Title: Survival after high dose chemotherapy for refractory and recurrent Ewing sarcoma

Running Head: High dose chemotherapy in Ewing sarcoma

Version + Date: V4 15.02.22

References 19 (max 40)

Tables/Figures: 4/2(max x)

Supplementary Appendix:

#	Author	Affiliation	Email address
	Rachael Windsor	University College London Hospitals NHS Foundation Trust	rachael.windsor@nhs.net
	Anthony Hamilton	University College London	tony.hamilton6@gmail.com
	Anne McTiernan	University College London Hospitals NHS Foundation Trust	anne.mctiernan@nhs.net
	Palma Dileo	University College London Hospitals NHS Foundation Trust	palma.dileo@nhs.net
	Maria Michelagnoli	University College London Hospitals NHS Foundation Trust	maria.michelagnoli@nhs.net
	Beatrice Seddon	University College London Hospitals NHS Foundation Trust	beatrice.seddon@nhs.net
	Sandra J Strauss	University College London Hospitals NHS Foundation Trust; University College London	s.strauss@ucl.ac.uk
	Jeremy Whelan	University College London Hospitals NHS Foundation Trust	jeremy.whelan@nhs.net

Acknowledgements: Thank you to Menelaos Pavlou for his statistical advice.

Correspondence: Rachael.windsor@nhs.net

Word count:

Section	Word count	Target
Abstract	227	**250**
Background	279	
Methods	485	
Results	1107	
Discussion	1056	
Total	**3154**	**2500**

Abstract

Background: Outcome of primary refractory or recurrent Ewing sarcoma (RRES) is poor and the role of high-dose therapy (HDT) remains uncertain. We retrospectively reviewed all patients treated for RRES in the London Sarcoma Service (LSS) over a 22-year period with the aim of adding to current literature and developing a prognostic risk score to aid clinical decision-making.

Methods and Results: One hundred and ninety-six patients were included; 64 patients received HDT, 98 standard non-HDT chemotherapy and 34 no systemic therapy. At RRES, median age was 20 years and seventy-four percent of patients had progressed or relapsed within 24 months. Median overall survival for HDT and non-HDT patients was respectively 76 months (95% CI 34.8-117.2) and 10.5 months (95% CI 8.9-12.1). Two and five-year post-relapse survival (PRS) for HDT patients was 67.9% (SE 5.9) and 52.7% SE 6.5) and for non-HDT patients, 20.5% (SE 4.2) and 2% (SE 1.5). Four prognostic factors significant on multivariate analysis were assigned a score of 1 point each, creating good (score 0), intermediate (score 1-2) and poor (score 3-4) prognosis groups. Increased score was significantly associated with reduced PRS.

Conclusion: Our study demonstrates that in RRES, HDT is associated with superior outcomes compared with non-HDT chemotherapy. RRES patients can be risk-stratified according to a predictive prognostic index we have developed, with potential benefit of HDT observed even in patients with poor prognostic scores.

Keywords: Sarcoma, Ewing; autologous stem-cell transplant;

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

Multimodality therapy has significantly improved the outcome for patients with Ewing sarcoma (ES). However, 40% with localised disease, and 60-80% of patients with metastatic ES will develop progressive or recurrent disease within 5 years [1-3]. After these events, outcomes are poor, (reported 5-year post-relapse survival (PRS) rates of 10-19%) [2-4]. The most significant prognostic factor consistently reported for PRS is time to disease recurrence with

5-year survival of <10% within 24 months of diagnosis compared to 30% for those with a longer disease-free interval [2, 3].

The optimal management of primary refractory or recurrent Ewing sarcoma (RRES) remains uncertain. Several combination chemotherapy regimens have been investigated, with agents including cyclophosphamide, topotecan, irinotecan, temozolomide, cis- and carboplatin, gemcitabine, docetaxel and etoposide. Response rates between 30-71% are reported but sustained responses are unusual [4-7]. rEECur is a randomised controlled multi-arm chemotherapy trial recruiting internationally to define standard of care for RRES [8]. Novel agents are being explored but are not yet standard of care.

High-dose chemotherapy with haematopoietic stem cell rescue (HDT) has been investigated as a treatment for poor-prognosis ES. As a component of first-line therapy in patients with high-risk localised disease, HDT was associated with a significant improvement in survival [9] although no survival advantage was demonstrated in patients with pulmonary metastases [10]. No randomised trials of HDT in RRES have been undertaken but published literature reflects widespread use of this treatment modality.

In this retrospective report, we examined the therapeutic efficacy of HDT in patients with RRES treated in the London Sarcoma Service (LSS) over a 22-year period. We used prognostic factors identified in this group of patients to develop a prognostic index that predicted outcome after RRES.

Materials and methods

All patients treated for RRES between 01/01/1995 and 31/12/2017 were included, identified from the LSS patient database (established in 2011) and prior to this, by manual interrogation of histopathology records. This HDT dataset extended that previously reported by McTiernan et al and so for completeness, four additional patients were included although they were treated in 1993-94 [11,12].

Refractory disease was defined as disease progression during first-line induction or consolidation chemotherapy. All diagnostic biopsies were reported primarily or reviewed within LSS, including confirmation of characteristic chromosomal translocations. Re-biopsy was not routinely undertaken at recurrence or disease progression as unequivocal new radiological lesions were accepted as proof of recurrent disease in the absence of another plausible explanation.

Patient demographics and treatment information were obtained from hospital case notes. At primary diagnosis, data recorded included gender, age, primary site (axial, extremity) site of metastases (pulmonary, extra-pulmonary, combined) and time to RRES. Treatment information included chemotherapy regimen; VDC/IE (vincristine (V), doxorubicin (D), cyclophosphamide (C), ifosfamide (I), etoposide (E)) VIDE/VAI/VAC (actinomycin (A)), E/VAIA (A - actinomycin and doxorubicin) and "Other"; local therapy - surgery with radical intent, radical radiotherapy, surgery plus radiotherapy, or palliative (non-radical dose for symptom management only).

Data recorded at RRES included site of recurrence (isolated pulmonary, isolated local, local and pulmonary and extrapulmonary NB. patients with local and extrapulmonary recurrence were recorded as extrapulmonary), position in treatment (during induction chemotherapy, during consolidation chemotherapy and after completion of treatment (less than or more than 24 months). Treatment information included chemotherapy regimen; high-dose ifosfamide regimen (including VIDE, ifosfamide/etoposide, ICE (ifosfamide/carboplatin/etoposide) and single-agent ifosfamide), cyclophosphamide/topotecan, irinotecan/temozolomide, "Other iv", "Other oral" and "No systemic therapy"; surgery; excision of local recurrence or pulmonary metastatectomy; radiotherapy; radical, adjuvant (post-operative), whole lung, palliative, or total body irradiation. For local recurrence, "definitive local therapy" was defined as surgical excision and/or radical dose radiotherapy (doses ≥ 50Gy). High-dose therapy (HDT) was defined as myeloablative chemotherapy with autologous stem cell rescue (ASCR). HDT conditioning regimen was recorded as Busulfan/Melphalan; Treosulfan/Melphalan and "Other" (including combinations of melphalan, etoposide, cyclophosphamide and total body irradiation) and any chemotherapy regimen that did not require an ASCR was defined as 'non-HDT'. We attempted to access retrospective radiology reports to assess response to re-induction chemotherapy. Response was recorded as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD).

Statistical analysis

Analysis was completed using IBM SPSS Statistics version 27.0. The primary outcome was post-relapse survival (PRS), defined as the time from progression or recurrence until death or censorship on 16th June 2021. Progression-free survival (PFS) was defined as the time from initial diagnosis until first disease progression or recurrence. Survival analysis was conducted using the Kaplan-Meier method and the Log Rank test. The Cox-Regression method was used for univariate and multivariate analysis, including hazard ratio (HR) calculations. Pre-defined subgroup analyses examined HDT regimen and site of relapse. Development of the prognostic index is presented below.

Results

Patient demographics and disease presentation:

One hundred and ninety-six patients were included for analysis. Median follow-up (reverse Kaplan-Meier) was 233 months (range 4-387). At initial diagnosis, median age was 18.3 years (Range 4.5 - 66.2), male to female ratio (113 vs 83) and 184/196 (93.9%) had osseous primaries; 84 with extremity and 100 with central/axial tumours. Disease was localised in 105 patients with pulmonary metastases in 39 and extra-pulmonary metastases in 52. The majority were treated with high-dose ifosfamide regimens in standard use at the time of diagnosis (Table 1).

Characteristics at diagnosis of recurrent or refractory ES (Table 1)

The median age at diagnosis of RRES was 20 years. One hundred and forty-five patients (74%) progressed or relapsed within 24 months. Isolated local recurrence was seen in 35 patients (17.9%), isolated pulmonary recurrence in 53 (27.0%), combined local and pulmonary in 22 (11.2%), and extra-pulmonary in 86 patients (43.9%).

Sixty-four patients received HDT, 98 no HDT and 34 no systemic therapy. In the HDT group, seven patients (11%) had primary refractory disease, twenty-three (36%) relapsed after completion of initial treatment but within 24 months and 34 (53.1%) relapsed after 24 months. In the non-HDT group, 22 patients (22.5%) had primary refractory disease, 61 (62.2%) relapsed after completion of initial treatment but within 24 months and 15 (15.3%) relapsed after 24 months. Sixteen of 34 (47.1%) patients in the “no systemic therapy” group had primary refractory disease, 16 (47%) relapsed after completion of initial treatment but within 24 months and 2 (5.9%) after 24 months. Almost three-quarters of this group (25 patients, 73.5%) had extrapulmonary disease at recurrence. Isolated pulmonary or isolated local recurrence was more frequent in HDT compared to non-HDT patients (40.6% vs 25.5%) and (25% vs 13.3%) respectively whereas extra-pulmonary recurrence was less frequent (23.4% vs 46.9%, $p<0.0005$).

Seventy-four patients (74/196, 37.8%) underwent definitive local treatment; 46/64 (71.9%) of HDT patients, 23 (23.5%) non-HDT and 5/34 (14.7%) of patients with no systemic therapy. Thirty-nine patients (39/196, 19.8%) underwent radical dose radiotherapy (definitive or adjuvant) (23 (35.9%) HDT patients, 14 (14.2%) non-HDT patients and 2 (5.8%) in the “no systemic therapy” group). Three patients (1.5%) had whole lung radiotherapy (two HDT patients and one non-HDT) and two HDT patients had Total-Body Irradiation as part of their

conditioning regimen. Forty-four (68.8%) HDT patients were re-challenged with a high-dose ifosfamide regimen compared to 23 (23.5%) in the non-HDT group. Prior to transplant, fifty (78%) HDT patients recieved one chemotherapy regimen and 14 (22%) were treated with further second-line iv or oral chemotherapy. Of the non-HDT group, 47 patients (48%) received one chemotherapy regimen and 51 (52%) further second-line therapy.Busulfan/melphalan (BuMel) (n=39) and treosulfan/melphalan (TreMel) (n=14) were the most common regimens. Eleven patients were treated with alternative regimens ("other HDT"). (Table 1).

Of the 34 patients who did not receive systemic treatment, 32 had relapsed within 24 months. Extra-pulmonary metastatic disease was more frequent, (61.8% at primary diagnosis and 73.5% at RRES). Five of six patients with a local relapse had definitive local treatment only and a further nineteen patients had some form of palliative surgery and/or radiotherapy.

Survival

For all patients, median PRS was 12.9 months (range 0.3-349; 95% CI 9–16.8). Two-year PRS and 5-year PRS were 33% (Standard error SE 3.4%) and 18% (SE 2.9%) respectively.

Survival of patients according to site of relapse/progression is shown in Figure 1a. The 2-year PRS was 53.9% for patients with isolated pulmonary disease, 38.7% for isolated local relapse, 21.8% for extra-pulmonary recurrence, and 16.7% for combined local and pulmonary recurrence (LogRank p<0.0005).

Median PRS for HDT patients was 76 months (95% CI 34.8-117.2) compared with 10.5 months (95% CI 8.9-12.1) for non-HDT (LogRank p<0.0005) (Figure 1b). Two and 5-year PRS for HDT and non-HDT were 67.9% (SE 5.9%) and 20.5% (SE 4.2%), and 52.7% (SE 6.5%; 6 censored) and 2% (SE 1.5%). Treatment with HDT was associated with significantly longer PRS across all relapse sites (Table 2). There was a suggestion that HDT regimen influenced survival (Median (months): Bu/Mel 89.8; Treo/Mel 48.3; Other 25.4. p 0.09).

Due to logistical challenges in accessing historical imaging, data on response to induction chemotherapy was available only for 55 HDT patients. At the time of HDT, 21 (38%) patients were in complete remission (CR), 28 (51%) in partial remission (PR), 4 (7%) had stable disease (SD), and 2 (3%) had progressive disease. Patients in CR showed non-significant superior 2-year PRS rates to those in PR (71.4% vs 65.8%). In the patients with SD, the longest survivor (PRS 141 months) had a local and pulmonary recurrence <24 months from initial treatment, one relapsed with isolated pulmonary recurrence >24 months off treatment (PRS 89 months), one had local disease progression on primary treatment and underwent

Imasurgery plus HDT (PRS 11.7 months) and one had a local recurrence >24 months off treatment (PRS 17.5 months). Both patients with PD had isolated pulmonary recurrence (one on treatment and one >24 months) and were alive at five years post-HDT. One subsequently died of disease 89.6 months post-relapse and the other remained alive and disease-free at census (PRS 123.0 months).

Univariate/Multivariate analysis (MVA) and Prognostic Score (Table 3).

Univariate and multivariate analysis of potential prognostic factors is displayed in Table 3. Four prognostic factors were identified on MVA: relapse ≤24 months, presence of extra-pulmonary metastases at primary diagnosis, relapse site and primary refractory disease.

To develop the prognostic score, proportional weighting was allocated to each factor identified on MVA. As two variables related to timing of relapse (primary refractory disease and relapse <24 months), this was scored as one variable (0 – relapse >24 months, 1 – relapse after treatment but <24 months, 2 – relapse during treatment). Relapse site was scored as a maximum of 1 (both extrapulmonary and combined local/pulmonary were significant on MVA). This created a maximum score of 4 (relapse site score maximum of 1). Prognostic groups were then defined as good (score 0), intermediate (score 1-2) and poor (score 3-4).

Table 4 shows categorisation of patients according to prognostic groups. Twenty-six patients were categorized as good prognosis (score 0), 126 as intermediate (score 1-2) and 56 as poor prognosis (score 3-4).

Prognostic group was significantly associated with PRS (Figure 2). Five-year PRS was 51.9% (SE 10.1) for good and 19.1% (SE 3.8) for intermediate prognosis ($p<0.0005$). Fifty-two of fifty-four high-risk patients died, the longest survivor at 25 months; two were censored, the last at 58.9 months. Risk of death was significantly associated with increasing score (Intermediate (score 1-2), HR 2.4, (95% CI 1.4-4.2); High (score 3-4), HR 10.3, (95% CI 5.7-18.6), LogRank $p<0.0005$). Two and 5-year PRS according to treatment is shown in Table 4.

Discussion

This study analysed 196 patients with RRES treated at a single centre over a 22-year period and includes the largest published report of HDT outcomes. HDT for primary refractory or recurrent disease was associated with significantly increased post-relapse survival was even in those with disseminated disease. We also developed a prognostic score that predicted post-relapse survival and may aid clinical decision-making.

The five-year PRS of 52.7% in the HDT group is comparable to that reported by Tenneti et al in a systematic review of seven studies of HDT in relapsed ES. For all studies, 3-5 year EFS ranged from 20-61% and corresponding OS, 33-77% [13]. Rasper et al reported two-year EFS of 45% and 47% for fifty-three patients with relapsed ES treated with BuMel and TreMel HDT respectively, compared to 10% for non-HDT patients [14], somewhat lower than our findings (2-year PRS of 67.9% and 52.7% for HDT and non-HDT respectively). There are some notable factors that may account for the difference in survival, in particular the distribution of disease at recurrence. Local recurrence was broadly comparable (25% and 20%) but the classification of distant disease differed between the studies. Almost three-quarters of the Rasper HDT patients were recorded as distant relapse, not subdivided further into pulmonary/extrapulmonary. Of our HDT patients, 40% had pulmonary metastatic disease only, a factor known to confer a better long-term outcome. Furthermore, 47% of our HDT patients had relapsed within 24 months compared to 80% of BuMel and 53% TreMel patients in the Rasper cohort. A disease-free interval of greater than 2-years is recognised to be one of the most consistent prognostic factors in recurrent ES.

Rasper and Tenneti et al did not report the distribution of disease at initial diagnosis [13-14]. We noted a high proportion of bone primaries, possibly reflecting the superior prognosis of extra-skeletal Ewing sarcoma [15]. Similarly, we noted a high proportion of central or axial tumours, likely reflecting their poorer prognosis and increased risk of recurrence [16].

Ferrari et al evaluated post-relapse survival in 107 patients with recurrent ES [17], 80 of whom received second-line chemotherapy (50 high-dose ifosfamide, 30 other regimens), 17 definitive local therapy only and 10 palliative treatment. Twenty of the 50 high-dose ifosfamide patients proceeded to consolidation with HDT, with a 5-year PRS of 50% whereas 5-year PRS for other regimens was 5% and for local therapy alone, 31%.

Consistent with published literature, we observed post-relapse survival was significantly influenced by extra-pulmonary or combined local and pulmonary relapse and a relapse-free interval <24 months [2,3,18,19]. The presence of extra-pulmonary metastases at primary diagnosis remained significant at relapse although other studies have reported conflicting results [2, 20].

We developed a prognostic index based on the four significant disease-related characteristics, similar to that previously reported for primary disseminated ES [1] and metastatic rhabdomyosarcoma [21]. The hazard ratio increased with increasing score, suggesting prognosis can be predicted depending on disease characteristics at recurrence or progression. We observed superior PRS for moderate-risk patients treated with HDT

compared with non-HDT but patient numbers make the comparison of HDT effect in low and high-risk patients less reliable. However, of 20 low risk patients treated with HDT, 64.6% of were alive at 5-years compared to zero of six who did not receive HDT.

Only two high-risk patients received HDT but it is notable that one has achieved long-term survival approaching five years at the time of censorship (56.9 months). High-risk patients in the non-HDT group had a very poor prognosis with 6.9% alive at 2-years. A potential prognostic index would require external validation before use in clinical practice.

There is no doubt this study has significant limitations, the main one being retrospective data collection over a prolonged period, during which multiple different treatment protocols and imaging techniques will have been used. We tried to ameliorate this by grouping high-dose ifosfamide-containing regimens but cannot quantify any potential impact on our results.

In our centre, multidisciplinary discussion of potential treatment options for all patients with RRES is standard. Due to the nature of ES, clinical circumstances are highly individualised, but the majority of patients will receive systemic therapy and appropriate therapy for local recurrence with personalised decision-making guided by time to recurrence, extent of disease and performance status both at first and subsequent episodes of disease progression. The centre's goal was to offer HDT to patients in whom the disease burden had been reduced to a minimum and although consideration of HDT was not guideline-led, the overall guiding principles were uniform, taking account of the apparent importance of characteristics identified in our previous analyses, of favourable response, low disease burden and longer interval from primary treatment. Naturally, there is an inherent bias in patients treated with HDT as those with the greatest perceived benefit will be selected, for example, limited metastatic disease or local recurrence, longer time to relapse and good response to induction chemotherapy and / or definitive local control. It is important to note that none of our HDT patients with SD or PD had extra-pulmonary metastases. Ferrari et al [17] did not show a significant benefit for HDT treatment but did demonstrate the importance of achieving a second complete remission (CR2). Unfortunately, we were unable to access historical imaging to assess response for non-HDT patients and could not therefore investigate the influence of CR2 in that group. It is important to reflect also whether the MDT decision-making process changed over the study period and in view of the survival benefit we observed even in poor prognosis or progressive disease could be more open in future.

Conclusion

The use of HDT for the treatment of RRES has remained controversial and the lack of prospective studies mean clinical decision making is based on best-available retrospective analyses. Due to its size, we believe our study adds significantly to existing data and whilst the evidence is strongest for the intermediate-prognosis group, HDTis a treatment option that should be considered for patients with good risk and could potentially be considered for thosewith poor prognostic factors. With validation, our proposed prognostic index could aid clinical decision-making at relapse or progression of ES, in judging the balance of risk and benefit of HDT for individual patients. rEECur [8] will establish the gold standard for initial post-relapse chemotherapy in RRES, and whilst there remains no appetite for HDT randomisation, these data are more important than ever.

Declaration of interest: None declared

References

1. Ladenstein, R., et al., Primary Disseminated Multifocal Ewing Sarcoma: Results of the Euro-EWING 99 Trial. Journal of Clinical Oncology, 2010. 28(20): p. 3284-3291.

2. Leavey, P.J., et al., Prognostic Factors for Patients with Ewing sarcoma (EWS) at First Recurrence Following Multimodality Therapy – A Report from the Children's Oncology Group. Pediatric blood & cancer, 2008. 51(3): p. 334-338.

3. Stahl, M., et al., Risk of recurrence and survival after relapse in patients with Ewing sarcoma. Pediatric Blood & Cancer, 2011. 57(4): p. 549-553.

4. Palmerini E, Jones RL, Setola E, Picci P, Marchesi E, Luksch R, Grignani G, Cesari M, Longhi A, Abate ME, Paioli A, Szucs Z, D'ambrosio L, Scotlandi K, Fagioli F, Asaftei S, Ferrari S. Irinotecan and temozolomide in recurrent Ewing sarcoma: an analysis in 51 adult and pediatric patients. Acta Oncol. 2018 Jul;57(7):958-964. doi: 10.1080/0284186X.2018.1449250. Epub 2018 Mar 13. PMID: 29533113.

5. Yoon JH, Kwon MM, Park HJ, Park SY, Lim KY, Joo J, Park BK. A study of docetaxel and irinotecan in children and young adults with recurrent or refractory Ewing sarcoma family of tumors. BMC Cancer. 2014 Aug 28;14:622. doi: 10.1186/1471-2407-14-622. PMID: 25164234; PMCID: PMC4155244.

6. Raciborska A, Bilska K, Drabko K, Chaber R, Pogorzala M, Wyrobek E, Polczyńska K, Rogowska E, Rodriguez-Galindo C, Wozniak W. Vincristine, irinotecan, and temozolomide in patients with relapsed and refractory Ewing sarcoma. Pediatr Blood Cancer. 2013 Oct;60(10):1621-5. doi: 10.1002/pbc.24621. Epub 2013 Jun 15. PMID: 23776128.

7. Kebudi R, Cakir FB, Gorgun O, Agaoglu FY, Darendeliler E. A modified protocol with vincristine, topotecan, and cyclophosphamide for recurrent/progressive ewing

sarcoma family tumors. Pediatr Hematol Oncol. 2013 Apr;30(3):170-7. doi: 10.3109/08880018.2013.767868. PMID: 23484903.

8 EuroEwingConsortium. rEECur - International Randomised Controlled Trial of Chemotherapy for the Treatment of Recurrent and Primary Refractory Ewing Sarcoma. McCabe et al. Results of the second interim assessment of rEECur, an international randomized controlled trial of chemotherapy for the treatment of recurrent and primary refractory Ewing sarcoma (RR-ES).DOI: 10.1200/JCO.2020.38.15_suppl.11502 Journal of Clinical Oncology 38, no. 15_suppl (May 20, 2020) 11502-11502.

9. Whelan J et al. High-Dose Chemotherapy and Blood Autologous Stem-Cell Rescue Compared With Standard Chemotherapy in Localized High-Risk Ewing Sarcoma: Results of Euro-E.W.I.N.G.99 and Ewing-2008. J Clin Oncol 2018; 36(31): JCO2018782516 Epub ahead of print

10. Dirksen et al. High-Dose Chemotherapy Compared With Standard Chemotherapy and Lung Radiation in Ewing Sarcoma With Pulmonary Metastases: Results of the European Ewing Tumour Working Initiative of National Groups, 99 Trial and EWING 2008. J Clin Oncol 2019; 37(34): 3192-3202

11 McTiernan, A., et al., High dose chemotherapy with bone marrow or peripheral stem cell rescue is an effective treatment option for patients with relapsed or progressive Ewing's sarcoma family of tumours. Annals of Oncology, 2006. 17(8): p. 1301-1305.

12. McTiernan, A.M., et al., Improving Outcomes After Relapse in Ewing's Sarcoma: Analysis of 114 Patients From a Single Institution. Sarcoma, 2006. 2006: p. 83548.

13. Tenneti P, Zahid U, Iftikhar A, Yun S, Sohail A, Warraich Z, Anwer F. Role of High-Dose Chemotherapy and Autologous Hematopoietic Cell Transplantation for Children and Young Adults with relapsed Ewing's Sarcoma: A Systematic Review. Sarcoma 2018, Article ID 2640674).

14 Rasper, M., et al., The value of high-dose chemotherapy in patients with first relapsed Ewing sarcoma. Pediatric Blood & Cancer, 2014. 61(8): p. 1382-1386.

15 Abboud A, Masrouha K, Saliba M, Haidar R, Saab R, Khoury N, Tawil A, Saghieh S. Extraskeletal Ewing sarcoma: Diagnosis, management and prognosis (Review).Oncology letters 2021; 21:354].

16 Zhan H, Mo F, Zhu M, Xu X, Zhang B, Liu H, Dai M. A SEER-based nomogram accurately predicts prognosis in Ewing's sarcoma. Sci Rep. 2021 Nov 22;11(1):22723. doi: 10.1038/s41598-021-02134-0.

17 Ferrari, S., et al., Post-relapse survival in patients with Ewing sarcoma. Pediatric Blood & Cancer, 2015. 62(6): p. 994-999.

18 Bacci, G., et al., Therapy and survival after recurrence of Ewing's tumors: the Rizzoli experience in 195 patients treated with adjuvant and neoadjuvant chemotherapy from 1979 to 1997. Annals of Oncology, 2003. 14(11): p. 1654-1659.

19 Pawlowska A. et al., Long-Term Follow-up of High-Dose Chemotherapy with Autologous Stem Cell Transplantation in Children and Young Adults with Metastatic

or relapsed Ewing Sarcoma: A Single-Institution Experience. Transplant and Cell Ther 2021; 27:72.e1-72.e7

20 Barker L, Pendergrass T, Sanders J, Hawkins D. Survival after recurrence of Ewing's Sarcoma family of tumors. J Clin Oncol 2005; 23(19): 4354-4362]

21 Oberlin, O., et al., Prognostic Factors in Metastatic Rhabdomyosarcomas: Results of a Pooled Analysis From United States and European Cooperative Groups. Journal of Clinical Oncology, 2008; 26(14): p. 2384-2389.

Table 1: Patient demographics – primary disease and recurrence

Characteristic	Non-HDT n (%)	HDT n (%)	No systemic therapy n (%)
Total patients	98	64	34

Gender			
Male	66 (67.3)	35 (54.7)	12 (35.3)
Female	32 (32.7)	29 (45.3)	22 (64.7)
Median age at diagnosis (years)	18.4	19.9	21.8
Primary disease			
Primary tumour location			
Extremity	31 (31.6)	40 (62.5)	13 (38.2)
Central/axial	59 (60.2)	24 (37.5)	17 (50)
Other	8 (8.2)	0	4 (11.8)
Metastasis at diagnosis			
None	49 (50)	46 (71.9)	10 (29.4)
Isolated pulmonary	20 (20.4)	16 (25)	3 (8.8)
Extra-pulmonary	29 (20.6)	2 (3.1)	21 (61.8)
Primary chemotherapy			
VIDE/VAC/VAI	70 (71.4)	39 (60.9)	27 (79.4)
E/VAIA	9 (9.2)	18 (28.1)	0
VDC/IE	18 (18.4)	2 (3.1)	5 (14.7)
Other	1 (1)	5 (7.8)	2 (5.9)
Recurrent/progressive disease			
Relapse site			
Isolated pulmonary	25 (25.5)	26 (40.6)	2 (5.8
Isolated local	13 (13.3)	16 (25)	6(17.6)
Extra-pulmonary	46 (46.9)	15 (23.4)	25(73.5)
Local and pulmonary	14 (14.3)	7 (10.9)	1(2.9)
Timing of progression or relapse			
During induction chemotherapy	9 (9.2)	4 (6.3)	4 (11.8)
During consolidation chemotherapy	13 (13.3)	3 (4.7)	12 (35.3)
After completion of treatment:			
<24 months	64 (65.3)	25 (39)	16 (47)
>24 months	12 (12.2)	32 (50)	2 (5.9)
Definitive local therapy			
No	75 (76.5)	18 (28.1)	29 (85.3)
Yes	23 (23.5)	46 (71.9)	5 (14.7)
Relapse chemotherapy (first-line)			
None	0	3 (4.7)	-
High-dose ifosfamide regimen	23 (23.5)	44 (68.8)	-
Cyclophosphamide/Topotecan	34 (34.7)	5 (7.8)	-
Irinotecan/Temozolomide	4 (4.1)	1 (1.6)	-
Other IV	20 (20.4)	11 (17.2)	-
Other oral	17 (17.3)	0	-
High-dose regimen			
Busulfan/Melphalan	-	39 (61)	-
Treosulfan/Melphalan	-	14 (22)	-
Other	-	11 (17)	-

HDT: High-dose chemotherapy; VIDE/VAC/VCD: Vincristine, Ifosfamide, Doxorubicin, Etoposide/Vincristine, Actinomycin, Cyclophosphamide/Vincristine, Cyclophosphamide, Doxorubicin; E/VAIA: Etoposide/Vincristine, Actinomycin, Ifosfamide, Adriamycin; VDC/IE: Vincristine, Doxorubicin, Cyclophosphamide/Ifosfamide, Etoposide

Characteristic	N (Censored)	PRS % (SE)		Median months (95% CI)	p
		2 year	5 year		
All patients					
HDT	64	67.9(5.9)	20.5(4.2)	76 (34.8-117.2)	<0.0005
Non-HDT	96(6)	52.7(6.5)	2(1.5)	10.5 (8.9-12.1)	
Isolated pulmonary					
HDT	26 (11)	81 (7.7)	57.7 (9.7)	89.7(0-201)	<0.0005
Non-HDT	25 (1)	29.3 (9.3)	0	15(5.7-24)	
Isolated local					
HDT	16 (9)	67.7 (11.9)	67.7 (11.9)	-	<0.0005
Non-HDT	13 (1)	23.1 (11.2)	0	10.5(8.6-12.5)	
Extra-pulmonary					
HDT	15 (3)	50.6 (13.4)	36.1(12.9)	25.4(18.3-32.5)	0.003
Non-HDT	46 (0)	21.7 (6.1)	4.3 (3)	10.1(6.4-13.8)	
Local and pulmonary					
HDT	7 (3)	53.6 (20.1)	35.7 (19.8)	27(11.8-42.2)	0.001
Non-HDT	14 (1)	0	0	4.7(1.6-7.7)	

Table 3: Univariate and multivariate analysis and prognostic score

	Univariate		Multivariate		Prognostic score
Characteristic	**PRS HR (95% CI)**	**P (HR)**	**HR (95% CI)**	**P (HR)**	**Risk points**
Gender					
Male	1				
Female	0.8 (0.6-1.1)	0.3			
Age group at diagnosis					
0-13	1				
>13-20	1.1 (0.7-1.7)				
>20	1.3 (0.8-1.9)	0.5			
Primary disease					
Primary tumour location					
Extremity	1		1		
Central/axial	1.4 (1-1.9)	0.06	1 (0.7-1.5)	0.9	-
Other	3.3 (1.7-6.1)	0.002	1 (0.5-2)	0.9	-
Metastasis at diagnosis					
None	1		1		0
Isolated pulmonary	1.3 (0.8-1.9)	0.2	1.6 (0.9-2.6)	0.1	0
Extra-pulmonary	3.9 (2.7-5.7)	<0.0001	12.1 (1.3-3.5)	0.002	1
Primary chemotherapy					
VIDE/VAC/VCD	1		1		-
E/VAIA	0.5(0.3-0.9)	0.01	1.2 (0.7-2.1)	0.4	-
VDC/IE	1.5 (0.9-2.3)	0.1	0.8 (0.6-1.8)	0.8	-
Other	0.6 (0.3-1.4)	0.2	1.2 (0.5-2.9)	0.6	-
Recurrent/progressive disease					
Timing of progression or relapse					
During induction chemotherapy	3.6 (1.9-6.7)	<0.0001	2.3 (1.2-4.3)	0.01	1
During consolidation chemotherapy	4.3 (2.5-7.3)	<0.0001	3.3 (1.6-6.8)	0.001	1
After completion of treatment:					
<24 months	2.3 (1.5-3.4)	<0.0001	1.6 (1-2.5)	0.04	1[b]
>24 months	1		1		
Relapse site					
Isolated pulmonary	1		1		
Isolated local	1.1 (0.7-1.8)	0.7	1.1 (0.6-1.9)	0.7	
Extra-pulmonary	2.4 (1.7-3.6)	<0.0001	1.8 (1.1-2.8)	0.009	1[a]
Local and pulmonary	2 (1.2-3.5)	0.01	2.1 (1.1-4)	0.02	1
Relapse chemotherapy					
HDT	1			1	
Non-HDT	5.4 (3.5-8.3)	<0.0001	4.3 (2.6-7)	<0.0001	-
No chemotherapy	14.9 (8.7-25.5)	<0.0001	11.7 (6.3-22)(5-18)	<0.0001	-

HDT: High-dose chemotherapy; VIDE/VAC/VCD: Vincristine, Ifosfamide, Doxorubicin, Etoposide/Vincristine, Actinomycin, Cyclophosphamide/Vincristine, Cyclophosphamide, Doxorubicin; E/VAIA: Etoposide/Vincristine, Actinomycin, Ifosfamide, Adriamycin; VDC/IE: Vincristine, Doxorubicin, Cyclophopsphamide/Ifosfamide, Etoposide

a relapse site score maximum 1

b time to progression score maximum 2

Table 4: Outcome according to Prognostic Group

Prognostic group (n)	**PRS (%, SE)**		**p**
	2 years	**5 years**	
Good (26)			
HDT (20)	80 (8.9)	64.6 (10.8)	<0.0005
Non-HDT (6)	62.5 (21.3)	0	
Intermediate (156)			
HDT (42)	62.8 (7.6)	46.9 (8)	<0.0005
Non-HDT (63)	23 (5.4)	3.3 (2.3)	
No chemotherapy (11)	9 (8.7)		
Poor (56)			
HDT (2)	50 (35.4)	Censored at 56mths	
Non-HDT (29)	6.9 (4.7)	0	
No chemotherapy (23)	0	0	

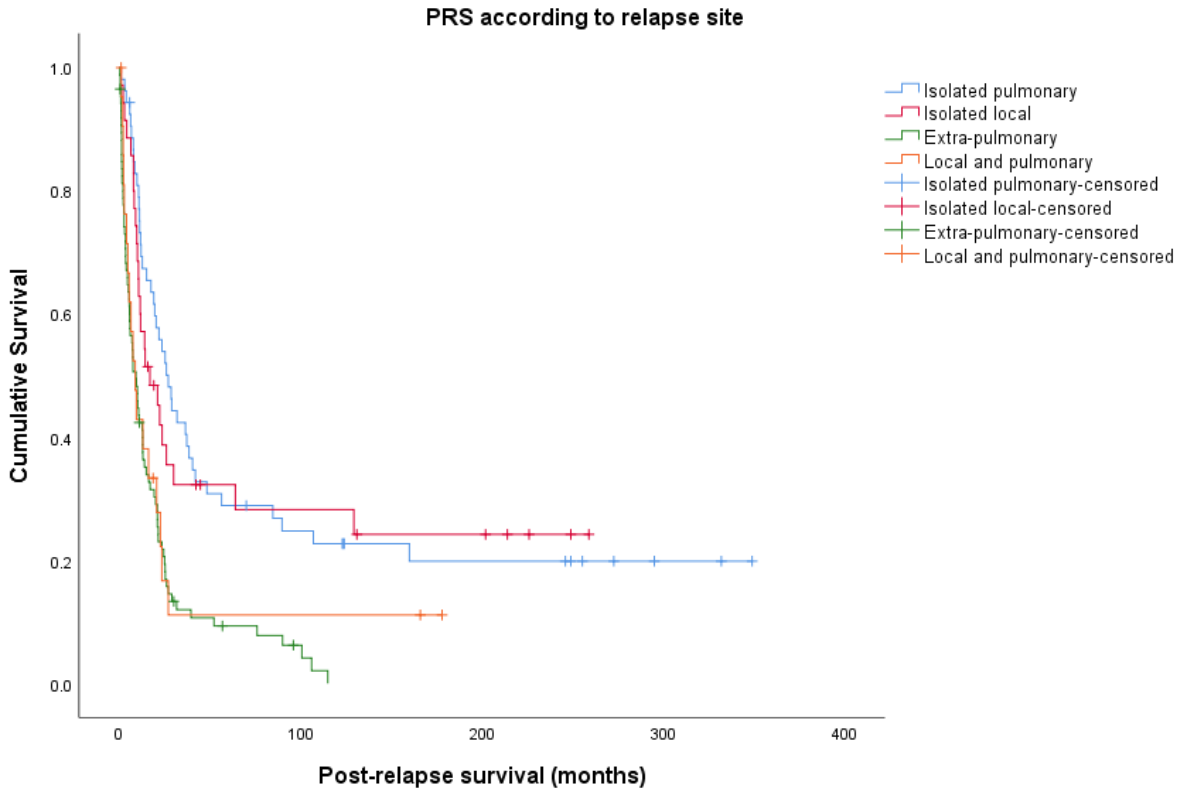
HDT – high-dose chemotherapy

Figure list

- Figure 1a: Kaplan-meier Post-relapse Survival according to relapse site
- Figure 1b: Kaplan-meier Post-relapse Survival according to HDT vs non-HDT
- Figure 2: Kaplan-meier Post-relaspe Survival according to prognostic group (A)Good risk (B) Intermediate risk (C) Poor risk

Figure 1: Post-Relapse Survival

1a: PRS according to relapse site



1b: PRS according to HDT/non-HDT

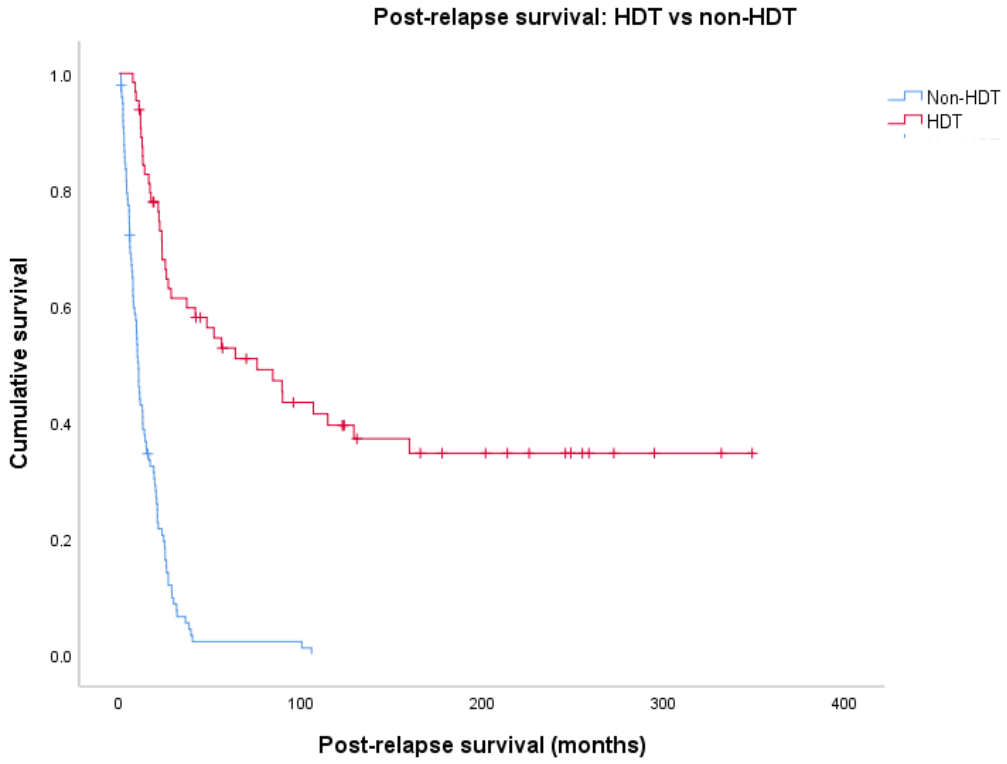
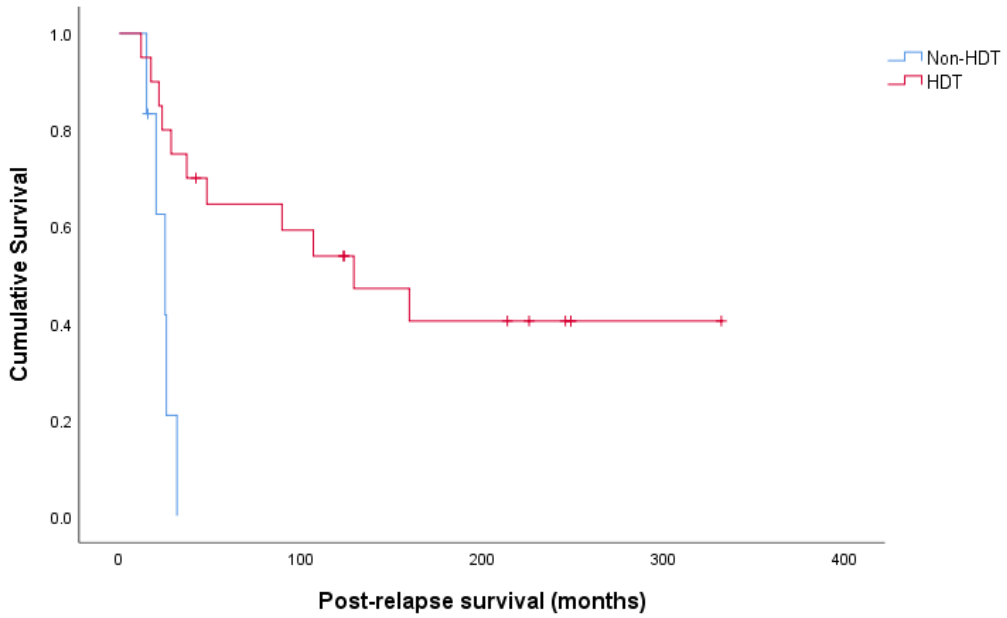


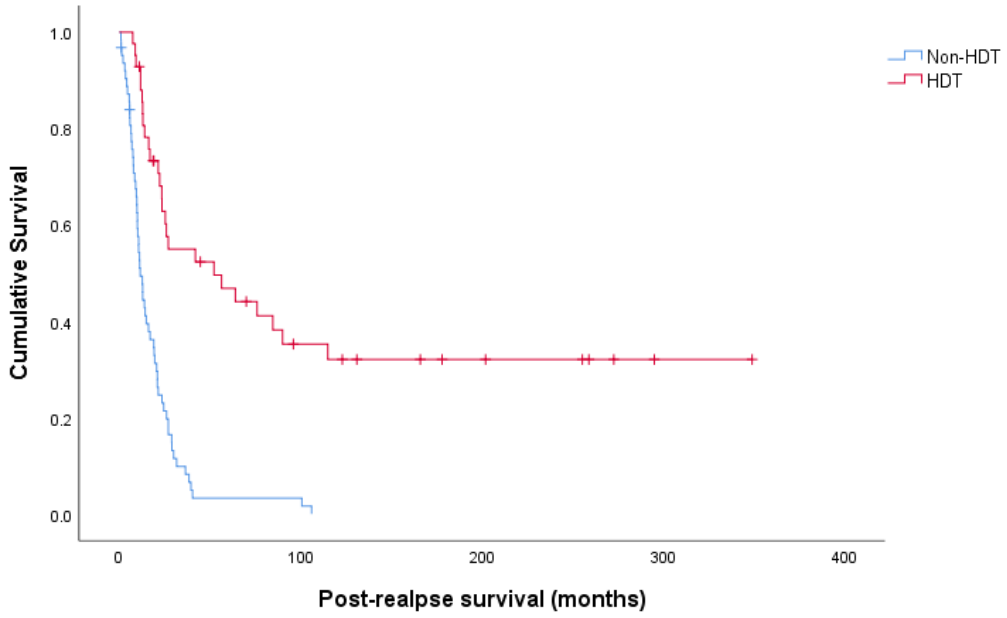
Figure 2: Post-relapse Survival according to prognostic group

A Good-risk



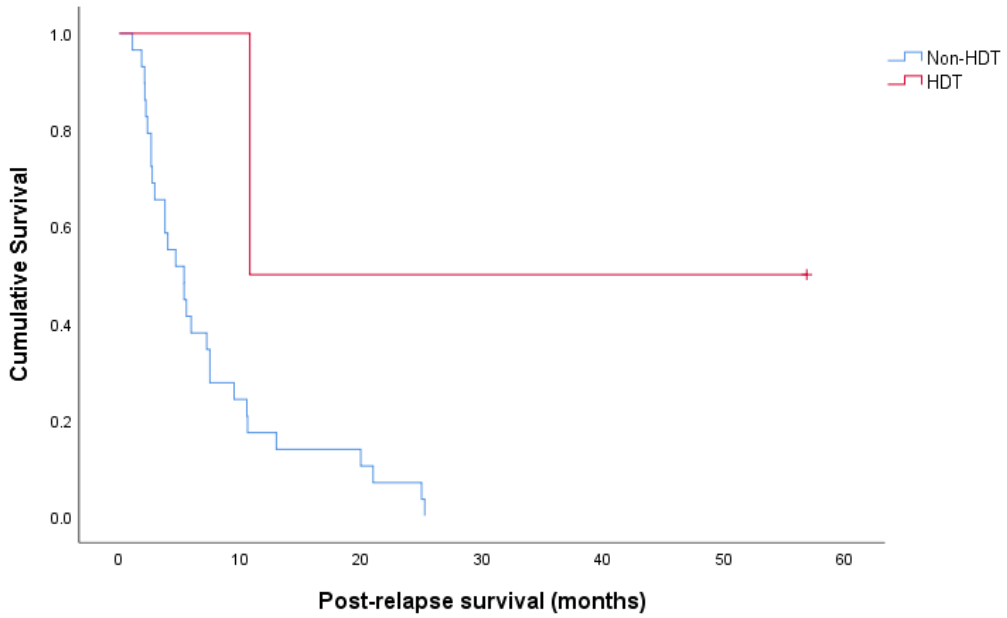
HDT – high-dose chemotherapy

b) Intermediate risk



HDT – high-dose chemotherapy

c) High risk



HDT – high-dose chemotherapy