherapy. Individual planning and discussion with the respective reference centre is advised.

23.14.11 Urogenital Non-Bladder/Prostate Site

Patients in subgroup C (favourable histology) with complete secondary resection may not receive postoperative radiotherapy (see chapter 14.4)
Incompletely resected paratesticular RMS need to be irradiated. In order to avoid late sequelae all non mutilating surgical possibilities should be exhausted. In case radiotherapy is necessary (microscopically complete resection not possible), the dose according to the general guidelines should be given with a PTV margin of 2 cm around the initial tumour volume. The contralateral testicle should be positioned out of the treatment volume if possible (orchidopexy). Radiotherapy to lymph node sites is performed according to the general recommendations. When there is scrotal involvement, the infiltrated scrotal area must be treated with a PTV margin of 2 cm.

RMS of the vagina with favourable histology (embryonal RMS) do not receive radiotherapy if in clinical complete remission after chemotherapy. Patients with unfavourable histology (alveolar RMS) and patients who are not in complete clinical remission after chemotherapy need to be treated with radiotherapy. Depending on the extent and infiltration of the disease these patients may be treated with afterloading techniques/brachytherapy. Individual planning and discussion with the respective reference centre is advised. Oophoropexy has to be considered in order to avoid radiation doses at the ovary in all girls treated for pelvic tumours.

23.14.12 Abdomen

Intraperitoneal RMS or RMS of small and large bowel should be resected and only rarely irradiated. Abdominal structures most often prevent high radiation doses.
If radiotherapy to the abdomen is performed, the kidney and liver tolerance doses have to be respected (see paragraph 2.12). In growing patients, a radiation dose gradient through vertebral bodies should be avoided because of the risk of scoliosis. Vertebral bodies and pedicles should either be included in or, if feasible from the tumour extension, be excluded from the radiation fields.
Whole abdominal radiotherapy is performed only when there is malignant ascites or gross tumour spillage during surgery. These patients will be treated in the protocol for metastatic RMS.

23.14.13 Pelvis

Small bowel/iliocoecal bowel may be displaced from the pelvis by treating the patient in prone position and by using a belly board. In some cases, bowel can be spared with special surgical techniques using a tissue expander.
Tumours with non-infiltrating extension into the preformed pelvic cavity often show a large intrapelvic mass which shrinks dramatically after chemotherapy. Irradiating the pre-treatment volume would mean that large volumes of normal tissue (bowel and bladder) are in the radiation field. In these cases, the target volume in the areas of non-infiltrating tumour encompasses only the residual mass after chemotherapy at the beginning of radiotherapy and a 2 cm safety margin. For all other parts of the tumour (infiltrated muscle or bone), the general safety margins according to the initial tumour extension are to be applied.

23.14.14 Retroperitoneum

RMS of the retroperitoneum should be irradiated as outlined in the general radiotherapy guidelines and treatment planning should be CT-based. Tolerance doses of organs in this region need to be respected (i.e. kidneys, bowel, spinal cord). Dose volume histograms for these organs are strongly recommended. In order to avoid scoliosis in growing patients the vertebral bodies should either be irradiated symmetrically or shielded.

23.14.15 Chest wall

The doses and target volume definitions follow the general guidelines.
Tumours with non-infiltrating extension into the preformed thoracic cavity often show a large intrathoracic mass which shrinks dramatically after chemotherapy. Irradiating the pre-treatment volume would mean that large volumes of lung tissue are in the radiation field. In these cases, the target volume in the areas of non-infiltrating tumour encompasses only the residual mass after chemotherapy at the beginning of radiotherapy and a 2 cm safety margin. For all other parts of the tumour (infiltrated muscle or bone), the general safety margins according to the initial tumour extension are to be applied.
Radiotherapy of the hemithorax is performed only when there is malignant pleural effusion or gross tumour spillage during surgery. These patients will be treated in the protocol for metastatic RMS.

23.15 QUALITY ASSURANCE OF RADIOTHERAPY

Radiotherapy documentation forms will be completed and submitted via the relevant data office for review by the Radiotherapy Committee. Simulator films, plans and diagnostic films which determined treatment volume will be requested in all cases who fail locally after radiotherapy and in randomly selected cases of those who do not fail as part of a quality assurance assessment. This will be co-ordinated by the Radiotherapy Committee who will contact centres for films from individual patients as requested.

23.16 REFERENCES – RADIOTHERAPY

1. Wolden S, J Anderson, W Crist et al.: Indications for radiotherapy and chemotherapy after complete resection in rhabdomyosarcoma: A report from the Intergroup Rhabdomyosarcoma Studies I to III. J Clinical Oncology 17 (11):3468-75; 1999

2. Schuck A, A Mattke, D Kunz et al.: IRS-Group II rhabdomyosarcoma and rhabdomyosarcoma-like tumors: Is radiotherapy necessary? JCO, 22(1):143-9; 2004

3. Arndt C, S Donaldson, J Anderson et al.: What constitutes optimal therapy for patients with rhabdomyosarcoma of the female genital tract? Cancer 91:2454-68; 2001

4. Martelli H, O Oberlin, A Rey et al.: Conservative treatment for girls with nonmetastatic rhabdomyosarcoma of the genital tract: A report from the study committee of the International Society of Pediatric Oncology. JCO 17:2117-2122; 1999.

5. Koscielniak E, B Schmidt, R Knietig et al.: Effectivity of a 32 Gy radiation dose in children with RMS: Report of the German Cooperative Soft Tissue Sarcoma Studies (CWS). Med Pediatr Oncol 37: 186; 2001.

6. Koscielniak E, M Morgan, J Treuner: Soft tissue sarcoma in children. Prognosis and management (review). Paediatr Drugs 4:21-28;2002.

7. Regine WF, J Fontanesi, P Kumar et al. Local tumor control in rhabdomyosarcoma following low-dose irradiation: comparison of group II and select group III patients. Int J Radiat Oncol Biol Phys. 31(3):485-91; 1995

8. Donaldson S, J Meza, JC Breneman et al.: Results from the IRS-IV randomized trial of hyperfractionated radiotherapy in children with rhabdomyosarcoma--a report from the IRSG. Int J Radiat Oncol Biol Phys. 51 (3):718-28; 2001.

9. Oberlin O, A Rey, J Anderson et al. Treatment of orbital rhabdomyosarcoma: survival and late effects of treatment-results of an international workshop. JCO 19:197-204;2001

24. Chemotherapy guidelines

All the drugs used are licensed in Europe and have passed clinical phase II trials.

24.1 CHEMOTHERAPY STARTING/ STOPPING RULES

The following chemotherapy courses should not be started unless all these conditions are present:

- 2 x10^9/l WBC, or 1 x10^9/l neutrophils
- 80 x10^9/l platelets are reached.
- absence of any relevant organ dysfunction (especially heart, kidney or liver)

24.2 GENERAL GUIDELINES

24.2.1 Drug modulation during IVADo treatment

Dose/time intensity is regarded to be an essential aspect of the IVADo strategy. In case of relevant (≥ CTC grade III) toxicity, actinomycin D (ACT-D) is the first drug to be reduced.
It is suggested, that in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay ≥ 1 week due to neutropenia-related toxicity the use of G-CSF with subsequent courses is recommended.
In case of severe mucositis or hepatotoxicity or treatment delay due to ACT-D related cause, ACT-D shall be reduced by 25% for the subsequent course.
If further episodes of treatment delay and/or severe mucositis/neutropenic infections should occur, the dose of actinomycin D should be further reduced or even omitted.
The dose of Doxorubicin should not be modified unless there is evidence of cardiac toxicity (see also paragraph 24.4.2)

24.2.2 Drug modulation in the Maintenance phase

In case of neutropenia (<1 x10^9/l neutrophils) and/or thrombocytopenia (< 80 x10^9a/l platelets) stop Cyclophosphamide administration until count recovery and consider withholding the third vinorelbine dose in the following course.

In case of further haematological toxicity, vinorelbine will be administered at 66% dose at day 1 and 8 (skip the third dose).

24.3 DRUGS INFORMATION AND MODE OF ADMINISTRATION

ACTINOMYCIN D (ACT)
Mechanism of action: inhibition of DNA synthesis

Side effects: gastrointestinal irritation (nausea, vomiting, diarrhoea, ulcerative stomatitis, gastroenteritis), hepatotoxicity (veno-occlusive disease, particularly in young children), bone marrow depression, alopecia, exanthema. It is a radiosensitizer and may enhance radiotherapy damage when given concomitantly.
Extravasation may cause severe local and regional ulceration.

Dose and mode of administration in this protocol:
ACT-D: 1,5 mg/m² iv. as bolus injection. Single doses should not exceed 2 mg.
The drug can be given by peripheral iv. cannula or central line with appropriate precautions against extravasation.

CYCLOPHOSPHAMIDE (CPM)
Mechanism of action: alkylating agent (CPM has to be activated by hepatic hydroxylation)

Side effects: bone marrow depression (nadir 8-14 days), haemorrhagic cystitis (Mesna uroprotection), gastrointestinal irritation (nausea, vomiting, diarrhoea, stomatitis), alopecia, dermatitis, infertility, immunosuppression. Rarely cardiotoxicity and SIADH have been reported.

Dose and mode of administration in this protocol:
CPM: 25 mg/m^2 per os every day (no rest between cycles)
Oral cyclophosphamide is only available in capsules of 50 mg which cannot be cut in smaller capsules so the doses should be divided over more days.
For example, in the case of a patient with a body surface of 1.3 m^2, the daily dose should be 32.5 mg, corresponding to about 100 mg every 3 days: therefore one entire tablet (50 mg) for two consecutive days followed by one day off should be given.
Another option would be to ask the pharmacist to prepare smaller capsules (i.e. 10 mg) from the powder contained in the IV bottles (this has been performed at the Institut Gustave Roussy and the powder resulted stable for 2 months).

It is advised to administer CPM capsules early in the day to decrease the amount of drug remaining in the bladder overnight. During the treatment, an adequate fluid intake (at least 1 L/m^2) is recommended in order to minimize damage of transitional epithelium.

DOXORUBICIN (ADRIAMYCIN) (DOXO)
Mechanism of action: inhibition of DNA synthesis

Side effects: bone marrow depression, acute and late cardiotoxicity, gastrointestinal irritation (nausea, vomiting, ulceration), allergic reactions with skin rash and fever, alopecia.
Extravasation causes local ulceration.

Dose and mode of administration in this protocol:
Doxo: 30 mg/m^2 day 1 and 2 (60 g/m^2 total) in 4 hour infusion.

Longer infusion does not seem cardioprotective and may increase the risk of mucositis, especially if Doxo is administered along with actinomycin.
The drug can be given by peripheral iv. cannula or central line with appropriate precautions against extravasation.

IFOSFAMIDE (IFO)
Mechanism of action: alkylating agent (IFO has to be activated hepatic hydroxylation)

Side effects: haemorrhagic cystitis (Mesna uroprotection), nephrotoxicity (tubulopathy with glucosuria, aminoaciduria, loss of phosphate and Ca, full range of tubulopathies from subclinical changes to a full Fanconi syndrome), bone marrow depression, gastrointestinal irritation (nausea, vomiting, diarrhoea, stomatitis), alopecia, neurotoxicity with transient somnolence and mental disturbance, infertility, immunosuppression

Dose and mode of administration in this protocol:
IFO: 3 g/m² day 1 and 2 (6 gram total) as iv. infusion over 3 hours in each block.
Hyperhydration 3 L/m^2/day and Mesna 3 g/m^2 , day 1 and 2, are required until 12 hrs after completion of IFO .

VINCRISTINE (VCR)
Mechanism of action: mitotic inhibitor; block microtubule polymerization

Side effects: peripheral neuropathy (including constipation and/or paralytic ileus, ptosis, vocal cord paralysis, jaw pain, areflexy, paresthesia, muscular weakness, ataxia), central neurotoxicity (including hallucinations, convulsions, SIADH), arthralgia, myalgia, minimal bone marrow depression, alopecia.
Extravasation causes local ulceration.

Dose and mode of administration in this protocol:
VCR: 1,5 mg/ m^2 iv. as bolus injection day 1 of each cycle (weekly during the first 7 weeks).
Single doses should not exceed a maximum of 2 mg.
The drug should be given by peripheral iv. cannula or central line with appropriate precautions against extravasation.

VINORELBINE (VNL)
Mechanism of action: mitotic inhibitor; block microtubule polymerization

Side effects: myelosuppression, alopecia, mucositis, neurotoxicity. Vesicant.

Dose and mode of administration in this protocol:
VNL: 25 mg/m^2 i.v. day 1,8,15 of each cycle
The drug is given on an outpatient basis, diluted in isotonic solution to a concentration between 1.5 and 3 mg/dl and infused over 5 to 10 minutes into either a large central vein or a free-flowing infusion of 0.9% sodium chloride or 5% dextrose into a fixed peripheral venous infusion device. In patients who receive vinorelbine in a peripheral vein, the vein should be then flushed with a rapid infusion of at least 75 to 125 ml of normal saline solution to reduce the risk of chemical phlebitis.

24.4 DOSE MODIFICATIONS

24.4.1 Age and weight

Age ≤ 1 month
These patients are eligible for the protocol but they are not eligible for the randomised study and should be initially treated with VA at doses calculated *by weight* without further reduction. Doses are reported in Table 9.
Ifosfamide should be added when the child is > 1 month old.
Anthracyclines should be avoided in the initial(s) cycle(s), but should be administered when the child is >3 months old with doses calculated by weight. Doses are reported in Table 9.

Age > 1 month and ≤ 3 months
These patients are eligible for the protocol but they are not eligible for the randomised study and should be initially treated with VA or IVA, according to the risk group. Vincristine and Actinomycin D doses will be calculated *by weight* without further reduction. Ifosfamide dose will be calculated *by weight and then reduced to 50%.*
Anthracyclines should be avoided in the initial(s) cycle(s), but should be administered when the child is >3 months old with doses calculated by weight.

Age > 3 months and ≤ 6 months
These patients are eligible for the protocol but they are not eligible for the randomised study. Drug doses will be calculated *by weight* without further reduction. Doses are reported in Table 9.

Age > 6 months and ≤ 12 months (or ≤ 10 kg body weight)
These children are eligible for the protocol and randomised study according to the risk stratification. Drug dose should be calculated *by weight* without further reduction.

Table 9 - Age and Drug dose calculation

Age	Eligibility	Drugs and dose calculation	Regimen
0 – ≤ 1 months	Not eligible to the randomised trial	Drug dose calculated by body weight. The resulting dose is: - VCR 0.05 mg/kg/dose - ACT-D 0.05 mg/kg/dose No IFO or Doxo administration. Add IFO when the child is > 1 month	VA only (to be modified when the child > 1 month)
1- 3 months	Not eligible to the randomised trial	VCR and ACT-D: drug dose calculated by body weight. The resulting dose is: - VCR 0.05 mg/kg/dose - ACT-D 0.05 mg/kg/dose IFO: dose calculated by body weight and then reduced to 50%. The resulting dose is - IFO 50 mg/kg/dose No Doxo administration.	VA or IVA
> 3 - ≤ 6 months	Not eligible to the randomised trial	VCR, ACT-D, IFO and Doxo: drug dose calculated by body weight. The resulting dose is: - VCR 0.05 mg/kg/dose - ACT-D 0.05 mg/kg/dose - IFO 100 mg/kg/dose - Doxo: 1 mg/kg/dose	VA or IVA (IVADo only for very high risk group)
> 6 – ≤ 12 months > 6 months and/or ≤ 10 kg	Eligible to the randomised trial if in the high risk Group	VCR, ACT-D, IFO and Doxo: drug dose calculated by body weight. The resulting dose is: - VCR 0.05 mg/kg/dose - ACT-D 0.05 mg/kg/dose - IFO 100 mg/kg/dose - Doxo: 1 mg/kg/dose	VA, IVA or IVADo (depending on risk group)
> 12 months and > 10 kg	Eligible to the randomised trial if in the high risk Group	Full m² dose	VA, IVA or IVADo (depending on risk group)

Note:

a) if tolerated, drug dose should be increased by 25-30% at each cycle to full dose by body weight.
b) IFO should not be given in children less than 3 months in the initial cycle(s), however it should be administered in the subsequent courses as the child grows up and providing the chemotherapy is well tolerated
c) Doxo should not be administered in children age less than 3 months at diagnosis. Therefore they will be treated initially with VA and subsequently with IVA.

In patients with body surface area (BSA) > 2 m^2 the chemotherapy dose should not exceed the dose calculated for a BSA of 2 m^2 (observe maximum single dose 2 mg for VCR and ACT-D).
The dose given to obese patients should be calculated based on regular body weight.
The chemotherapy doses must be recalculated for each course of chemotherapy according to the actual weight and surface area.

24.4.2 Toxicity

HAEMATOLOGICAL TOXICITY

Recovery of neutrophils > 1.0 x 10^9/l and Platelets > 80 x 10^9/l is required before the start of each course of chemotherapy.
For neutropenia management during IVADo see chapter 24.2.1, for the Maintenance phase see Chapter 24.2.2. For the other cases if count recovery is delayed more than 5 days after the planned start of the next course of chemotherapy on more than one occasion, consider the use of growth factors (see) or dose reduction of all drugs in the subsequent course to 75% of previous dose (except vincristine).

BLADDER TOXICITY

Haemorrhagic cystitis with ifosfamide is rare if hydration and mesna are utilised appropriately. Microhaematuria usually can be tolerated. In case of macrohaematuria it is important to continue (or re-implement) hydration. In case of cystic bleeding under or within 24 hours of completion of IFO-infusion mesna protection should be continued or started again. Only recurrent macroscopic haematuria is an indication for discontinuing IFO, in which case CPM at a dose of 1500 mg/m² per course may be substituted.

RENAL TOXICITY

Serious renal toxicity may occur with exposure to IFO. A prospective monitoring is therefore necessary (see Appendix A.9) and is more likely to occur with an increasing cumulative dose. If nephrotoxicity (tubular or glomerular toxicity > grade 2) occurs discontinue IFO and substitute CPM at a dose of 1500 mg/m² per course for the remaining courses of treatment.
Be careful because increased excretions of tubular enzymes, amino acid or proteins may be evident shortly after IFO infusion. This tubular dysfunction is usually transient, and does not require dose modification.

CARDIOTOXICITY

In this protocol the total cumulative dose of doxorubicin is 240 mg/m^2, therefore lower than the threshold dose for late cardiotoxicity reported in most studies. However, careful monitoring for possible acute or late cardiotoxicity is recommended.
Significant deterioration in cardiac function is indicated by a shortening fraction (SF) <28%. In this event, temporarily withdraw Doxo.
A fall in shortening fraction by an absolute value of >10 percentile units but with an actual SF value >28% (i.e. from SF 42% to SF 31%) may also represent a significant deterioration in function. In this event omit Doxo in the next course.
If the decrease is not persistently proven, i.e. if repeated investigations (after a week) cannot reproduce the dysfunction, Doxo can be recommenced (and the omitted dose of Doxo should be supplied instead of ACT with the first possible cycle).
If persistent deterioration of myocardial function occurs, e.g. persistent decrease in fractional shortening by an absolute value of 10 percentile points from previous tests or a persistent fractional shortening below 28%, consider further avoidance of Doxo and the patient should be referred to a cardiologist.

LIVER TOXICITY AND VOD

Liver dysfunction related to chemotherapy or abdomen irradiation may occur. Patients with signs of liver dysfunction should be monitored carefully.
A particular type of hepatic toxicity is represented by the veno-occlusive disease (VOD). VOD appears related to the administration of different drugs and ACT-D in particular. No specific predisposing factor has been found to identify the patient at risk. A prior persistent or slow recovery of thrombocytopenia may be an indicator of VOD.
In case of VOD actinomycin D should not be given until the main abnormalities have returned to normal and half the dose should be given for the first following course. If tolerated ACT-D dose may be increased progressively in the following cycles.
If the symptoms reappear during ACT-D treatment, this drug should be withdrawn permanently.

VOD of any grade is considered a serious adverse event and must be reported immediately (see chapter 26) using the RDE system

Criteria for diagnosis and grading of VOD are reported in appendix A.8.

NEUROLOGICAL TOXICITY

Serious neurological toxicity from IFO is rare but more likely to occur in patients with impaired renal excretion of the drug, either from an obstructed urinary tract at initial diagnosis or from renal impairment later in treatment. Evidence of IFO encephalopathy may be mild initially but should be considered in any patient who demonstrates altered level of consciousness during or shortly after the drug infusion.
In case seizures occur methylen-blue may be given: 30 mg/m^2 (max 50 mgs) as a 2% aqueous solution, give by slow i.v. injection. The reversal of encephalopathic features should occur over the next 30-60 minutes.
If grade 3 or 4 central neurotoxicity occurs (somnolence > 30% of the time, disorientation / hallucination / echolalia / perseveration / coma) consider to avoid further IFO and substitute with cyclophosphamide 1500 mg/ m^2 per cycles.

Peripheral neurotoxicity from vincristine is a common but usually mild side effect. If grade 3-4 peripheral neurotoxicity occurs (intolerable paresthesia, marked motor loss, paralysis or paralytic ileus) one or two injections of vincristine should be omitted and restarted at a 50% dose.
Laxatives should be prescribed when weekly vincristine is given and thereafter if needed to prevent constipation.

25. Toxicity Monitoring

Patients having adverse events will be monitored with relevant clinical assessments and laboratory tests as determined by the Investigator. All adverse events must be followed to satisfactory resolution or stabilisation of the event(s).
Any action taken and follow-up results must be recorded either on the appropriate page of the Case Report Form, as well as in the subject's source documentation. Follow-up laboratory results should be filled with the subject's source documentation.

For all adverse events that require the subject to be withdrawn from the study, relevant clinical assessment and laboratory tests will be repeated on at least a weekly basis (if possible), until final resolution or stabilisation of the event(s).

26. Serious Adverse Event reporting

26.1 ADVERSE EVENTS

Adverse events are illnesses, signs of illnesses or symptoms which occur or aggravate after the patient has been included in E*p*SSG protocol. Fluctuations of pre-existing illnesses or ailments including the illness which lead to treatment in E*p*SSG protocol do not need to be documented as AE.

The investigator must try to assess the relationship of any adverse event to the use of study drugs, based on available information, using the following guidelines:

1. not connected to the Protocol treatment = Unlikely-no temporal association, or the cause of the event has been identified, or the drugs cannot be implicated
2. possibly connected to Protocol treatment = Possible-temporal association, but other etiologies are likely to be the cause; however involvement of the drug cannot be excluded
3. definitely or most probably connected to Protocol treatment = Probable-temporal association, other etiologies are possible, but unlikely to be the cause of the event.

Severity of adverse event must be classified as

- *Mild:* Awareness of any sign, symptom or event, but easily tolerated, and not requiring intervention.
- *Moderate:* Discomfort enough to cause interference with usual activity and may warrant intervention
- *Severe:* Incapacitating with inability to do usual activities or significantly affecting clinical status, and warrants intervention.
- *Life threatening:* Serious adverse event (SAE: see below)

26.2 SERIOUS ADVERSE EVENT (SAE)

A serious adverse event is any event that:

- Is fatal
- Is life threatening
- Is significantly or permanently disabling
- Is a congenital anomaly or birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse drug experience when, based upon appropriate medical judgement, they may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalisation, or the development of drug dependency or drug abuse. In addition, laboratory value(s) changes may require reporting unless otherwise specified in the protocol.
- Second malignancy

A list of the most common SAE follows:

- Septic shock (febrile neutropenia is not a SAE)
- Hemorrhagic cystitis (grade 4)
- Severe nephrotoxicity (see appendix A.9)
- VOD (any grade, see appendix A.8)
- Acute or late cardiotoxicity (shortening fraction <28%)
- Seizures
- Central neurotoxicity (somnolence > 30% of the time, disorientation / hallucination / echolalia / perseveration / coma)
- Second malignant neoplasm
- Death if derived from any kind toxicity (not from tumour progression)

Please note that it is known and accepted that the intensive chemotherapy adopted for patients with RMS may cause important toxicity. “Expected” toxic events, even if they cause hospitalization, but are not life threatening, should not be regarded as a SAE and does not need to be reported with the SAE form.

Examples of "expected" toxicities are:
- febrile neutropenia without septic shock
- blood or platelets transfusions
- mucositis
- constipation
- haematuria
- nausea and vomiting

Note: the RDE system will assist the clinician to define a SAE

All serious adverse events, whether or not deemed drug-related, must be reported *immediately (within 24 hours of knowledge of the event)*. The remote Data entry system provides a special form denominated “SAE form” that should be filled. An automatic email alert will be generated and sent to the national and protocol coordinators.

In case the system is not used, the notification must be done by fax to the national coordinator that will be in charge to inform the protocol coordinator as soon as possible.

Moreover the Investigator must provide documentation of a serious adverse reaction in compliance with local laws.
All serious adverse events, including cases of death, must also be communicated to the local Ethical Committee by the Investigator, according to regulations in force.
A specific document regarding safety has been issued (Standard Operative Procedures (SOPs) for managing Serious Adverse Events (SAEs) and Suspect Unexpected Serious Adverse Reactions (SUSARs) throughout the EpSSG RMS 2005 Trial). Investigators are requested to refer to this document for the management of safety aspects.

27. Supportive Care

The treatment of patients with RMS requires a multidisciplinary approach with a high degree of medical competence existing only in institutions familiar with the administration of intensive chemotherapy and adequate infrastructure to provide the necessary supportive care.

27.1 HEMATOLOGICAL TOXICITY

- Anaemia should be treated by transfusion if necessary (Hb 7-8 g/l) according to national or centre guidelines but is not an indication to modify the treatment schedule.
- Thrombocytopenia: should be treated by transfusion if platelets count <10 $x10^9$/l or in hemorrhagic patients with thrombocytopenia.

27.2 USE OF GROWTH FACTORS (G-CSF)

Primary prophylaxis with G-CSF is not required for the chemotherapeutical regimen outlined in E*p*SSG RMS 2005.
During IVADo treatment, in case of life-threatening neutropenic infection, or treatment delay $\geq$ 1 week due to neutropenia, the use of G-CSF (lenograstim 150 μg/m^2/day) with subsequent courses is recommended (see also chapter 24.2.1).
In other cases if infection complications (neutropenic fever) or prolonged neutropenia develops administration of growth factors will be considered according to Centre guidelines.
G-CSF should be continued until WBC > 1 $x10^9$/l for 3 consecutive days.

27.3 NAUSEA AND VOMITING

These symptoms are expected with all drug combinations of E*p*SSG RMS2005 except single dose vincristine. Antiemetic therapy according to the institutional policy should be given with each major block of therapy.

27.4 INFECTIONS

Neutropenic Fever

Episodes of neutropenic infection are likely to occur after EpSSG RMS2005 cycles of chemotherapy. All participating Institutions must be familiar with managing such problems instituting promptly all necessary investigations (e.g. blood culture) and empiric antibiotic treatment according to centre guidelines.

Pneumocystis carinii pneumonia

Patients treated in the standard, high and very high risk arm may receive cotrimoxazole according to the centre guidelines. The usual dose is 5 mg trimethoprim/kg/day in two divided doses or 10 mg trimethoprim/kg (in two divided doses per day) given twice weekly.

Varicella or herpes

Patients who develop varicella or herpes should receive Aciclovir and chemotherapy should not be restarted until one week after the resolution of the rash.

27.5 CONSTIPATION

Approximately 10% of patients suffered from grade 3-4 constipation during the IVADo pilot study. Laxatives should be prescribed when weekly vincristine is given and thereafter if needed to prevent constipation.

27.6 CENTRAL LINE

The use of central lines is recommended (apart from patients treated in the low risk regimen).

28. Follow up recommendations

Post therapy all patients should be followed for possible tumour relapse and treatment side effects monitoring.

28.1 TUMOUR RELAPSE SURVEILLANCE

	1st year	2nd year	3rd year	4th and 5th year
Clinical examination	Every 3 months	Every 4 months	Every 4 months	Every 12 months
Ultrasound ± CT scan or MRI of the primary tumour site				
Chest x-ray				

Bone marrow aspiration and bone scintigraphy should be performed in case of clinical suspicion

28.2 LATE EFFECTS SURVEILLANCE

1. General studies for all patients

a. Height and weight at 6 months to 1 year intervals. Any child showing a growth deceleration of 20-25 percentile units on standard growth charts from the pretreatment height, should be eva1uated for thyroid and pituitary function.

b. Annual blood pressure measurement.

c. Annual Tanner Staging for girls and boys till maturity. If there is delayed appearance of secondary sexua1 maturation, the patient warrants eva1uation of gonadal hormone values, i.e., at 12-14 years for girls (FSH, LH and estradiol) and boys (FSH, LH and testosterone).

d. Record annual measurement of testicular size in boys using volume measured by Prader orchidometer if possible. The vast majority of patients on this study will receive alkylating agents and may accrue damage to the germinal epithelium of the testis.

e. Record the onset of menses in girls and regularity of periods. Because of local radiotherapy or alkylating agents therapy, ovarian failure may occur in some patients.

f. History should include **school performance and behavioural disturbances** so that early intervention can be made for recognized problems.

2. Studies in children receiving specific chemotherapeutic agents.

a. Doxorubicin. If cardiac toxicity occurred while on therapy (decreased ejection fraction on MUGA scan or decreased shortening fraction on Echocardiogram), annual evaluation of cardiac function should be made for at least 5 years. Histories should include reference to exercise tolerance or shortness of breath.

b. Cyclophosphamide, Ifosfamide. Surveillance of testicular growth in boys at annual visits and initial screening of gonadal hormone values at 14 years of age (FSH, LH and testosterone). Adult values for these hormones are expected at 16-17 years of age. High FSH values suggest damage to the germinal epithelium.
Semen analysis can be done if requested by the patient or if the patient is receptive to the suggestion by a physician.
In girls, evidence of ovarian dysfunction should be investigated by getting values for FSH, LH and estradiol.
If hemorrhagic cystitis occurred while on therapy, urinary should be followed till clear for 2 years. Bladder function can best be assessed by voiding cysto- urethrograms.

3. Studies for specific primary sites

A. HEAD/NECK

1**) Annual growth measurements** plotted on standard growth curves for all patients (see 1.a.).

2) **Annual ophthalmologic exam** by an ophthalmologist if eye was in radiotherapy field.

3) **Annual dental exam** if maxillary/mandibular sites were in radiotherapy field.

4) **Auditory examination every year** if the ears were in the irradiated field.

6) **Thyroid function** (TSH, T3, T4) must be verified every 2 years in case of irradiation on the neck.

B. TRUNK

1) If radiotherapy was given to primary tumours of the chest or to pulmonary metastases, take **history for exercise intolerance or shortness of breath**. If part of heart was in radiotherapy field and patient also received doxorubicin, follow for cardiac toxicity (see 2.a.).
2) Studies appropriate to investigate problems fol1owing **abdominal/pelvic irradiation** which may include bowel obstruction, chronic diarrhoea, inadequate absorption, recta1 stenosis, and sphincter problems.

3) **Kidney function** should be followed annually in patients receiving para-aortic node irradiation or other abdominal sites encroaching on the kidneys.

4) If radiotherapy port included the **upper femurs/hip joints**, slipped capita1 femora1 epiphyses may occur severa1 years after therapy. Symptoms are limp or pain.

5) If radiotherapy was given to primary tumours of the chest or to pulmonary metastases consider the risk of breast cancer and give screening advice (self palpation, mammography).

C. G-U SITES

1) **Children without a bladder** and with various types of urinary diversion should have kidney function evaluated with imaging studies every 1-2 years for hydronephrosis, evidence of pyelonephritis and rena1 function. Contrast studies of ilea1 loops may be necessary to detect kinking, stenosis or reflux of the ureters.

2) Girls with **uterine or vaginal tumours** should be followed for sexual maturation and ovarian failure as in l.b., 1.e. and 2.b. Vaginal examination under anaesthesia until 5 years follow-up and after depending on the treatment received.

3) Boys treated for bladder, prostate or paratesticular primaries should be followed as in l.b. and c. and 2.b.

4) If radiotherapy was given to the bladder, the volume and function should be assessed by voiding cysto-urethrograms or other imaging studies if indicated.

5) History in teen-age boys should include questions of normal **ejaculatory function**, particularly in patients with bladder/prostate or paratesticular primaries.

6) Semen analysis as described in l.b.

D. EXTREMITY SITES

1) If radiotherapy was given, appropriate **bilateral limb length measurements** should be done annually.

2) History should address **limp, evidence of pain and other dysfunction** of the involved extremity.

Pain in the primary site 5-10 years after therapy warrants investigation for the development of secondary bone tumours. This is applicable to all radiation treated sites.

The development of **a second malignant neoplasm,** either leukaemia, lymphoma or solid tumour, should be reported immediately (see SAE report, section 26.2).

29. Pathology guidelines

29.1 E*p*SSG PATHOLOGY PANEL

Professor Vito Ninfo/Dr. Anna Kelsey – Co-Chairpersons

Dr. Rita Alaggio

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29.2 GENERAL REMARKS

Pathology Protocol

All rhabdomyosarcomas diagnosed up to the age of 21 years should be registered.

Role of the pathologist in a participating centre:

The local pathologist has an essential role in both the clinical trial and the prospective study.

1. The diagnosis and sub-typing of rhabdomyosarcoma is made by the local pathologist.

2. Patient stratification is dependent on a number of factors but the diagnosis and subtyping is critical to the management of the patient.

3. Material needs to be sent to the national coordinators as soon as possible following the biopsy or resection.

4. The local pathologist needs to liaise with the molecular biology laboratories so that appropriate molecular characterisations are carried out; it is important for the study that molecular studies are carried out on all rhabdomyosarcomas.

5. The local pathologist needs to be involved in/coordinate tissue banking.

THE NATIONAL COORDINATORS AND THE EpSSG PANEL OF PATHOLOGISTS ARE WILLING TO OFFER REAL TIME REVIEW OF ALL SOFT TISSUE SARCOMAS. THE SUBTYPING OF RHABDOMYOSARCOMA IS IMPORTANT IN PLANNING MANAGEMENT AND RANDOMISATION.

SLIDES, BLOCKS AND FORMS SHOULD BE SENT DIRECTLY TO THE NATIONAL COORDINATORS AS SOON AS POSSIBLE TO AVOID DELAYS. THE REVIEW DIAGNOSIS WILL BE COMMUNICATED DIRECTLY TO THE REFERRING PATHOLOGIST.

29.3 CLASSIFICATION AND DIAGNOSIS OF RHABDOMYOSARCOMA

This is not meant to be a comprehensive review. For full description refer to:

1. Soft Tissue Tumours, Enzinger & Weiss, 4th. Edition.
2. Diagnostic Soft Tissue Pathology, Markku Miettinen.
3. Pathology and Genetics. Tumours of Soft Tissue and Bone. WHO Classification of Tumours.

Soft tissue sarcomas constitute approximately 7% of malignant tumours in children, 15 years old or less at diagnosis. For the morphologic-based classification refer to WHO Histologic Typing of Soft Tissue Tumours.

29.4 RHABDOMYOSARCOMA

RMS is the most common STS in children, accounting for up to 60% of soft tissue tumours (data from Manchester Children's Tumour Registry) with an overall survival exceeding 65%. The clinicopathologic classification proposed by the Intergroup Rhabdomyosarcoma Study is currently used:

1. Superior Prognosis
   - A. Botryoid Embryonal RMS
   - B. Spindle cell RMS
2. Intermediate Prognosis
   - Embryonal RMS
3. Poor Prognosis
   - Alveolar RMS including
   - Solid Variant RMS

Embryonal rhabdomyosarcoma and its subtypes, botryoid and spindle cell, occur most often in the head and neck region, genitourinary tract and body cavities, and they have an intermediate to highly favourable prognosis. Alveolar rhabdomyosarcoma, including the solid variant, has a predilection for the extremities and a poor prognosis. Prognosis is determined by histological classification, stage and risk group, age and site of origin.

29.4.1.1 BOTRYOID EMBRYONAL RMS

This tumour is characterised macroscopically by its polypoid (grape-like) growth. Most are found in mucosa-lined hollow regions such as the nasal cavity, vagina and urinary bladder.

The consensus criterion for the diagnosis of Botryoid Embryonal RMS is the demonstration of a **cambium layer** beneath an **intact epithelium**, in at least one microscopic field – irrespective of the gross description, and therefore supersedes the gross demonstration of a 'grape-like' tumour. The degree of differentiation of rhabdomyoblasts may vary from slight to well differentiated

29.4.1.2 SPINDLE-CELL RMS

Spindle cell RMS is a rare subtype of RMS accounting for approximately 4.4% of RMS (data from the German-Italian Cooperative STS Study Group). This tumour is commonly seen in the paratesticular region, followed by the head and neck region, but can occur in other sites. Grossly the tumour is firm and well circumscribed but not encapsulated. The cut surface shows a nodular pattern often with a whorled appearance. Histologically the tumour is composed almost exclusively of spindle cells with cigar-shaped nuclei and prominent nucleoli. At least 80% of the tumour should consist of spindle cells for a tumour to warrant a diagnosis of spindle cell RMS. Some tumours are rich in collagen and have a storiform or whorled pattern, whereas the more cellular and collagen-poor tumours have a fascicular pattern.

Please note this diagnosis should not be made on a trucut biopsy as the sample may not be representative.

29.4.1.3 EMBRYONAL RMS

Embryonal RMS form poorly circumscribed, fleshy, pale masses that may show areas of haemorrhage, necrosis and even cyst formation.

These tumours have a variable pattern ranging from poorly differentiated tumours to well differentiated neoplasms. There are a number of features common to all these tumours:

a. a myxoid stroma
b. a mixture of small cells with hyperchromatin-rich or spindle shaped cells and other cells showing variable degrees of rhabdomyoblastic differentiation.
c. Variable degree of cellularity with dense areas usually around vessels alternating with loose hypocellular myxoid areas.

Note – foci of immature cartilage can be seen in some Embryonal RMS

Two differential diagnoses that can cause problems:

Fetal Rhabdomyoma
Pseudosarcomatous Myofibroblastic Tumour

29.4.1.4 ALVEOLAR RMS

Alveolar RMS is a rapidly growing, soft-tissue tumour with a fleshy, grey tan appearance. ARMS displays a nesting alveolar or solid pattern of cells in a fibrous stroma. The cells have monotonous, round to oval nuclei and inconspicuous nucleoli, but some can have prominent nucleoli. Multi-nucleated tumour cells with eosinophilic cytoplasm and nuclei arranged in a 'wreath-like' fashion are seen in the alveolar structures and can be helpful in the diagnosis of alveolar RMS especially in small biopsies.

The characteristic "alveolar" pattern is well recognised. In MMT'95 the diagnosis of Alveolar RMS was made even if the tumour shows **focal** alveolar **histology**. However, there still remains a degree of discordance in the diagnosis and definition of Solid Variant Alveolar RMS. At present there does not appear to be a different prognosis for Solid versus Classic Alveolar RMS.

Definition of Solid Variant RMS:

A poorly differentiated, cellular tumour composed of sheets of cells with no fibrous septa or may have thin, fibrous septa or fibrovascular septa running through the tumour, but lacking well defined alveolar spaces. Reticulin staining can be helpful in highlighting this sub-type.

Some alveolar RMS present with bone marrow infiltration and the only material available for diagnosis is a trephine biopsy. The same criteria for making the diagnosis apply.

29.4.1.5 RMS N.O.S. – subtype cannot be determined.

Please note, RMS N.O.S. (not otherwise specified) is not a subtype; it indicates that a diagnosis of RMS can be made but no further subtyping is possible. This usually arises when the biopsy is very small, sometimes with crushing artifact; it is only possible for the histopathologist to make a diagnosis of RMS. When clinically feasible, a re-biopsy is indicated to ensure subtyping and molecular characterization.

When subtyping is not possible, as a pragmatic decision and to avoid possible undertreatment patients the risk group will be decided as per Alveolar RMS.

29.4.1.6 Undifferentiated Soft tissue sarcoma

These are a rare group of tumours associated with a poor prognosis similar to alveolar rhabdomyosarcoma.
The histologic appearance is that of a high grade, cellular tumour with no specific differentiation by light microscopy, immunohistochemistry or electron microscopy. They usually express Vimentin.
Patients with undifferentiated soft tissue sarcoma will be treated according to this protocol with the same strategy as unfavourable RMS. These patients are not elegible to the randomised trial and will receive chemotherapy according to Arm A (9 cycles of IVA) if categorized in the High Risk Group

Be aware that undifferentiated (embryonal) sarcoma of the liver is not part of this entity.

29.4.1.7 Ectomesenchymoma

These are rare tumours occurring most commonly in young male patients, Histologically this tumour combines a rhabdomyosarcoma (embryonal, spindle cell or alveolar) with variable neurons or neuroblasts. Immunohistochemistry is important as the neural component may be focal and scarce. Positive immunostaining with S100, Synaptophysin, Neurofilament, Glial Fibrillary Acidic Protein or Protein Gene Product 9.5 is seen.
Patients with ectomesenchymoma will be treated according to this protocol with the same strategy as unfavourable RMS. These patients are not elegible to the randomised trial and will receive chemotherapy according to Arm A (9 cycles of IVA) if categorized in the High Risk Group.

29.5 IMMUNOHISTOCHEMISTRY

It is recommended that a panel of antibodies be used for the diagnosis of RMS:

- Vimentin	- Cam5.2	- CD3
- Desmin	- S100	- CD79a
- Sarcomeric Actin	- EMA	- Mic2**
- MYF4 or MyoD1 *	- LCA (CD45)	- Fli-1

* MYF4 or MyoD1: Nuclear positivity. % of tumour cells positive is higher in Alveolar>Embryonal
**Mic2: Although some RMSs demonstrate immunopositivity to Mic2, it is often weakly granular and intra-cytoplasmic, as opposed to the distinct plasma membrane staining seen in extra-osseous Ewing's/peripheral primitive neuroectodermal tumour. Mic2 immunostain should always be done as part of a panel of antibodies that includes specific myogenic markers.

Immunostaining with monoclonal antibodies against the intranuclear myogenic transcription factors Myogenin (MYF4)) and MyoD1 is recommended for all RMS subtypes. These are excellent markers showing high sensitivity and specificity. Myogenin seems to give more consistent results.
Also note that a small % of RMS can show focal positivity with Cam5.2 (cytokeratin).

29.6 HANDLING OF SPECIMENS

The type of surgical procedure influences the handling of the specimen and the extent of information that can be gained from its pathological examination. **Important - Please note, specimens should be received fresh in the laboratory. It is important that the surgeon/oncologist liaises with the pathologist to ensure that specimens can be received fresh in the laboratory.**

Biopsy - Open biopsy is recommended to ensure sufficient material is available for:

1. Diagnosis
2. molecular characterisation/research (see schematic diagram)

Resected specimens (Read surgical guidelines for full definition)

Primary resection: example orchidectomy for paratesticular tumours.

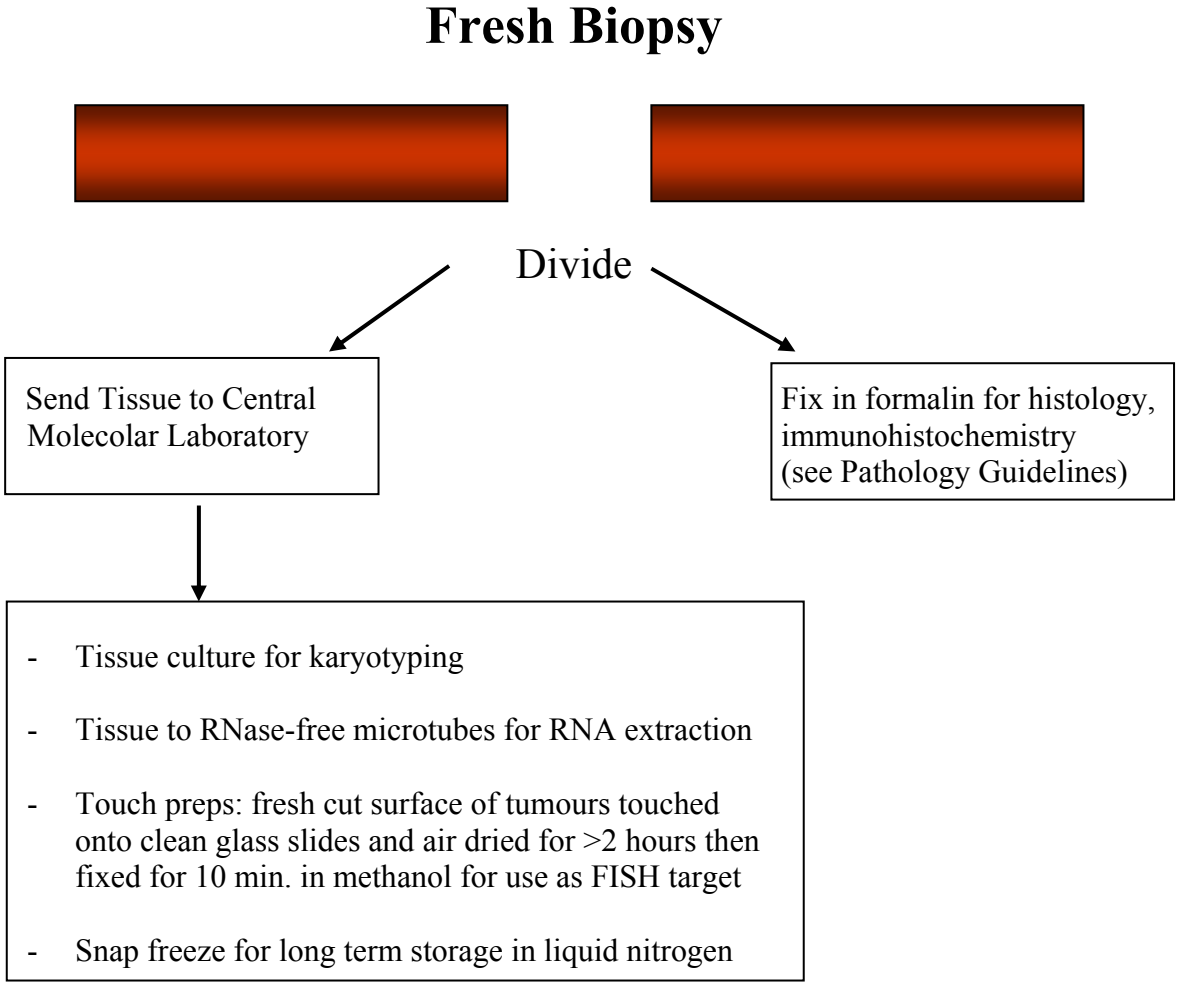
Primary re-operation: to achieve resection in patients with microscopic disease before other therapy.

Secondary operation (post chemotherapy): to achieve complete resection of a residual mass after chemotherapy e.g. bladder/prostate.

All primary and post-chemotherapy resection specimens need margins to be evaluated by the pathologist.

1. Surface of specimen should be inked before incision.
2. Specimen should be weighed and measured (in 3 dimensions).
3. Orientation of specimen is important – this may need to be done with the surgeon. The distance of tumour from the minimum nearest resection margin is important. In resected specimens, tumour depth e.g. dermal, subcutaneous, subfascial, intramuscular, needs to be specified macroscopically and microscopically.
4. Ideally the specimen should be photographed, including the cut surface, and a block guide prepared.
5. At least a block per centimetre of greatest tumour diameter needs to be sampled. However, it is strongly recommended that, where feasible, the entire specimen should be processed to ensure adequacy of excision and for accurate sub-typing of the RMS, for example in mixed embryonal/alveolar RMS.
6. The cut surface(s) should be examined and the pathologist should sample as above as well as taking blocks from areas which look macroscopically different in consistency or texture from other areas, in particular, take note of nodularity and sample.
7. Document macroscopic % of necrosis – sample areas of necrosis.
8. The pathologist should assess what tissue has been kept for molecular diagnostics/research. This can be done in one of two ways, either A – do a frozen section from the cut surface to assess i) tumour is present and ii) tumour is not necrotic, or B – a paraffin section, identified as representative section of tissue sent for molecular diagnostics/research can be taken and assessed as per frozen section.
9. Lymph nodes - please note – site of lymph nodes sampled should be documented as this is important in staging. All lymph nodes received by the pathologist should be examined. The entire lymph node or lymph nodes should be processed to ensure accurate assessment. Multiple levels need to be examined to exclude micro metastases.
10. Molecular characterisation (see schematic diagram).

29.6.1 Biopsy



NB: When handling small biopsies it may be important to prioritise type of biological study to be undertaken. Although we would recommend taking material for RTPCR and FISH, the pathologist should liaise with the central molecular laboratory that the material is being sent to.

In some national group's pathology and biology labs may be organized differently than in other countries and this may influence the procedures for optimizing biological studies and/or collection and storage of specimens

NB: The pathologist needs to document what tissue has been centralized for molecular characterization/research. The pathologist should be informed by the oncologist if consent has been obtained for storage of material for research. **It is strongly recommend that each centre has a system set up whereby the pathologist is informed in writing that consent has been given. It is up to individual centers to ensure that this is taking place. It is also strongly recommend that consent is obtained prospectively and not retrospectively.**

In most cases the pathologists will receive biopsy material. It is important that such specimens are received fresh and promptly in the laboratory and handled only by pathologists who will decide on how the specimen can be divided. Please note treatment depends on good histological diagnosis and therefore this should not be compromised for molecular studies. This, however, is at the discretion of the local pathologists.

29.6.2 Resected Specimens

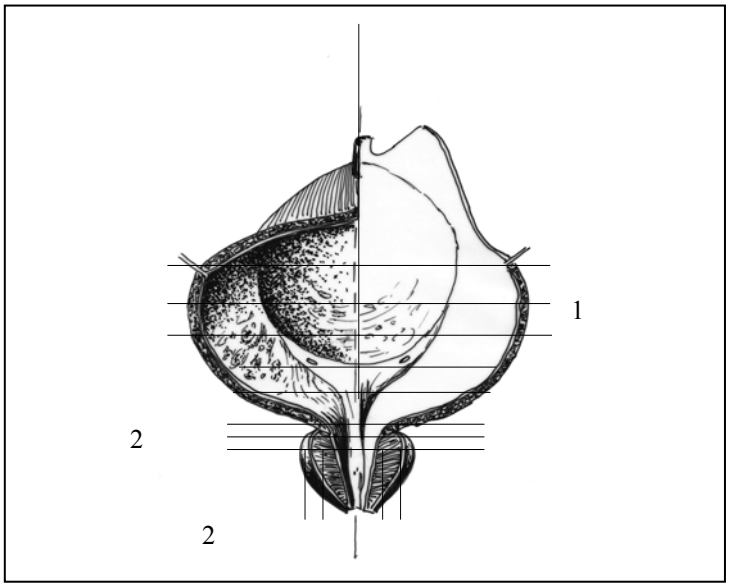
- The surface of the specimens should be inked before opening/bisecting.

- Tumour for molecular characterization/research/storage should be taken ensuring that the margins are not affected by this procedure.

- Same protocol should be followed as for open biopsy.

- In resected specimens, photographs and documentation of blocks taken (block guide) is necessary. The following are examples:

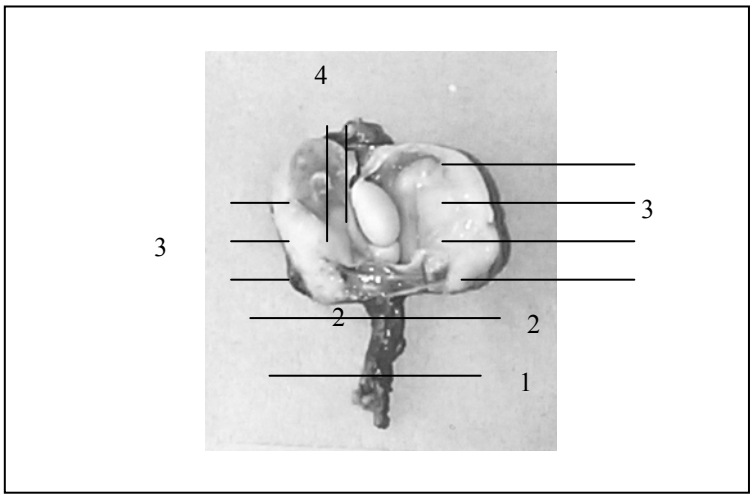
Fig. 1 Bladder/prostate



Procedure:
- Paint the whole external surface of bladder and prostate, if present, with India ink.
- Follow your preferred procedure to open the bladder; we recommend to open it through the anterior wall with a Y-shaped cut.
- Fix overnight in formalin.

Sampling:
1. Tumour: include most of it.
2. Bladder neck and prostate: include all with cuts as shown in figure 1.
3. Bladder wall: anterior and posterior wall, at least two sections each, if not involved by tumour.
4. Urethral orifices.
5. Perivisceral lymph nodes, if present.
6. Any abnormal area.

Fig. 2 Orchidectomy for paratesticular RMS



Procedure:
1. Ink surface of specimen.
2. Cut specimen sagitally while it is in the fresh state and put in formalin.
3. Take photographs of specimen and use for block guide.

Description:
Weight and dimension of tumour.
Extent of tumour involvement.
Length of spermatic cord.
Features of tumour, in particular presence of nodularity, haemorrhage and necrosis.

Sections for histology:
1. Spermatic cord and surrounding soft tissue at time of resection - one cross section.
2. Spermatic cord and surrounding soft tissue at about 1 cm from testicle - one cross section.
3. Tumour – at least one section for each centimeter. The sections should include the tunica albuginea. Always take sections from hemorrhagic and necrotic areas of tumour as well as from solid or fleshy areas. In addition, any nodules or vague nodularity should be sampled. Each block should be identified separately and linked to photograph.
4. Uninvolved testicle – at least two sections.
5. Epididymis – one section, if identified.

29.6.3 Bone Marrow Trephine Biopsy

1. Should be fixed in buffered formalin and decalcified according to the laboratory protocol.

2. Multiple levels (H&E stain) should be examined to exclude metastatic RMS. Reticulin stain is helpful in highlighting small foci of tumour.

3. It is also important that when cutting levels intermediate sections are kept for immunostaining to avoid cutting out of micrometastases.

4. Routinely stain for Desmin, MYF4 and MyoD1.

5. Please note: in cases where bone marrow aspirates/peripheral blood is sent for the detection of MRD, then the corresponding trephine biopsy needs to be sent for central review.

29.7 THE PATHOLOGY REPORT

The following need to be included:

Macroscopic:

Specimen type:
Biopsy – excision or trucut – please state.
Primary resection
Primary re-operation
Secondary operation (post chemotherapy).

Specimen site:
Head/neck
Bladder/prostate
Genitourinary (not bladder/prostate)
Cranial
Extremity
Orbit
Parameningeal
Other – specify (include trunk, retroperitoneum, etc.).
Not specified.

Laterality (as appropriate)

Tumour size:
Three dimensions – specify maximum diameter.

Microscopic:

Histologic type:

Embryonal – botryoid
Embryonal – spindle cell
Embryonal – not otherwise specified
Alveolar - classic
Alveolar – solid variant
Mixed alveolar /embryonal

Rhabdomyosarcoma NOS – subtype cannot be determined.
Please note, RMS – NOS is not a subtype; it indicates that a diagnosis of RMS can be made but no further subtyping is possible. This usually arises when the biopsy is very small, sometimes with crushing artifact, it is only possible for the histopathologist to make a diagnosis of RMS. When clinically feasible, a re-biopsy is indicated to ensure subtyping and molecular characterization.

Anaplasia:

Absent
Focal
Diffuse
Indeterminate

Necrosis:

Absent
Present
Extent %

Mitotic rate:

(x 40 objective).
-/10 high-power fields.

Regional lymph nodes:

None sampled.
No regional lymph node metastases.
Regional lymph node metastases – specify:
Site of lymph node
Number examined.
Number involved.

Venous/lymphatic invasion:

Present
Absent
Cannot be assessed

Molecular characterization:
Please note: if molecular characterization has been undertaken, then this should either be included in the main body of the report or set out as a separate report. A copy of this report needs to be sent to the national coordinator together with the copy of the histology report and form.

Post-chemotherapy specimens:

Same procedure as above. It is important to specify the following:

- % of necrosis
- % of fibrosis
- presence of rhabdomyoblasts - % of tumour cells
- Presence of anaplasia

29.8 MATERIAL TO BE SENT TO NATIONAL COORDINATORS.

1. In the case of **trucut biopsies**, both primary and post-chemotherapy, **1 H&E** and **15uss** (or the loan of the block).

2. In the case of **open biopsies/resected specimens**, including post-chemotherapy specimens – **1 H&E** from each block, and at least **20 uss** from representative block(s) (or the loan of the blocks). In the case of specimens in which there are focal areas of alveolar histology it is important that H&E and uss from these areas are sent to the local co-coordinator.

3. The uss should be on coated slides to be used for immunohistochemistry.

4. It is important that material from primary biopsy/resection and post-chemotherapy biopsy/resection and biopsy/resection of metastases is sent for review by the local co-ordinator. If bone marrow aspirate/peripheral blood is sent for the detection of Minimal Residual Disease (MRD), then the corresponding trephine biopsy (H&E + 5 uss) needs to be sent for central review.

5. If in the case of a very small biopsy there is not sufficient material left in the block, please send **1 H&E** to be kept by the local coordinator and the original H&E and immunohistochemistry slides, which will be returned.

6. It is understandable that these requests create more work for the pathologist and laboratory staff. Therefore, it is possible to send blocks to the local coordinator. These will be returned.

7. The local pathologist report and the form need to be sent with the slides.

8. The histological subtyping of RMS is important for patient stratification and management. The national coordinators and E*p*SSG panel of pathologists are offering real time review.

9. The slides/block and forms should be sent directly to the national coordinators.

NB: It is very important that the results of the molecular characterisation are collected prospectively. Each oncology centre/pathology lab. should ensure that, if this cannot be carried out in their centre/lab., arrangements should be made with other laboratories to ensure that, whenever possible, molecular diagnostics are carried out.

29.9 PATHOLOGY STUDIES

Anaplastic RMS

A recent study[5] based on IRS I-III patients, looking at the significance of anaplasia, showed that the presence of diffuse anaplasia was of prognostic significance.

Definition of Anaplasia:
The presence of anaplasia needs to be documented. Anaplasia is diagnosed if RMS (both embryonal and alveolar) contains cells with large, lobulated hyperchromatic nuclei (at least 3 times the size of neighbouring nuclei) and atypical (multipolar) mitoses. Furthermore, it is important to document whether these cells are focal or diffuse.

Current study: - The presence of anaplasia will not be used for stratification of patients. However, in this study we will be prospectively assessing the incidence of anaplasia as well as the distribution of the anaplastic cells in both biopsies and resected specimens.

Presence of maturation
In MMT'95, the presence of maturation was defined as the presence of 10% or more of tumour cells showing rhabdomyoblastic differentiation on haematoxylin and eosin stained sections. We will continue to assess the presence of maturation prospectively in E*p*SSG RMS2005.

Tissue Microarrays
As part of the collection of biological specimens, it is intended that the pathology coordinators for this European study of rhabdomyosarcomas also coordinate collection of blocks for the preparation of tissue microarrays. This will only be undertaken if:
1. there is consent for research
2. consent from the local pathologists
3. process does not compromise any future diagnostic process.

The national pathology coordinators will supervise the process.

29.10 REFERENCES - PATHOLOGY

1. Newton WA Jr, Gehan EA, Webber BL, et.al. Classification of rhabdomyosarcomas and related sarcomas. Pathologic aspects and proposal for a new classification – an Intergroup Rhabdomyosarcoma Study. Cancer 1995;76:1073-85.
2. Qualman SJ, Coffin CM, Newton WA, et.al. Intergroup Rhabdomyosarcoma Study: update for pathologists. Pediatr.Dev.Pathol. 1998;1:550-61.
3. Cavazzana AO, Schmidt D, Ninfo V et al. Spindle cell rhabdomyosarcoma. Am.J.Surg.Pathol.16:229, 1992.
4. Leuschner I, Newton WA Jr, Schmidt D, et.al. Spindle cell variants of embryonal rhabdomyosarcoma in the paratesticular region, a report of the Intergroup Rhabdomyosarcoma Study. Am.J.Surg.Pathol. 1993;17:221-230.
5. Kodet R, Newton WA Jr, Hamoudi A, Asmar L, Jacobs DL, Maurer H. Childhood rhabdomyosarcoma with anaplastic (pleomorphic) features, a report of the Intergroup Rhabdomyosarcoma Study. Am.J.Surg.Pathol. 1993;17:443-453.
6. Coffin C. The new international rhabdomyosarcoma classification, its progenitors and considerations beyond morphology. Adv.Anat.Pathol.1997;4:1-16.

30. Biological aspects

(see paragraph 29.6.1 for tissue handling)

General considerations.

The knowledge of biological phenomena involved in solid tumours is becoming increasingly relevant for the understanding of the behaviour of a variety of cancers. This, together with the availability of recent powerful technologies and new reagents for cellular and molecular biology studies, makes the field of sarcoma biology particularly attractive and challenging.
Recent molecular studies have contributed to an expanding list of genetic abnormalities in paediatric solid tumours, including chromosomal translocations and inversions, amplification of proto onco-genes and gene-deregulation.
The group of malignancies known as "small round cell tumours" of childhood are still a diagnostic problem due to the relative lack of differentiation in these tumours. Traditionally included in this group is the alveolar (aRMS) and embryonal (eRMS) rhabdomyosarcoma and the Ewing's sarcoma family, including PNET. Other entities also entering this differential diagnosis include intra-abdominal desmoplastic small round cell tumour (DSRCT) and, among fibrous or spindle cell malignancies, synovial sarcoma (SS) and congenital infantile fibrosarcoma (CIFS).
Cytogenetic studies of several childhood sarcomas have identified reciprocal chromosomal translocations which correlate with specific tumour types. Molecular cloning of the translocation breakpoints have identified fusions between genes located at the breakpoints of each partner chromosome and which result in the expression of chimeric oncoproteins.
From a clinical perspective, some of the genetic abnormalities represent tumour associated markers that can be used to confirm the histological diagnosis or to assess biological characteristics that may have clinical impact. Furthermore, they can be used as tumour markers to detect minimal dissemination of disease with a much higher sensitivity than standard histopathological approaches.

Common molecular targets in paediatric sarcomas

Several RT-PCR protocols were recently established to specifically detect transcripts that can be used for the identification of paediatric sarcomas. Among others, PAX-FKHR transcripts characterize alveolar rhabdomyosarcoma; EWS-FLI1 and EWS-ERG are expressed in the Ewing's family of tumours; ETV6-NTRK3 in congenital infantile fibrosarcoma; EWS-WT1 in desmoplastic sarcoma, whereas SYT-SSX1 and SYT-SSX2 are found in synovial sarcoma.
Other transcripts may be useful in the detection of tumour cells: MyoD1 and Myogenin transcripts are present in the vast majority of RMS, independently of the histological subtype, and they can be used in the study of minimal bone-marrow (BM) infiltration. New molecular markers may be identified in the future that could have clinical applications

Role of biological studies in paediatric sarcomas

The new clinical trials of the European paediatric Soft tissue sarcoma Study Group (E*p*SSG) represent an unique opportunity to conduct prospective clinical and biological studies in the context of uniform diagnostic and therapeutic strategies. Moreover, the relatively large patient accrual in reasonable time periods, would give biologists and clinicians the possibility of translating into the clinical setting any relevant findings that may emerge from collaborative studies.
Thus, a great effort is warranted by all the national participating groups and each clinical Institution in collecting biological samples to conduct selected and potentially relevant biological studies.

A Biology Panel has been created in which representatives from each national groups should participate and collaborate both in identifying specific priorities and methods to make the collaboration most fruitful and translatable into clinical relevant information as well as in collecting biological samples for further studies.

Cytogenetics

Although characteristic genetic abnormalities have been reported in specific types of sarcomas, in some cases no specific genetic tumour marker can be identified. For this reason cytogenetic analysis should be performed in any solid tumour and results should be collected prospectively: this will allow us to learn about yet unknown genetic alterations that may be associated to specific tumours or subgroups of patients and to identify recurrent complex alterations that cannot be determined by molecular methods. Cytogenetic studies are only possible on fresh tumour tissue.

FISH

Fluorescent-in-situ hybridisation is a rather recent technique that, making use of specific labelled DNA fragments, can detect genetic abnormalities both with regard to gene/chromosome structure and number. By this technique specific chromosomal translocations, including reciprocal translocations of the most common paediatric sarcomas, can be identified. Amplification or loss of genetic material can also be determined. Similarly to cytogenetics, fresh tumour tissue or cells are the optimal starting material for the assay.

Reverse transcriptase polymerase chain reaction for chimeric transcripts

Cytogenetic studies of childhood sarcomas have identified chromosomal translocations which are correlated with specific tumour types. These genetic abnormalities give rise to fusion genes that are transcribed into specific chimeric RNA that can be revealed by Reverse transcriptase polymerase chain reaction (RT-PCR). Often chimeric transcripts represent tumour associated markers that can be used as a diagnostic tool. Identical fusion transcripts can be found in specific subgroups of tumours with the similar histologic appearance or, alternatively, identical tumours can harbour different chimeric genes. The prognostic implication of the presence or absence of specific reciprocal translocations are not known.

In addition, not only the presence of a genetic abnormalities may be of relevance for the biology of a cell, but the level of expression may be important as well. Quantitative PCR (Real-time PCR) is a technique that allows not only the identification of specific genetic characteristics, but also can determine their level of expression.

Other molecular markers of disease

Tumour cells may possess entirely new genetic markers, such as fusion genes, but they may also express genes that are silent in normal cells. Moreover, genes may represent a tool to identify lineage specific characteristics that may be relevant to identify tumour cells in the context of cells of different origin/lineage. From this viewpoint the expression of that specific gene represents a tumour marker. This is the case for MyoD1 and Myogenin which are expressed in cells of skeletal muscle lineage.

30.1 BIOLOGICAL CHARACTERIZATION OF RHABDOMYOSARCOMA

Among the soft tissue sarcomas of childhood, RMS represents one of the best characterised malignancies from the biological point of view. Although the progress in this field has elucidated relevant biological features and mechanisms in RMS, not many results have been achieved in terms of biological research that can potentially be translated into the clinical setting. This is the case for the group of RMS as a whole, but also for selected subgroups of this diseases, such as the aRMS which are still characterised by a poor prognosis.

In particular, despite some reports in the literature suggesting that aRMS may differ biologically and prognostically, based on the presence or absence of the reciprocal chromosomal translocation t(2;13)(q35;q14) or its rarer variant t(1;13)(p36;q14), there is a lack of prospective studies conducted in the context of homogeneous treatment protocols that may give a clear insight in this issue. Furthermore, although RMS, specially aRMS, have a proneness to metastasise or are metastatic at diagnosis, very little information is available on the prevalence and potential clinical impact of circulating tumour cells at diagnosis or of microdissemination of rhabdomyoblasts to the bone marrow.

These and other reasons suggested to us the need to strongly pursue few and well defined translational biological studies, in an attempt to optimise the diagnostic and therapeutic approach to children with RMS.

Below are some of the major objectives that have been discussed and are to be implemented in a prospective biological study of RMS patients enrolled in this study. Any medical professional involved in the care of children with solid tumours should make every effort to achieve the biological specimen collection in order for the nations' laboratories to ensure that all tumours banked are well characterised. We are convinced (based on previous experience) that this goal is achievable, but only if we can establish a strong and coordinated interaction among oncologists, pathologists, surgeons and biologists who, at different points in time are involved in the management of these patients.

Aims of the Study

As it might be clear from the general introduction on the biological studies in paediatric STS and from the more specific considerations on RMS, within this E*p*SSG protocol for the diagnosis and treatment of RMS, we selected the following studies/activities to be conducted prospectively:

- Expression of tumour associated molecular markers
- Analysis of specific ARMS chromosomal translocations
- Prevalence and kinetics of minimal disseminated disease
- Cytogenetics and FISH
- Collection of biological specimens for further analysis

This may appear rather limited in terms of goals to be achieved within the current clinical trial, but we have tried to keep the approach as simple as possible, with the further aim of setting the basis for more ambitious biological studies for the next generation of collaborative trials.

Expression of tumour associated molecular markers.

We and others have suggested that selected markers, including *MyoD1* and *Myogenin*, are expressed by virtually all RMS. Although both markers can be determined at the protein level, there are some technical draw-backs that make the interpretation of immunohistochemical analysis rather difficult (and this is the case of MyoD1, especially). MyoD1 and Myogenin expression can be

studied at the RNA level as well as by reverse transcriptase polymerase reaction (RT-PCR). We and others have observed that, at least 95% of RMS are positive for MyoD1 and Myogenin transcript. Expression of MyoD1 and Myogenin will be studied in each suspect RMS, independently from its histological subtype.

Analysis of Alveolar RMS specific chromosomal translocations.

Several reports have described the reciprocal chromosomal translocations t(2;13)(q35;q14) or the less frequent t(1;13)(p36;q14) in aRMS. They give rise to the chimeric genes PAX3-FKHR and PAX7-FKHR, respectively, whose products possess transcriptional activity and are involved in the tumourigenesis of aRMS. Recent data suggests that, the translocation is not only characteristic of aRMS subtype, but may have prognostic significance. Although it needs to be further confirmed in a large prospective study and multivariate analysis, the presence of t(2;13) may be associated to a worse prognosis compared to the negative aRMS, whereas the t(1;13) positive aRMS might possess an intermediate outcome.
The translocations will be determined by RT-PCR, but whenever possible also by FISH analysis, and molecular findings would be compared to the standard cytogenetics results.

Prevalence and response kinetics of minimal disseminated disease.

One of the aims of the biological studies in this protocol would be the assessment of the prevalence of minimal disseminated disease (MDD) and the response kinetics of BM tumour infiltration during treatment.
Based on previous experience and in an attempt to study MDD in aRMS, as well as in eRMS, we elected MyoD1 and Myogenin as tumour associated markers that, if expressed in the tumour biopsy, should be evaluated in the BM and peripheral blood of each patient with RMS. At present we have concluded a pilot analysis of MyoD1 and Myogenin expression by RT-PCR and have established a method for the quantitative analysis of MDD by Real Time PCR.
This study will be extended to as many patients as possible, through the optimisation of specific protocols to this aim. Although the study of PAX-FKHR chimeric transcripts (by RT-PCR), in our preliminary experience, may be less sensitive than MyoD1, in case of positivity of a RMS for these genetic markers, they should also be assessed in the BM by RT-PCR.
Identical studies would be conducted on peripheral blood.

Cytogenetics and FISH.

Cytogenetics and FISH analysis are important assessments that should be performed in any malignancy. While FISH analysis would be possible in the great majority of the cases (on touch preps - see “handling of specimens” flow chart), cytogenetics needs a larger amount of “very good quality” viable tumour cells and might be more difficult to accomplish. *Every* effort though should be made to perform standard cytogenetics studies.

Collection of biological specimens for further analysis.

Willingly, a limited number of biological studies have been selected that realistically can and should be accomplished in a prospective manner during the course of this E*p*SSG-collaborative trial. Nevertheless, the biological characterisation of paediatric RMS should be conducted more

extensively and in greater depth. To this goal it is of foremost importance that tumour tissue and other biological specimens be collected and stored appropriately to make them available for future research. As an example, each participating Group and each single Institution should be aware that tumour genetic profiling by oligo or cDNA microarrays, is already pursued in selected Centres and will very likely become a more readily available and affordable technology for the biological studies of RMS. This though would imply that tumour tissue should be collected, carefully characterised and stored for this aim. Similarly, studies on the expression profile of proteins might be accomplished by the combination of different techniques as part of the proteomic approach. Lastly, the availability of serum from patients with RMS might allow future studies of soluble tumour related molecules that may be used as markers of disease, possibly related to prognosis.

Table 10 - Summary of sample collection for minimal disseminated and minimal residual disease studies

	Fresh tumour in medium	Bone marrow in NaCitrate 3 ml	Blood in NaCitrate 5-7 ml	Serum 2-3 ml
Diagnosis°	X*	X	X	X
Before cycle II		X**	X**	X**
Assessment of tumour response (after the initial three cycles of CT°°)		X**	X**	X**
In case of delayed surgery	X*	X**	X**	X**
End of treatment		X**	X**	X**

° Whenever possible, bone marrow aspirate and peripheral blood should be obtained prior to the initiation of any surgical approach to any tumour mass (to prevent a possible tumour cell dissemination caused by surgery)

*Fresh tumour tissue must be received in the laboratory at latest within 24 hours from surgery: viable cells are needed for cytogenetic analysis.

** Except at diagnosis any other BM or blood/serum examination should be performed only if positive at the previous assay or in case of a suspected relapse or based on other clinical conditions

BM, peripheral blood and serum should also be obtained in case of relapse in a patient with positive molecular markers (MyoD1, myogenin, PAX-FKHR transcripts). MDD should also be determined in case a relapsed patient would enter an intensified treatment program with autologous peripheral blood stem cell (PBSC) rescue. In this case an aliquot of PBSC harvest should also be tested.

Details on methods, protocols and technical questions will be discussed and decided within the Biological Committee of the Trial. Standards and quality controls for the biological studies will be set.
It is strongly recommend that biological studies be conducted in a single laboratory for each participating National Group.

31. Diagnostic problems

31.1 BONE MARROW INFILTRATION ON MOLECULAR BIOLOGY ONLY

Morphology: Bone marrow negative for RMS infiltration
Molecular biology: positive for RMS markers
The tumour should be considered as a localised RMS and treated accordingly, provided there are not signs of distant lesions in other organs.
In such cases it is strongly requested:
a) to send BM slides for urgent central review
b) to perform follow up bone marrow aspirate as summarised in table 10.

31.2 ALVEOLAR TRANSLOCATIONS FOUND IN EMBRYONAL RMS

Morphology: embryonal RMS
Molecular biology: positive for t(1;13) or (2;13) translocation.
The presence of a t(2;13) or t(1;13) translocation is strongly correlated with alveolar RMS. In a case where the local pathologist has made a diagnosis of embryonal RMS, but a translocation is identified, rapid central review of the case is mandatory. If the diagnosis of embryonal RMS is confirmed on central review, a local decision has to be made with regard to patient management taking into consideration the possibility of sampling issues in mixed tumours and clinical information (i.e. age and site of tumour). Moreover, it may also be appropriate that the molecular characterization be repeated. In these cases molecular characterization should also be undertaken in post-chemotherapy specimens if viable tumour is present.

As general recommendations these patients should be treated as alveolar RMS.

In such cases it is strongly requested:
a) to ask the local pathologist to carefully review the tumour material
b) to send slides for urgent central review
c) to register the case appropriately (a special items for such cases will be prepared in the database)

32. Statistical considerations and analysis

32.1 RANDOMISED TRIAL - PATIENTS IN HIGH RISK GROUP

Design of the trial

This study is a prospective phase III international, multi-institutional, non blinded double-randomised clinical trial.
The aims of the trial are to evaluate the addition of Doxorubicin to the standard therapy with Ifosfamide, Vincristine and Actinomycin (IVA) in paediatric patients with rhabdomyosarcoma in high risk group, as defined in chapter a 15 – *intensification question,* and the role of a maintenance therapy with Vinorelbine and Cyclofosfamide in the same category of patients who have achieved a complete remission at the end of first line treatment – *maintenance question.*
The expected accrual period is 5 years followed by a minimum follow up period of 3 years.
First and second randomisation are provided centrally, by a computer-based service (supplied by CINECA, Casalecchio ITALY) that is accessible via Internet, for all countries and Institutions. Access to the randomisation system is managed according to specific policies adopted by each country (both direct local site and mediated by national data centre access are possible). All eligibility criteria (see chapter 15) and requirements for randomization have to be fulfilled prior to the randomization process.
Randomization is stratified according to participating country and risk subgroup (E, F and G). To reduce possible imbalances in the number of treatment assignments, a randomised blocked design will be used.

End points

Intensification question

1. Primary end point for the intensification question is event free survival, measured as time from date of first randomisation up to an event. Event is defined as: death for all reasons, progression of a residual tumour, relapse following previous complete remission, appearance of a new tumour and switch for a second line chemotherapy in patients without good response. Patients without an event at the end of the study or lost to follow up will be censored at the date of last observation.
2. Secondary end points are:

- Overall survival, measured as time from date of first randomisation up to death for all reasons. Patients still alive at the end of the study or lost to follow up will be censored at the date of last observation.
- Progression free survival, measured as time from date of first randomisation up to tumour progression. Patients without a progression at the end of the study or lost to follow up will be censored at the date of last observation.
- response rate in according to classification criteria reported in chapter 19.
- toxicity according to NCI-CTC version 3 (see appendix A.7)

Maintenance question

1. Primary end point for the maintenance question is disease free survival, measured as time from date of second randomisation up to relapse or death. Patients still alive and without relapse at the end of the study or lost to follow up will be censored at the date of last observation.

2. Secondary end points are:
- overall survival, measured as time from date of second randomisation up to death for all reasons. Patients still alive at the end of the study or lost to follow up will be censored at the date of last observation.
- toxicity according to NCI-CTC version 3 (see appendix A7)

Population Analysis

All efficacy analysis will be carried out according to the intention to treat principle. It foresees that all randomised subjects, whether or not they received any study medication, will be analysed in the arm to which they were assigned.
Patients will also be analysed according to the treatment they actually received only for explorative purposes. This per-protocol population is defined as all subjects who fulfil all inclusion and exclusion criteria and who receive the planned doses of chemotherapy and radiotherapy according to protocol indications for dose delivery and modifications (i.e., patients who were eligible and who received treatment as planned).
Analysis of toxicity will be based on the safety population that consists of all the subjects who received at least one dose of chemotherapy analysed according to the actual treatment received.

Description of patient population

The number and percentage of patients included, completed, withdrawn and lost to follow-up will be summarised using descriptive statistics.
The patient population will be described by descriptive statistics as follows:
1. Demography Variables
    - Co-operative group and Country of provenience
    - Age (≤1 year, 1-10 years, ≥10 years)
    - Gender
2. Prognostic Factors
    - Pathology (favourable, unfavourable)
    - Post-surgical stage (IRS group)
    - Site of disease
    - Node stage
    - Size of the tumour (maximum diameter ≤ or > 5 cm)

Description of treatment exposure

The number of treatment cycles administered will be summarised using descriptive statistics. Treatment delays will be summarised using counts and percentages. The cumulative dose and actual dose intensity (mg/m^2/wk) and the relative dose intensity (actual dose/planned dose) of Doxorubicin and IVA regimen will be summarised using descriptive statistics (median, range).

Survival Analysis

Event Free Survival, Disease Free Survival, Progression Free Survival and Overall Survival in the two treatment arms (standard vs intensification and maintenance vs. control, respectively) will be plotted as a function of time using Kaplan-Meier product limit method. The two-sided log rank test will be used to compare the treatment arms on a significance level of 5%. Summary statistics (3-yr and 5-yr, EFS, DFS, PFS and OS) will be reported together with their 95% confidence interval.

In addition, the Cox regression model will be used to adjust the treatment comparison for possible prognostic factors, whenever all assumptions will be satisfied.
The p-value corresponding to the secondary questions will be regarded as explorative.

Safety evaluation and analysis

The safety evaluation will be based on the NCI-CTC Version 3 and will be displayed in summary tables according to category and grade (all grades, grade 3 and grade 4) for the worst grade documented. Tables will be generated on a per cycle and an overall basis. All comparisons will be performed using two-sided chi squared test on a significance level of 5%. Adjustments for multiplicity will not be made.

Sample size

On the basis of the accrual of the latest studies carried out by SIOP and ICG-CWS (MMT 95 and RMS-CWS 96) a minimum enrolment rate of 100-125 patients per year may be expected. Taking into account previous experience and that new therapeutic strategies may not be available in the near future, the accrual period could be prolonged for at least five years, so that the dimension of the study will be 600 patients roughly.
The 3-years EFS in the high risk group treated with the IVA regimen should be approximately 50%. The minimal difference that the study should be able to detect is an absolute increase in 3-yrs EFS from 50% to 60%, corresponding to a 26.3% relative reduction in event rates in the IVADo regimen (HR=0.737). In order to detect a difference of this magnitude, under the assumption of exponential distributed EFS, with an 80% power at the 5% significance level (2-sided), 343 events must be observed, and approximately 119 patients per year will have to be enrolled for 5 years, and followed for about 3 more years, taking into account a drop out rate of 5%.
Assuming an 80% of complete remission rate at the end of first period of treatment, 15% of refusal to second randomisation and 5% drop out rate during the recruitment period, 388 patients roughly will be available for maintenance comparison. This sample size allows to detect an absolute increase in 3-yr DFS from 55% to 67% corresponding to a relative reduction in relapse rate of 33% in the maintenance arm with 80% power and alpha 5% (two sided test). The patients, after the enrolment period of 5 years, should be followed for further 3 years until 200 relapses have occurred.
The sample sizes for the intensification and maintenance questions were calculated for a one-step design with nQuery Advisor 5.0 and adapted to a three-step group sequential design (two interim analyses plus the final analysis) (Jennison C., Turnbull BW. Group sequential methods with applications to clinical trials. Chapman & Hall / CRC (2000))

Interim Analysis and stopping rules

Two formal interim analyses will be performed after 1/3 and 2/3 the expected events occurred, unless the trial is stopped before to reject the hypothesis of no treatment difference.

Intensification question
With an accrual period of 5 years, a minimum follow up period of 3 years, an accrual rate of 119 patients per year, a 3-year EFS of 50% for the IVA regimen and 60% for the IVADo regimen, and a drop-out rate of 5%, a total number of 343 events is expected. Therefore, the first interim analysis for the intensification question will be conducted after 114 events and the second after 229 events. The trial will be terminated after an interim analysis if the main question is answered.
The O'Brien & Fleming stopping boundaries will be used to monitor the study.

Maintenance question
To answer the maintenance question, a total number of 200 relapses is needed. Therefore, the first interim analysis will be performed after 67 relapses and the second after 133 relapses. The trial will be terminated after an interim analysis if the hypothesis of no treatment difference is rejected. The O'Brien & Fleming stopping boundaries will be used to monitor the study.

Sample size amendment (*May 2012*)

The EpSSG RMS 2005 randomised trial planned to randomise about 600 patients in 6 years thus, considering that the study started in 2006, it should have been closed by the end of 2011. Six years from the start of the study, the randomisation rate of about 60 patients per year (364 randomised patients at the end of 2011), as well as clinical, ethical and administrative considerations, have induced the Independent Data Monitoring Committee to suggest prolonging the accrual period until 2013. The Trial Management Committee approved the amendment of the sample size during the 2012 EpSSG spring meeting.
With a total sample size of about 500 patients, the minimal difference that the study will be able to detect, with an 80% power, is about a 35% relative reduction in event rates in the IVADo regimen (HR=0.65), compared to the standard IVA regimen. In order to detect a difference of this magnitude, under the assumption of exponential distributed EFS, at the 5% significance level (2-sided), 169 events must be observed. The enrolment of about 500 patients over 8 years with a further follow-up period of 2 years should generate the required total number of events.
Since about 60% of patients randomised at first randomisation have been randomised at the second randomisation, a sample size of about 300 patients will be available for the comparison maintenance versus no maintenance. This sample size will allow a detection of a relative reduction in the relapse rate of 50% in the maintenance arm, with an 80% power testing at the 5% significance level (2-sided). Final analysis will be performed when 65 relapses have occurred.
The sample sizes for the intensification and maintenance questions were calculated for a one-step design with nQuery Advisor 6.0 and adapted to a two-step group sequential design (one interim analyses plus the final analysis) (Jennison C., Turnbull BW. Group sequential methods with applications to clinical trials. Chapman & Hall / CRC (2000))

Interim Analysis and stopping rules amendment (*May 2012*)

One formal interim analysis will be performed after half the expected events occurred.

Intensification question 2012
Therefore, the first interim analysis for the intensification question will be conducted after 85 events. The trial will be terminated after an interim analysis if the main question is answered.
The O'Brien & Fleming stopping boundaries will be used to monitor the study.

Maintenance question 2012
To answer the maintenance question, a total number of 65 relapses is needed. Therefore, the first interim analysis will be performed after 33 relapses. The trial will be terminated after the interim analysis if the hypothesis of no treatment difference is rejected. The O'Brien & Fleming stopping boundaries will be used to monitor the study.

33. Organisational and administrative issues

The E*p*SSG is an inter-group structure which represents an evolution of a well established situation in Europe. It is based on the already existing national and international organisations built with the efforts of the participants to CWS, STSC and SIOP MMT studies over many years.
The E*p*SSG takes into account the differences in the study management and regulations that may exist in the different European countries and co-operative Groups and try to harmonise them.

33.1 PARTICIPATING CENTRES

All clinical centres previously part of the SIOP, CWS or STSC Co-operative Group are expected to participate in the E*p*SSG study.
New clinical centres, whose national group does not take part as a whole, who wish to participate must demonstrate their ability to participate in the study.

All participating centres are expected to:
- confirm in writing the intention to participate before starting to recruit patients
- name a clinician who will be responsible for communication with the data office.
- obtain approval for the study from their local Research Ethical Committee
- obtain patient's/parents' written consent to inclusion into the randomised trial (if applicable), data processing and sending diagnostic material to reference institution
- register all patients with non-metastatic Rhabdomyosarcoma
- randomise all eligible patients for the duration of their participation in the study
- submit in a timely and accurate manner clinical data on paper to their reference Co-ordinating Centres or directly via a Remote Data Entry System
- provide diagnostic material for central pathology review, and for related biological studies

33.2 CO-OPERATIVE GROUPS AND CO-ORDINATING CENTRES

Each Co-operative Group will keep its existent Co-ordinating Centre.
All existing Co-ordinating Centres are expected to:
- promote the study within their group and obtain specific study commitment by the clinical centres
- distribute the protocol, the forms and all pertinent material to the participating centres within their Group
- manage the data collection and implement procedures for data quality control within their group
- be a referring Centre for the Clinicians from participating centres to address clinical questions
- collaborate with the E*p*SSG Co-ordinating Centre to update regularly the data

Other National Co-ordinating Centres may be added or created on purpose to support the work of E*p*SSG if reputed necessary.

33.3 E*p*SSG CO-ORDINATING CENTRE

The E*p*SSG Co-ordinating Centre is the trial unit in charge of harmonisation and co-ordination of the study related activity of each Group.
In detail it is expected to:
- co-ordinate the development of the common data base in co-operation with CINECA (Bologna, Italy) and the Co-ordinating Centres
- guarantee the functionality of the data base during the whole study period

- supervise the data collection and data quality to ensure the validity of interim and final analyses on the common data
- be a referring Centre for the Co-ordinating Centres to address technical and operative questions regarding the data management of the study
- be responsible for the statistical analysis within the trial at given time periods in collaboration with the panel of statisticians from individual groups
- update regularly the protocol committees on the ongoing trial

E*p*SSG Co-ordinating Centre contact details:

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33.4 PROTOCOL AND FORMS

One common protocol will be used by the three Groups and all participating Centres. The master protocol will be in English. Translations of the master protocol will be prepared if required by each Co-ordinating Centre.

Any amendments to the protocol must be agreed by all the participating Groups and notified in writing. Addenda may be added independently by any of the national groups to address local needs, provided they have no bearing on the essential aims of the international protocol and they have been previously discussed and approved by the protocol Committee.

Each Co-ordinating Centre will be responsible for distribution of protocols to the Institutions within their Group.

The latest version of the protocol with all the amendments will be accessible online via the E*p*SSG website to all the participating Investigators.

Identical data forms will be used by all co-operative groups. The master version will be in English and each Co-ordinating Centre is responsible for translating the document for the national Centres. Additional forms may be produced within each Co-operative group for data collection that are specific for that group and exceed the international data set.

33.5 DATA MANAGEMENT

Data flow

The E*p*SSG RMS trial will be managed via a web based system. It is expected that each Co-ordinating centre will utilise the Remote Data Entry system hosted at CINECA to perform the data management of the study.
Centers may enter directly the data into the electronic data base via Internet or may use the traditional paper based flow of data within their Co-ordinating Centre.
If paper based flow is chosen, forms returned from the treating Institutions will be stored at the respective Co-ordinating Centres for time periods conforming to national law.
On receipt of forms at each Co-ordinating Centre, common range and logical checks will be carried out on the data prior to entering into the web-based national database.
Errors noted in the national and/or master data base will be reported back to the Co-ordinating centre or to the institution of origin.
Standard Operating Procedures for the electronic data management will be agreed on and followed by the Co-ordinating Centres. These SOPS will be described in a specific document.

Patient Registration procedure

Patients with a diagnosis of localised RMS must be registered only after he/she and/or his/her legal guardian has consented to registration and data handling. Patients must be registered before treatment is started by the participating Institutions using the Remote Data Entry (RDE) system.
If the access to the RDE system is not possible for whatever reason a fax must be sent to the corresponding Co-ordinating Centre. The Co-ordinating Centre will register the patient using the RDE system.

Randomisation procedure

First randomisation:
Every patient with a diagnosis of localised RMS who fulfils the trial eligibility criteria must be randomised before treatment is started using the RDE system.

Second randomisation
Patients eligible to the first randomisation will also be eligible to the second randomisation if in CR after the completion of standard treatment (i.e. 9 cycles of chemotherapy+ surgery/radiotherapy).
Patients not eligible for the first randomisation because younger than 6 months at diagnosis are eligible to the second randomisation if older than 6 months at the end of standard treatment.
Patients achieving CR after second line treatment are still eligible to second randomisation.
Patients must be randomised within 8 weeks after the end of treatment.
The end of treatment is defined as the last day of the 9th chemotherapy cycle. However:

- if surgery is performed after the 9th chemotherapy cycle, the date of surgery will be considered;
- if radiotherapy is administered after 9 cycles of chemotherapy, the date of the end of RT will be considered. Since maintenance CT should be started within 8 weeks from the last day of the 9th CT cycle, it would be better to start the maintenance CT during irradiation.

If the access to the RDE system is not possible for whatever reason a fax must be sent to the corresponding Co-ordinating Centre. The Co-ordinating Centre will randomise the patient using the RDE system and will communicate the randomisation results to the treating Centre.

Access to data from E*p*SSG Central Database

The collected data will be available to all the research staff involved in the trial with different access profiles, in real time and with the possibility of multiple concurrent access, despite geographical location.
The Co-ordinating Centre of each group, for example, could have access to all data from its Clinical Centres; instead the principal investigator of each participating Centre may have access only to his centre's data.
Data relating to the present study must not be reported or published without prior consultation of the Protocol Committee.

Data analysis and monitoring

Reports on the study progress will be prepared twice yearly, describing accrual of the patients, group allocations, local therapy modalities and toxicity of the treatments given. This report will be circulated to the Principal Investigators. Data will be published as abstracts at each SIOP meeting if considered appropriate.
The Protocol Committee shall meet as appropriate to consider patient accrual, eligibility, treatment allocation and outcome and ensure a smooth conduct of the study.
Results of the interim analysis shall be reported to the International Data Monitoring Committee (IDMC) as scheduled by the protocol. The IDMC may recommend early stopping, continuation or extension of the study to the international study committee.

Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) composed of 3 international experts will be designated to monitor the progress of the study from an ethical and scientific point of view (names are reported in the EpSSG Administrative organization section)

The role of IDMC will be:

- to review the patient accrual and to be involved with all interim analysis according to the statistical plan. These interim analyses will remain confidential. On the basis of these analyses, the IDMC will recommend whether the study can continue, whether it has to be extended or changed or terminated prematurely.
- to monitor toxicity of all treatments but especially toxicity of the experimental arms and serious adverse events. Every 6 months a report of toxicity will be prepared by the E*p*SSG Co-ordinating centre and circulated among the participating national groups and to the IDMC. The IDMC will review these interim toxicity data and any relevant information will be forwarded to each co-operative group. Problems and patterns of major toxicity shall be analysed to prevent major toxicity endangering the conduct of the study.
- to examine other pertinent trials. The IDMC will review reports of related studies performed by other groups or organisations to determine whether such information materially affects the aims or preliminary findings of the trial. In case that interim analyses or the results of other studies imply that the study questions have been answered, the IDMC has to decide in conjunction with the Protocol Committee about the continuation of the current study.
- to review any major modification to the study proposed by the Protocol Committee prior to its implementation.

Protocol modification

Any modification 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. Such amendment will be agreed upon by the Protocol Committee and reviewed prior to implementation.
A formal approval by the Ethics Committees for minor administrative changes of the protocol which have no impact on the conduction of the study will not be required.

33.6 INSURANCE

The study should be covered by a specific insurance against damage ensuing from the organisation of the trial, if requirements are stated in the national laws.
These aspects will be dealt with on a country basis.

33.7 FINANCING

Each Co-operative Group and Co-ordinating Centre will provide its own financing. E*p*SSG will not pay for the expenses sustained by the clinicians involved in the study.
The E*p*SSG Co-ordinating Data Centre and the Remote Data Entry system (provided by CINECA) will be supported by a Research Grant from the Fondazione Città della Speranza ONLUS, via Pasubio 17 - 36034 Malo (Vicenza), www.cittadellasperanza.org**.**

33.8 PUBLICATION POLICY

Participating centres or national Groups may publish details of their own cases but will agree to allow the committee the exclusive right to publish the results of the Protocol EpSSG RMS2005, in part or in total.
Similarly each Cooperative Group forming the E*p*SSG agrees that the results of the Protocol RMS2005 should not be published separately.
All publications using data from the E*p*SSG central data bank are considered to be official E*p*SSG papers and these should be agreed by the main author of the project with the E*p*SSG Protocol RMS 2005 Committee before starting the work, so that authorship can be discussed within this group prior to preparation of any publication.
All such publications will be presented on behalf of the E*p*SSG and will acknowledge the contribution of the participating clinicians.
All persons designated as authors should qualify for authorship. Every other author should have participated sufficiently in the work to take public responsibility for the content.
All manuscripts and abstracts (including abstracts for presentation at meetings) and other documents that contain data from the central E*p*SSG data bank must be submitted to the Protocol Committee at least 21 days prior to the deadline for conference submission.
All abstracts must have written approval from the executive committee prior to final submission.

39. Ethical issues

The protocol will be submitted, before patients enrolment, to the Ethics Committee of each participating Centre for review and approval according to in force law.

39.1 INFORMED CONSENT

The patient's and/or parent's written consent to participate in the study must be obtained after a full explanation has been given of the treatment options including the conventional and generally accepted methods of treatment and the manner of treatment allocation.
If the patient is a minor, the treatment must be explained to and consent received from his/her guardian. Additionally the child should receive an explanation as to his/her means of understanding and should give consent as well, if he/she is able to do so. Enough time and the opportunity to discuss participation before the decision for and start of treatment have to be given. The right of a patient to refuse to participate without giving reasons must be respected.
Consent for participation in the study, for data management and biology material handling will be also obtained.
The patient must remain free to withdraw at any time from the study and the protocol treatment or to withdraw his/her data from the study without giving reasons and without prejudicing his/her further treatment.
All patients and/or their parents must give written consent to inclusion into the trial, data processing and – if applicable – to sending diagnostic material to reference institutions, which in all participating countries has to conform to the national data protection legislation.
Examples of Information sheet/Consent Form are provided in Appendix A.11.
Administrative documents, consent forms and copies of the study documentation of a study patient have to be kept according to set archival terms.

39.2 DECLARATION OF HELSINKI

The investigator agrees, by signing the protocol, to adhere to the principles of Good Clinical Practice. A copy of the Declaration of Helsinki in its latest form is provided in Appendix A10.

39.3 CONFIDENTIALITY/SECURITY

A high standard level of data confidentiality and security should be guaranteed throughout the study.
In detail:

- The International common data base will not contain individual personal information
- Patients will be identified by a code, not by full name
- All traffic with the server will be encrypted.
- Each user at each site will have a personal User ID and Password.

The system will ensure:

- appropriate and regular backup on electronic media of all data, to permit restoration in case of loss or damage of the data base,
- operation tracking log (for each user: registration of any operation),
- electronic data audit trails (creation of a data base of original entries/modifications with identification of date, time, source and user identity),
- disaster recovery procedures.

40. Appendix

- A1. TNM Classification and Grouping
- A2. IRS Grouping
- A3. pTNM and Grouping System
- A4. Definition of sites
- A5. Regional lymph Nodes definition
- A6. Radiology guidelines
- A7. Toxicity grading
- A8. Veno-Occlusive Disease of the liver - Grading
- A9. Nephrotoxicity Grading
- A10. Declaration of Helsinki
- A11. Information sheets/Consent Form
    - **A. treatment according to the "observational study" (that includes low, standard and very high risk strategy):**
      A1 - INFORMATION SHEET FOR PARENTS
      A2 – INFORMATION SHEET FOR OLDER PATIENTS
      A3 - INFORMATION SHEET FOR YOUNGER PATIENTS
    - **B. randomisation into high risk strategy.**
      B1 - ADDITIONAL INFORMATION SHEET FOR PARENTS OF PATIENTS WITH HIGH RISK TUMOURS
      B2 - ADDITIONAL INFORMATION SHEET FOR older PATIENTS WITH HIGH RISK TUMOURS
      B3 - INFORMATION SHEET FOR YOUNGER CHILDREN

A.1 TNM CLASSIFICATION

Pre treatment TNM

Tumour:

T0: No evidence of tumour

T1: Tumour confined to organ or tissue of origin
- T1a: Tumour ≤ 5 cm in greatest dimension
- T1b: Tumour > 5 cm in greatest dimension

T2: Tumour not confined to organ or tissue of origin
- T2a: Tumour ≤ 5 cm in greatest dimension
- T2b: Tumour > 5 cm in greatest dimension

TX: No information on size and tumour invasiveness

Lymph nodes:

N0: No evidence of lymph node involvement

N1: Evidence of regional lymph node involvement

NX: No information on lymph node involvement

Metastasis:

M0: No evidence of metastases or non-regional lymph nodes

M1: Evidence of distant metastasis or involvement of non-regional lymph nodes

MX: No information on metastasis

pTNM: Post surgical TNM classification

pT

pT0: No evidence of tumour found on histological examination of specimen.

pT1: Tumour limited to organ or tissue of origin.
Excision complete and margins histologically free.

pT2: Tumour with invasion beyond the organ or tissue of origin.
Excision complete and margins histologically free.

pT3 Tumour with or without invasion beyond the organ or tissue of origin.
Excision incomplete.
- pT3a: Evidence of microscopic residual tumour.
- pT3b: Evidence of macroscopic residual tumour.
- pT3c: Adjacent malignant effusion regardless of size.

pTX: Tumour status may not be assessed.

pN

pN0: No evidence of tumour found on histological examination of regional lymph nodes

pN1: Evidence of invasion of regional lymph nodes
- pN1a: Evidence of invasion of regional lymph nodes
  Involved nodes considered to be completely resected
- pN1b: Evidence of invasion of regional lymph nodes
  Involved nodes considered not to be completely resected

pNX: N status may not be assessed due to lack of pathological examination or inadequate information on pathological findings.

pM

pM0: No evidence of metastasis found on histological examination of non-regional lymph nodes

pM1: Evidence of metastasis on histological examination

pMX: M status may not be assessed due to lack of pathological examination or inadequate information on pathological findings.

For evaluations NX and pNX will be regarded as N0 and pNX, MX and pMX will be regarded as M0 and pM0

A.2 IRS CLINICAL GROUPING CLASSIFICATION

Group I: Localized disease, completely resected
(Regional nodes not involved – lymph node biopsy or dissection is required except for head and neck lesions)

(a) Confined to muscle or organ of origin

(b) Contiguous involvement – infiltration outside the muscle or organ of origin, as through facial planes.

Notation: This includes both gross inspection and microscopic confirmation of complete resection. Any nodes that may be inadvertently taken with the specimen must be negative. If the latter should be involved microscopically, then the patient is placed in Group IIb or IIc (See Below).

Group II: Total gross resection with evidence of regional spread

a) Grossly resected tumour with microscopic residual disease.
(Surgeon believes that he has removed all of the tumour, but the pathologist finds tumour at the margin of resection and additional resection to achieve clean margin is not feasible.) No evidence of gross residual tumour. No evidence of regional node involvement. Once radiotherapy and/or chemotherapy have been started, re-exploration and removal of the area microscopic residual does not change the patient's group.

b) Regional disease with involved nodes, completely resected with no microscopic residual.

Notation: Complete resection with microscopic confirmation of no residual disease makes this different from Groups IIa and IIc.
Additionally, in contrast to Group IIa, regional nodes (which are completely resected, however) are involved, but the most distal node is histologically negative.

c) Regional disease with involved nodes, grossly resected, but with evidence of microscopic residual and/or histologic involvement of the most distal regional node (from the primary site) in the dissection.

Notation: The presence of microscopic residual disease makes this group different from Group IIb, and nodal involvement makes this group different from Group IIa.

Group III: Incomplete resection with gross residual disease

a) After biopsy only

b) After gross or major resection of the primary (>50%)

Group IV: Distant metastasic disease present at onset
(Lung, liver, bones, bone marrow, brain, and distant muscle and nodes)

Notation: The above excludes regional nodes and adjacent organ infiltration which places the patient in a more favourable grouping (as noted above under Group II).

The presence of positive cytology in CSF, pleural or abdominal fluids as well as implants on pleural or peritoneal surfaces are regarded as indications for placing the patient in Group IV.

A.3 IRS AND pTNM GROUPING SYSTEM

IRS Group	**Definition**	**pTNM**
I	Tumour macroscopically and microscopically removed	
(IA)	Tumour confined to organ or tissue of origin	pT1
(IB)	Tumour not confined to organ or tissue of origin	pT2
II IIA IIB	Macroscopic complete resection but microscopic residuals Lymph nodes not affected Lymph nodes affected but removed	pT3a
III	Macroscopic complete resection but microscopic residuals and lymph nodes affected and not removed	pT3a
	Macroscopic residuals after resection or biopsy With malignant effusion	pT3b pT3c
IV	Metastasis present or non-regional lymph nodes involved	pT4

A.4 DEFINITION OF SITES

To define the site of origin may be difficult in some cases of RMS. A correct site assignation is of importance in the choice of treatment. The following definitions are given to facilitate the clinician in the appropriate site classification.
We acknowledge the permission given by the IRSG to modify and use their original document on site definitions,

ORBIT

1. Eyelid
This site is sometimes erroneously designated as "eye". Although there may occasionally be a case arising from the conjunctiva of the eye, the globe itself is not a primary site. The eyelid is much less frequent than the orbit itself.

2. Orbit
This refers to the bony cavity, which contains the globe, nerve and vessels and the extra-ocular muscles. Tumour in this site will only rarely invade the bony walls and extend into the adjacent sinuses. This is why this tumour which is clearly adjacent to the skull base and its meninges is not by its natural history appropriate to include in the parameningeal sites unless there is invasion of bone at the base of the skull.

PARAMENINGEAL

1. Middle ear
This refers to a primary that begins medial to the tympanic membrane. This tumour is often advanced at presentation and because of extension laterally may present with a mass in front of or under the ear suggesting a parotid origin. It may also extend through the tympanic membrane and appear to be arising in the ear canal. When there is doubt about the site of origin, the "middle ear" designation should be picked as it implies the more aggressive therapy required of parameningeal sites.

2. Nasal Cavity and Para nasal Sinuses
The three Para nasal sinuses are the maxillary sinuses, the ethmoid sinuses, and the sphenoid sinus. These surround the nasal cavity, and a primary in one will frequently extend to another. It can be difficult to determine the exact site of origin, but the choice is academic as the treatment is not affected. The site designation will have a bearing on the design of radiotherapy portals. Tumour arising in the maxillary or the ethmoid sinuses may invade the orbit. This is much more likely than a primary in the orbit invading one of the sinuses. When the distinction between orbit and Para nasal sinus is unclear, the site selected should be Para nasal sinus as it is the more likely primary site and requires appropriately more aggressive therapy. A primary arising in the sphenoid sinus (rare) may extend inferiorly to involve the nasopharynx.

3. Nasopharynx
This refers to the superior portion of the pharynx which is bounded anteriorly by the back of the nasal septum, superiorly by the sphenoid sinus, inferiorly by a level corresponding to the soft palate, and laterally and posteriorly by the pharyngeal walls.

4. Infratemporal Fossa/Pterygopalatinand Parapharyngeal Area
This refers to the tissues bounded laterally by the medial lobe of the parotid gland and medially by the pharynx. Large tumours in this region may extend through the parotid gland and present as a mass of the lateral face, sometimes extending even to the cheek. Where there is doubt as to the primary, the parameningeal designation should be chosen as it confers appropriately more aggressive treatment. The superior boundary of this tissue volume is the base of skull just under the temporal lobe, hence the term "infratemporal". The distinction between this and the "parapharyngeal" area is academic.

5. Orbital tumours with bone erosion
Tumours arising in the orbit but with intracranial extension or important bone erosion are included in the parameningeal group.

In addition the following are classified as parameningeal tumours:
- Tumours involving vessels or nerves with direct intracranial connection (Arteria carotis interna, vertebralis, N. opticus, trigeminus, facialis etc).
- All intracranial and intraspinal tumours (but tumours arising from the paraspinal muscles with intraspinal extension should be designated as paraspinal, see "Other site" definition)
- All tumors with cranial nerve paresis (excluding parotid tumours with facial nerve palsy)
- CSF Tumour cell positive patients

HEAD AND NECK

1. Scalp
This site includes primaries arising apparently in, or just below, the skin of all the tissues of the face and head that are not otherwise specified below. This usually means the scalp, external ear and pinna, the nose and the forehead, but not the eyelids or cheek.

2. Parotid
The parotid gland lies just in front of, and under, the ear and may surround both sides of the posterior aspect of the ascending ramus of the mandible. As noted above, large primaries in the infratemporal fossa may erode through the parotid. A true parotid primary should not, on radiographic studies, reveal a mass in the infratemporal fossa.

3. Oral Cavity
This includes the floor of the mouth, the buccal mucosa, the upper and lower gum, the hard palate, the oral tongue (that portion of the tongue anterior to the circumvallate papillae). A primary arising in the buccal mucosa can be impossible to distinguish from one arising in the cheek, but the distinction is academic. This would also include those lesions arising in or near the lips.

4. Larynx
This refers to primaries arising in the subglottic, glottic, or supraglottic tissues. Tumours of the aryepiglottic folds can be impossible to distinguish from the hypopharynx, but the distinction is academic.

5. Oropharynx
This includes tumours arising from the anterior tonsillar pillars, the soft palate, the base of the tongue, the tonsillar fossa, and oropharyngeal walls. Tumours arising in the parapharyngeal space may indent the oropharyngeal wall. In this circumstance, the primary should be considered parameningeal. If the mucosa of the oropharynx actually contains visible tumour as opposed to being bulged by it, the primary would be oropharynx. Primaries arising in the tongue base, soft palate, or tonsillar region may extend into the oral cavity. The oropharynx designation is preferred.

6. Cheek
This refers to the soft tissues of the face that surround the oral cavity. Tumours arising in the parotid may invade the cheek. As noted above, the distinction between this and the buccal mucosa is academic.

7. Hypopharynx
This refers to the pyriform sinus and may be difficult to distinguish from larynx although the designation is academic.

8. Thyroid and Parathyroid
Primaries arising in these two sites are exceedingly rare, if they exist at all, and should those structures be involved, it would more likely be from a primary arising in an adjacent structure such as the neck or, rarely, the trachea.

9. Neck
This refers to the soft tissues of the lateral neck between the mastoid tip and the clavicle. It does not include those medial structures such as hypopharynx and larynx noted above. Unfortunately this site overlaps with the designation "paraspinal" included under the site group "trunk". Primaries arising in the neck can and frequently do behave as a paraspinal primary with direct invasion into the spinal extra dural space, especially if posteriorly placed.

GENITO-URINARY BLADDER AND PROSTATE

1. Bladder
Our criteria for identifying the bladder as a primary site has included the appearance of tumour within the bladder cavity, which can be biopsied under cystoscopy or occasionally at laparotomy. We do not recognize as primary bladder tumours those that simply displace the bladder or distort its shape. The latter are ordinarily primary pelvic tumours, unless otherwise specified.

2. *Prostate*
It is important to differentiate true prostatic tumours from pelvic tumours.

3. Bladder/Prostate
In approximately 20% of males with bladder or prostatic tumours, the precise site cannot be determined even at autopsy. The histologic features are similar. Although it is desirable to have an indication of the "most probable" site from the institution, and one should try to get this, it may not be possible.

GENITO-URINARY NON BLADDER AND PROSTATE

1. Paratesticular
The tumours arises from mesenchymal elements of the spermatic cord, epididymis, and testicular envelopes, producing a painless scrotal mass.

2. Testis
This designation is wrong because the tumours arise from paratesticular structures and may invade the testis.

3. Uterus
A tumour in this primary site may be difficult to differentiate from a primary vaginal site, because a tumour originating in the uterus (corpus or cervix) may fill the vagina. After a therapeutic response, the distinction is usually clear. In general there is a wide separation of age range between these two groups, with the vaginal cases occurring in infancy or early childhood and uterine primaries in adolescents or young adults.

4. Vagina
A patient with a primary vaginal lesion must have evidence of a visible tumour on the vaginal surfaces which can be biopsied through the vagina. Displacement or distortion of the vagina is not sufficient.

5. Vulva
Primary lesions in this site arise in the labia minora or majora.

EXTREMITIES

1. Hand
Refers to the area from the top of the fingers to the wrist

2. Forearm
Refers to the area from the wrist to the elbow joint

3. Arm
Refers to the area from the elbow joint to the shoulder joint. Tumours arising in the axilla are considered as extremity lesions.

4. Shoulder
The posterior aspect of the shoulder, i.e., the scapular area, is an extremity site.

5. Foot
Refers to the area from the toes to the ankle

6. Leg
Refers to the area from the ankle to the knee

7. Thigh
Refers to the area from the knee to the hip joint

8. Buttocks
These are extremity lesions.

OTHER SITES

This term conventionally groups tumours originating from the sites not mentioned above. Prognosis is similar and usually not satisfying.
The following specific sites have been defined:

1. Thorax
Includes tumours arising in the following sites:

a) Thoracic wall:
includes tumours arising from the thoracic muscles and the parietal pleura

b) Mediastinum:
occasionally a primary rhabdomyosarcoma may arise form trachea, heart or nearby areas.

c) Lung:
includes tumours arising form the lung parenchyma, bronchus and visceral pleura

d) Breast

e) Diaphragm

2. Abdomen

a) Abdominal Wall (including Lumbar or lumbo-sacral wall)
This refers to the anterior abdominal wall from the inferior costal margins superiorly to the inguinal ligaments and symphysis pubis, inferiorly, and extends laterally between the costal margin and posterior iliac crests to the paraspinal region.

b) Liver
True liver rhabdomyosarcoma are less frequent than bile duct tumours.

c) Bile duct

Bile Duct is a specific site and can be recognised as such at surgery. This might also be called "choledochus" or "biliary tract". There is probably no way one can distinguish an intrahepatic bile duct site from a primary liver site except by examining the excised specimen.

d) Pancreas

e) Bowel

f) Abdomen

The term abdominal refers to tumours arising in the intraperitoneal cavity, when a specific organ of origin such as liver, bile duct, pancreas or intestine cannot be determined.

g) Retroperitoneum

The term retroperitoneal is reserved for those posteriorly situated abdominal tumours in which there does not seem to be a more specific site. Tumours in a retroperitoneal site are in the posterior aspect of the abdominal and/or pelvis. The term "psoas" as a site is not very specific, as the muscle extends through the posterior lower abdomen, pelvis and into the leg.

3. Paraspinal

When tumours are described as adjacent to the vertebral column, arising from the paraspinal muscles. This designation is preferable to "abdominal wall" or "trunk" or "neck". They often show an intraspinal component and this should be specified.

4. Pelvis

It is difficult to define the site of origin when there is a large tumour in the abdomen. The pelvis designation is reserved for lesions involving the lower part of the abdomen when no more specific site is appropriate.

5. Perianal

These sites are ordinarily "perirectal" or "perianal". They are distinguished with difficulty from perineal and vulval sites; but the latter distinction is important.

6. Perineum

This should include the site which appear between the anus and the scrotum in males and the labia in females. It extends anteriorly to the base of the scrotum in males and to the introitus in females. It must be distinguished from labial and perianal sites.

A.5 REGIONAL LYMPH NODES DEFINITION

Regional lymph node involvement is defined N1 according to TNM system.
Regional lymph nodes are defined as those appropriate to the site of the primary tumour, for example:

Head & Neck :	ipsilateral cervical and supraclavicular lymph nodes; bilateral adenopathy may be present with centrally situated tumours
Orbit :	ipsilateral jugular, pre-auricular, cervical
Intrathoracic:	internal mammary, mediastinal nodes
Thoracic wall:	axillary, internal mammary, infraclavicular nodes

Intraabdominal & Pelvic : Sub diaphragmatic, intra abdominal and iliac lymph nodes according to site.

Abdominal wall:	inguinal, femoral nodes
Genito-urinary:	
Bladder Prostate:	iliac nodes (external, internal and common chains; note that paraaortic nodes are second level nodes).
Cervix and Uterus	iliac nodes (external, internal and common chains)
Paratesticular :	external iliac and para-aortic (retroperitoneal) lymph nodes at renal artery or below (inguinal if the scrotum is interested)
Vagina:	iliac nodes (external, internal and common chains; notes that paraaortic nodes are second level nodes).
Vulva:	inguinal nodes
Perineum:	inguinal and iliac (may be bilateral)
Upper Limbs :	axillary lymph nodes (epitrochlear rarely involved)
Lower Limbs :	inguinal lymph nodes (popliteal rarely involved)

Evidence of nodal involvement different than those listed above must be interpreted as distant metastasis and the patient must be treated according to the protocol for patients with metastatic disease at diagnosis .
Examples:
- perineal tumour with nodes above the pelvis
- thigh tumour with iliac or periaortic nodes
- intrathoracic tumour with subdiaphragmatic nodes
- Unilateral tumour with controlateral involved lymph nodes (except in the head and neck).

A.6 MRI AND CT SCAN TECHNICAL RECOMMENDATIONS

MRI protocol

- Intravenous gadolinium administration (0,2 ml/kg - 0,1 mmol/kg) is mandatory for all MRI examinations (post-contrast T1-weighted sequences should ideally be performed with fat saturation)
- Tumour measurements should be performed on post-gadolinium T1 or T2-weighted sequences (but not on STIR or non-enhanced T1-weighted sequences).
- Fast dynamic sequences (e.g. spoiler 3D T1 : FLASH 3D, VIBE, FSPGR, 3D-FFE, volume RF-FAST) to assess early tumour vascularity are recommended at diagnosis (can help differentiation between vascularized and necrotic areas), after biopsy (helps differentiation between residual disease and fibrosis), and also after chemotherapy (depiction of residual disease) and for suspected relapse (helps differentiation between residual disease and fibrosis).
- Sedation or general anaesthesia for children 6 months-5 years according to local procedures.
- A cutaneous localiser for small superficial lesions or in front of scars on limbs is good practice.

Additional recommendations according to primary location :

Orbit	Bilateral study Thin slice width 2-4 mm
Head and Neck	No sedation if airway obstruction
Limbs	Surface coil Cutaneous localiser
Cranio-spinal MR	from C0 to S3 Anterior presaturation

Technical recommendations for CT scanning

- Apnea if possible for chest and abdominal CT
- 3 to 5 mm reconstruction slice width
- 100 - 120 kV
- mAs adjusted according to patient size, pitch and rotation time
- Recommended CTDI vol : 5 to 15 mGy according to age, location and local technical options
- Reconstruction filters for soft tissue, bone and lung
- Oral contrast opacification is recommended for all abdominal and pelvic studies.
- Intravenous contrast injection : 1,5-2ml/Kg of iodinated agent (300 or 350 mg Iodine/l); rate : 0,7 to 2 cc/sec, scan delay: 35 - 40 sec.

A.7 TOXICITY GRADING

This is a short version of the NCI CTC only containing the most common side effects. The full text version can be downloaded from: http://ctep.cancer.gov/reporting/ctc.html.

ALLERGY/IMMUNOLOGY

Adverse Event	1	2	3	4
Allergic reaction/ hypersensitivity (including drug fever)	transient flushing or rash; drug fever < 38°C (<100.4°F))	Rash; flushing; urticaria, dyspnea; drug fever ≥ 38°C (≥100.4°F),	Symptomatic bronchospasm, with or without urticaria parenteral medication(s) idicated; allergy-related edema/angioedema; hypotension	anaphylaxis

REMARK: Urticaria with manifestations of allergic or hypersensitivity reaction is graded as Allergic reaction/hypersensitivity (including drug fever).

AUDITORY/EAR

Hearing: patients without baseline audiogram and not enrolled in a monitoring program	-	Hearing loss not requiring hearing aid or intervention (i.e., not interfering with ADL)	Hearing loss requiring hearing aid or intervention (i.e., interfering with ADL)	Profound bilateral hearing loss (>90 dB)

BLOOD/BONE MARROW

Haemoglobin	< LLN - 10.0 g/dl < LLN – 6.2 mmol/L < LLN – 100 g//L	<10.0 – 8.0 g/dL <6.2 – 4.9 mmol/L <100 – 80 g/L	<8.0 – 6.5 g/dL <4.9 – 4.0 mmol/L <80 – 65 g/L	< 6.5 g/dl <4.0 mmol/L <65 g/L
Leukocytes (total WBC)	< LLN - 3.0 x 10^9 /L < LLN - 3000/mm^3	<3.0 - 2.0 x 10^9 /L <3000 - 2000/mm^3	<2.0 - 1.0 x 10^9 /L <2000 - 1000/mm^3	< 1.0 x 10^9 /L < 1000/mm^3
Neutrophils/granulocytes (ANC/AGC)	<1.5 – 1.5 x 10^9 /L <1500/mm^3	<1.5 - 1.0 x 10^9 /L <1500 - 1000/mm^3	<1.0 – 0.5 x 10^9 /L <1000 - 500/mm^3	< 0.5 x 10^9 /L < 500/mm^3
Platelets	< LLN - 75.0 x 10^9 /L < LLN – 75.000/mm^3	<75.0 - 50.0 x 10^9 /L <75.000 – 50.000/mm^3	<50.0 - 25.0 x 10^9 /L <50.000 – 25.000/mm^3	< 25.0 x 10^9 /L < 25.000/mm^3

CARDIAC ARRHYTHMIA

Conduction abnormality/ atrioventricular heart block	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Incompletely controlled medically or controlled with device (e.g., pacemaker)	Life – threatening (e.g., arrhytmia associated with CHF, hypotension, syncope, shock)
Prolonged QTc interval	QTc>0.45 – 0.47 second	QTc >0.47 - 0.50 second; ≥0.06 second above baseline	QTc >0.50 second	QTc >0.50 second; life-threatening sings or symptoms (e.g., arrhythmia, CHF, hypotension, shock syncope); Torsade de pointes
Supraventicular and nodal arrhythmia	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
Ventricular arrhythmia	Asymptomatic, no intervention indicated	Non-urgent medical intervention indicated	Asymptomatic and incompletely controlled medically or controlled with device (e.g., defibrillator)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)

CARDIAC GENERAL

Cardiac Ischemia/infarction	Asymptomatic arterial narrowing without ischemia	Asymptomatic and testing suggesting ischemia; stable angina	Symptomatic and testing consistent with ischemia; unstable angina; intervention indicated	Acute myocardial infarction
Cardiopulmonary arrest,	-	-	-	
Hypertension	Asymptomatic, transient (<24 hrs) BP increase >ULN; intervention not indicated	Recurrent or persistent (>24 hrs) BP >ULN; monotherapy may be indicated	Requiring more than one drug or more intensive therapy than previously	Life-threatening consequences (e.g. hypertensive crisis)

REMARK: Use age and gender-appropriate normal values >95th percentile ULN for pediatric patients

Hypotension ALSO CONSIDER: Syncope (fainting)	intervention not indicated	Brief (<24 hrs) fluid replacement or other therapy; no physiologic consequences	Sustained (≥24 hrs) therapy, resolves without persisting physiologic consequences	Shock (e.g., acidemia; impairment of vital organ function)
Left ventricular diastolic dysfunction	Asymptomatic diagnostic finding; intervention not indicated	Asymptomatic, intervention indicated	Symptomatic CHF responsive to intervention	Refractory CHF, poorly controlled; intervention such as ventricular assist device or heart transplant indicated
Left ventricular Systolic dysfunction	Asymptomatic , resting ejection fraction (EF) <60-50%; shortening fraction (SF) <30 – 24%	Asymptomatic, resting EF <50 – 40% SF <24 – 15%	Symptomatic CHF responsive to intervention; EF <40 – 20% SF <24 – 15%	Refractory CHF or poorly controlled;<20%; intervention such as ventricular assist device , ventricular reduction surgery, or heart transplant indicated

COAGULATION

Adverse Event	1	2	3	4
DIC (disseminated intravascular coagulation)	__	Laboratory findings with no bleeding	Laboratory findings and bleeding	Laboratory findings, life-threatening or disabling consequences (e.g., CNS haemorrhage, organ damage, or hemodynamically significant blood loss)

REMARK: DIC (disseminated intravascular coagulation) must have increased fibrin split products or D-dimer

CONSTITUTIONAL SYMPTOMS

Fatigue (asthenia, lethargy, malaise)	Mild fatigue over baseline	Moderate or causing difficulty performing some ADL	Severe fatigue interfering with ADL	Disabling
Weight gain	5-<10% of baseline	10 - <20% of baseline	≥20% of baseline	__

REMARK: Oedema, depending on aetiology, is graded in the CARDIAC GENERAL or LYMPHATICS CATEGORIES.
ALSO CONSIDER: Ascites (non-malignant); Pleural effusion (non-malignant).

Weight loss	5 to <10% from baseline; intervention not indicated	10 - <20% from baseline; nutritional support indicated	≥20% from baseline; tube feeding or TPN indicated	__

DERMATOLOGY/SKIN

Burn	Minimal symptoms; Intervention not indicated	Medical intervention; minimal debridement indicated	Moderate to major debridement or reconstruction indicated	Life-threatening consequences

REMARK: Burn refers to all burns including radiation, chemical, etc.

Injection site reaction/extravasation changes	Pain; itching; erythema	Pain or swelling, with inflammation or phlebitis	Ulceration or necrosis that is severe; operative intervention indicated	__

ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever); Ulceration

Rash/desquamation	Macular or papular eruption or erythema without associated symptoms	Macular or papular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area (BSA)	Severe, generalized erythroderma or macular, papular or vescicular eruption; desquamation covering ≥50% BSA	Generalized exfoliative, uklcerative, or bullous dermatits.

REMARK: Rash/desquamation may be used for GVHD.

Rash: dermatitis associated with radiation -	Faint erythema or dry desquamation	Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate oedema	Moist desquamation other than skin folds and creases; bleeding induced by minor trauma or abrasion	Skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site
Ulceration	__	Superficial ulceration <2 cm size; local wound care; medical intervention indicated	Ulceration ≥2 cm size; operative debridement, primary closure or other invasive intervention indicated (e.g., hyperbaric oxygen)	Life-threatening consequences; major invasive intervention indicated (e.g., complete resection, tissue reconstruction, flap, or grafting)
Urticaria (hives, welts, wheals)	Intervention not indicated	Intervention indicated for <24 hrs	Intervention indicated for≥24 hrs	__

ALSO COSIDER: Allergic reaction/hypersensitivity (including drug fever).

ENDOCRINE

Neuroendocrine: ADH secretion abnormatity (e.g., SIADH or low ADH)	Asymptomatic	Symptomatic, not interfering with ADL; intervention indicated	Symptoms interfering with ADL	Life-threatening consequences

GASTROINTESTINAL

NAVIGATION NOTE: Abdominal pain or cramping is graded as pain-*Select* in the PAIN CATEGORY.

Anorexia	loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); IV fluids, tube feedings or TPN indicated	Life-threatening consequences
Ascites (non-malignant)	Symptomatic, medical intervention indicated	Symptomatic, invasive procedure indicated	Life-threatening consequences	

REMARK: Ascites (non-malignant)refers to documented non-malignant ascites or unknown aetiology, but unlikely malignant, and inclused chylous ascites.

Colitis	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences (e.g., perforation, bleeding, ischemia, necrosis, toxic megacolon)
Constipation	requiring stool softener or dietary modification	Requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon

ALSO CONSIDER:Ileus, GI (functional obstruction of bowel, i.e., neurocostipation);

Adverse Event	1	2	3	4
Diarrhoea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4-6 stools per day over baseline; IV fluids indicated <24 hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL	Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL	Life-threatening consequences (e.g., hemodynamic collapse)

REMARK: Diarrhoea includes diarrhoea of small bowel or colonic origin, and/or ostomy diarrhoea.

Enteritis (inflammation of the small bowel)	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal sings	Life-threatening consequences (e.g.., perforation, bleeding, ischemia, necrosis)

Esophagitis	Asymptomatic pathologic, radiographic, or endoscopic findings only	Symptomatic; altered eating/swallowing (e.g., altered dietary habits, oral supplements); IV fluids indicated <24 hrs	Symptomatic and severely altered eating/swallowing (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥ 24 hrs	Life-threatening consequences

REMARK: Esophagitis includes reflux esophagitis.

Ileus, GI (functional obstruction of bowel, i.e., neuroconstipation)	Asymptomatic, radiographic findings only	Symptomatic; altered GI function (e.g., altered dietary habits); IV fluids indicated <24 hrs	Symptomatic and severely altered GI function; IV fluids, tube feeding, or TPN indicated ≥ 24 hrs	Life-threatening consequences

REMARK: Ileus, GI is to be used for altered upper or lower GI function (e.g., delayed gastric or colonic emptying).

Mucositis/Stomatitis (clinical exam)	Erythema of the mucosa	Patchy ulcerations or pseudomembranes	Confluent ulcerations or pseudomembranes; bleeding with minor trauma	Tissue necrosis; significant spontaneous bleeding; life-threatening consequences
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated < 24 hrs.	Inadequate oral caloric or fluid intake; IV fluids, tube feedings or TPN indicated > 24 hrs.	Life-threatening consequences
Vomiting	1 episode in 24 hrs	2-5 episodes in 24 hrs; IV fluids indicated <24 hrs	≥6 episodes in 24 hrs; IV fluids, or TPN indicated ≥24 hrs	Life-threatening consequences

Gastrointestinal-Other (Specify__)	Mild	Moderate	Severe	Life-threatening; disabling

HEMORRHAGE/BLEEDING

Hematoma	Minimal symptoms, invasive intervention not indicated	Minimal invasive evacuation or aspiration indicated	Transfusion, interventional radiology, or operative intervention indicated	Life- threatening consequences; major urgent intervention indicated

REMARK: Hematoma refers to extravasation at wound or operative site or secondary to other intervention. Transfusion implies pRBC.

Haemorrhage/bleeding associated with surgery, intra-operative or postoperative	—	—	Requiring transfusion of 2 units non-autologous (10cc/Kg for pediatrics) pRBCs beyond protocol specification; postoperative interventional rediology, endoscopic, or operative intervention indicated	Life-threatening consequences

REMARK: Postoperative period is defined as ≤72 hrs after surgery. Verify protocol-specific acceptable guidelines regarding pRBC transfusion.
ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PPT (Partial Thromboplastin Time).

Hemorrhage, CNS	Asymptomatic, radiographic findings only	Medical intervention indicated	Ventriculostomy, ICP monitoring, intraventicular thombolysis, or operative intervention indicated	Life-threatening consequences; neurologic deficit or disability

ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PPT (Partial Thromboplastin Time).

Haemorrhage, GI	Mild, intervention (other than iron supplements) not indicated	Symptomatic and medical intervention or minor cauterization indicated	Transfusion, interventional radiology, endoscopic, or operative intervention indicated; radiation therapy (i.e., hemostasis of bleeding indicated)	Life-threatening consequences; major urgent intervention indicated

REMARK: Transfusion implies pRBC.

Haemorrhage/Bleeding –Other (Specify__)	Mild without transfusion	—	Catastrophic bleeding, requiring major non-elective intervention	

HEPATOBILIARY/PANCREAS

Liver dysfunction/failure (clinical)	—	Jaundice	Asterixis	Encephalopathy or coma

REMARK: Jaundice is not an AE, but occurs when the liver is not working properly or when a bile duct is blocked. It is graded as a result of liver dysfunction/failure or elevated bilirubin.

Pancreatitis	Asymptomatic, enzyme elevation and/or radiographic findings	Symptomatic, medical intervention indicated	Interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., circulatory failure, haemorrhage, sepsis

INFECTION

Adverse Event	1	2	3	4
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection) (ANC <1.0 x 1.0^9/L fever ≥ 38.5°)	—	—	Present	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)

ALSO CONSIDER: Neutrophilis/granulocytes (ANC/AGC).

Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophilis (ANC <1.0 x 1.0^9/L)	—	Localized, local interventio indicated	IV antibiotic, antifungal, or antiviral intervention indicated; interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)
Infection with normal ANC or Grade 1 or 2 neutrophilis	—	Localized, local intervention indicated	IV antibiotic, antifungal, or antiviral intervention indicated; interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)
Infection – Other (Specify, __)	Mild	Moderate	Severe	Life – Threatening; disabling

METABOLIC/LABORATORY

ALT; SGPT (serum glutamic oxaloacetic)	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
AST, SGOT (serum glutamic oxaloacetic transaminase) (ANC < 1.0 x 10^9/L)	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
Bilirubin (hyperbilirubinemia)	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 x ULN	>10.0 x ULN

REMARK: jaundice is not an AE, but may be a manifestation of liver dysfunction/failure or elevated bilirubin. If jaundice is associated with elevated bilirubin, grade bilirubin.

Calcium, serum-low (hypocalcaemia)	<LLN – 8.0 mg/dL <LLN – 2.0 mmol/L lionized calcium: <LLN – 1.0 mmol/L	<8.0 – 7.0 mg/dL <2.0 – 1.75 mmol/L lionized calcium: <1.0 – 0.9 mmol/L	<7.0 – 6.0 mg/dL <1.75 – 1.5 mmol/L lionized calcium: <0.9 – 0.8 mmol/L	<6.0 mg/dL <1.5 mmol/L lionized calcium: <0.8 mmol/L

REMARK: Calcium can be falsely low if hypoalbuminemia is present. Serum albumin <4.0 g/dL, hypocalcaemia is reported after the following corrective calculation has been performed: Corrected Calcium (mg/dL)= Total calcium (mg/dL) – 0.8 [Albumin (g/dL) - 4][4]. Alternatively, direct measurement of ionized calcium is the definitive method to diagnose metabolically relevant alterations in serum calcium.

Calcium, serum-high (hypocalcaemia)	>LLN – 11.5 mg/dL >LLN – 2.9 mmol/L lionized calcium: >LLN – 1.5 mmol/L	>11.5 – 12.5 mg/dL >2.9 – 3.1 mmol/L lionized calcium: >1.5 – 1.6 mmol/L	>12.5 – 13.5 mg/dL >3.1 – 3.4 mmol/L lionized calcium: >1.6 – 1.8 mmol/L	>13.5 mg/dL >3.4 mmol/L lionized calcium: >1.8 mmol/L

NEPHROTOXICITY

Creatinine	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 6.0 x ULN	>6.0 x ULN

REMARK: Adjust to age-appropriate levels for pediatric patients.
ALSO CONSIDER: Glomerular filtration rate.

Glomerular filtration rate	<75 – 50% LLN	<50 – 25% LLN	<25%LLN, chronic dialysis not indicated	Chronic dialysis or renal transplant indicated

ALSO CONSIDER: Creatinine.

Proteinuria	1+ or 0.15 – 1.0 g/24 hrs	2+ to 3+ or >1.0 – 3.5 g/24 hrs	4+ or >3.5 g/24 hrs	Nefrotic syndrome

Nephrotoxicity garding : totak score see appendix A.9

NEUROLOGY

Ataxia (incoordination)	Asymptomatic	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL; mechanical assistance indicated	Disabling

REMARK: Ataxia (incoordination) refers to the consequence of medical or operative intervention.

CNS cerebrovascular ischemia	—	Asymptomatic, radiographic findings only	Transient ischemic event or attack (TIA) ≥24 hrs duration	Cerebral vascular accident (CVA, stroke), neurologic deficit >24 hrs

Adverse Event	1	2	3	4
Cognitive disturbance	Mild cognitive disability; not interfering with work/school//life performance; specialized educational services/devices not indicated	Moderate cognitive disability; interfering with work/school/life performance but capable of independed living; specialized resources on part-time basis indicated	Severe cognitive disability, significant impairment of work/school/life performance	Unable to perform ADL; full-time specialized resources or institutionalization indicated
Confusion	Transient confusion, disorientation, or attention deficit	Confusion, disorientation, or attention deficit interfering with function, but not interfering with ADL.	Confusion or delirium interfering with ADL	Harmful to other or self; hospitalization indicated
Dizziness	With head movements or nystagmus only; not interfering with function	Interfering with function, but not interfering with ADL	Interfering with ADL	Disabiling

REMARK: Dizziness includes diequilibrium, light-headedness, and vertigo.

Encephalopathy	__	Mild simgs or symptoms; not interfering with ADL	Sings or symptoms interfering with ADL; hospitalization indicated	Life-threatening; disabiling

ALSO CONSIDER: Cognitive disturbance; Confusion; dizziness; Memory impairment; Mental status; Mood alteration – *Select* Psychosis (hallucinations/delusions)

Extrapyramidal/involuntary movement/ restlessness	Mild involuntary movements not interfering with function	Moderate involuntary movements interfering with function, but not interfering with ADL	Severe involuntary mevements or torticollis interfering with ADL	Disabiling
Mood alteration -*Select* -Agitation -Anxiety -Depression -Euphoria	Mild mood alteration not interfering with function	Moderate mood alteration interfering with function, but not interfering with ADL; medication indicated	Severe mood alteration interfering with ADL	Siucidal ideation; danger to self or other
Neuropathy: Cranial -Select -CN I Smell -CN II Vision -CN III Pupil, upper eyelid, extra ocular movements CN IV Downward, inward movement of eye CN V Motor-jaw muscles; Sensory-facial CN VI Lateral deavition of eye CN VII Motor-face; Sensory-taste CN VIII Hearning and balance CN IX Motor-pharynx; Sensory-ear, pharjnx, larynx CN X Motor-palate; pharynx, larynx CN XI Motor-sternomastoid and trapezius CN XII Motor-tongue	Asymptomatic, detected on exam/testing only	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL	Life-threatening; disabiling
Psychosis (hallucinations/delusion)	__	Transient episode	Interfering with ADL; medication, supervision or restraints indicated	Harmful to other or self; life-threatening consequences
Seizure	__	One brief generalized seizure; seizure (s) well controlled by anticonvulsants or infrequent focal motor seizures not interfering with ADL	Seizures in which consciousness is altered; poorly controlled seizure disorder, with breakthrough generalized seizures despite medical intervention	Seizures of any kind which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)
Somnolence/depressed Level of consciousness	__	Somnolence or sedation interfering with function, but not interfering with ADL	Obtundation or stupor; difficult to arouse; interfering with ADL	Coma
Neurology-Other (Specify__)	Mild	Moderate	Severe	Life-Threatening; disabling

PAIN

Pain-Other (Specify__)	Mild pain not interfering with function	Moderate pain; pain or analogesics interfering with function, but not interfering ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling

PULMONARY/UPPER RESPIRATORY

Adult respiratory Distress Syndrome (ARDS)	__	__	Present, intubation not indicated	Present, intubation indicated
Pleural effusion (non-malignant)	Asymptomatic	Symptomatic, intervention such as diuretics or up to 2 therapeutic thoracenteses indicated	Symptomatic and supplemental oxygen, >2 therapeutic thoracenteses, tube drainage, or pleurodesis indicated	Life-threatening (e.g, causing hemodynamic instability or ventilatory support indicated)

OCULAR

Ocular/Visual-Other (Specify, _____)	mild	Moderate	severe	unilateral or bilateral loss of vision (blindness)

A.8 VENO-OCCLUSIVE DISEASE OF THE LIVER - GRADING

VOD appears related to the administration of actinomycin among different drugs. No specific predisposing factor has been found to identify the patient at risk. A prior persistent or slow recovery of thrombocytopenia may be an indicator of VOD.

We considered hepatic toxicity as compatible with the clinical diagnosis of VOD when no other causes of liver disease were identified and at least 2 of the following features are present:
a) jaundice
b) hepatomegaly ($\geq$ 2 cm below the costal margin) and/or right upper quadrant pain,
c) ascites and/or sudden weight gain (> 2% of baseline body weight) due to fluid retention.

Doppler study of the liver may document retrograde portal venous flow.

Grading Criteria for VOD.

Mild VOD:
Total bilirubin $\leq$ 6 mg/dL
Weight gain of $\leq$ 5% of baseline of noncardiac origin
Reversible hepatic dysfunction

Moderate VOD:
Total bilirubin > 6 mg/dL and < 20 mg/dL
Weight gain > 5% of baseline of noncardiac origin
Clinical or image documented ascites
Reversible hepatic dysfunction

Severe VOD:
Total bilirubin > 20mg/dL and/or
Ascites compromising respiratory function and/or
Renal deterioration and/or
Hepatic encephalopathy which may not be reversible

Therapy modifications:
In case of VOD actinomycin should not be given until the main abnormalities have returned to normal and half the dose should be given for the first following course. If tolerated actinomycin dose may be increase progressively in the following cycles.
If the symptoms reappear during actinomycin treatment, this drug should be withdrawn permanently.

VOD is considered a serious adverse events and must be reported within 24 hours of knowledge of the event (see chapter 26) using the RDE system.

A.9 NEPHROTOXICITY GRADING

Table 11 - Nephrotoxicity Grading: Values

Toxicity Grade	GFR	Tm_p/GFR		HCO_3		EMUO
		Age <1 yr	Age ≥1 yr	Age <1 yr	Age ≥1 yr	
0	≥ 90	≥ 1.10	≥ 1.00	≥ 18	≥ 20	≥ 600 or normal response to DDAVP if tested
1	60-89	0.90 – 1.09	0.80 – 0.99	15.0 – 17.9	17.0 – 19.9	500 – 599
2	40-59	0.70 – 0.89	0.60 – 0.79	12.0 – 14.9	14.0 – 16.9	400 – 499
3	20-39	No symptoms but 0.60 – 0.69	0.50 – 0.59	No symptoms but 10.0 – 11.9	12.0 – 13.9	No symptoms 300 – 399 with no response to DDAVP if tested
4	≤ 19	HR or Myopathy or < 0.60	< 0.50	HCMA or < 10	< 12	NDI or < 300 with no response to DDAVP if tested

Tm_p/GFR = Renal threshold for Phosphate (mmol/l) which is calculated as

$$Tm_p / GFR = PO_{4(Plasma)} - \frac{PO_{4(Urine)} \times Creatinine_{(Plasma)}}{Creatinine_{(Plasma)}}$$

EMOU: Early Morning Urine Osmolarity (mOsm/kg)

HR: Hypophosphatemic Rickets: Defined by biochemistry (moderate or severe hypophosphatemia: < 0.90 mmol/l at < 1 year of age, < 0.80 at ≥ 1 year) with either clinical signs (genu valgus, bow legs, rickets rosary, cranial tabes, swollen wrists and ankles, abnormal gait, painful limb) or radiological features (wide epiphysal plate, expanded metaphysis, reduced bone density, secondary hyperparathyroidism with subperiostal erosion) or with both.

HCMA: Hyperchloremic Metabolic Acidosis: Defined by biochemistry (moderate or severe metabolic acidosis: HCO_3 < 15.0 at < 1 year of age, < 17.0 at ≥ 1 year; usually with moderate or severe hyperchloremia ≥ 112 mmol/l) with or without clinical symptoms (e.g. Kussmaul respiration)

NDI: Nephrogenic Diabetes Insipidus: Defined by clinical symptoms/signs (polyuria, polydipsia, dehydration) with or without biochemistry (moderate or severe hypernatremia < 150 mmol/l) with lack of response to DDAVP (a normal response is defined as a urine osmolality ≥ 800 mOsm/kg).

Table 12 - Nephrotoxicity Grading: Total Score

Sum scores	Total Score	Extent of nephrotoxicity
GFR + Tm_p/GFR + HCO_3 + EMUO	0	No nephrotoxicity
	1-3	Mild nephrotoxicity
	4-7	Moderate nephrotoxicity
	≥ 8	Severe nephrotoxicity

A.10 DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION
The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH
It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
The subjects must be volunteers and informed participants in the research project.
The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be informed on legally competent persons.
When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

A.11 Information Sheet /Consent Form

The following text is a suggested form of information suitable for parents of children who are being approached for

C. treatment according to the "observational study" (that includes low, standard and very high risk strategy):

A1 - INFORMATION SHEET FOR PARENTS
A2 – INFORMATION SHEET FOR OLDER PATIENTS
A3 - INFORMATION SHEET FOR YOUNGER PATIENTS

D. randomisation into high risk strategy.

B1 - ADDITIONAL INFORMATION SHEET FOR PARENTS OF PATIENTS WITH HIGH RISK TUMOURS
B2 - ADDITIONAL INFORMATION SHEET FOR OLDER PATIENTS WITH HIGH RISK TUMOURS
B3 - INFORMATION SHEET FOR YOUNGER CHILDREN

The text can be modified for use in discussions with older children/adolescents who may be giving their own consent. Local Research Ethical Committees may demand differing levels of written information as a part of the process of obtaining informed consent. Witnessed signed consent must be obtained for all patients who are entering the randomised study. The provision of written consent for patients allocated to the observational study (low, standard and very high arms), is a matter for individual institutions to agree in the context of their local ethical approval policies.
It is also advisable to have the family consent to the storage of biological material for future studies according to the rules existing in different countries.

European paediatric Soft tissue sarcoma Study Group
RMS 2005 Protocol

STUDY OF THE TREATMENT OF NON-METASTATIC RHABDOMYOSARCOMA IN CHILDHOOD AND ADOLESCENCE

A1 - INFORMATION SHEET FOR PARENTS (Observational Study)

Version 1.1, December 2004

BACKGROUND

Your child has recently been diagnosed with a tumour called a Rhabdomyosarcoma. This is a form of cancer that can occur almost anywhere in the body and of which there are many different types. The treatment your child will need is influenced by several factors including his or her age, where the tumour has arisen, whether or not it has already spread, the exact subtype of the tumour and the extent to which it can be removed by operation at the start of treatment.

A complete removal is usually not possible and it is often better to try to bring the tumour under control with drug treatment first. This drug treatment is called chemotherapy. Children with Rhabdomyosarcoma almost always need chemotherapy (anti-cancer drug treatment), it may then be necessary to follow this by (further) surgery and/or radiotherapy (x-ray treatment). Many children with Rhabdomyosarcoma can be cured but it is still necessary to collect further information about the treatment they have received, whether that is chemotherapy, surgery or radiotherapy to learn more about the best way of treating such patients in the future. Some patients with a more aggressive form of the tumour, or in whom the tumour has spread, may need more intensive therapy. These patients will be invited to take part in a different (randomised) trial, if your child is within this sub-group your doctor will inform you of this and give you further information about the trial.

1. What is the purpose of this study?

For many patients the purpose is to treat children in a systematic way according to an internationally agreed treatment protocol and to document their response to treatment in order to identify in a large number of patients, how the treatment of Rhabdomyosarcoma can be optimised. For some patients who have tumours that are thought to be more difficult to treat, they will be invited to take part in the randomised part of this study and further information will be given on an additional information sheet.

2. Why has my child been chosen?

Your child has been diagnosed with a Rhabdomyosarcoma and fulfils the eligibility criteria for this study.

3. Does my child have to take part?

It's up to you and your child whether or not to take part. If you decide to take part you will be given these information sheets to keep and asked to sign a consent form. If you and your child decide to take part, you are free to withdraw at any time without having to give a reason. Your doctor may wish to withdraw your child from the study if it is felt to be in his/her best interest. A decision to withdraw or not take part at all will not affect the standard of your child's care or the relationship with your child's doctor. You may take part in the clinical study without agreeing to have your child's tumour stored for a biological study (details to be given in an attached sheet).

4. What will happen to my child if we take part?

Your child will be treated according to the E*p*SSG protocol appropriate for your child's tumour depending on where it has occurred, whether it has spread, how large it is and what sub-type of Rhabdomyosarcoma it is. All chemotherapy has side effects. Your doctor will discuss these in detail with you. The commonest side effect of chemotherapy is a temporary poor functioning in the bone marrow. This causes an increased

susceptibility to infection for the whole duration of treatment. You will be instructed what to do if your child has a fever or appears unwell during this time. This side effect is temporary and your child's ability to fight infection will return to normal by six months from the end of treatment. Your child may also need blood and platelets transfusions during the course of treatment. There are also some drug specific side effects, some of which can be permanent (e.g. kidney damage from Ifosfamide) but the risk of these problems is low and your doctor will explain them in more detail. For some patients who receive a high dose of chemotherapy or who receive radiotherapy that includes the pelvis, there may be a possibility of infertility in later life. Your doctor will discuss your child's individual risk

The treatment is likely to last approximately six/seven months. Your child may also receive radiotherapy or further surgery. The details and side effects of which will be explained to you by the treating doctor.

For all children with Rhabdomyosarcoma we would like to store a small amount of blood together with a small piece of tumour that is left over after making the diagnosis and/or at a further operation to remove the tumour after treatment. These stored specimens will be used for scientific research to improve our understanding of Rhabdomyosarcoma. Both frozen and standard pathology wax blocks of tumour will be stored. Any research studies using your child's sample will only be undertaken once they have received full ethical approval.

5. Will there be any inconveniences?

We do not anticipate there will be any inconveniences over and above the normal treatment for Rhabdomyosarcoma from taking part in this study.

6. What are the possible benefits of taking part?

Whether or not you decide to take part your child will receive the best possible medical care. By taking part in this study and by looking in more detail to the different risk factors for Rhabdomyosarcoma (age at diagnosis, size of tumour, sub-type of tumour and whether it has spread), we will learn about how to optimize treatment it in the future. We hope to learn more about why some tumours do well and where - for those who do less well - to improve treatment for children with Rhabdomyosarcoma in the future. We are asking your permission to keep records of your child's treatment.

7. What are the possible risks of taking part?

There are usually no extra risks involved in collecting data or samples for storage for research. We are asking your permission to collect detailed information about your child's treatment.

8. Will my child taking part in the study be kept confidential?

With your consent we will be informing your GP about your child's participation in the study. If you agree your child's medical notes may be inspected by authorised professionals other than those involved directly in your child's care. Information on all patients entered into this study will be kept at the _____ (the relevant data centre, i.e. for the UK the United Kingdom Children's Cancer Study Group Data Centre in Leicester) where it will be retained. Data including your child's initials, date of birth, his/her diagnosis and the extent of the tumour, details of the treatment, any side effects and tumour response, and whether tissues have been stored will be recorded. Information relating to your child's treatment will then be forwarded electronically to an international database in Italy. No personally identifiable information will be released in this way. Only limited clinical information on your child's diagnosis and response to treatment will be sent to the central tumour office, in accordance with normal standards of medical confidentiality and data protection. Similar limited clinical information may be passed onto researchers in other countries, but this information will be anonymous.

9. What will happen to the results of the research study?

The results of this study will be published in a medical journal once the study has been completed and all patients have been followed up for at least one year. Your child will not be identified in any publication. The data are also examined every year by an independent data monitoring committee of experts in the field of childhood cancer. They can recommend early closure of the study if there are concerns about side effects of treatment or if some patients appear to do better than others.

10. Who is organising and funding the research?
This research is being organised by the European paediatric Soft tissue sarcoma Study Group. This group includes experts from a number of countries throughout Europe and other collaborating centres who have considerable experience in the treatment of this tumour. The decision to recommend this study in this country has been made by ….. (the relevant country oganisation, i.e. for the UK the United Kingdom Children's Cancer Study Group) which represents all the doctors in this country who treat children with soft tissue sarcomas.

11. What if I have any other concerns?
If you have any concerns or other questions about this study or the way it has been carried out you should contact the investigator [name, details] or you may contact the hospital complaints department.

Thank you for taking the time to read this information sheet and for taking part in the study if you agree to do so.

Contact details:

European paediatric Soft tissue sarcoma Study Group
RMS 2005 Protocol

STUDY OF THE TREATMENT OF NON-METASTATIC RHABDOMYOSARCOMA IN CHILDHOOD AND ADOLESCENCE

A2 - INFORMATION SHEET FOR OLDER PATIENTS (Observational Study)

Version 1.1, December 2004

BACKGROUND
You have recently been diagnosed with a tumour called a Rhabdomyosarcoma. This is a form of cancer that can occur almost anywhere in the body and of which there are many different types. The treatment you will need is influenced by several factors including where the tumour has arisen, whether or not it has already spread, the exact subtype of the tumour and whether it can be removed by operation at the start of treatment.
A complete removal is usually not possible and it is often better to try to bring the tumour under control with drug treatment first. This drug treatment is called chemotherapy. The vast majority of people with Rhabdomyosarcoma need chemotherapy (anti-cancer drug treatment),. It may then be necessary to follow this by (further) surgery and/or radiotherapy (x-ray treatment). We would like to collect information about the treatment you receive, whether that is chemotherapy, surgery or radiotherapy to learn more about the best way of treating patients like you in the future. Some patients with a more aggressive form of the tumour, or in whom the tumour has spread, may need more intensive therapy. If this applies to you, you will be invited to take part in a different randomised trial, you will receive further information about the trial.

1. What is the purpose of this study?
For many patients the purpose is to treat the tumour in a systematic way according to a treatment protocol that has been agreed in many different countries. If the response to treatment is documented in a large number of patients, it is hoped that we will better understand how the treatment of rhabdomyosarcoma can be improved.

2. Why have I been chosen?
You have rhabdomyosarcoma and are therefore eligible for this study.

3. Do I have to take part?
It is up to you whether or not you take part. If you decide to take part you will be given these information sheets to keep and asked to sign a consent form. You are free to withdraw from the study at any time without having to give a reason. Your doctor may also want to withdraw you from the study if it is felt to be in your best interest. A decision to withdraw or not take part at all will not effect the standard of your care or your relationship with your doctor and nurses.

4. What will happen to me if I take part?
You will be treated according to the E*p*SSG protocol appropriate for your type of tumour, depending on where it is, whether it has spread, how large it is and what subtype it is.
All chemotherapy has side effects, your doctor will discuss these with you in detail. The commonest side effect of chemotherapy is a temporary poor functioning in your bone marrow. This then reduces your ability to fight infection throughout your whole treatment. You will be told that if you have a temperature or you feel unwell that you must contact your doctor straight away. This is a temporary side effect and once the treatment is finished your ability to fight infection will return to normal within 6 months.
You may also need blood and platelet transfusions during the treatment because your bone marrow will not be making these properly.
There are other specific side effects some of which can be permanent, eg. kidney damage from the Ifosfamide, but the risk of these problems is low and your doctor will explain them in more detail. For some

patients who receive high dose chemotherapy or radiotherapy that includes the pelvis there may be a possibility of infertility in later life, again your doctor will discuss your individual risks.
The treatment is likely to last approximately 6 months. You may also receive radiotherapy or further surgery depending on the size and place of your original tumour. The details of the surgery and radiotherapy will again be explained to you by the treating doctor.

For all people with rhabdomyosarcoma we would like to store a small amount of blood, together with a small piece of the tumour that is left over after making the diagnosis and / or a further operation to remove the tumour. These stored specimens will be used for scientific research to improve our understanding of rhabdomyosarcoma. Any research studies, using your sample will only be undertaken once they have received full ethical approval.

5. Will there be any inconveniences?
We do not anticipate any inconveniences over and above the normal treatment for rhabdomyosarcoma from taking part in this study. We simply want to record the details of your treatment at a central database.

6. What are the possible benefits of taking part?
Whether or not you decide to take part, you will receive the possible medical care. By taking part in this study and allowing us to look in more detail at the different risk factors for rhabdomyosarcoma (such as where the tumour is placed, whether it has spread and what subtype it is) we hope that we will learn more about how to best treat it in the future. We are asking your permission to keep your records although these will be anonymised outside this hospital.

7. What are the possible risks of taking part?
There are usually no risks involved in collecting data or samples for storage for research.

8. Will my taking part be kept confidential?
We will be letting your GP know that you are taking part in this study with your consent and if you agree, your notes may be inspected by authorised professionals other than those directly involved in your care. Information on all patients entered into this study are kept at the _____
(the relevant data centre, i.e. for the UK the United Kingdom Children's Cancer Study Group Data Centre in Leicester) where it is kept and anonymised. Information relating to your treatment will then be forward electronically to an International Database in Italy. No personally identifiable information will be released in this way (i.e. it will all be anonymised). Only limited clinical information on your diagnosis and response to treatment will be sent to the Central Tumour Office, in accordance with normal standards of medical confidentiality and data protection.

9. What will happen to the results of the research study?
The results of this study will be published in a medical journal once the study has been completed and all patients who have been followed up for at least one year. You will not be identified in any publication. There is also a committee that monitors the study on a yearly basis, again your information will not be identifiable in this work.

10. Who is organising and funding the research?
This research is being organised by the European paediatric Soft tissue sarcoma Study Group. This group includes experts from a number of countries throughout Europe and other collaborating centres who have considerable expertise in the treatment of this tumour.

11. What if I have any other concerns?
If you have any concerns or questions about this study or the way it has been carried out you should contact the investigator in your centre [name, details] or you may contact the hospital complaints department.

Thank you for taking the time to read this information sheet and for taking part in the study if you agree to do so.
Contact details:

European paediatric Soft tissue sarcoma Study Group
RMS 2005 Protocol

STUDY OF THE TREATMENT OF NON-METASTATIC RHABDOMYOSARCOMA IN CHILDHOOD AND ADOLESCENCE

A3 - INFORMATION SHEET FOR YOUNGER PATIENTS (Observational Study)

Version 1.1, December 2004

This information sheet can be given or read to children as appropriate

Dear Patient

You have a lump or tumour called a rhabdomyosarcoma (RMS). We do not know why it has happened to you but we do know ways of trying to make you better. You will need a combination of treatments, these include medicines called 'chemotherapy', an operation to try and remove the tumour and also x-ray treatment called 'radiotherapy'.

We are trying to make the treatment for rhabdomyosarcoma better by lots of doctors in Europe working together to plan the best treatment for this tumour. We keep a register of all the children having treatment for RMS and this register is kept at another place in the UK. Some of the information from your treatment will also be sent by a computer to Italy.

If you want to know about the details of treatment, you can ask your nurse, doctor or your mum or dad to explain it some more.

European paediatric Soft tissue sarcoma Study Group
RMS 2005 Protocol

STUDY OF THE TREATMENT OF NON-METASTATIC RHABDOMYOSARCOMA IN CHILDHOOD AND ADOLESCENCE

B1 - ADDITIONAL INFORMATION SHEET FOR PARENTS OF PATIENTS WITH HIGH RISK TUMOURS (Research Study)

Version 1.1, December 2004

INTRODUCTION

You would have received an additional information sheet with background information about Rhabdomyosarcoma and other details of taking part in this study all of which applies to your child. This information sheet provides further details of a research study for those children with tumours that are more difficult to treat. This is a research study and before you decide whether to take part or not it is important to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. We would like you to take time to decide whether or not you wish to take part, however, a decision needs to be made before treatment is commenced.

1. What is the purpose of the study?

The purpose of the study is to find out whether the treatment for non-metastatic high risk Rhabdomyosarcoma can be improved by

a) the addition of an extra drug called Doxorubicin and/ or
b) the addition of "maintenance" chemotherapy following initial standard treatment.

2. Why have I been chosen?

You have been chosen because your child has a tumour that has been identified as higher risk (i.e. more difficult to treat because of various factors which may include, site, size, your child's age and the subtype of the Rhabdomyosarcoma).

3. Do I have to take part?

It is up to you and your child to decide whether or not to take part. If you both do decide you will be given this information sheet to keep and would be asked sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. Your doctor may wish to withdraw your child from the study if it is felt to be in their best interest. A decision to withdraw, or not to take part at all, will not affect the standard of your child's care or the relationship with your child's doctor.

4. What will happen to my child if we take part?

This is the randomised part of the study. Sometimes doctors do not know which is the best way to treat a particular tumour and we need to make comparisons. To make these comparisons patients are put into two groups with different treatment regimens. The groups are selected by a computer which has no information about the individual, i.e. by chance. This means that there is a 50/50 chance that your child will be allocated to one of two treatment regimens. In this study this can happen at two stages of the treatment.

a) At the beginning of treatment.

The aim of this part of the study is to find out whether the addition of a drug called Doxorubicin to the standard treatment regimen (this is three drugs, Ifosfamide, Actinomycin D and Vincristine) will improve the number of children cured with high risk Rhabdomyosarcoma. We do not now whether it will have any

impact on the tumour and the addition of Doxorubicin may increase the risk of infection to your child (through the increase in bone marrow suppression). It may also cause heart problems. The heart complications can happen both in the short term and longer term but the risk is small and your child will be monitored throughout treatment. If there is any evidence that there are problems with the heart, a decision will be made as to whether the doxorubicin needs to be stopped.

b) Once the initial treatment for your child is over

We also want to find whether extending the treatment for RMS beyond the standard treatment period (approximately 27 weeks) is of value in improving survival. There will therefore be a further randomisation between stopping treatment at the normal time (standard approach) and continuing maintenance chemotherapy for a further six month period.

The maintenance chemotherapy will consist of two chemotherapy drugs: Cyclophosphamide (which is given orally on a daily basis) and Vinorelbine, which is given intravenously (through your child's central line) three weeks out of every four.

There are two main side effects associated with this treatment the first is that your child will be immuno-suppressed for a longer period (a further six months) and the risk of infertility for your child in the long term may be increased. There may also be a risk of bleeding within the bladder due to the cyclophosphamide, this is usually reversible.

5. What are the alternatives for diagnosis or treatment?

If you or your child decide not to take part in the study then your child will be given the current standard treatment which is Ifosfamide, Vincristine and Actinomycin D.

6. Will there be any inconveniences?

It is likely that those patients who receive the additional Doxorubicin will spend longer in hospital as the treatment will be more intensive, and there may be a greater risk of infection during the treatment. All other treatment will be according to the standard protocol and not cause additional inconvenience over and above standard expected complications of treatment for Rhabdomyosarcoma.

7. What are the possible benefits of taking part?

There may not be individual benefits for your child in taking part, but it is hoped that information from the study overall will help to improve the treatment for children with rhabdomyosarcoma.

8. What are the possible risks of taking part?

There may be an increased risk of infective complications and need for blood products in patients receiving the additional doxorubicin and the other complications of the additional chemotherapy as detailed above.

9. What if I have any other concerns?

If you have any concerns or other questions about this study or the way it has been carried out you should contact the investigator [name, details] or you may contact the hospital complaints department.

Thank you for taking the time to read this information sheet and for taking part in the study if you agree to do so.

Contact details:

European paediatric Soft tissue sarcoma Study Group
RMS 2005 Protocol

STUDY OF THE TREATMENT OF NON-METASTATIC RHABDOMYOSARCOMA IN CHILDHOOD AND ADOLESCENCE

B2 - ADDITIONAL INFORMATION SHEET FOR OLDER PATIENTS WITH HIGH RISK TUMOURS (Research Study)

Version 1.1, December 2004

INTRODUCTION
You should have received an another information sheet with the background information about Rhabdomyosarcoma and other details of taking part in this study.

This information sheet provides further details of a research study for those people with tumours that are more difficult to treat. This is a research study and before you decide whether to take part or not it is important to understand why the research is being done and what it will involve. Please take time to read the following information and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. We would like you to take time to decide whether or not you wish to take part, however, a decision needs to be made before treatment is commenced.

1. **What is the purpose of the study?**
The purpose of the study is to find out whether the treatment for non-metastatic high risk Rhabdomyosarcoma can be improved by

a) the addition of an extra drug called Doxorubicin and/ or
b) b) the addition of "maintenance" chemotherapy following initial standard treatment.

2. **Why have I been chosen?**
You have been chosen because you have a tumour that has been identified as higher risk (i.e. more difficult to treat because of various factors which may include: where it is, the size of the tumour, your age or the subtype of the rhabdomyosarcoma).

3. **Do I have to take part?**
It is up to you whether or not to take part. If you do decide you will be given this information sheet to keep and will be asked sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. Your doctor may wish to withdraw you from the study if it is felt to be in your best interest. A decision to withdraw, or not to take part at all, will not effect the standard of your care or your relationship with your doctor.

4. What will happen to me if I take part?
This is the randomised part of the study. Sometimes doctors do not know which is the best way to treat a particular tumour and we need to make comparisons. To make these comparisons patients are put into two groups with different treatment regimens. The groups are selected by a computer which has no information about the individual, i.e. by chance. This means that there is a 50/50 chance that you will be allocated to one of two treatment regimens. In this study this computer allocation can happen at two stages of the treatment.

a) At the beginning of treatment.
The aim of this part of the study is to find out whether the addition of a drug called Doxorubicin to the standard treatment regimen (this is three drugs, Ifosfamide, Actinomycin D and Vincristine) will improve the number of people cured with high risk Rhabdomyosarcoma. We do not now whether it will have any impact on the tumour and the addition of Doxorubicin may increase the risk of infection (through the increase in bone marrow suppression). It may also cause heart problems. The heart complications can happen both in the short term and longer term but the risk is small and will be monitored throughout treatment. If there is any evidence that there are problems with the heart, a decision will be made as to whether the Doxorubicin needs to be stopped.

b) Once your initial treatment is over
We also want to find whether prolonging the treatment for RMS beyond the standard treatment period (approximately 27 weeks) is of value in improving cure rates. There will therefore be a further randomisation between stopping treatment at the normal time (standard approach) and continuing maintenance chemotherapy for a further six month period.

The maintenance chemotherapy will consist of two chemotherapy drugs: Cyclophoshamide (which is given orally on a daily basis) and Vinorelbine, which is given intravenously (through your central line) three weeks out of every four.

There are two main side effects associated with this treatment, the first is that you will be immuno-suppressed for a longer period (a further six months) and the risk of infertility for you in the long term may be increased. There may also be a risk of bleeding within the bladder due to the Cyclophosphamide, this is usually reversible.

5. What are the alternatives for diagnosis or treatment?
If you decide not to take part in the study then you will be given the current standard treatment which is Ifosfamide, Vincristine and Actinomycin D.

6. Will there be any inconveniences?
It is likely that those patients who receive the additional Doxorubicin will spend longer in hospital as the treatment will be more intensive, and there may be a greater risk of infection. For those patients randomised to receive maintenance chemotherapy there again may be more admissions to hospital because of infection. However, we do not know whether this will be balance against better cure rates in the long term. All other treatment will be according to the standard protocol and not cause additional inconvenience over and above the standard expected complications of treatment for Rhabdomyosarcoma.

7. What are the possible benefits of taking part?
There may not be individual benefits for you, but it is hoped that the information from the study overall, helps to improve the treatment for children for people with rhabdomyosarcoma.

8. What are the possible risks of taking part?
There may be an increased risk of infective complications and need for blood products in patients receiving the additional Doxorubicin and the other complications of the additional chemotherapy as detailed above.

9. What if I have any other concerns?
If you have any concerns or other questions about this study or the way it has been carried out you should contact the investigator [name, details] or you may contact the hospital complaints department.

Thank you for taking the time to read this information sheet and for taking part in the study if you agree to do so.

Contact details:

European paediatric Soft tissue sarcoma Study Group
RMS 2005 Protocol

STUDY OF THE TREATMENT OF NON-METASTATIC RHABDOMYOSARCOMA IN CHILDHOOD AND ADOLESCENCE

B3 - INFORMATION SHEET FOR YOUNGER CHILDREN (Research Study)

Version 1.1, December 2004

This information sheet can be given or read to children as appropriate

Dear Patient

This is an invitation to take part in a research study. Research means finding out about things and finding better ways of giving medicines. We have talked to your mum and dad about this research but we think it is important that you also understand what it is about. You do not have to agree to the research, you can say no and no one will get upset. If you want to know more about the research then you ask your doctor, nurse or mum or dad.

1. WHAT WILL HAPPEN IF I TAKE PART?

The normal chemotherapy treatment lasts about 6 months when treating rhabdomyosarcoma (RMS). We want to know 2 things about RMS:

1. We want to find out whether adding an extra chemotherapy medicine (Doxorubicin) to your normal treatment will help your tumour go away more easily.
2. We want to know if adding 6 months of mild chemotherapy treatment to your normal treatment will help the tumour to go away. We do not know whether the extra treatments will help and so, some patients in the research study will get the extra treatments and some patients will not.

2. ARE THERE ANY SIDE EFFECTS OF THE EXTRA TREATMENT?

There is more chance that you will be in hospital if you have the extra treatments, (either the Doxorubicin or the extra 6 months of therapy) but we hope that they will also be helpful in treating your tumour.

3. DO I HAVE TO TAKE PART?

No you do not, it is up to you or your mum or dad if you do not wish to take part and that is okay and no one will be upset.

If you do not take part, then we will give you the normal treatment that we are already using to make your tumour go away.

Thank you for reading this information sheet.

E*p*SSG-RMS-MET 2008: Treatment Arm for Metastatic Disease

BACKGROUND

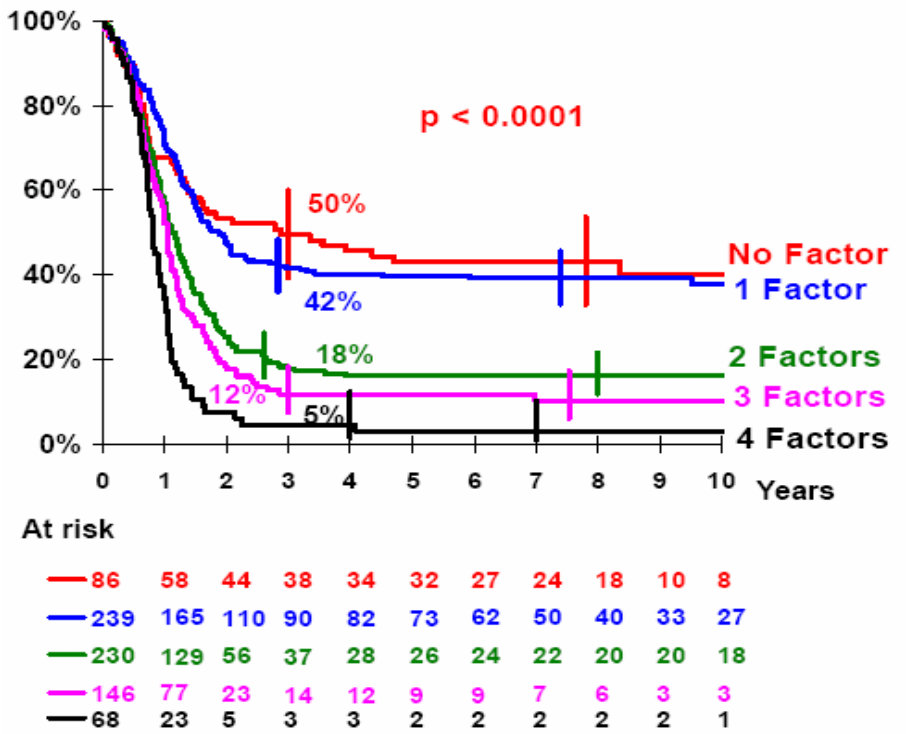
Although major accomplishments have been achieved during the last three decades for localized RMS (overall survival at 5 years is now 70%), overall survival of patients with metastatic RMS remains very poor. Despite impressive response rates observed with induction chemotherapy in various regimens [1-3], these tumors are difficult to cure, and the long-term event-free survival (EFS) of all series is below 30%, even after high dose chemotherapy with hematopoietic stem cell rescue [4,5].

Prognostic factors of clinical outcome of metastatic RMS have been investigated in several studies [4-7]. A recent pooled analysis of data from European and American studies since 1984 showed that the event-free survival of 788 patients with metastatic rhabdomyosarcoma reached a plateau of 27% at 3 years [8]. The univariate analysis showed that event-free survival (EFS) was correlated with several independent risk factors, among them being:

- Age
- Histology
- Site of the primary tumor
- Number of metastatic sites
- Presence/absence of bone or bone marrow involvement

The presence or absence of these prognostic factors defines the high risk group, as compared to the low risk group with a significant difference in outcome. The three-year EFS of patients who had none or one risk factor was 58% or 40%, respectively, whereas it was 22% or less for patients who had two or more risk factors (see Figure 1) [8]

Figure 1 Event-free survival of patients according to number of unfavorable prognostic factors



The standard chemotherapy for high risk RMS remains combination therapy with an alkylating agent (cyclophosphamide or ifosfamide), vincristine and actinomycin D. Despite clinical trials demonstrating the efficacy of individual drugs such as ifosfamide and cisplatin in the classical phase II setting and others such as melphalan, topotecan, irinotecan [9-14] in the phase II window setting, the value of adding other drugs to the standard combination regimen has not been fully demonstrated in terms of survival benefit for children with rhabdomyosarcoma [14,15]. Previous European studies in stage IV RMS often used the combination of 6 drugs for induction treatment in stage IV RMS [4,16-18]. For reasons described in chapter 8.4 (Rationale for high risk patients) the currently ongoing E*p*SSG study in localized RMS addresses the question of the value of dose intense doxorubicin combined with the standard combination IVA (IVADo). In this protocol for stage IV RMS we propose to offer metastatic patients the most intensive investigational treatment arm as standard induction treatment.

The vinorelbine-cyclophosphamide combination, which will be used during maintenance therapy, has shown activity in soft-tissue sarcoma in a pilot study (ORR 38% in recurrent or progressive RMS) [19] and is currently being evaluated in the phase II setting in relapsed rhabdomyosarcoma, Ewing's sarcoma, neuroblastoma, and medulloblastoma with an encouraging response rate in relapsing RMS. The efficacy of the maintenance regimen and its possibility for long-term treatment are conducive to the use of this combination for one year after the nine IVADo/IVA cycles of induction therapy in this patient population who are at high risk of early relapse.

The CWS group showed promising results with maintenance treatment in stage IV RMS [16]. They used standard chemotherapy in children with metastatic soft tissue sarcoma followed by high dose chemotherapy (thiotepa + cyclophosphamide and melphalan + etoposide) or an oral treatment with trofosfamide + idarubicine. The results in 62 patients are very promising with 3-year EFS above 50% for patients taking oral treatment (and EFS 20% after high dose). Since the comparison was not randomised a risk bias between the two groups must be taken into consideration. It seems though that oral maintenance therapy might have a greater benefit for group IV patients than does high dose chemotherapy.

The duration of treatment has been progressively decreased over years without apparently impairing the results. However, stage IV disease patients might benefit from a longer duration of treatment. Although in the European Intergroup Studies [4] 73% of patients achieved complete remission with the combination of intense induction treatment and local therapy, many patients suffered from relapse (5-year EFS 20%). A longer duration of maintenance treatment might be effective in treating minimal residual disease especially in the metastatic patient group. Therefore we propose maintenance treatment for a duration of 1 year.

1. Pappo A, Lyden E, Breneman J, et al. Up-front window trial of topotecan in previously untreated children and adolescents with metastatic rhabdomyosarcoma: an intergroup rhabdomyosarcoma study. J Clin Oncol 2001;19: 213-219.

2. Pappo A, Lyden E, Breitfeld P, ey al. Vincristine and irinotecan: A highly active combination in metastatic rhabdomyosarcoma. A report from the Soft Tissue Sarcoma Committee of the Children's Oncology Group (STSCOG). J Clin Oncol 2005;23: 8509 (abstract).

3. Lager J, Lyden E, Anderson J, et al. Pooled analysis of phase II window studies in children with contemporary high-risk metastatic rhabdomyosarcoma: a report from the Soft Tissue Sarcoma Committee of the Children's Oncology Group. J Clin Oncol 2006;24: 3415-3422.

4. Carli M, Colombatti R, Oberlin O, et al. European Intergroup Studies (MMT4-89 and MMT4-91) on Childhood Metastatic Rhabdomyosarcoma: Final Results and Analysis of Prognostic Factors. J Clin Oncol 2004;22: 4787-4794.

5. Koscielniak E, Rodary C, Flamant F, et al. Metastatic rhabdomyosarcoma and histologically similar tumors in childhood: a retrospective European multi-center analysis. Med Pediatr Oncol 1992;20: 209-214.

6. Anderson J, Ruby E, Lin M, et al. Identification of a favorable subset of patients (pts) with metastatic (MET) rhabdomyosarcoma (RMS): A report from the Intergroup Rhabdomyosarcoma Study Group (IRSG). Proc Am Soc Clin Oncol 1997;16: 1836 (abstract).

7. Breneman J, Lyden E, Pappo A, et al. Prognostic Factors and Clinical Outcomes in Children and Adolescents With Metastatic Rhabdomyosarcoma - A Report From the Intergroup Rhabdomyosarcoma Study IV. J Clin Oncol 2003;21: 78-84.

8. Prognostic Factors in Metastatic Rhabdomyosarcomas. The results of a pooled analyze from US and European cooperative groups. Odile Oberlin, Annie Rey, Elizabeth Lyden, Gianni Bisogno, Michael Stevens, William Meyer, Modesto Carli, James Anderson. Submitted to J Clin Oncol,

9. Hays DM. Rhabdomyosarcoma. Clin Orthop Relat Res. 1993; 289:36-49.

10. Rodriguez-Galindo C, Crews KR, Stewart CF, Furman W, Panetta JC, Daw NC, Cain A, Tan M, Houghton PH, Santana VM. Phase I study of the combination of topotecan and irinotecan in children with refractory solid tumors. Cancer Chemother Pharmacol. 2006; 57(1): 15-24.

11. Walterhouse DO, Lyden ER, Breitfeld PP, Qualman SJ, Wharam MD, Meyer WH. Efficacy of topotecan and cyclophosphamide given in a phase II window trial in children with newly diagnosed metastatic rhabdomyosarcoma: a Children's Oncology Group study. J Clin Oncol. 2004; 22(8): 1398-403.

12. Raney RB, Anderson JR, Barr FG, Donaldson SS, Pappo AS, Qualman SJ, Wiener ES, Maurer HM, Crist WM. Rhabdomyosarcoma and undifferentiated sarcoma in the first two decades of life: a selective review of intergroup rhabdomyosarcoma study group experience and rationale for Intergroup Rhabdomyosarcoma Study V. J Pediatr Hematol Oncol. 2001; 23(4): 215-20.

13. Pappo AS, Lyden E, Breneman J, Wiener E, Teot L, Meza J, Crist W, Vietti T. Up-front window trial of topotecan in previously untreated children and adolescents with metastatic rhabdomyosarcoma: an intergroup rhabdomyosarcoma study. J Clin Oncol. 2001; 19(1): 213-9.

14. Stevens M, Rey A, Bouvet N, et al. SIOP MMT 95: Intensified (6 drug) versus standard (IVA) chemotherapy for high risk non metastatic rhabdomyosarcoma (RMS). J Clin Oncol 2004;22(14S): 8515 (abstract).

15. Crist W, Gehan E, Ragab A, et al. The Third Intergroup Rhabdomyosarcoma Study. J Clin Oncol 1995;13:610-630.

16. T. Klingebiel et al, SIOP XXXV Annual Conference-Abstracts, MPO,Volume 41, Issue 4, page 278 (O.085), 2003

17. H. McDowell et al, SIOP XXXVII Annual Conference-Abstracts, PBC, Volume 45, Issue 4, page 411 (O.110), 2005

18. G. Bisogno et al., SIOP XXXV Annual Conference-Abstracts, MPO,Volume 41, Issue 4, page 278 (O.086), 2003

19. Casanova M, Ferrari A, Bisogo G, et al. Vinorelbine and low-dose cyclophosphamide in the treatment of pediatric sarcomas: pilot study for the upcoming European Rhabdomyosarcoma Protocol. Cancer 2004;101(7): 1664-1671.

OBJECTIVE

To improve the results in this poor prognosis group of patients administering the more intensive treatment IVADo plus 1 year of maintenance chemotherapy.

PATIENTS AND TREATMENT

Patients with the following criteria are eligible for the EpSSG-RMS-2005 protocol for Metastatic disease:

- A pathologically proven diagnosis of Rhabdomyosarcoma.
- Evidence of metastatic lesions, i.e. presence of any distant lesion other than regional lymph node involvement, e.g. bone or bone marrow disease, lung metastases, liver metastases, distant lymph node involvement (for definitions see Appendix 5), or patients with malignant effusion (i.e. tumour cell in peritoneal or pleural fluid) or malignant cells in the spinal fluid)
- Age less than 21 years (20 years and 364 days) of age.
- Previously untreated except for primary surgery.
- No pre-existing illness preventing treatment, in particular renal function must be equivalent to grade 0-1 nephrotoxicity, no prior history of cardiac disease and normal shortening fraction (> 28%) and ejection fraction (> 47%).
- No previous malignant tumours.
- Interval between diagnostic surgery and treatment no longer than 8 weeks.
- Diagnostic material available for pathology review.
- Available for long term follow up through the treatment centre.
- Written informed consent for treatment available.

Patients with a diagnosis of RMS not satisfying the above criteria will be registered, but not evaluated for the purpose of this study.

Patients with RMS N.O.S, Undifferentiated STS and Ectomesenchymoma are eligible for EpSSG-RMS-2005 protocol: see paragraph 29.4

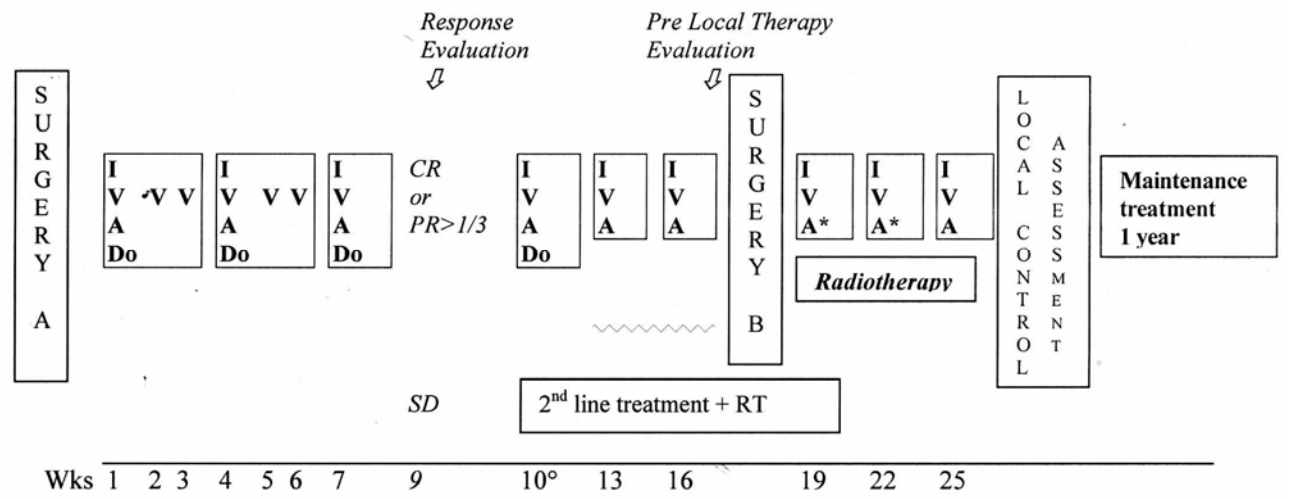
Notes
- Adults with RMS (> 21 years) may be eligible for registration and treatment on study (according to institutional preference)

After the diagnostic surgery primary re-operation can be considered, before chemotherapy starts, in selected cases (see paragraph 22.4).

Risk Groups

All patients with metastatic RMS (as defined in the inclusion criteria) are eligible for the EpSSG-RMS-2005 metastatic study. This includes the high risk and standard risk subgroups of metastatic RMS (see Section 16A). However, other treatment options may be available within the framework of national or international studies, especially for high risk patients. It is strongly recommended that each patient should be discussed with the national study centre in order to be aware of any other relevant studies.

Metastatic patients: Intensive Treatment



I Ifosfamide 3 g/m^2 is given as a 3 hour intravenous infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment. (Total IFO dose/course = 6 g/m^2).

V Vincristine 1.5 mg/m^2 (maximum single dose 2 mg) is given as a single intravenous injection on day 1 of each course and weekly, for a total of seven consecutive doses, from week 1 to 7.

A Actinomycin D 1.5 mg/m^2 (maximum single dose 2 mg) as a single intravenous injection on day 1 of each course of treatment.

Do Doxorubicin 30 mg/m^2 given as a 4-hour intravenous infusion daily on days 1 & 2 for courses 1-4 of treatment (total dose per course = 60 mg/m^2).

** Actinomycin should be given at the very beginning of RT (week 19) but may be omitted during RT (week 22). Caution is needed in the administration of week 25 ACT-D.*
For more details see chapter 23.11)

Interval between courses is 3 weeks and chemotherapy courses should not be started unless all these conditions are present: 2 x10^9/l WBC (or 1 x10^9/l neutrophils) + 80 x10^9/l platelets + absence of any relevant organ dysfunction.

For children $\leq$ 1 month VA only should be administered in the 1st cycle. For children $\leq$ 1 year (or $\leq$ 10 kg body weight) first cycle doses will be calculated by body weight and increased in the following cycles if tolerated. See chapter 24.4.1.

Growth factors may be used at the physicians' discretion. It is suggested to use them in case of life-threatening neutropenic CTC grade III-IV infection, or treatment delay $\geq$ 1 week due to toxicity after previous cycles.
For the use of growth factors see also chapter 27.2.

ASSESSMENT OF TUMOUR RESPONSE AND TREATMENT DECISIONS

- *1st assessment*: after the initial 3 cycles of chemotherapy (week 9) a full clinical and radiological assessment of the tumour response will be evaluated.

  Patients in CR or tumour volume reduction > 1/3 will continue the treatment they have been allocated at diagnosis.
  Patients with stable disease (SD: tumour volume reduction ≤ 1/3), will be eligible for 2nd line treatment (see chapter 20).

- *2nd assessment*: after 6 cycles of chemotherapy (week 18) a full clinical and radiological assessment of all tumour sites will be performed to plan local treatment. Any patient with progressive disease must proceed to 2nd line treatment.

➲ At this time local control modality must be decided

Surgery
In patients with metastatic disease surgery should be performed after cycle 6, so around week 19.

Where residual masses are demonstrated or in case of doubt, surgical resection should be done (surgery B), although there may be certain anatomical sites, particularly in the head and neck, where this may not be feasible and the final decision in these cases is left to the discretion of the individual Surgeon. Secondary operations are not indicated if clinically and radiologically (CT and/or MRI) there is no visible tumour (see chapter 22.5).
Secondary operations should, as a rule, be conservative but demolitive operations may be appropriate in certain circumstances. "Debulking" is not recommended. Particular care must be taken to ascertain completeness of resection.
Radical lymph node dissections are not indicated and involved lymph nodes should be irradiated, whether resected or not. There are rare occasions when, if radiotherapy is contraindicated (e.g. age ≤ 3 years), a lymph node dissection may be considered as definitive local treatment.
Week 19 chemotherapy (7th cycle) should begin after recovery from surgery B, and radiotherapy should start with the 7th chemotherapy cycle.

For general surgical guidelines see Chapter 22.

Radiotherapy Patients in local IRS Group II and III must have the primary tumour irradiated. Different doses will be delivered according to chemotherapy response and delayed surgery results (see Chapter 23 for details). Radiotherapy must be performed concomitantly with the 7th cycle (week 19).
If Surgery B is not possible and radiotherapy is decided this must be delivered beginning at week 19, after the administration of the 6th cycle.
Radiotherapy to the involved lymph node sites should be performed independently of histology, response to therapy, and surgical resection. (see paragraph 23.5)

Radiotherapy should also be given to all sites of metastatic disease, if feasible, regardless of response to therapy. Discretion by the treating clinician, with advice from the study's radiotherapy coordinator, if required, will be needed when multiple metastatic sites are present.

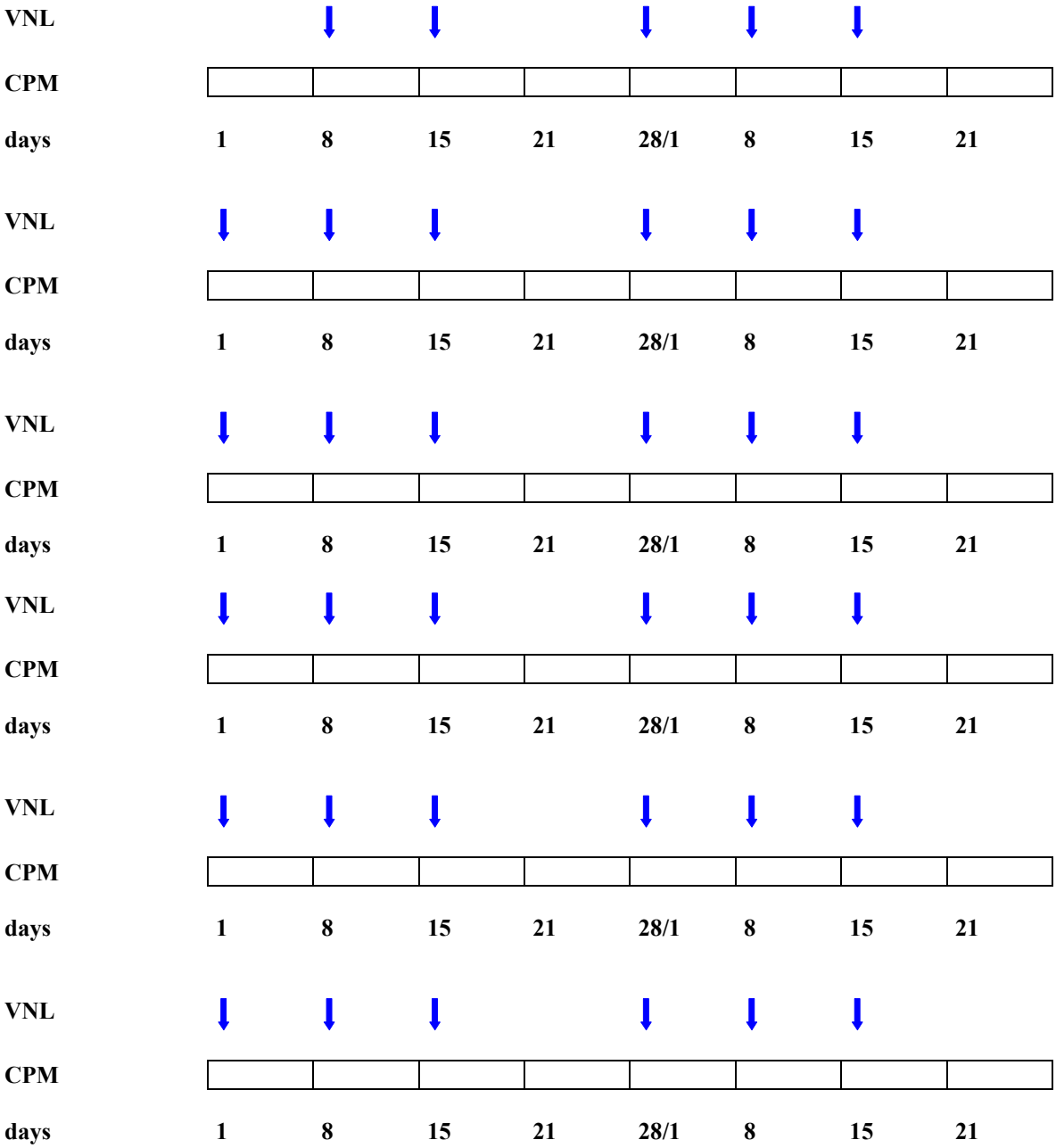
For general guidelines concerning radiotherapy see Chapter 23.

Radiation doses for distant metastases:
The number and localization of metastatic sites can be very variable, and the doses given here are a guide only. Patients have to be considered individually within the local multidisciplinary team, and if necessary discussed with the study's radiotherapy coordinator. In each case, consideration has to be given to the age of the patient, the normal tissues involved, the volume of disease and any medical co-morbidity. Surgery for metastatic disease also needs to be considered as adjunctive treatment. Treatment to distant metastatic sites will normally be given at the same time as primary and nodal radiotherapy.
For one or more lung metastases, whole lung radiotherapy is given. The usual dose will be 15 Gy in ten fractions with lung correction.
For bone metastases, and metastases at other sites, 30 Gy in up to 20 fractions depending on site, age and volume will usually be given.
In rare circumstances of very limited metastatic disease, where small volumes could safely be treated at higher doses of 40-50 Gy, this may be considered. Such exceptional cases should be discussed with the study's radiotherapy coordinator.
As these are all aggressive tumours, radiotherapy should also be considered in children less than 3 years of age, unless unacceptable sequellae are anticipated. General guidelines for irradiation of patients less than 3 years of age are given in paragraph 23.12.
Adjustments to the chemotherapy schedule are necessary during radiotherapy in particular for the administration of doxorubicin and actinomycin (see paragraph 23.11).

- *3rd assessment:* a third assessment must be performed after 9 courses of chemotherapy (end of standard treatment).
  At this point surgery should be reconsidered (Local control assessment) in case of residual tumour.

Metastatic patients: Maintenance Treatment

Following the 9th block of chemotherapy, surgery or a biopsy of what appears to be a possible residual tumour may be performed. Patients may not continue with the maintenance treatment if viable tumour is found and the clinician thinks that more intensive chemotherapy would be appropriate. However in presence of limited quantity of viable tumour maintenance treatment should be adopted.



VNL: Vinorelbine 25 mg/m^2 i.v. over 5-10 minutes day 1,8,15 of each cycle
CPM: Cyclophosphamide 25 mg/m^2 per os every day (no rest between cycles)

This treatment is given on an outpatient basis.

N.B. Cyclophosphamide is only available in capsules of 50 mg, which cannot be cut in smaller capsules so the doses should be divided over more days. Capsules should be administered early in the day and followed by adequate fluid intake to minimize bladder toxicity.
For drug administration details see also paragraph 24.2 and 24.3.

Figure

Figure 1 – EpSSG risk stratification for non metastatic rhabdomyosarcoma

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
Low Risk	**A**	Favourable	I	Any	N0	Favourable
Standard Risk	***B***	Favourable	I	Any	N0	Unfavourable
	C	Favourable	II, III	Favourable	N0	Any
	D	Favourable	II, III	Unfavourable	N0	Favourable
High Risk	***E***	Favourable	II, III	Unfavourable	N0	Unfavourable
	F	Favourable	II, III	Any	N1	Any
	G	Unfavourable	I, II, III	Any	N0	Any
Very High Risk	***H***	Unfavourable	I, II, III	Any	N1	Any

- **Pathology:**

Favourable = all embryonal, spindle cells, botryoid RMS
Unfavourable = all alveolar RMS (including the solid-alveolar variant)

- **Post surgical stage** (according to the IRS grouping, see appendix A.2):

Group I = primary complete resection (R0);
Group II = microscopic residual (R1) or primary complete resection but N1;
Group III = macroscopic residual (R2);

- **Site:**

Favourable = orbit, GU non bladder prostate (i.e. paratesticular and vagina/uterus) and non PM head & neck
Unfavourable = all other sites (parameningeal, extremities, GU bladder-prostate and "other site")

- **Node stage** (According to the TNM classification, see appendix A1 and A.5):

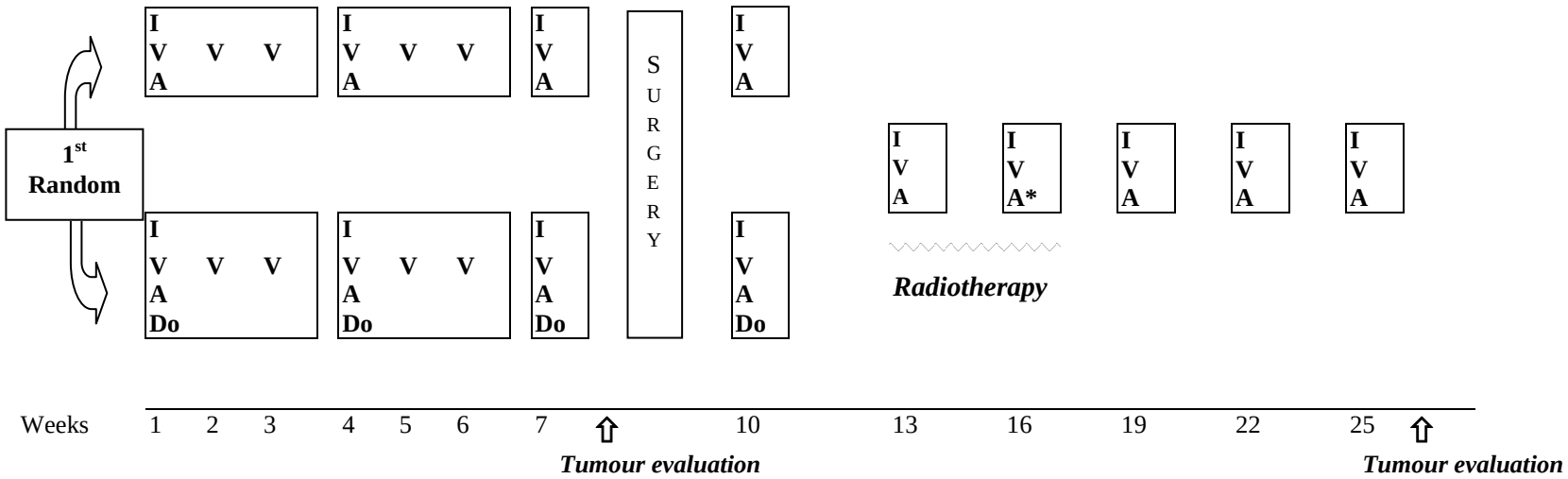
N0 = no clinical or pathological node involvement
N1 = clinical or pathological nodal involvement

- **Size & Age:**

Favourable = Tumour size (maximum dimension) $\leq$5cm *and* Age <10 years
Unfavourable = all others (i.e. Size >5 cm ***or*** Age $\geq$10 years)

Figure

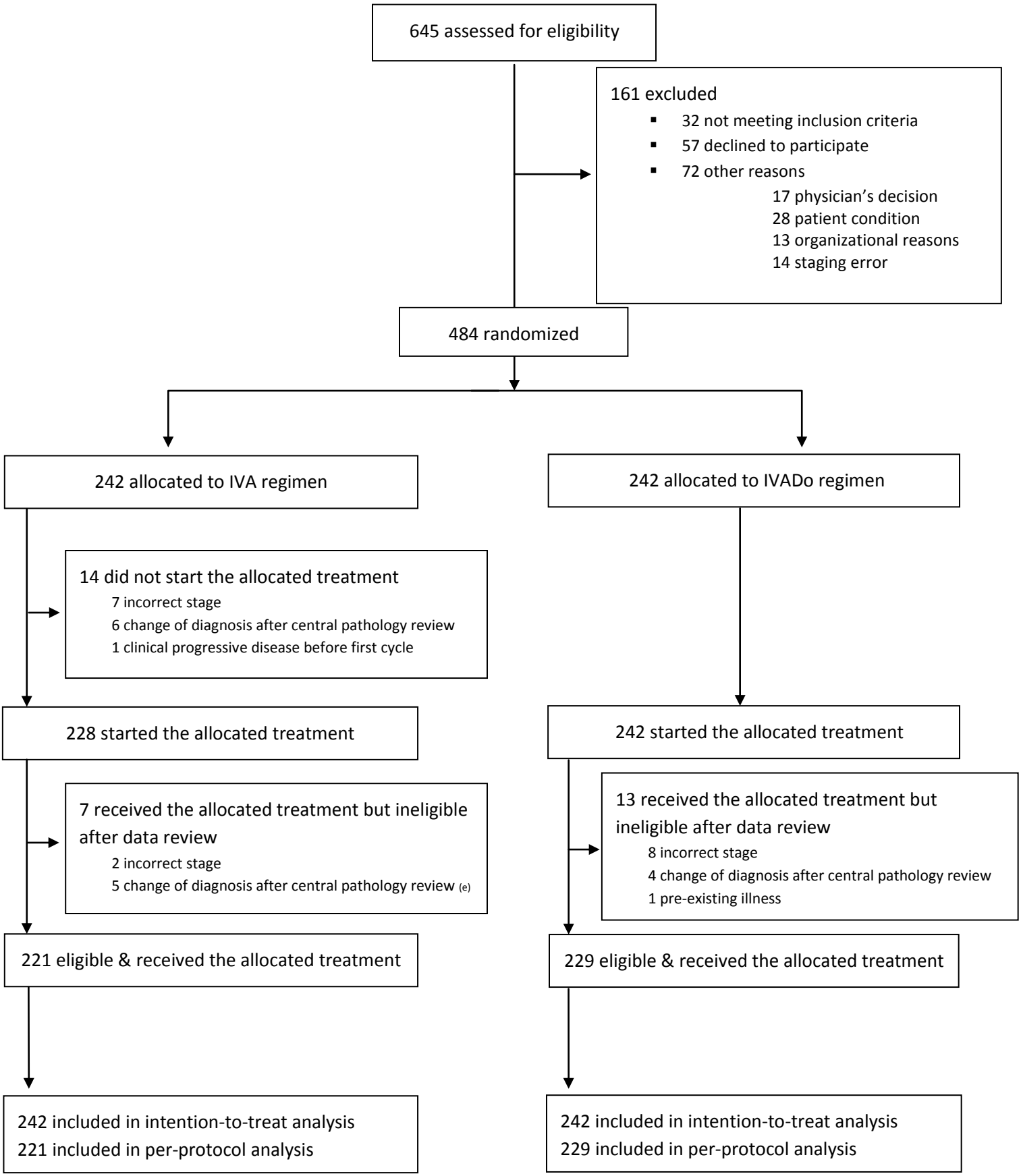
Figure 2 – Treatment plan for patients with high risk rhabdomyosarcoma



Legend:
I: Ifosfamide 3 g/m^2 is given as a 3 hour i.v. infusion daily, with Mesna (3 g/m^2) and hydration, on days 1 & 2 for each course of treatment, V: Vincristine 1.5 mg/m^2 (max. single dose=2 mg) is given as a single i.v. injection on day 1 of each course and weekly for a total of 7 consecutive doses, from week 1 to 7, A: Actinomycin D 1.5 mg/m^2 (maximum single dose = 2 mg) as a single i.v. injection on day 1 of each course of treatment, Do: Doxorubicin 30 mg/m^2 given as a 4-hour i.v. infusion daily on days 1 & 2 for courses 1-4 of treatment.
**Actinomycin may be given at the very beginning of RT (week 13) but is omitted during RT (week 16).*

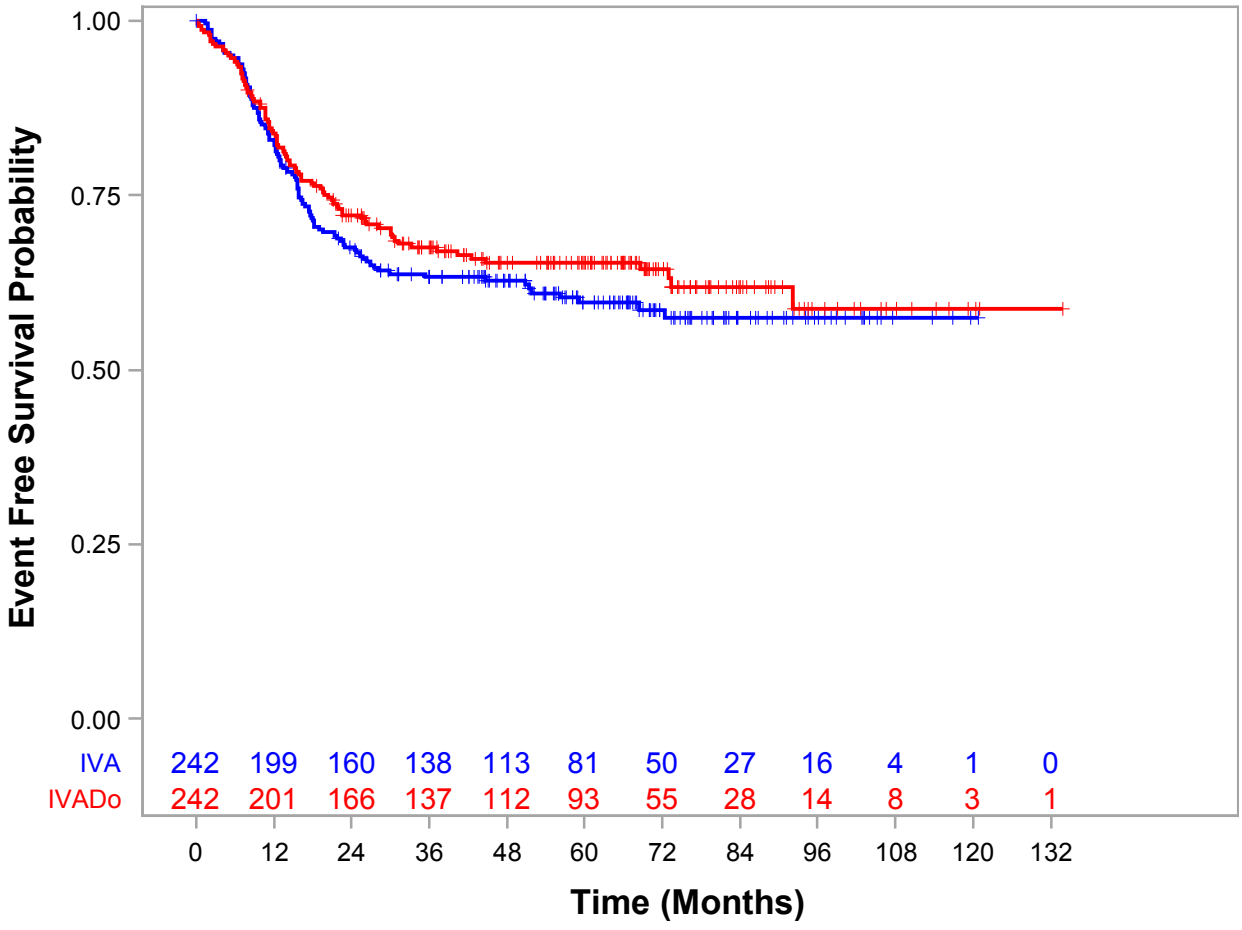
Figure

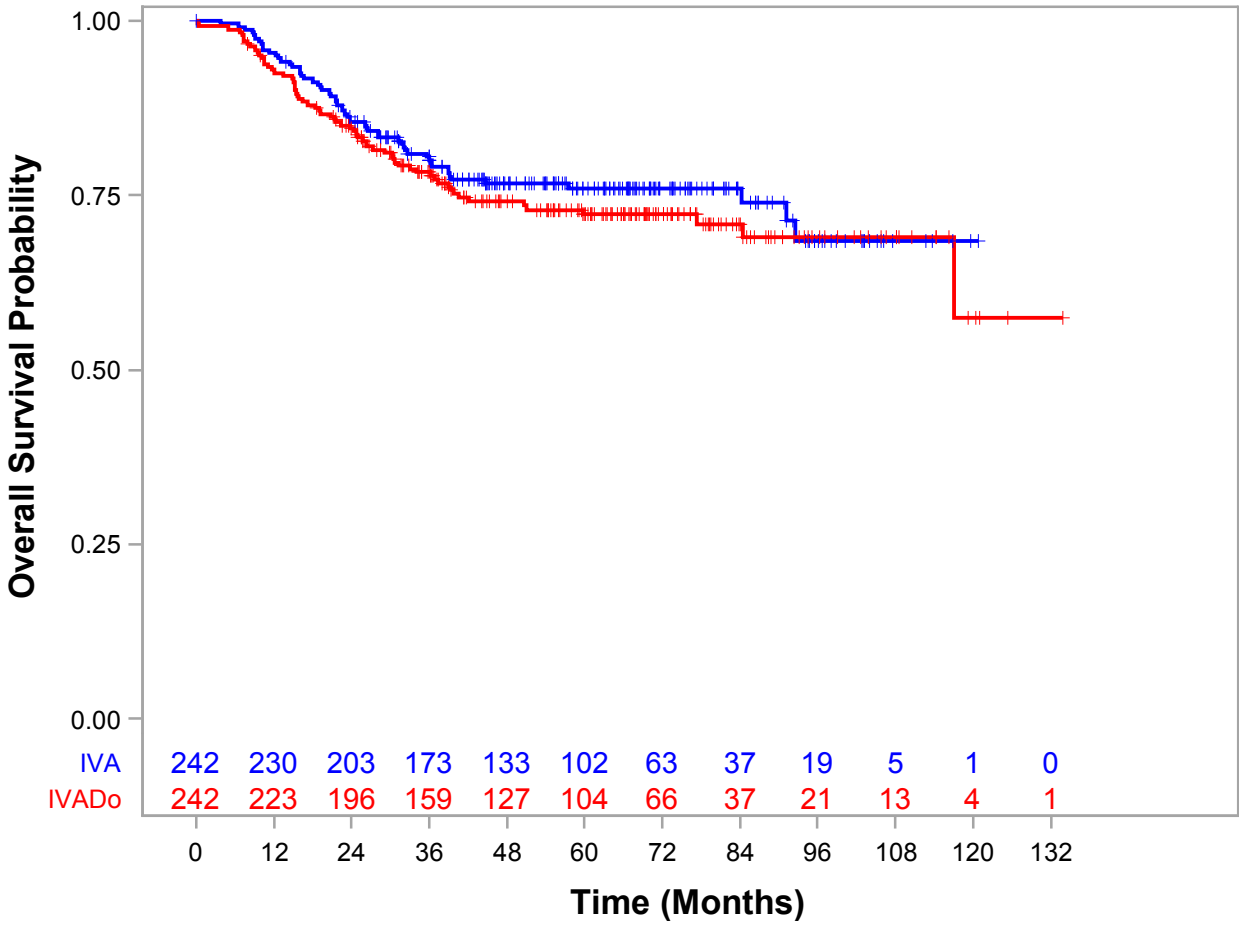
Figure 3: CONSORT diagram



Figure

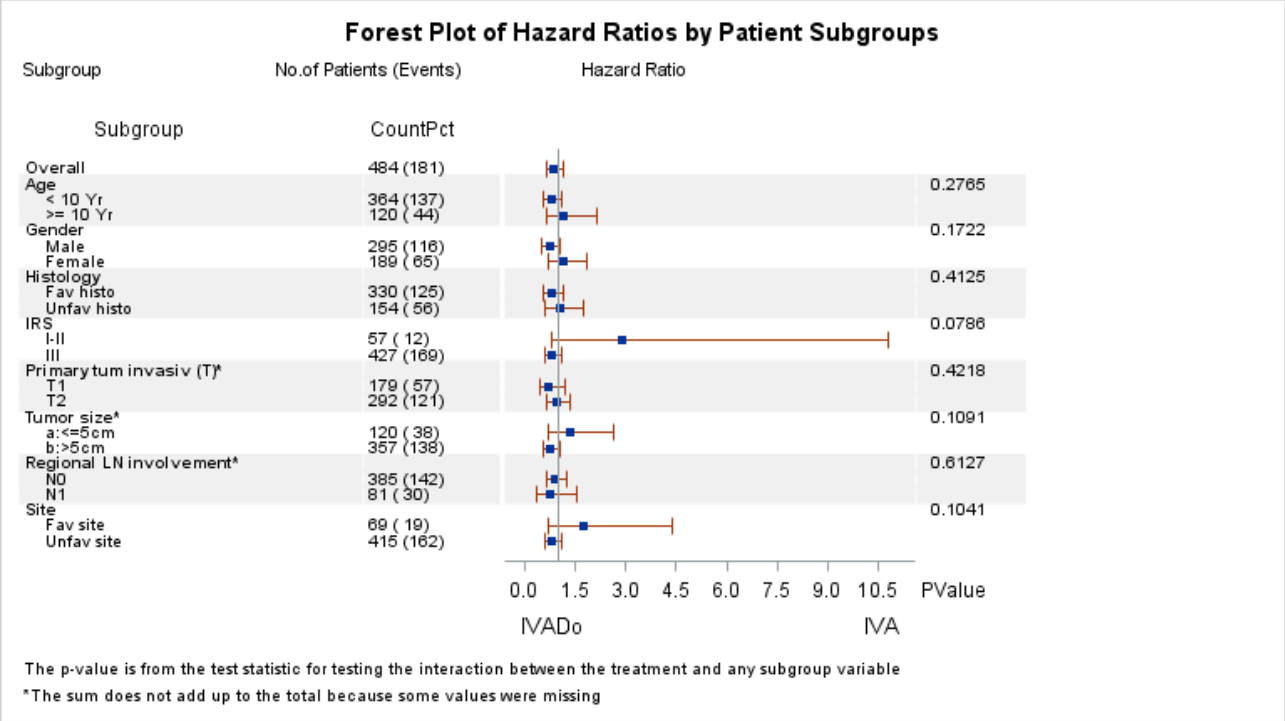
Figure 4: Kaplan-Meier plots for event-free survival and overall survival


Event Free Survival Probability
1.00
0.75
0.50
0.25
0.00
IVA 242 199 160 138 113 81 50 27 16 4 1 0
IVADo 242 201 166 137 112 93 55 28 14 8 3 1
0 12 24 36 48 60 72 84 96 108 120 132
Time (Months)


Overall Survival Probability
1.00
0.75
0.50
0.25
0.00
IVA 242 230 203 173 133 102 63 37 19 5 1 0
IVADo 242 223 196 159 127 104 66 37 21 13 4 1
0 12 24 36 48 60 72 84 96 108 120 132
Time (Months)

Figure

Figure 5. Event Free Survival by subgroups



The p-value is from the test statistic for testing the interaction between the treatment and any subgroup variable

*The sum does not add up to the total because some values were missing

Table

Table 1: Clinical characteristics of randomised patients by treatment arm

	Random A Arm IVA (n=242)	Random B Arm IVADo (n=242)	Total (n=484)
Country			
Belgium	13 (5.4%)	11 (4.5%)	24 (5·0%)
Brazil	5 (2.1 %)	6 (2.5%)	11 (2·3%)
Czech Republic	7 (2.9%)	8 (3.3%)	15 (3·1%)
France	66 (27.3%)	65 (26.9%)	131 (27·1%)
Israel	6 (2.5%)	4 (1.7%)	10 (2·1%)
Italy	64 (26.4%)	64 (26.4%)	128 (26·4%)
Norway	5 (2.1%)	2 (0.8%)	7 (1·4%)
Slovakia	1 (0.4%)	-	1 (0·2%)
Slovenia	-	1 (0.4%)	1 (0·2%)
Spain	16 (6.6%)	18 (7.4%)	34 (7·0%)
Switzerland	-	3 (1.2%)	3 (0·6%)
The Netherlands	10 (4.1%)	10 (4.1%)	20 (4·1%)
UK & EIRE	49 (20.2%)	50 (20.7%)	99 (20·5%)
Age (years) at diagnosis			
≤ 1 year	14 (5·8%)	4 (1·7%)	18 (3·7%)
>1 and <10 years	175 (72·3%)	171(70·7%)	346 (71·5%)
≥10 and <18 years	48 (19·8%)	60 (24·8%)	108 (22·3%)
≥ 18 years	5 (2·1%)	7 (2·9%)	12 (2·5%)
Gender			
Female	94 (38·8%)	95 (39·3%)	189 (39·0%)
Male	148 (61·2%)	147 (60·7%)	295 (61·0%)
Histology			
Alveolar RMS	76 (31·4%)	71 (29·3%)	147 (30·4%)
Botryoid RMS	11 (4·5%)	11 (4·5%)	22 (4·5%)
Embryonal RMS	153 (63·2%)	149 (61·6%)	302 (62·4%)
Not Otherwise Specified RMS*	1 (0.4%)	6 (2.5%)	7 (1·4%)
Spindle cells/Leiomiomatous RMS	1 (0.4%)	5 (2.1%)	6 (1·2%)
Pathology			
Favourable	165 (68·2%)	165 (68·2%)	330 (68·2%)
Unfavourable	77 (31·8%)	77 (31·8%)	154 (31·8%)
Post surgical tumour staging (IRS)			
Group I	2 (0·8%)	9 (3·7%)	11 (2·3%)
Group II	24 (9·9%)	22 (9·1%)	46 (9·5%)
Group III	216 (89·3%)	211 (87·2%)	427 (88·2%)
Primary tumour Invasiveness (T)			
T1 – Localized to the organ or tissue of origin	84 (34·7%)	95 (39·3%)	179 (37·0%)
T2 – Extending beyond the tissue or organ of origin	149 (61·6%)	143(59·1%)	292 (60·3%)
Tx – Insufficient information about the primary tumour	9 (3·7%)	4 (1·7%)	13 (2·7%)
Tumour size			
a: ≤ 5 cm	54 (22.3%)	66 (27.3%)	120 (24·8%)
b:> 5 cm	187 (77.3%)	170 (70.2%)	357 (73·8%)
x: not evaluable	1 (0.4%)	6 (2.5%)	7 (1·4%)
Regional lymph node involvement			
N0 – No evidence of lymph node involvement	191 (78.9%)	194 (80.2%)	385 (79·5%)
N1 – Evidence of regional lymph node involvement	42 (17.4%)	39 (16.1%)	81 (16·7%)
Nx – No information on lymph node involvement	9 (3.7%)	9 (3.7%)	18 (3·7%)
Site of origin of primary tumour			
Orbit	8 (3.3%)	8 (3.3%)	16 (3·3%)

	Random A Arm IVA (n=242)	Random B Arm IVADo (n=242)	Total (n=484)
Head neck	21 (8.7%)	13 (5.4%)	34 (7·0%)
Parameningeal	80 (33.1%)	81 (33.5%)	161 (33·3%)
Bladder Prostate	47 (19.4%)	39 (16.1%)	86 (17·8%)
Genito-urinary non Bladder Prostate	5 (2.1%)	14 (5.8%)	19 (3·9%)
Extremities	35 (14.5%)	36 (14.9%)	71 (14·7%)
Other sites	46 (19.0 %)	51 (21.1%)	97 (20·0%)
Subgroup risk			
High Risk (Subgroup e)	123 (50.8%)	126 (52.1%)	249 (51·4%)
High Risk (Subgroup f)	42 (17.4%)	39 (16.1%)	81 (16·7%)
High Risk (Subgroup g)	77 (31.8%)	77 (31.8%)	154 (31·8%)

Table 2: Type of event by randomised arm

	Random A Arm IVA (n=242)	Random B Arm IVADo (n=242)	Total (n=484)
Local relapse	30	29	59
Regional lymph-node relapse	2	6	8
Regional lymph-node relapse and metastases relapse	1	-	1
Local and regional lymph-node relapse	7	2	9
Local and regional lymph-node and metastases relapse	1	2	3
Local relapse and metastases	3	2	5
Metastases	15	17	32
Progressive disease	13	17	30
Progressive disease and regional lymph-node relapse	1	-	1
Toxic death	1	2	3
Switch to second line therapy (no due to PD)	19	5	24
Second Tumour	2	3	5
Death	1*	-	1
Total	**96**	**85**	**181**

* 1 patient died due to suicide

Table 3: Grade 3-4 adverse events experienced during the initial 4 cycles of chemotherapy

	IVA n=227	IVADo n=249	p-value°
Haematological Toxicity			
Haemoglobin	111 (48·9%)	195 (78·3%)	<0·0001
Leukocytes	194 (85·5%)	232 (93·2%)	0·0061
Neutrophilis	208 (91·6%)	236 (94·8%)	0·1706
Platelets	59 (26·0%)	168 (67·5%)	<0·0001
Non Haematological Toxicity			
Cardiac	3 (1·3%)	5 (2·0%)	0·5606
Hepatobiliary/pancreas	4 (1·8%)	1 (0·4%)	0·1459
Infection	128(56·4%)	198 (79·5%)	<0·0001
Renal	3 (1·3%)	-	0·0688
Neurology	18(7·9%)	15 (6·0%)	0·4137
Nausea/vomiting	33(14·5%)	51 (20·5%)	0·0893
Gastrointestinal	19(8·4%)	78 (31·3%)	<0·0001
Allergy	-	1 (0·4%)	0·3392
Skin	-	1 (0·4%)	0·3392

°Chi-square for comparison of grade 3-4 in arm A and B

***Reply to Reviewers Comments**

Manuscript reference number: THELANCETONCOLOGY-D-18-00297

We would like to thank you for considering the manuscript for publication in Lancet Oncology.
We have modified the paper according to the reviewers' suggestions, as outlined below point by point (in red our answer).

Reviewers' comments:

Reviewer #1: This article presents results from a randomised trial assessing the use of doxorubicin in patients with previously untreated rhabdomyosarcoma.

Major comments
*Randomisation was stratified by country and risk subgroup. The appropriate method of analysis is then to adjust for the stratification factors in the analysis (e.g. by including them as covariates in a Cox regression model) - failure to adjust for the stratification factors will lead to incorrect p-values and confidence intervals (e.g. see Improper analysis of trials randomised using stratified blocks or minimisation, Statistics in Medicine). The analyses in this paper were not adjusted for the stratification factors, and so may not have correct p-values. It would be useful to present a sensitivity analysis adjusting for these factors to ensure results are valid.
We agree with the reviewer and we have re-done the analysis as suggested but the final results did not change (and p values resulted exactly the same).
In the method section (page 8) we have also specified that "For the primary end point analysis HR was adjusted for the stratification factors at randomization."

Other comments
*It would be helpful to report treatment effect estimates and confidence intervals in the abstract
Done, as suggested

*It is not clear why the data monitoring committee suggested reducing the sample size from 600 to 500 - was this due to slower than expected recruitment, or for another reason?
EpSSG was funded by 3 Groups: 1) SIOP Malignant mesenchymal Tumors 2) Italian Soft Tissue Sarcoma Committee and 3) the German Cooperative Group (CWS). The initial sample size was therefore calculated taking into account the number of patients enrolled by these 3 different cooperative Groups in the previous protocols. For internal reasons CWS was not able to participate the EpSSG study in the end and this caused a slower than planned patients recruitment obliging us to reduce the total sample size. We are not sure this is of interest for the reader so we have added "The patients recruitment was slower than expected" in the statistical analysis paragraph page 7.

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*Page 7: "The estimate of the HR was 1.024:1.00" - it's not clear what this means.
To make this clear we have modified the sentence as follow: “The estimate of the HR was 1·024, IVADo vs IVA, with a p-value of 0·89

*In the methods section you state you obtained a p-value for futility, which led to the study being stopped for futility. It's not entirely clear how you obtained this p-value for futility. A clearer explanation would be useful.
We calculated the test statistic T as the ratio of the difference between the log relative risk estimate and the log relative risk of the alternative hypothesis and the standard error of the log relative estimate, being (0.02414-(-0.4308))/0.17288=2.631. The p-value associated with this statistical test was 0.004. Fleming, Harrington and O’Brien, suggested that a p-value less than 0.005 would be sufficient to stop early a trial and declare futility.
We have simplified the following sentence in the method section (page 7-8):
“Repeated testing of the alternative hypothesis has been performed to assess futility (log relative risk estimate= 0·02414, standard error = 0·17288, log relative risk β = -0·4308, T=2·631) obtaining a p-value of 0·004, suggesting the study could be stopped for futility.”

*It would be helpful to state explicitly what is meant by 'intention-to-treat', as this term is often used differently by different groups.
Patients have been analyzed according to the randomized treatment not according to the treatment they actually received according to the Consort statement definition.
We have added on page 8: “i.e. including patients in the group to which they were assigned, whether or not they received the allocated treatment”

*The CONSORT diagram lists the per-protocol analysis population, however this is not mentioned in the main text - was a per-protocol analysis performed?
Yes, but the analysis did not add any meaningful information so we did not mention it in the paper.
We added the following sentence in the method section (page 8):
A sensitivity efficacy analysis for the per protocol population, i.e. eligible patients that received the allocated treatment, was performed.
In addition the per protocol analysis has been reported in the Results section (page 10):
“450 patients met the criteria for the per-protocol analysis: the 3-yr EFS was 68·8% (95% CI 62·3 – 74·4) in the IVADo arm and 63·1% (95% CI 56·4 – 69·1) in the IVA arm (HR 0·82, 95%CI 0·60-1·10; p=0·1924). The 3-yr OS was 79·2% (95% CI 73·3 – 84·0) and 81·1% (95% CI 75·2 – 85·7) in the IVADo vs. IVA arm (HR 1·13, 95%CI 0·78-1·65, p= 0.5101).”

*In table 1 it would be helpful to give both the number and % within each treatment group.
Done, as suggested

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*For figure 4, it would be useful to change the y-axis to label to something other than 'survival', as this may lead readers to erroneously conclude the outcome is overall survival.
The labels have been changed.

*The methods section discusses some methods for subgroup analyses, but these are not presented in the results.
We have added the following sentence in the Result section and a new figure: “An analysis taking into account the most relevant clinical variables including age at diagnosis, gender, histological subtype, nodal involvement, primary tumour invasiveness, size, and site, did not show any difference among the two arms in any subgroup of patients (Figure 5).”

Reviewer #2: Bisogno G et al report the results of a randomized phase III study evaluating the addition of doxorubicin to standard IVA chemotherapy in children with high-risk RMS. Their study was well designed and provides the most convincing evidence to-date that doxorubicin does not improve outcome when included in multi-agent chemotherapy for RMS. The manuscript is well-written, and the data are presented clearly.

Major comments:
1) The abstract (page 2, line 22) states that IVA remains the standard therapy for localized RMS in Europe, but a similarly strong statement is missing from the discussion section. The authors should clarify how their results influence their recommendations for standard treatment.
We have added the same statement at the end of the discussion: “The IVA regimen remains the standard of care for patients with localised RMS in Europe.”

2) The observed 3-year EFS (65-68%) was substantially better than anticipated (page 7, line 11: 50%). The authors should comment upon the apparent improvement in outcome compared to the historic experience.
We agree with the reviewer. It is difficult however to find a clear explanation for the continuous survival improvement. We have added the following sentence in the discussion (page 12):
“Since the seventies a series of randomized clinical trials have been performed with the aim of improving the treatment of children with high risk RMS. None of the trials performed so far has been able to identify a chemotherapy regimen more effective than the standard VAC or IVA. Despite these “negative” results the survival of children with RMS has progressively increased over the years. The same has happened with this trial: we were not able to demonstrate that the “new” IVADo was more effective than IVA but the observed 3-year EFS for the whole population was substantially better than anticipated. This can been explained by a general improvement of care with better imaging, surgery and radiotherapy planning but one major reason may rely on the higher number of patients that received radiotherapy during first line treatment (85·1%) in comparison with previous European studies (approximately 60%) (5)”.

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3) Patients in this analysis were eligible for a second randomization at the completion of initial therapy to either stop therapy or receive maintenance. Although the results of the second randomization are not provided in this manuscript, the authors should report whether there was an imbalance in the use of maintenance chemotherapy (either as part of the second randomization or by physician choice) between the IVA and IVADo arms. If there was an imbalance in the use of maintenance chemotherapy, do the authors believe it could have influenced the primary results?

We have checked this possibility but 60 patients in *IVA* arm and 61 in the IVADo arm were randomized to receive maintenance. In addition 27 patients in the IVA arm and 28 patients in the IVADo arm received maintenance by physician choice (i.e. outside the randomized trial). Therefore no unbalance among the 2 groups was evident. The number of patients receiving maintenance have been added in the Result section (page 9): "Patients were also balanced considering the treatment received after the 9 cycle of chemotherapy: 87 patients in the IVA arm and 89 in the IVADo arm received maintenance because included in the second randomized trial or by physician choice."

4) According to the methods section (page 4, lines 18-19), patients with paratesticular alveolar RMS were excluded, but the EpSSG RMS 2005 protocol document does not include this exclusion (protocol page 32). The authors should explain this discrepancy.

Sorry the protocol was not clear on this point. Paragraph 8.4.4 of RMS2005 protocol is dedicated to Alveolar Paratesticular tumors and it is written they are not eligible for the randomized trial

5) If data regarding the use of G-CSF use was collected, it would be helpful to report its frequency by treatment arm in order to compare to other treatment regimens for which G-CSF use is common.

G-CSF was more often used in patients assigned to the IVADo arm. We reported this data in the Results section (page 10): "The higher rate of myelotoxicity in the IVADo arm prompted the investigators to a more frequent use of G-CSF that was administered in the 37·7% of cycles in the IVADo arm vs 22·5% in the IVA arm

Minor comments:

1) The abstract (page 2, line 7-8) states that patients with localized alveolar RMS without nodal involvement were eligible, but the methods section does not refer to exclusion of patients with alveolar RMS and nodal involvement. The methods section should be revised for clarity.

Figure 1 shows the EpSSG stratification where alveolar RMS and nodal involvement are assigned to the very high risk group and therefore they were not eligible to the randomized trial where only high risk patients were included.

2) Page 4, line 9: recommend revising to "...no evidence of DISTANT metastatic lesions..." for clarity.

Done

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3) Page 6, line 6: was the dose 51.4 Gy or 50.4 Gy for incomplete or no secondary surgery?

We have specified as follow: “51·4 Gy for cases of incomplete or unfeasible secondary resection”

4) VOD is only used once in the manuscript (page 10, line 4) and therefore does not need to be defined with an abbreviation.

done

Reviewer #3:

Major comments:

1. Although chemotherapy remains the mainstay of treatment for patients with rhabdomyosarcoma, the inability to control the bulk tumor at the primary site remains the by-far greatest challenge in children who present with radiographically localized tumors. The development of radiographically overt distant metastases at sites of previously occult disease, while not an insignificant issue, is a far lesser risk for most patients. The results of this study support these observations: the single most common cause of treatment failure in both chemotherapy arms was isolated local relapse (seen in just over 12% of all patients and representing a minimum of approximately one-third of all failure events), and either primarily or secondarily "uncontrolled" loco-regional disease (with or without the concurrent development of metastatic disease) accounted for nearly 80% of all "events". Fewer than 18% of treatment failures were isolated metastatic recurrences (32 of 181 events), and an additional 9 patients developed distant metastases in combination with local and/or loco-regional recurrence. Although 24 of the "events" involved "crossing over" to second-line therapy due to stable disease (of which 80% were cross-overs from IVA to IVADo), clearly the addition of 4 doses of doxorubicin to the neoadjuvant treatment regimen of children with radiographically localized "intermediate risk" rhabdomyosarcoma is insufficient to improve local control and has no impact on the already relatively-low risk of isolated metastatic treatment failure. Given the predominance of failure to achieve local control relative to all "failure" events, the magnitude of improvement in LOCAL CONTROL rates that would have been necessary to see in the doxorubicin arm would have been astronomical (and probably unrealistically so). Further confounding these results, is the observation that outcome for patients treated with the "standard" IVA regimen was substantially better than had been assumed for the purpose of study design (in fact, at 63% it exceeded the pre-determined improvement in 3-year EFS that would have been required to declare IVADo the "winner").

We agree with the reviewer that local events represent by far the most challenging aspect of RMS treatment and probably this explains why investigators have not been able to find a chemotherapy regimen more effective than standard VAC or IVA despite a series of randomized clinical trials run so far. It is also true that chemotherapy, as underlined also by the reviewer, remains a mainstay of RMS treatment and this is true not only for the control of distant disease.

In modern protocols local control measures are decided and implemented early in the treatment of RMS patients and this justify the intensification of the initial phase of

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chemotherapy with a not negligible dose of doxorubicin as planned in the RMS2005 trial. A better tumor response may allow a better (more complete and/or less aggressive) local control. In addition a better/more effective chemotherapy may have an impact reducing the rate of progressive disease (20.9% of tumor related event in our series). Finally in the 76 patients that showed local relapse as an event the local control of the tumor was achieved but the tumor later reappeared. A better chemotherapy regimen may contribute to avoid the persistence of minimal clinically undetectable disease and reduce the rate of relapse. For all these reason Oncologists are still committed to identify better chemotherapy regimens for RMS patients
As the reviewer underlines the EFS results in our trial were better than anticipated (see also reviewer 2). We have added a paragraph in the discussion (page 12) to comment this aspect.

2. The actual proportion of patients with "unfavourable" pathology (31.8%), almost all of whom had ARMS (either conventional or solid variant), is somewhere between 50-100% higher than the estimated proportion of patients with alveolar rhabdomyosarcoma cited in the Background section of the paper (15-20%). Separate figures (and statistical analyses) for 3-year EFS and OS for patients with ARMS (including by type of event) should be included. There should also be some commentary on why such an unexpectedly high proportion of patients with ARMS was seen and what impact, if any, this might have had on the results.
The 15-20% mentioned in the background section of the paper refers to the whole RMS population. Since a substantial part of patient with “favourable” (embryonal) RMS have been included in the low and standard risk arms of the RMS2005 protocol having one third of patients with ARMS in the high risk category is exactly as expected. ARMS were perfectly balanced in the 2 arms (31% in IVA and 29.3% in IVADO arm) so no impact on the randomized trial can be expected. The result according to clinical variables including histology have been added in the text (page 10) and a new figure (no. 5) has been included.

3. Please describe in more detail the reasons for and potential impact on the results of no radiation therapy being given to approximately 15% of the study subjects. The only Group I patients on this study were those with completely resected alveolar tumors who would have been scheduled to receive 41.4 Gy. Patients with Group III tumors of either histology that achieved a complete response (either to upfront chemotherapy or second-look surgery) were also to receive 41.4 Gy. It would also be worthwhile to know whether the proportion of patients receiving less than full-dose (50.4 cGy) OR boosted dose (55.8 cGy) XRT differed between the groups (i.e., was there any evidence that the use of IVADo chemotherapy allowed for comparable rates of local control to be achieved but with lower average radiation doses).

Please take into account that the number of irradiated patients is higher than in previous European trial and this may be one of the reasons because the overall results of the trial are better than anticipated.
Patients not receiving RT and the reason are reported in the following table:

	IVA	IVADo
	32	40
Young children (< 3 yrs)	14	13
Event before RT	5	8
histological complete remission (including amputation)	3	2
Change of diagnosis	3	1
Centre decision	7	16

So the number of not irradiated patients and the reasons were quite balanced in the 2 groups (the only minor unbalance was on Centre decision but number are small and it is difficult to further investigate the reason why the centre withhold radiotherapy). Based on these data we think there has not been any impact of no irradiation in the results achieved in the 2 arms of the randomized trial.

The median dose of external radiotherapy was 50.4 (range 35.2 – 64.8) in the IVA arm and 50.4 (range 35.0 – 61.2) in the IVADo arm.
We have found that 35% of patients received less than 50.4 Gy in the IVA arm and 46% in the IVADo arm. This may support the reviewer observation that the use of doxo may reduce the radiotherapy doses necessary to achieve a local control. This aspect may require further investigation from our radiotherapists, however the dose was administered according to protocol guidelines so any comparison may have some selection biases.

4. In Table 2, a type of event is described as "Progressive disease"; this appears to be described in the body of the manuscript (page 7, Outcome section) as "progression of a residual tumor" but this is somewhat confusing as many children with RMS (particularly ERMS) will have a period of marked shrinkage - but less than complete disappearance of their tumor - before there is evidence of local progression or relapse. While one can infer that this category refers to patients who never achieved apparent control of their primary site (i.e., a complete response), this should be clarified as distinct from local relapse.
These are common definitions for RMS: we define an event as a relapse when a complete tumor remission has been previously achieved and a progression of disease when the tumor increase its size (or the appearance of new lesions) in a case when complete remission has never been achieved. To make these concept more clear we have modified the definition on page 7, outcome section:
"…,progression of disease (complete tumor remission was never achieved)"

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1.Table 1: It's not clear why alveolar and solid alveolar RMS are listed as separate entities, what "mixed embryonal/alveolar" tumors are, or why "not otherwise specified RMS" appears to be included amongst those with "unfavorable" pathology.
We agree and we have modified the table according reviewer's suggestions: alveolar, solid alveolar and mixed embryonal/alveolar have been grouped together. How to treat patients with Not otherwise specified RMS has been a matter of discussion at the beginning of the study. See protocol RMS2005 chapter 29.4.1.5 RMS N.O.S. – subtype cannot be determined: "When subtyping is not possible, as a pragmatic decision and to avoid possible undertreatment of patients the risk group will be decided as per Alveolar RMS"

2.Introduction (page 3, line 20) : Inclusion of published results for outcome of patients with comparable-risk RMS, is on the low-side. The citation for the D9803 series (reference 4) actually understates the overall 4-year FFS (73% for VAC versus 68% for VAC/VTC).
It is in some way difficult make this comparisons but we have amended the survival results "increasing" them up to 70%

Patient
3.Methods (page 4, line 8): it's not clear what "pre-existing illness" would prevent treatment (on the next to last line of page 8, one child is described who had a "genetic syndrome" but this would not automatically preclude treatment).
This is a standard eligible criteria to avoid the inclusion of children that could not fully receive the treatment provided in the study. The child mentioned in the paper had Goldenhar syndrome with cardiovascular anomalies that prevented the administration of anthracyclines

4. Methods (page 4, line 15): it's not clear whether molecular confirmation of the presence of a FKHR translocation was required to classify a tumor as alveolar?
To make this point clear we have added the following sentence in the Method section (page 4): "Molecular confirmation of the presence of a FKHR translocation was not mandatory to classify a tumor as alveolar."

5. Methods (page 5, 8 lines from bottom): it remains not fully clear why patients with radiographic SD were considered the "same" as those with PD and taken off-study and switched to the other regimen.
This has been a common policies in all EU protocols to change the chemotherapy regimen after initial chemotherapy when the response is not considered satisfactory. We are aware this is not the strategy in use in north America but European studies are more in favor to consider the response to initial chemotherapy a prognostic factors with patients having a bad response having a poor outcome (Dantonello et al. Pediatr Blood Cancer. 2015 Jan;62(1):16-23). Therefore all Europena protocol advice to find a more effective chemotherapy in case of PD or SD after initial chemotherapy.

6. Methods (page 6, line 6): there is a typo in the dose given to those with gross disease (it should be 50.4 Gy).
Done, thank you

7. Statistical analysis (page 7, first line of section): check spelling of "enrol".
Done, thank you

8. Results (page 9): As above in Item 5, since neither of the Kaplan-Meier curve shows this measurement, it's not clear why Progression-Free Survival (distinct from Event-Free Survival) figures are described.
PFS figures are not provided but PFS results are described in the Results section page 9

9. Although the clinical trial clearly defines the groups that comprise the "high-risk" patients on this study, the use of this term to describe patients who would generally otherwise be considered to have "intermediate-risk" disease may be misleading. It might be helpful to specifically comment upon this difference in terminology.
Unfortunately different Cooperative Groups use different criteria and terminology to stratify patients. There is a substantial overlap between patienst included in the EpSSG high risk group and those included in the Children Oncology Group intermediate-risk group. Other Groups may use different terminology (for instance the German group uses the same definition used by EpSSG) so it is difficult to say that EpSSG high risk group patients are “generally” considered as intermediate-risk. There is an ongoing effort to have a common international definition to stratify patients. Discussing these aspects is however beyond the scope of the paper in our opinion.

Editorial comments

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1. Please ensure that the primary and secondary outcomes listed in the Methods and Results sections are consistent with those listed in your protocol, and trial registry, if applicable.
We confirm

2. Please could you provide an updated Research in context panel, in the new style with three sections:
Evidence before this study - This section should include a description of all the evidence that the authors considered before undertaking this study. Authors should briefly state: the sources (databases, journal or book reference lists, etc) searched; the criteria used to include or exclude studies (including any date restrictions of the search, ie, articles published between month/day/year and month/day/year), which should not be limited to English language publications; the search terms used; the quality (risk of bias) of that evidence; and the pooled estimate derived from meta-analysis of the evidence, if appropriate.
Added value of this study - Authors should describe here how their findings add value to the existing evidence.
Implications of all the available evidence - Authors should state the implications for practice or policy and future research of their study combined with existing evidence.

The research in the context panel has been updated with the new style

3. Please confirm that all authors who qualify for authorship for this manuscript (in adherence with ICMJE guidelines) are included in the authorship.
We confirm that all authors who qualify for authorship for this manuscript are included in the authorship.

4. Please confirm that all individuals who need to be acknowledged in this manuscript are in the Acknowledgments section.
We have added the following sentence: We would like to aknowlegde the contribution of Ilaria Zanetti for her valuable data management activities and Paola dal Bianco for her advices on the statistical design and analysis.

5. If your research is funded by a body with an Open Access agreement in place with Elsevier (ie, by one of the Research Councils UK, Wellcome Trust, Cancer Research UK, Arthritis Research Council, British Heart Foundation, UK Department of Health, UK Chief Scientist Office, Austrian Science Fund, or Parkinson's UK), please consider now which licence you would opt for, should the paper be accepted for publication. There are two options - gold Open Access and green Open Access. Further details can be found at http://www.thelancet.com/lancet-oncology-information-for-authors/open-access.

Our trial has not been funded by a body with an Open Access agreement in place with Elsevier.

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