Title

Metastatic rhabdomyosarcoma: results of the European *paediatric* Soft tissue sarcoma Study Group MTS 2008 study and pooled analysis with the concurrent BERNIE study

Running head: Metastatic rhabdomyosarcoma, pooled analysis MTS 2008 and BERNIE.

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Acknowledgements

The authors thank the patients, caregivers and medical staff involved in this study from the recruiting countries (United Kingdom, Ireland, France, Italy, The Netherlands, Spain, Israel, Argentina, Brazil, Belgium, Norway, Slovakia). The overall organisation of this study has been supported by Fondazione Città della Speranza. JCC is supported by the Giant Pledge through the Royal Marsden Cancer Charity

and this independent research is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at The Royal Marsden NHS Foundation Trust and the Institute of Cancer Research, London. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Previous presentations

The results of this study have been previously presented at the annual conference of the International Society of Paediatric Oncology (SIOP) in Lyon, October 2019, the annual Connective Tissue Oncology Society (CTOS) meeting in Tokyo, November 2019, and the European Society of Paediatric Oncology (SIOPE) annual meeting (virtual) in 2021. The Event Free Survival analyses for the BERNIE cohort have been reported previously (PMID: 28738258). In retrospect 1 patient was not eligible from the cohort described by Chisholm et al. and is excluded from the current analyses.

Context Summary

Key objective

To evaluate the efficacy of the addition of doxorubicin to standard chemotherapy (ifosfamide, vincristine, actinomycin D; IVADo) and the introduction of one year maintenance chemotherapy (cyclophosphamide, vinorelbine) in patients with metastatic rhabdomyosarcoma.

Knowledge generated

Outcomes in this study seem improved compared to historical cohorts, but owing to the design of the study it remains unclear if this is attributable to the addition of doxorubicin or maintenance chemotherapy, or may be explained by more consistent application of local therapy. Outcome for metastatic patients with adverse prognostic factors remains poor.

Relevance

The IVADo regimen followed by one year of maintenance chemotherapy is the current standard for metastatic rhabdomyosarcoma patients in Europe, but further studies are needed to validate the role of doxorubicin and role and duration of maintenance chemotherapy. Introduction of new strategies in frontline treatment is needed, to reduce treatment failure in patients with metastatic rhabdomyosarcoma.

Abstract

PURPOSE

Outcome for patients with metastatic rhabdomyosarcoma (RMS) is poor. This study presents results of the MTS 2008 study with a pooled analysis including patients from the concurrent BERNIE study.

PATIENTS AND METHODS

In MTS 2008, patients with metastatic RMS received 4 cycles of ifosfamide, vincristine, actinomycin D (IVA) plus doxorubicin, 5 cycles of IVA and 12 cycles of maintenance chemotherapy (low-dose cyclophosphamide and vinorelbine). The BERNIE study randomised patients to the addition or not of bevacizumab to the same chemotherapy. Local therapy (surgery/radiotherapy) was given to the primary tumour and all metastatic sites when feasible.

RESULTS

MTS 2008 included 270 patients (median age 9.6 years, range 0.07-20.8). With a median follow-up of 50.3 months, 3-year event free survival (EFS) and overall survival (OS) were 34.9% (95% CI 29.1-40.8%) and 47.9% (95%CI 41.6-53.9%) respectively. In pooled analyses on 372 patients with a median follow-up of 55.2 months, 3-year EFS and OS were 35.5% (95% CI 30.4-40.6%) and 49.3% (95% CI 43.9-54.5%) respectively. Patients with ≤2 Oberlin risk factors had better outcome than those with ≥3 Oberlin risk factors: 3-year EFS was 46.1% vs. 12.5% ($p<0.0001$) and 3-year OS 60.0% vs. 26.0% ($p<0.0001$). Induction chemotherapy and maintenance appeared tolerable; however, about 2/3 of patients needed dose adjustments during maintenance.

CONCLUSION

Outcome remains poor for patients with metastatic RMS and multiple Oberlin risk factors. Due to the design of the studies, it was not possible to determine if the intensive induction regimen and/or the addition of maintenance treatment resulted in apparent improvement of outcome compared to

historical cohorts. Further studies, with novel treatment approaches are urgently needed, to improve outcome for the group of patients with adverse prognostic factors.

Introduction

Rhabdomyosarcoma (RMS) is a very aggressive tumour with a strong tendency to metastasize. Outcome in patients with localised disease is generally good (1,2), but outcome for patients with metastatic RMS remains poor with 3-year overall survival (OS) of 34-56% (3,4). Various attempts to increase treatment intensity failed to improve survival (e.g. high dose chemotherapy with stem cell support) (5-8) or resulted in very limited improvement in selected subgroups of patients (dose compressed chemotherapy) (4).

The European *paediatric* Soft tissue sarcoma Study Group (E*p*SSG) has collaborated in three studies in newly diagnosed RMS in recent years. The E*p*SSG RMS 2005 study (conducted from 2005 to 2016) explored the added value of dose intense doxorubicin in combination with standard ifosfamide, vincristine and Actinomycin-D (IVADo) chemotherapy, and the role of six months of maintenance chemotherapy following completion of standard therapy in high risk localised disease (1,2). Concurrently with the opening of RMS 2005, the E*p*SSG and Innovative Therapies for Children with Cancer (ITCC) collaborated with Roche in the BERNIE study, a pharma-sponsored study for patients with metastatic soft tissue sarcoma (9). In this open-label, randomised phase II study (conducted from 2008 to 2013), patients received standard induction chemotherapy followed by a year of maintenance treatment with vinorelbine and low-dose cyclophosphamide. Patients were randomised to receive or not receive bevacizumab. The BERNIE study recruited 152 patients, including 102 with RMS. No benefit of bevacizumab on event free survival (EFS) was demonstrated (9).

Since the BERNIE study was open in a limited number of sites and had stringent inclusion/exclusion criteria, the single-arm E*p*SSG MTS 2008 study was introduced as an amendment to RMS 2005, utilising the same induction and maintenance chemotherapy as the BERNIE study but without bevacizumab, to capture data on patients with metastatic RMS who did not enter the BERNIE study.

The current study reports treatment, toxicity and outcome of metastatic RMS patients treated within the MTS 2008 study. As a secondary objective, in order to address potential selection bias introduced

by the concurrent BERNIE study, we performed a pooled analysis of MTS 2008 and BERNIE study results. For the purpose of the current analysis, results from the BERNIE study were updated and mature overall survival data were reported for the first time.

Patients and methods

Study design and participants

MTS 2008 was an academic, international, prospective study (NCT00379457) involving 74 hospitals across 11 countries. Patients <21 years with a histological diagnosis of RMS (excluding pleomorphic RMS) with distant metastatic disease, <8 weeks between diagnostic surgery/biopsy and start of chemotherapy, who had received no prior chemotherapy or radiotherapy were eligible.

Concurrent with the MTS 2008 study, patients were recruited to the BERNIE study (BO20924/ITCC-006; NCT00643565) (10). Updated data from the final BERNIE Clinical Study Report were used for the current overall survival analyses.

Detailed eligibility criteria for both studies can be found in Supplemental Table 1.

Both studies were conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. All participating centres were required to obtain approval from their local authorities and ethics committees, and written informed consent from patients and/or their parents or legal guardians.

MTS 2008 Treatment

Induction chemotherapy comprised 9 x 3-weekly cycles including 4 cycles of IVADo and 5 cycles of IVA (1) (Supplemental Figure 1a). Maintenance chemotherapy comprised 12 x 28 day cycles of intravenous vinorelbine and low-dose oral cyclophosphamide (2) (Supplemental Figure 1b). Chemotherapy was identical to the standard treatment arm of the BERNIE study, where in the

investigational arm patients received the same chemotherapy treatment with the addition of bevacizumab every 3 weeks on day 1 of each cycle and every 2 weeks during maintenance (9). Growth factors were allowed at the physicians' discretion. Adverse events (AEs) were graded according to the Common Terminology Criteria for Adverse Events (v3.0). Only the following AEs ≥ grade 3 were recorded: infection (proven or suspected), cardiomyopathy, neuropathy, mucositis or venoocclusive disease (VOD).

Surgical resection of residual primary tumour was considered after the 6th chemotherapy course (week 19 onwards), generally avoiding mutilating surgery. Resections were only recommended if a R0 (microscopically margin-negative resection) or R1 (macroscopic resection with positive microscopic margins) resection seemed feasible. R2 resection (macroscopic residual) and radical lymph node dissections were not recommended.

Radiotherapy was recommended to the primary tumour site and, if feasible, to all metastatic sites, regardless of response to chemotherapy, starting concomitantly with the 7th chemotherapy cycle. Dose to the primary tumour was adapted to primary tumour response and histology (Supplemental Table 2). Whole lung radiotherapy was recommended for patients with one or more lung metastases. Since the number of metastatic sites and the size of the metastases can vary and can be very extensive, the local multi-disciplinary teams considered each patient individually, involving the study's radiotherapy coordinator if needed.

<u>Response assessment</u>

First response assessment was scheduled after three cycles of chemotherapy (week 9). In case of insufficient response (≤1/3 volume reduction), patients were eligible for second line treatment. Alternatively, participation in the VIT-0910 study was considered, evaluating the addition of temozolomide to the combination of vincristine and irinotecan (10). A second response assessment was scheduled preceding local treatment, after six cycles of chemotherapy (week 18). Response of

the primary tumour was measured as volume reduction; response of metastatic lesions was measured according to the Response Evaluation Criteria in Solid Tumours (RECIST v1.0) (11,12).

Statistical methods

Differences between cohorts were compared with the Chi square or Fisher's exact test, depending on frequency distribution of each variable. Survival probabilities were estimated by use of the Kaplan-Meier method and the log-rank test. Event free survival (EFS) was defined as time between diagnosis and disease progression, recurrence, refusal of therapy, suspension of treatment due to toxicity or death due to any cause. Overall survival (OS) was defined as time from date of diagnosis up to death for any reason. Patients still alive at the end of the study or lost to follow up were censored, both in the EFS and OS analyses, at the date of last observation. EFS and OS were evaluated by prognostic factors identified in the joint European-Children's Oncology Group study published by Oberlin et al. (3) (Oberlin risk factors), being: age, site, bone or bone marrow involvement and number of metastatic sites. Of note: parameningeal primary tumour site was grouped as favourable by Oberlin et al. (3).

Results

Patient characteristics MTS 2008

Between October 2010 and December 2016, 324 patients were registered in the MTS 2008 study; 54 patients were reported not eligible for the following reasons: age >21 years (n=11), included in another protocol (n=6), other previous treatments (n=16), no written informed consent (n=7), pathology not available for central review (n=5), interval from surgery to chemotherapy start exceeded eight weeks (n=7), staging error (n=1) or rhabdomyosarcoma diagnosis not confirmed by pathology (n=1) (Supplemental Figure 2). The remaining 270 patients (median age 9.6 years, range 0.1-20.8 years, unfavourable histology 57%) were included in the MTS2008 analysis (Table 1).

Treatment characteristics

Standard induction chemotherapy was completed as scheduled in 218/259 patients (missing data, n=11); 12 were switched to second line treatment for stable disease (SD) (n=11) or serious adverse event (SAE) (n=1) and in 29 patients induction chemotherapy was discontinued because of progressive disease (PD) (n=25), death (n=3) or treatment refusal (n=1). Of the 218 patients completing induction treatment, 181 (83%) commenced maintenance chemotherapy and 103/181 (57%) completed all 12 cycles. Reasons for discontinuation of maintenance chemotherapy were: death or disease recurrence (n=60), toxicity (n=4), error (n=7), patient's choice (n=4), change in diagnosis (n=1) or unknown (n=2).

Data on primary tumour response were available for 248/270 patients; the majority of patients (228/248, 92%) achieved sufficient response (≥33% volume reduction of the primary tumour) during induction chemotherapy, including 17 complete remissions (CR). Response of metastatic lesions was not available for all sites, but overall, 182/560 (33%) metastatic sites were in CR after 2-4 courses of induction chemotherapy (Supplemental Table 3).

Local treatment included delayed (i.e. week 19) resection of the primary tumour in 66 patients; 40 patients had R0, 17 R1 and 9 R2 resection. In 20 patients, loco-regional lymph node exploration was performed at delayed surgery, by surgical biopsy (n=13), lymphadenectomy (n=6) or both (n=1). Data on radiotherapy were available for 256/270 patients; radiotherapy was administered in 211 patients; 45 patients were not irradiated. Reasons for withholding radiotherapy were: early disease progression (n=14), physicians' decision (n=13), very young age (n=7), parental refusal (n=3), early death (n=3), widespread disease at diagnosis (n=2) or reason unknown (n=3). In total, 194 patients received radiotherapy to the primary tumour with a median dose of 50.4 Gy (range 18-68.6 Gy) and 89 patients were irradiated at one or more metastatic sites (median dose 30 Gy, range 9-59.4 Gy).

Toxicity

During induction chemotherapy, most common grade 3/4 adverse events (evaluated in 218 patients) were infection (grade 3; n=118, grade 4; n= 5), followed by mucositis (grade 3; n=66, grade 4; n=9)

and neuropathy (grade 3; n=22, grade 4; n=2). Venoocclusive disease (VOD) and cardiac adverse events were rare, with 3 and 2 patients developing grade $\geq$3 toxicity, respectively. During induction chemotherapy, courses were modified in about 20% of the patients (see Table 2 for details). During maintenance therapy: 22/100 (22%) patients had grade 3 infection, 2 (2%) grade 3 neuropathy and 1 (1%) a grade 3 cardiac adverse event. Chemotherapy was modified according to protocol guidelines; in approximately 40% of patients during the first maintenance cycle, up to 60% during the 2nd cycle and remained stable around 60% thereafter (Table 2). Reasons for treatment reduction were mostly myelotoxicity or infection.

Outcome

Median follow-up duration was 50.3 months (range 6.3-110.7). For the 173 patients who experienced an EFS event, the median time from diagnosis was 11.6 months (range 0.2-63.8). The 3-year EFS was 34.9% (95% CI 29.1-40.8%) and 3-year OS was 47.9% (95% CI 41.6-53.9%) (Figure 1). Of 270 patients, 125 (46%) developed progressive disease, had insufficient response, relapsed or died during (or at completion of) induction (n=65) or maintenance (n=60) treatment.

Pooled analysis

Overall, 102 consecutive treated patients from the BERNIE cohort (50 were randomised to the experimental bevacizumab arm) were analysed. Patients <6 months and ≥18 years and patients with brain metastases were ineligible for the BERNIE study, introducing a difference in age distribution and the number of patients with brain metastases between cohorts (Table 1). In addition, more patients with locoregional lymph node involvement (p=0.0008) and a large primary tumour (>5 cm) (p=0.02) were included in the MTS 2008 study. Median follow-up duration for patients in the BERNIE study was 71.8 months (range 0.03- 117.6). The 3-year EFS was 37.0% (95% CI 26.2-47.8%) and 3-year OS was 53.1% (95% CI 42.4-62.6%). OS for both BERNIE arms was comparable (Figure 2).

Outcome data were available for 365/372 patients (98%) in the pooled analysis. At last follow-up, 164 patients (45%) were alive. With a median follow-up of 55.2 months (range 0.03- 117.6 months),

the 3-year EFS and 3-year OS for the pooled cohort were 35.5% (95% CI 30.4-40.6%) and 49.3% (95% CI 43.9-54.5%) respectively (Table 3). The 3-year EFS was similar for patients in the MTS 2008 and BERNIE study (p=0.54), 3-year OS was lower for patients in the MTS 2008 study compared to the BERNIE study (p=0.03) (Figure 2).

We performed subgroup analyses, excluding patients <1 or ≥18 years old (not enrolled in the BERNIE study) or with brain metastases (exclusion criterion; Supplemental Table 1), to adjust for the difference in patient characteristics between the MTS 2008 and BERNIE cohort. There was no significant difference in 3-year OS between MTS 2008 (3-year OS 51.8% (95% CI 44.9-58.2%) and BERNIE (3-year OS 53.1% (95% CI 42.4-62.6%)) for this specific patient subgroup (p=0.14). Overall, 106/372 (28.3%) of patients were <10 years with embryonal histology. Follow up data were available for 103/106 patients; the 3-yr EFS was 54.3% (95% CI 43.9-63.3%) and 3-yr OS was 63.5% (95% CI 53.2-72.2%). EFS and OS by Oberlin risk factors are shown in Figure 3. Patients who had 0-2 Oberlin risk factors had a significantly better outcome than those with 3-4 Oberlin risk factors: 3-year EFS 46.1% vs. 12.5% (p<0.0001) and 3-year OS 60.0 vs 26.0% (p <0.0001, Figure 2b, Supplemental Figure 3a and 3b).

Discussion

This study suggests a moderate improvement in outcome for patients with metastatic disease compared to historical cohorts, similar to the results described for the COG ARST0431 study, which employed a dose intense multi-agent schedule and radiotherapy sensitisation with irinotecan (4). Owing to the design of the studies, it was not possible to determine if the addition of doxorubicin or the introduction of maintenance treatment contributed to the apparent improvement. Additionally, we present for the first time the mature OS for metastatic RMS patients treated in the BERNIE study (9), confirming that the addition of bevacizumab to the MTS 2008 backbone did not improve OS for this group of patients. The pooled analysis, with data from the concurrent BERNIE study, undertaken to overcome potential selection bias, further confirmed the results presented for MTS 2008.

Both EFS and OS in the MTS 2008 and BERNIE study seem to be better than previously reported in a pooled analysis of data from 788 patients included in 9 European and North American studies between 1984-2000 (Table 3) (3). The authors reported 3-year EFS of 27% (95% CI 24-30%) and 3-year OS of 34% (95% CI 31-38%), compared to 36% (95% CI 30-41%) and 49% (95% CI 44-55%) respectively from the pooled analyses reported here. Results were similar to those achieved with the ARST0431 study (3-year EFS 38% (95% CI 29-48%) and 3-year OS 56% (95% CI 46-66%)) (2). MTS 2008, BERNIE and ARST0431 all introduced important changes to the treatment regimen, in particular the introduction of a years' maintenance treatment for both E*p*SSG studies and a dose-intensified, interval compressed regimen in ARST0431.

The concept of maintenance was suggested as a metronomic approach to kill residual tumour cells resistant to drugs given in prior standard chemotherapy (13). There is now convincing evidence for this approach in localised RMS. Recently, the E*p*SSG RMS 2005 trial showed that 24 weeks of maintenance with vinorelbine and low-dose cyclophosphamide improved OS in high risk localised RMS (8).

The possible contribution of prolonged vinorelbine and cyclophosphamide to the outcomes in the MTS 2008 cohort reported here is uncertain. For those patients who experienced an EFS event, the median time from diagnosis to event was 11.6 months (range 0.2-63.8) for MTS 2008 patients and 60/181 patients had an event during maintenance therapy, suggesting early failure. This remains a significant issue and enhanced induction strategies are needed for such patients. By contrast, in localised high risk RMS patients, the median time from randomisation to relapse was delayed from 6.9 months (interquartile range (IQ) 3.0-16.1) to 10.1 months (IQR 6.9-15.4) by the addition of 24 weeks of maintenance vinorelbine and cyclophosphamide, with the majority of events in both groups taking place after the 24 week window for maintenance treatment (2). The steep decrease in the survival curves presented in this report underlines the problem that the current systemic treatment approach of induction plus maintenance chemotherapy is insufficient to control the disease in many

patients with metastatic RMS, especially in patients with adverse prognostic factors (i.e. with 3-4 Oberlin risk factors).

Anthracyclines were part of previous European regimens for metastatic disease (7,8) and localized disease (1). The dose intense addition of doxorubicin to the IVA backbone, did not improve outcome in RMS 2005 in patients with high risk localised RMS (1). Anthracyclines were also incorporated in two COG studies for patients with metastatic RMS (ARST0431, ARST08P1, Table 3) (4,14). Although ARST08P1 contained the same dose-dense chemotherapy backbone, including doxorubicin, and prolonged duration as the (historical comparison) ARST0431 study, outcome was inferior and failed to reveal the same trend in outcome improvement observed with both ARST0431 and the current study. This difference may be explained by the adjusted eligibility criteria in ARST08P1, where patients with favourable characteristics (age <10 years, embryonal histology) were not eligible until safety was established, with a resulting different distribution of patient characteristics and Oberlin risk factors. Nevertheless, these outcomes underline the limitations of comparisons between sequential studies. Although doxorubicin is an active drug in newly diagnosed metastatic rhabdomyosarcoma (15) the value of adding doxorubicin to a dose dense chemotherapy backbone remains debatable.

Due to the design of the studies, the exact contributions of dose intense doxorubicin and maintenance chemotherapy remain uncertain and alternative explanations for the moderate survival improvement (compared to the historical cohort described by Oberlin et al.) should be considered. Firstly, more rigorous application of local treatment (i.e. surgery and radiotherapy) may have improved outcome (16,17,18). Secondly, the systematic implementation of more effective second line treatment (19) may have prolonged post-recurrence survival. Lastly, over the last decades staging techniques and risk stratification have further evolved in addition to better supportive care treatments.

Previous studies in metastatic RMS categorised patients into 'poor' and 'better' outcome groups by comparing patients with 0-1 Oberlin risk factors with patients having ≥2 Oberlin risk factors (3,4). In the analyses presented in this study, this difference remained, but the EFS curves by Oberlin risk factors were distributed differently from the curves presented previously: patients with 2 Oberlin risk factors seemed to do better and group with the EFS curves for patients with 0 or 1 Oberlin risk factor. Although outside the scope of this study, it could be hypothesised that development in staging procedures, such as the increased use of 18-fluoro-2-deoxyglucose (FDG) positron emission tomography/computed tomography (^{18}F-FDG-PET/CT), may have resulted in the detection of more metastatic sites, moving patients with extensive disease, who previously may have been underdiagnosed and grouped as having 2 Oberlin risk factors, to the group of patients with 3 or 4 Oberlin risk factors. This may have resulted in improved survival figures for patients with 2 Oberlin risk factors in the current pooled studies.

Unexpectedly, 3-year OS was lower in the MTS 2008 study compared to the BERNIE study. Any effect of bevacizumab can be discounted as it improved neither EFS (9) nor OS (Figure 2) for RMS patients within the BERNIE study. The BERNIE study was open in selected sites only, whereas the MTS 2008 study was open in all E*p*SSG centres. There were some minor differences between the studies in eligibility criteria (Supplemental Table 1) and the method of response assessment (volumetric assessments in MTS 2008, RECIST 1.0 in BERNIE). After adjustment for known confounders, such as different age categories and eligibility of patients with CNS metastases, the survival difference became statistically nonsignificant. Comparisons between different studies should be made cautiously; other potential confounding factors in this analysis may be variability in eligibility criteria, data collection or the limited number of patients in the BERNIE cohort (especially after three years of follow-up).

In conclusion, outcome for patients with high Oberlin scores remains very poor and new approaches are needed for this patient group. In the recently opened E*p*SSG Frontline and Relapse

Rhabdomyosarcoma study (EudraCT: 2018-000515-24) a phase 1b dose finding study in patients with metastatic RMS will set the recommended phase 2 dose of irinotecan for the dose-intense combination of IVA in week 1 with irinotecan in week 2 (I_RIVA) (20). Patients with metastatic disease will then be randomised to receive either IVADo or I_RIVA at recommended phase 2 dose. In a second randomised question, 12 months of maintenance chemotherapy will be compared to 24 months maintenance therapy. Furthermore, there will be three randomisations on radiotherapy related questions. Lastly, the relapse part of the study will introduce targeted agents in combination with backbone chemotherapy.

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Data sharing statement

The data used in this study will be made available via the International Soft Tissue Sarcoma Consortium (INSTRuCT; https://commons.cri.uchicago.edu/instruct). Data requests can be submitted to INSTRuCT.

Tables and Figures

Table 1: Patient characteristics for MTS 2008 and BERNIE study

		MTS 2008		BERNIE		
		N	%	N	%	p-value
Age at diagnosis (yrs)	≤1	5	1.9	-	-	0.002
	1-9	138	51.1	56	54.9	
	10-17	104	38.5	46	45.1	
	≥18	23	8.5	-	-	
Gender	Male	151	55.9	56	54.9	0.86
	Female	119	44.1	46	45.1	
Histology	Favourable	116	43.0	41	40.2	0.63
	Unfavourable	154	57.0	61	59.8	
Primary tumour site	Orbit	-	-	1	0.3	0.33
	PM	63	23.3	15	14.7	
	HN nPM	12	4.4	4	3.9	
	GU BP	28	10.4	12	11.8	
	GU non BP	18	6.7	6	5.9	
	Extremities	67	24.8	35	34.3	
	Other sites	77	28.5	27	26.5	
	Unknown	5	1.9	2	2.0	
Site classified by Oberlin[1]	Favourable	121	44.8	38	37.3	0.19
	Unfavourable	149	55.2	64	62.8	
Tumour size	≤ 5 cm	57	21.1	29	28.4	0.02[2]
	> 5 cm	203	74.1	55	53.9	
	Not evaluable	10	4.8	18	17.7	
Nodal site	N0	103	35.9	58	56.9	0.0008[3]
	N1	162	60.0	41	40.2	
	Nx	5	4.1	3	2.9	
Bone or BM	Yes	139	51.5	53	52.0	0.93
	No	131	48.5	49	48.0	
CNS metastases	Yes	10	3.7	0	0.0	
	No	260	96.3	102	100.0	
Nr. metastatic sites	single	127	47.0	41	40.2	0.24
	multiple	143	53.0	61	59.8	
	≤2	193	71.5	69	67.7	0.47
	≥3	77	28.5	33	32.3	

Yrs; years, N; number, PM; parameningeal, HN nPM; head and neck non-parameningeal, GU; genitourinary, BP; bladder prostate, N0; no evidence of lymph node involvement, N1; locoregional lymph node involvement, Nx; no information on lymph node involvement

[1]Favourable: orbit, HNnPM, PM, GUBP, GUnBP. Unfavourable: extremities, other/unknown.

[2]28 Patients with unknown size of the primary tumour were excluded from Fisher exact test.

[3]8 Patients with unknown nodal status were excluded from Fisher exact test.

Table 2: Modification of systemic treatment in MTS 2008

	Cycle											
Standard	1 N (%)	2 N (%)	3 N (%)	4 N (%)	5 N (%)	6 N (%)	7 N (%)	8 N (%)	9 N (%)			
N cycles assessed	259	258	255	246	241	239	229	222	218			
Total modified	49 (19)	53 (21)	58 (23)	56 (23)	55 (23)	49 (21)	42 (18)	47 (21)	43 (20)			
Ifosfamide*	20	19	19	16	18	18	18	20	21			
Vincristine*	28	41	41	32	37	38	28	31	29			
Actinomycin D*	6	20	31	24	20	21	16	12	12			
Doxorubicin*	10	15	21	16	4							
Maintenance	1 N (%)	2 N (%)	3 N (%)	4 N (%)	5 N (%)	6 N (%)	7 N (%)	8 N (%)	9 N (%)	10 N (%)	11 N (%)	12 N (%)
N cycles assessed	176	168	164	157	146	140	121	115	111	108	106	100
Total modified	78 (44)	100 (60)	97 (59)	90 (57)	88 (60)	86 (61)	72 (60)	64 (56)	65 (59)	65 (60)	65 (61)	61 (61)
Vinorelbine**	17	28	37	35	36	36	34	33	33	29	32	31
Cyclophosphamide**	30	19	9	6	4	6	3	1	2	2	2	1
Both reduced	11	22	19	26	22	19	16	15	14	14	13	13
Other modification	20	31	32	23	26	25	19	15	16	20	18	16

N; number

**reduced, omitted, delayed or replaced*
***reduced, omitted or stopped*

Table 3: Survival data in metastatic rhabdomyosarcoma cohorts

				≤ 1 Oberlin Risk Factor			≥ 2 Oberlin Risk Factors		
	N	3-year EFS (95%CI)	3-year OS (95%CI)	N	3-year EFS (95%CI)	3-year OS (95%CI)	N	3-year EFS (95%CI)	3-year OS (95%CI)
Oberlin[1]	788	27 (24-30)	34 (31-38)	325	44 (38-49)		444	14 (11-18)	
MTS 2008	263	35 (29-41)	48 (42-54)	113	50 (40-59)	61 (52-70)	150	24 (17-31)	37 (29-45)
BERNIE	102	37 (26-48)	53 (42-63)	44	45 (26-63)	72 (53-85)	58	31 (19-44)	39 (25-52)
MTS 2008/ BERNIE	365	36 (30-41)	49 (44-55)	157	49 (40-57)	64 (56-72)	208	26 (20-32)	38 (31-45)
ARST0431[2]	109	38 (29-48)	56 (46-66)	43	69 (52-82)	79 (62-89)	66	20 (11-30)	14 (11-18)
ARST08P1[3]	168	16 (8-23)	41 (32-50)	38	38 (14-62)	70 (51-88)	130	9 (3-15)	33 (24-43)

N; number, CI; confidence interval, EFS; event free survival, OS; overall survival, ORF; Oberlin risk factor

[1] The Oberlin analyses included patients from nine studies from three international cooperative groups treated between 1984 and 2000.

[2] ARST0431 was open for patient enrolment between July 17th 2006 and June 13th 2008.

[3]ARST08P1 was open for patient enrolment between January 19th 2010 and July 19th 2013. ARST08P1 consisted of two pilot studies: in pilot 1 (N=97) cixutumumab was added to the chemotherapy backbone, in pilot 2 (N=71) temozolomide was added to the same chemotherapy backbone.

Figure legends

Figure 1: Event free and overall survival of patients in MTS 2008

EFS; event free survival, OS; overall survival, CI; confidence interval.

Figure 2: Overall survival by treatment cohort

Beva; bevacizumab, chemo; chemotherapy, N; number, yr; year, OS; overall survival, CI; confidence interval.

Figure 3a: Event free survival by Oberlin risk factors for pooled MTS 2008 and BERNIE cohort

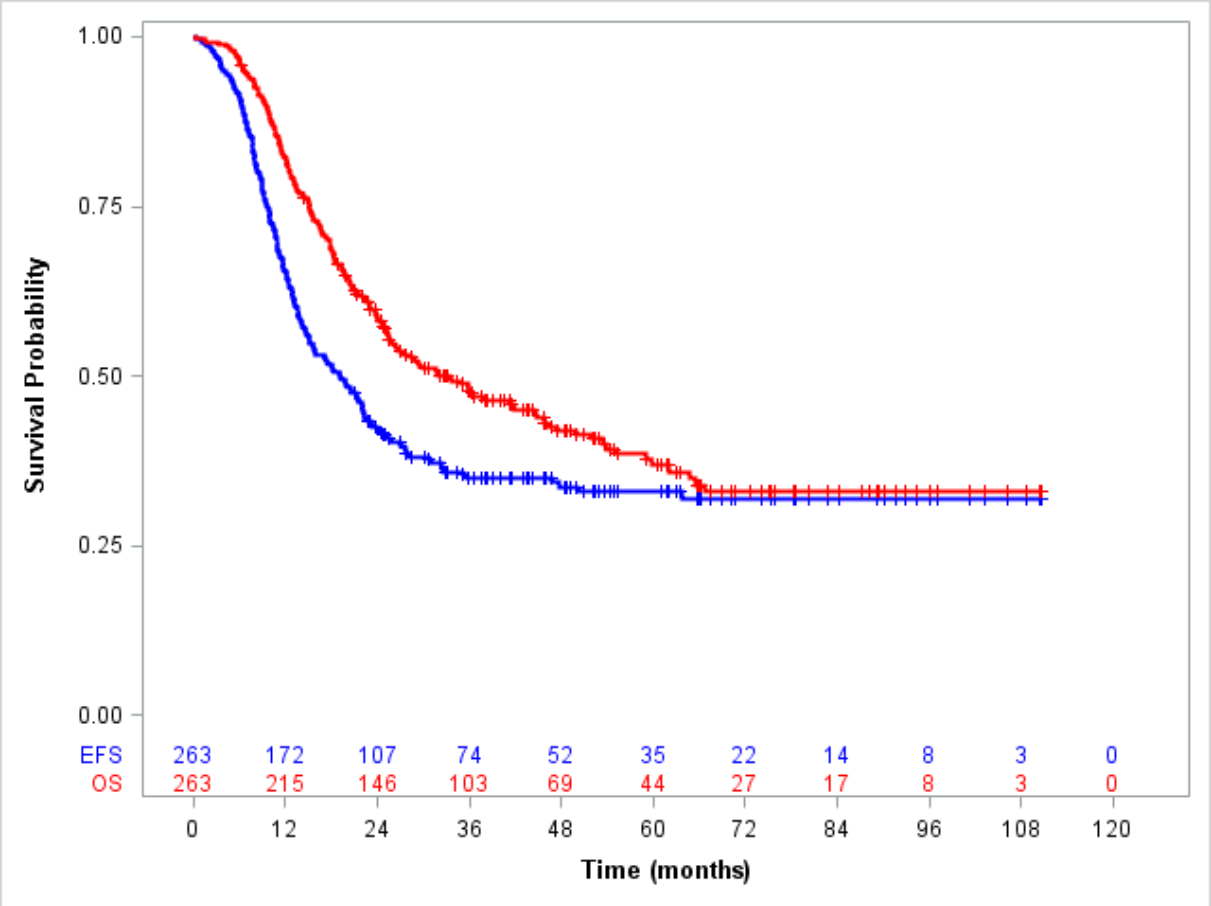
N; number, yr; year, EFS; event free survival, CI; confidence interval.

Figure 3b: Overall survival by Oberlin risk factors for pooled MTS2008 and BERNIE cohort

N; number, yr; year, OS; overall survival, CI; confidence interval.

Figures

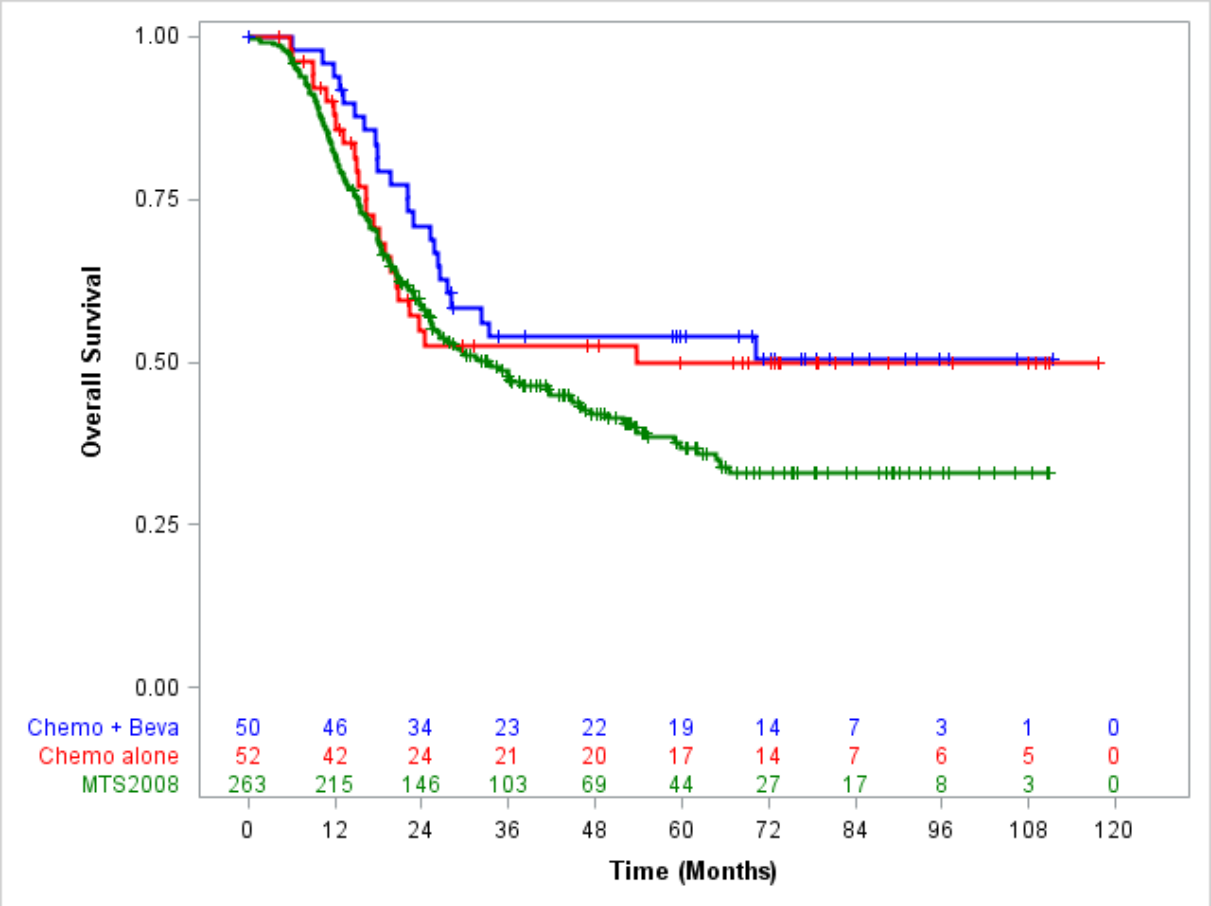
Figure 1: Event free and overall survival of patients in MTS 2008



	N	Failed	3-yr EFS (CI 95%)	Deaths	3-yr OS (CI 95%)
MTS2008 patients	263	173	34.9 (29.1-40.8)	155	47.9 (41.6-53.9)

EFS; event free survival, OS; overall survival, CI; confidence interval.

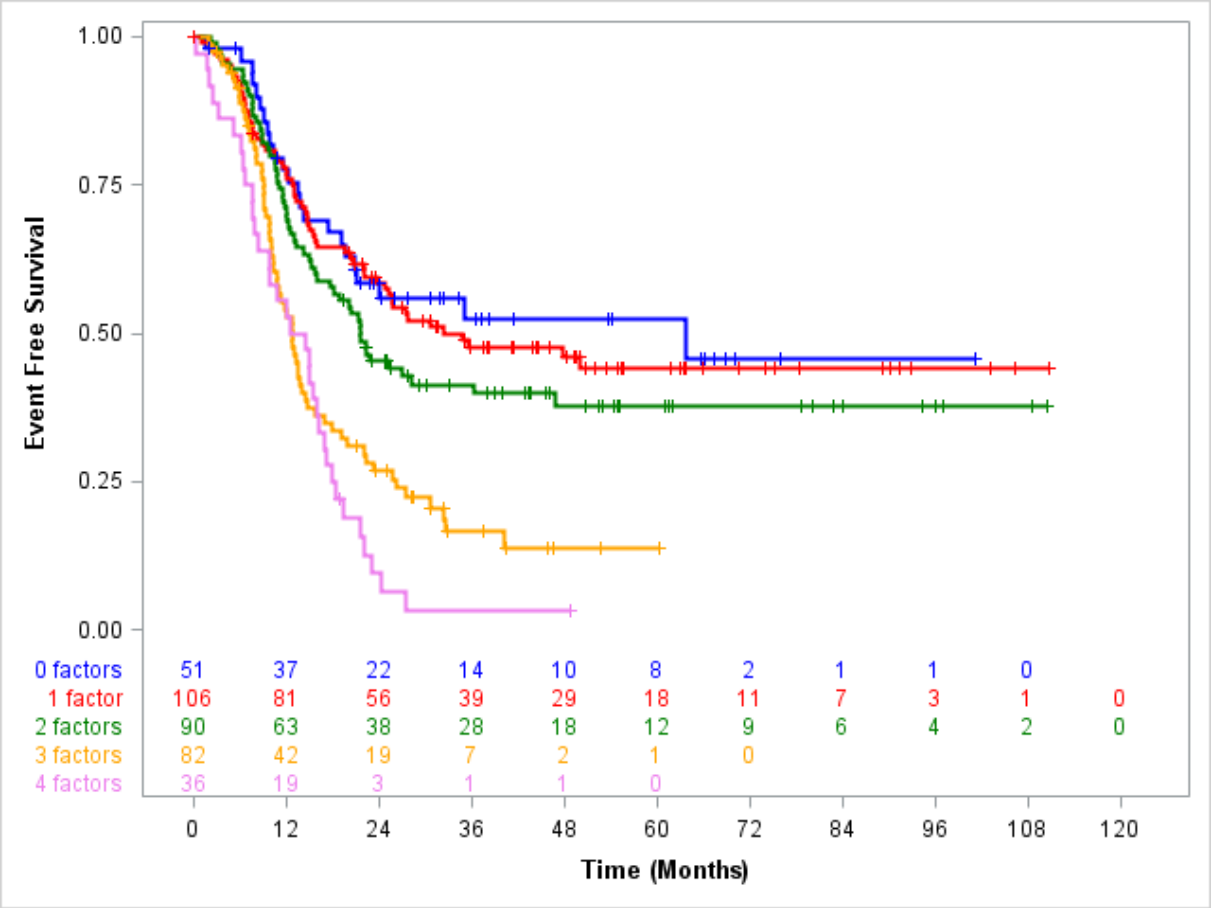
Figure 2: Overall survival by treatment cohort



	N	Deaths	3-yr OS (CI 95%)	p-value
Bernie – Chemo + Bevacizumab	50	23	53.9 (38.8-66.8)	0.08
Bernie – Chemo alone	52	23	52.6 (37.3-65.8)	
MTS 2008	263	155	47.9 (41.6-53.9)	

Beva; bevacizumab, chemo; chemotherapy, N; number, yr; year, OS; overall survival, CI; confidence interval.

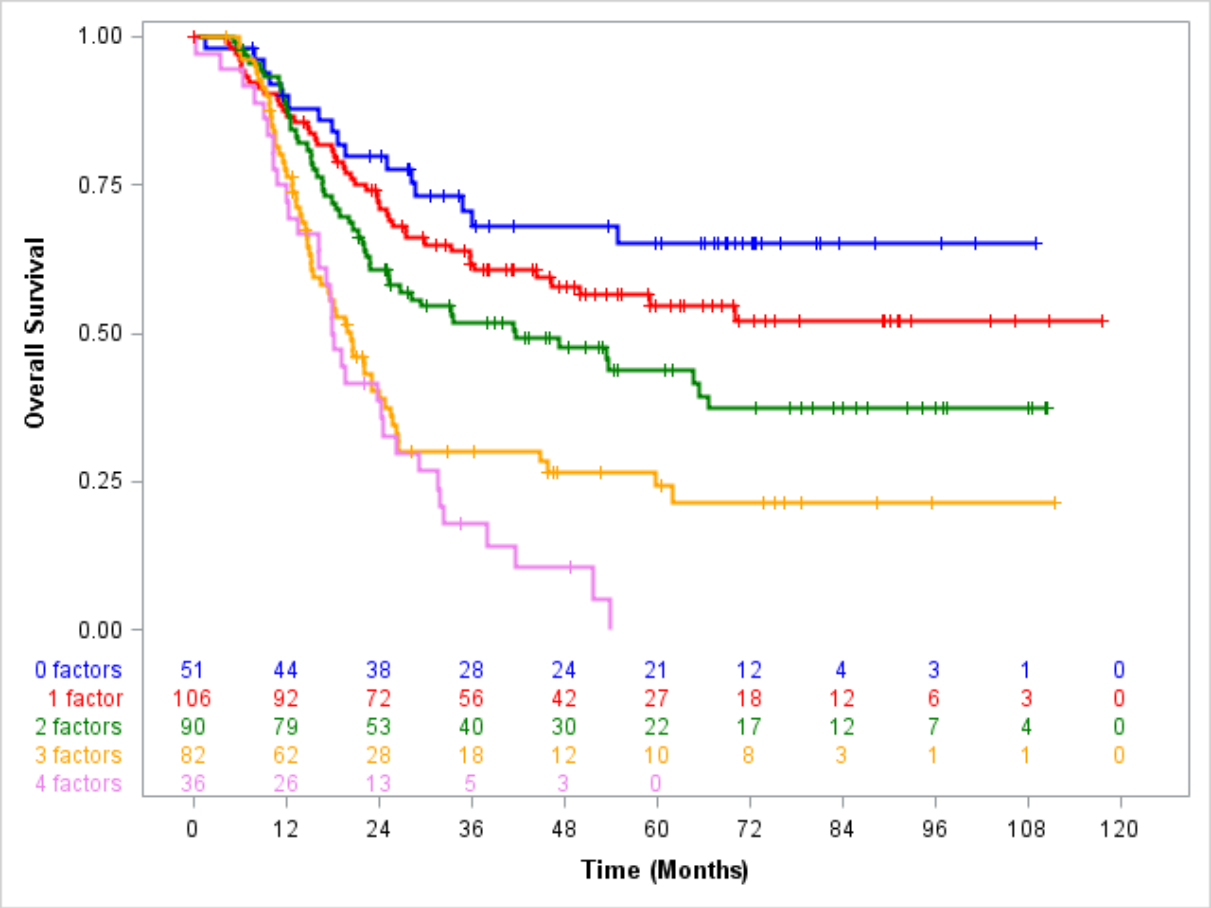
Figure 3a: Event free survival by Oberlin risk factors for pooled MTS 2008 and BERNIE cohort



	N	Failed	3-yr EFS (CI 95%)	p-value
0 factors	51	23	52.3 (36.5-66.0)	<0.0001
1 factor	106	55	47.6 (37.5-57.1)	
2 factors	90	54	41.4 (31.0-51.5)	
3 factors	82	64	16.5 (8.7-26.5)	
4 factors	36	34	3.2 (0.2-13.8)	

N; number, yr; year, EFS; event free survival, CI; confidence interval.

Figure 3b: Overall survival by Oberlin risk factors for pooled MTS2008 and BERNIE cohort



	N	Deaths	3-yr OS (CI 95%)	p-value
0 factors	51	16	70.6 (55.3-81.5)	<0.0001
1 factor	106	45	61.8 (51.6-70.5)	
2 factors	90	50	51.9 (40.9-61.8)	
3 factors	82	57	30.2 (20.2-40.9)	
4 factors	36	33	17.9 (7.3-32.1)	

N; number, yr; year, OS; overall survival, CI; confidence interval.