Systemic Treatment of Ewing Sarcoma: Current Options and Future Perspectives

Research Article

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Abstract: Ewing sarcoma (ES) is an uncommon malignant neoplasm, mostly affecting young adults and adolescents. Surgical excision, irradiation, and combinations of multiple chemotherapeutic agents are currently used as a multimodal strategy for the treatment of local and oligometastatic disease. Although ES usually responds to the primary treatment, relapsed and primarily refractory disease remains a difficult therapeutic challenge. The growing understanding of cancer biology and the subsequent development of new therapeutic strategies have been put at the service of research in recurrent and refractory ES, generating a great number of ongoing studies with compounds that could find superior clinical outcomes in the years to come. This review gathers the current available information on the treatment and clinical investigation of ES and aims to be a point of support for future research.

Keywords: Ewing sarcoma • chemotherapy • systemic treatment

1. Introduction

Ewing sarcoma (ES) is an aggressive osteolytic malignancy, first described in 1921 by the American pathologist James Ewing^[1]. It represents 14% of bone tumours among both genders, being the third most common intraosseous malignancy after chondrosarcoma and osteosarcoma^[2]. ES mostly affects patients during the second decade of life, and it rarely develops after the age of 30. Although 20% to 30% of cases do occur during the first decade of life, it only rarely affects children below the age of 5^[3]. Although any bone can host ES, its most common primary location is within the diaphysis of the long bones, like the humerus, femur, and tibia. It can also appear in the axial skeleton, affecting the pelvis, spinal vertebrae, and thoracic wall^[4, 5]. ES originating from soft tissues mostly appears in adults, and makes up about 30% of cases^[6].

According to the relevant literature, the five-year survival rate of patients with localised ES is about 50% to 70%, with the appropriate locoregional and systemic treatment. Unfortunately, metastatic patients have a worse prognosis, with five-year survival rates between 18% and 31%. Lung parenchyma is the most frequent site of distant metastases, and extrapulmonary metastatic disease seems to significantly aggravate prognosis^[1, 6]. Apart from metastatic disease, primary tumour volume above 100 ml, pelvic or spinal primary location, and increased LDH at diagnosis are additional negative prognostic factors^[6–9].

Histologically, ES belongs to the family of “small, blue, and round cell tumours,” also including peripheral primitive neuroectodermal tumours (PNET), and Askin tumours^[10–13]. These are mainly intraosseous malignancies, occasionally appearing also in soft

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tissues. Intriguingly, the true origin of ES remains to be elucidated, as various cell types, including endothelial, mesodermal, epithelial, neural, and mesenchymal have been regarded as its potential precursors^[13]. In fact, there is evidence that ES can originate from mesenchymal stem cells, given their shared genomic features^[14].

Chromosomal translocations harboured by ES cells are of special interest, as they serve as ES fingerprints in cases of ambiguous diagnosis, and could play a crucial role in the biologic behavior and evolution of the malignancy. The most representative molecular feature of ES, recognized in 85% of patients, is the t(11;22)(q24;q12) translocation, resulting in the chimeric transcript EWS-FLI1^[10–13]. Indeed, the EWS (EWSR1, Ewing sarcoma breakpoint region 1) gene on chromosome 22q12 can fuse with any member of the ETS (erythroblast transformation specific) gene family, including ERG, ETV1, ETV4, and FEV^[10–13]. The EWS-FLI fusion transcript is regarded as an oncogenic protein, as the activity of the FLI transcription factor is modified by the EWS domain, resulting in an aberrant transcriptional function, potentially promoting sarcomagenesis^[15]. Notably, it seems that individuals of Caucasian origin are more prone to develop ES compared to Africans and Asians, as the former host a microsatellite extension, enhancing the binding of EWS-FLI1 chimeric transcriptional factor on the regulatory locus of the growth factor ERG2. This results in increased ERG2 transcription, eventually promoting cellular growth^[16]. During recent decades, EWS translocations have been investigated as potential treatment targets, unfortunately failing to produce satisfactory results. A recent publication has shown that the small molecule TK216, which inhibits the EWSR1-FLI1 fusion, has promising activity^[17]. Nonetheless, downstream targets and related proteins continue to be explored in an attempt to produce specific, targeted therapeutic agents^[18].

The low survival rates and the age of onset of these patients demonstrate the significant need for further understanding and research of this disease. This review presents the current clinical knowledge of local and metastatic ES, and it is intended to be a point of support for further research. All four authors participated equally in both literature research and the preparation of the article. A search of the keywords “Ewing sarcoma” in PubMed and Cochrane libraries yielded more than 10,500 results. The search was narrowed to series of cases, clinical trials, and metaanalyses; publications not in English and duplicated publications were rejected. Finally, around 300 papers were double screened, and 184 were selected to be used as references for this review.

2. Locoregional treatment

Surgical excision, radiotherapy (RT), or both are the modalities employed for locoregional disease control. There is no evidence supporting the superiority of either method, as no head-to-head comparison has been conducted to date.

Selection of surgery, RT, or their combination does not seem to affect event free and overall survival (OS) rates. Nonetheless, data support that surgery plus RT achieves better local disease control. In the CESS 86 trial, irradiation therapy alone resulted in a local control rate of 85% compared to 100% and 95% attained by surgery alone and surgery plus irradiation, respectively^[19]. Furthermore, in a retrospective analysis of CESS 81, CESS 86, and EICESS 92 trials, addressing the impact of local treatment modality on patients' clinical course, it was found that definitive RT had a local failure rate of 26.3%, whereas surgical excision, with or without postoperative RT, was followed by local relapse in only 7.5% of treated patients ($p = 0.001$). Preoperative irradiation, mostly employed in the EICESS 92 trial, only decreased the local failure rate to 5.3%. Five-year event-free survival rates were 47%, 59%, and 61% for definitive RT, surgery after preoperative RT, and surgery alone ($p = 0.0001$)^[20].

Likewise, in the INT-0091 study protocol, patients with pelvic ES treated with either irradiation or surgery alone had a local failure rate of 25%, compared to a rate of 10.5% for patients treated with both modalities, although this difference was not statistically significant ($p = 0.46$)^[21]. In a more recent meta-analysis of Children's Oncology Group trials (INT-0091, INT-0154, AEWS0031), treatment solely by irradiation was independently associated with a higher risk of local failure compared to surgical treatment (2.41; 95% CI [1.24, 4.68]), and it was not shown to increase the risk of distant relapse or death in the multivariate analysis^[22].

Although the preceding evidence highlights the significance of surgical excision of the primary tumour, it should be noted that patients undergoing definitive RT represent a group of negatively selected patients, mainly with axial skeleton primaries (e.g., vertebrae, pelvis), proximal to radiosensitive structures imposing dose reductions that might compromise the irradiation antineoplastic effect^[20]. Regardless of its limitations, definitive RT represents the only feasible treatment in selected patients, such as patients with spinal cord primaries, who cannot be submitted to an operation^[23, 24]. As for postoperative irradiation, it seems to confer a local control benefit to patients having undergone

intralesional or marginal resection, reducing local relapse incidence^[20, 22].

3. Adjuvant chemotherapy

Adjuvant chemotherapy for ES is based on multiagent regimens combining mitotic spindle poisons, anthracyclines, and topoisomerase inhibitors, and has accomplished prolonged event-free survival of patients after locoregional treatment.

The IESS-I trial examined the addition of doxorubicin to VAC regimen (vincristine, dactinomycin, cyclophosphamide), combined with RT of the primary lesion in 342 nonmetastatic patients between 1973 and 1978. The five-year regression-free survival (RFS) rate among 148 patients receiving doxorubicin was 60%, compared with 24% in the 74 patients that did not ($p < 0.001$). Combination of a VACA regimen (vincristine, dactinomycin, cyclophosphamide, doxorubicin) with RT of the primary and bilateral pulmonary RT was also tested as a third alternative choice, resulting in 44% of the 109 treated patients being free of regression at five years of treatment. Notably, the addition of doxorubicin did not confer any benefit in patients with pelvic ES in terms of RFS ($p = 0.81$), although it had a clearly protective effect in nonpelvic cases ($p < 0.001$). Distant metastases developed in 30%, 72%, and 42% of patients receiving VAC, VACA, and VACA plus bilateral pulmonary RT, respectively. Nonetheless, probability of local recurrence was not affected by treatment choice, and was noted in 15% of patients overall^[1]. Life-threatening toxicity rates were not found to be increased by doxorubicin addition, as they were 70%, 57%, and 61% ($p = 0.12$) in patients treated with VAC, VACA, and VACA plus lung RT, respectively^[25].

In the IESS-II trial, initiated in 1978, the standard dose VACA regimen was compared to an intensified VACA, intermittently administered at high doses in 214 treatment-naïve, nonmetastatic patients, after surgical excision or radiation of the primary. Five year disease-free survival (DFS), RFS, and OS rates were 68%, 73%, and 77% for the intensified VACA, and 48%, 56%, and 63% for standard VACA, respectively ($p = 0.02$, 0.03, and 0.05). Importantly, intensified VACA prevented the appearance of lung metastases in 89% of patients at five years of treatment, compared to 78% of patients receiving the standard dose regimen ($p = 0.04$), but it did not achieve a greater prevention rate of bone metastases. Severe toxicity was equally high in both groups, affecting about 67% of patients, but severe cardiac adverse events (AEs) occurred more frequently in the intensified VACA group (8% vs. 2% for standard

VACA regimen, $p = 0.02$), also resulting in the only three treatment-associated deaths of the study^[26].

Alternating of VACA regimen with courses of ifosfamide and etoposide (VACA-IE regimen) was evaluated by a multicentric Phase III clinical trial, recruiting patients included in the National Cancer Institute protocol INT-0091 (CCG-7881 and POG-8850) between 1988 and 1992. A total of 398 nonmetastatic patients, up to 30 years old, were randomly assigned by 1:1 to receive either VACA-IE or VACA. VACA-IE resulted in a five-year event-free survival (EFS) and OS rate of 69%, whereas VACA alone resulted in a rate of 54% ($p = 0.005$). The addition of ifosfamide and etoposide courses also conferred an OS advantage to patients, increasing the five-year OS rate from 61% to 72% ($p = 0.01$). According to the subgroup analysis, the benefit of the addition of ifosfamide and etoposide courses was more prominent in patients younger than 17 years old. Nevertheless, in cases of pelvic primary location, the experimental VACA-IE regimen was found to be more efficient, resulting in a five-year EFS of 56% vs. 36% for the comparative VACA treatment, supporting that the VACA-IE regimen might compensate for the negative prognostic impact of pelvic location, or increased tumour size, but not for older age. In terms of toxicity, the experimental regimen significantly prolonged hospitalization of treated patients and was associated with higher incidence of leukopenia and infections^[27].

In a later subgroup analysis of the trial results, regarding only pelvic ES cases, the addition of IE courses to the VACA regimen showed a trend toward better locoregional disease control, even at a nonstatistically significant level (11% vs. 30%, $p = 0.06$), whereas the selection of local treatment modality, including surgery, radiation, or both did not seem to affect the risk of local disease reappearance^[21]. In addition, the five-year EFS was 54% and 45% for patients under VACA-IE and VACA treatment, respectively, with a p value of 0.36^[21], confirming the negative prognostic impact of the pelvic location.

An alternative regimen, eliminating dactinomycin, VDC-IE (vincristine, doxorubicin, and cyclophosphamide with alternating courses of ifosfamide and etoposide), was administered by a standard dose and an intensified schedule, in a randomized trial of 478 patients, with localized bone and soft tissue ES^[5]. Patients in the experimental arm with an intensified 30-week-long treatment and patients in the standard-dose 48-week-long regimen achieved similar five-year EFS rates (70% vs. 72%) and also equivalent five-year OS rates (80% vs. 77%), failing to support superiority of VDC-IE intensification.^[28]

In a later randomized Phase III clinical trial, an intensified VDC-IE given every two weeks was compared to the standard three-weekly regimen. In accordance with the preceding observations, the intensified every-two-weeks regimen resulted in 73% of patients being free of disease at five years of treatment, compared to 65% with the standard three-weekly regimen, a difference of borderline statistical significance ($p = 0.048$). Again, pelvic primary location and age older than 18 years were poor prognostic factors, but outcome in these patient subgroups in relation to treatment schedule was not examined in the trial report. Both treatment schedules showed similar toxicity rates, with grade 3–4 febrile neutropenia affecting about 6% to 7% of patients in both treatment arms^[29].

An alternative combination of vincristine, doxorubicin, cyclophosphamide, and ifosfamide was assessed by the EICESS-92 study^[30], published in 2008. The study population consisted of standard risk patients with localized disease, whose primary tumour was smaller than 100 ml, and high-risk patients with either localized but bulkier disease (primary larger than 100 ml) or distant metastases. Standard risk patients received induction treatment with four cycles of VAIA (vincristine, dactinomycin, ifosfamide, and doxorubicin), and then were randomized to receive ten cycles of either a VAIA ($n = 79$) or VACA regimen ($n = 76$). High-risk patients received 14 cycles of either VAIA ($n = 240$) or EVAIA (VAIA plus etoposide; $n = 252$).

In low-risk patients, there was no significant difference noted in the three-year EFS and OS rates (about 73% and 86–90%) as a result of treatment with either VAIA or VACA, although cyclophosphamide administration was associated with higher hematologic toxicity rates. As for the high-risk group, etoposide addition to the VAIA regimen was found to reduce risk of any event and death by 17% and 10%, respectively. The benefit of etoposide addition was more prominent in high-risk patients with nonmetastatic disease, both in EFS and OS, compared to metastatic patients (HR 0.80 for both EFS and OS vs. HR 0.96 and 1.02). Nonetheless, none of these differences was statistically significant. In conclusion, etoposide addition to the VAIA regimen might protect nonmetastatic patients with a large primary tumour from disease recurrence and death.^[30]

Euro-EWING99-R1^[31] was an international, noninferiority, randomized trial, attempting to prove that cyclophosphamide can replace ifosfamide in the consolidation phase of treatment of standard risk patients. Eligible patients had localised ES with good histologic treatment response (meaning less than 10% viable cells after induction treatment), size smaller than 200 ml, or initially treated by radiation alone. Specifically, patients

received intensive induction treatment with six cycles of vincristine, ifosfamide, doxorubicin, and etoposide (VIDE), plus one cycle of VAI (vincristine, dactinomycin, and ifosfamide) and then they continued with either seven cycles of VAC (vincristine, dactinomycin, and cyclophosphamide) or VAI. A total of 856 patients were included in the study protocol and were 1:1 allocated to either treatment arm. In the VAC arm, three-year EFS was 75.4%, compared to 78.2% in the VAI arm, with an HR of 1.12 (91.4% CI[0.89, 1.41]), supporting that cyclophosphamide can be an appropriate substitute for ifosfamide in the consolidation phase of standard risk patients, provided that the latter is included in the induction phase. Similarly, the three-year OS rates did not significantly differ between the two treatment arms (HR = 1.09, 91.4% CI[0.84, 1.42]). This is important, considering that cyclophosphamide use was associated with better tolerance and fewer regimen modifications due to toxicity, as well as a lower nephrotoxicity rate (acute tubular grade 2–4 toxicity in 16% with VAC vs. 31% with VAI). Nonetheless, patients in the VAC arm suffered more frequently from thrombocytopenia (45% vs. 35%).^[31]

High dose consolidation treatment of patients with localized ES, with a high relapse risk, was examined by the R2Loc trial^[32] conducted between 2000 and 2015. A total of 240 patients with primary tumour larger than 200 ml, or tumour irradiated prior to local excision, or with more than 10% viable cells in tumour biopsy after induction treatment with six courses of VIDE were randomized to receive consolidation treatment either with one cycle of high-dose busulfan and melphalan (BuMel) followed by autologous stem cell transplant or standard chemotherapy with VAI. The three-year EFS and OS rates were 69% and 78% in the BuMel arm, respectively, compared to 56.7% and 72.2% achieved by the standard VAI regimen. The protective effect of BuMel persisted beyond five years of treatment, with respective eight-year EFS and OS rates of 60.7% and 64.5%, vs. 47.0% and 55.6% reached in the VAI arm. As expected, a higher rate of severe AEs, involving hematologic toxicities, infections, and liver and gastrointestinal tract toxicities, was observed in the BuMel arm. Among the three treatment-related deaths, two occurred in the experimental treatment group, and only one of them was due to pancytopenia induced by chemotherapy.

In summary, adjuvant chemotherapy to reduce local or distant relapse risk is recommended to all ES patients after locoregional treatment with surgery, radiation, or both.

International guidelines recommend multiagent chemotherapy regimens, together with appropriate

growth factor support. VDC-IE, VAI, and VIDE are all acceptable choices, keeping in mind that etoposide addition has been found to especially benefit high-risk patients^[6]. High-dose chemotherapy followed by bone marrow transplant can also be considered as a strategy against high-risk disease, although associated with higher toxicity rates^[32]. Dose-dense regimen might offer a certain benefit, mainly in younger patients^[29]. We should keep in mind that old age and pelvic location of the primary tumour, as well as large size of the primary, are negative prognostic factors, prone to compromising the adjuvant treatment effect. Finally, about 30% to 40%^[6] of patients with ES will eventually experience relapse, a rate that has not been reduced since the 1980s. Taken together with the fact that affected patients are mostly children, teenagers, and young adults, this highlights the current need for further research, aiming to prolong the disease-free interval and eliminate the risk of relapse in the affected population.

4. Ongoing clinical trials

Locoregional disease control is also addressed in current clinical trials. Application of intensity modulated radiotherapy (IMRT) against ES of pelvis or spine, in the neoadjuvant or adjuvant setting, is being assessed by the IMRiS trial. The trial's main aim is to investigate reduction of radiation-induced toxicity, compared to conventional irradiation protocols (NCT02520128)^[33].

The addition of vincristine, topotecan, and cyclophosphamide in the standard initial treatment protocol against nonmetastatic, extracranial ES, in children and adults up to 50 years old, is currently being examined by a Children Oncology Group randomized Phase III trial. The control arm includes induction and consolidation treatment with alternating VDC-IE, and the experimental arm consists of vincristine, topotecan and cyclophosphamide (VTC) also alternating with etoposide and ifosfamide. The primary endpoint of the study is EFS rate at five years from study enrollment, and OS is secondarily investigated (NCT01231906)^[33].

An Italian sarcoma group trial is investigating the effect of an intensified three-agent protocol, involving high doses of vincristine, doxorubicin, and cyclophosphamide, compared to the standard doses of VACD-IE (ISG SSG III protocol), in the setting of induction treatment for stem cell transplant, with EFS being the primary trial endpoint (NCT02063022)^[33].

Furthermore, addition of irinotecan and temozolomide doublet sequentially to the standard VACD-IE regimen is going to be explored in patients up to 40 years old, with newly diagnosed ES, in a Phase II

trial (NCT01864109), organized by the Memorial Sloan Kettering Cancer Center.

Immunotherapy strategies have not been applied against ES to date. The combination of nivolumab and ipilimumab is going to be tested in patients with recurrent bone cancer, including relapsed ES (NCT02982486)^[33]. If immune checkpoint inhibitors (ICIs) prove to act effectively, this will reveal a new field of clinical investigation against ES, involving immunotherapeutic agents.^[33]

5. Long-term concerns

Secondary malignant neoplasms occurring in patients treated for localized ES are of specific interest, as affected patients are mostly young. According to reported data, secondary neoplasms occur in 1.1% to 3.1% of patients treated for localized ES^[27, 28–32].

The most common secondary malignancies are acute myeloid or lymphoblastic leukemias, appearing in 2.5% of the treated patient population^[5], and osteosarcomas developing within the tumour irradiation bed^[27, 30, 31]. Other secondary solid neoplasms are also reported in trials, including ovarian cancer, renal cell cancer, and malignant fibrous histiocytomas^[27–32]. Furthermore, in a follow-up observational study of patients treated for localized ES, the cumulative incidence of secondary cancer reached 9.0% (95% CI[5.8, 12.2]), proving that the occurrence of a subsequent neoplasm is a perpetual hazard for healed individuals^[34]. Consequently, provided that adjuvant chemotherapy cannot be omitted, treated patients should be followed up and regularly screened for secondary malignancies to ensure timely treatment.

In the long term, patients treated at a young age for localized ES might be concerned with functional impairment and rehabilitation issues, severely compromising their quality of life^[34–37]. Limb and pelvic reconstruction techniques have been developed within the last 40 years, aiming to preserve patients' mobility and independence^[35–37]. Encouragingly, ES patients have been reported to return to physical activity at rates of 83%, although the activity of patients with pelvic ES depended on the surgical reconstruction technique^[36]. Therefore, selection of local treatment modality, surgical technique, and rehabilitation exercises after treatment are of great significance in improving patients' physical performance and general condition^[36]. Indeed, an ongoing trial is assessing the grade of functional rehabilitation of affected extremities, after reconstruction treatments, as evaluated by patients, clinicians, and objective diagnostic tests (NCT03442465)^[33].

Finally, young patients who have undergone chemotherapy for ES are at risk of subfertility in their adult life due to chemotherapy with alkylating factors or pelvic irradiation^[6, 38]. Alarming, in a comparison of 341 treated sarcoma young adults with 2,878 healthy, untreated siblings^[39], the percentages of women achieving a pregnancy and men siring one, among former ES patients, were 29.7% and 11.3%, respectively, compared to 40.1% and 33.2% among their healthy siblings. After adjustment for social and other confounding factors, the probability of female ES survivors getting pregnant was 35% lower than for their healthy sisters ($p = 0.005$). Similarly, ES male survivors had also a 62% lower chance of siring a pregnancy, compared to healthy controls ($p < 0.001$).

These data underline the importance of fertility counseling and the selection of the appropriate fertility preservation strategy for each patient, with respect to national and local health care legislation^[38]. For male and female patients of reproductive age, storage of semen and oocytes, respectively, can be undertaken before treatment initiation, and cryopreservation of embryos can apply for both sexes. Female prepubertal patients can be offered cryopreservation of ovarian tissue, but cryopreservation of testicular tissue for prepubertal males is experimental and has no proven clinical value^[38, 39]. A promising strategy of ovarian reserve preservation might be the administration of gonadotropin analogues during chemotherapy, which has encouraging results in women receiving adjuvant treatment for breast cancer^[40], and is currently being assessed among ES female patients in an ongoing clinical trial (NCT02856048)^[33].

Further, it must be highlighted that only seven patients have been diagnosed with ES during their pregnancy. Five of them received chemotherapy during pregnancy and one patient experienced abortion due to oligohydramnion. Chemotherapy regimens given in the adjuvant setting were either VIDE or VAC^[41].

6. Options for refractory and relapsed disease

As already mentioned, disease relapse affects 30% to 40% of patients, and includes local or distant disease recurrence. Characteristics that increase treatment chances at disease recurrence include relapse beyond two years of initial treatment, excisable disease, and exclusively intrapulmonary metastases^[42, 43]. Indicatively, the five-year survival rate after disease relapse has been found to be 55% and 22% for local and distant relapse,

respectively^[44]. According to the relevant literature, only 2.5% to 12.0% of patients who relapse earlier than two years after initial diagnosis are still alive at five years of relapse, compared to 14% to 50% of patients having a later relapse^[42–44]. Chances of complete remission are slim (reported for about 13% of patients) and are often followed by a second recurrence^[44]. Additionally, although its frequency is not adequately reported, refractory ES, which is unresponsive to primary systematic treatment, poses a particular therapeutic challenge.

Several chemotherapeutic regimens have been approved for the treatment of refractory or relapsed ES (Table 1). However, long-term toxicity of drugs used in the perioperative or first-line setting, mainly secondary leukemia, limits their use^[45, 46]. Addition of carboplatin to ifosfamide and etoposide, in a later trial of relapsed bone sarcoma patients, had equivalent results^[46]. Studies of combinations of irinotecan and temozolomide with or without vincristine have shown the best results to date^[47–53], as it is associated with satisfactory response rates, acceptable toxicity, and the availability of outpatient oral treatment^[54]. In such cases, the dose of irinotecan is adjusted to achieve the expected bioavailability, and oral cephalosporines are coadministered to reduce the irinotecan-derived diarrhea^[55]. Notably, absent or low MGMT expression was correlated with higher response rates in a Phase I study of temozolomide and protracted irinotecan in refractory pediatric solid tumours^[56].

Docetaxel–gemcitabine^[57–59], ifosfamide–etoposide with or without carboplatin^[60–63], as well as cyclophosphamide–topotecan with or without vincristine addition^[64–68] are also commonly utilized in the same setting. High-dose ifosfamide, a regimen that requires durable hospitalization, has shown some modest activity in relapsed and refractory disease^[69–71].

Due to the lack of a standard of care in the second-line setting, safety and efficacy of the approved regimens have not been assessed in a randomized clinical trial. This question has been recently addressed by the rEECur study, which compares the efficacy of four regimens—cyclophosphamide–topotecan, gemcitabine–docetaxel, high-dose ifosfamide, and temozolomide–irinotecan—and aims to propose a standard approach to recurrent and refractory ES. Preliminary results have shown that the gemcitabine–docetaxel combination is the least effective one ($n = 66$, RR = 11.5% with 95% CI [4.4%, 23%], median PFS, 3 months with 95% CI [1.6, 8], median OS, 13.7 months with 95% CI [10.1, 23.9]), but is associated with fewer grade 3 and 4 toxicities (58% vs. 74% in the rest)^[72]. Second interim results proved that the irinotecan–temozolomide combination is less

Table 1: Approved chemotherapeutic regimens against treatment of refractory/relapsed Ewing's sarcoma.

Regimens	Cumulative patients	ORR %	PFS	OS	Main toxicities
Cyclophosphamide topotecan	49	32.6	N/A	61% at 1 year	Myelotoxicity Alopecia
Window therapy	17	36	> 9.6 months	N/A	
	13	23	8 months	N/A	
	37	57	N/A	N/A	
	14	50	N/A	15 months	Myelotoxicity Alopecia
Cyclophosphamide topotecan vincristine					
Irinotecan temozolomide +/- vincristine	25	64	5.5 months	N/A	Myelotoxicity Diarrhea
	14	28.5	5 months	N/A	
	22	54	3 months	2 year OS: 26.9% 55% at 25.7 months	
	19	63	8.3 months	55% at 1 year 30.9% at 2 years	
	51	34	3.9 months	54.2% at 1 year 13.9 months	
	15	40	6 months		
	20	55	5.5 months		
	118	20	4.7 months		
	17	94	N/A	N/A	Myelotoxicity, 97% Neutropenia
	18	16	N/A	4.8 months	
				N/A	
Ifosfamide etoposide	55	25	N/A		
	35	34	N/A	2-year OS: 29%	Myelotoxicity Neurotoxicity Alopecia Renal insufficiency
				4-year OS: 39%	
	30	N/A	4 year EFS: 27%	N/A	
High-dose ifosfamide			N/A		
	7	86			
	22	45	N/A	1 year: 43% 2 years: 33%	Myelotoxicity
Ifosfamide carboplatin etoposide					
Docetaxel gemcitabine	2	0	N/A	N/A	Myelotoxicity
	2	0	N/A	4.5 months	Neurotoxicity
				50% at 48 months	Alopecia
	6	67	10 months	13.7 months	Allergic reactions
	66	11.2	3 months		

Note: ORR = overall response rate; PFS = progression-free survival rate; OS = overall survival rate; EFS = event-free survival. Data from [47–53, 57–73].

effective than cyclophosphamide–topotecan and high-dose ifosfamide^[73] and the remaining two arms continue to recruit patients.

High-dose chemotherapy with autologous stem cell transplant (ASCT) has been also utilized in selected cases with recurrent ES, especially as a consolidation therapy for relapsed disease, able to attain two- and five-year EFS rates of 42.5% and 38.2%, respectively. Indeed, a retrospective analysis of 45 patients with high-risk ES treated with ASCT ($n = 20$) or conventional chemotherapy ($n = 25$) after induction with VAC-IE showed a three-year OS of 59% vs. 34%, favoring ASCT^[74]. However, there are insufficient data to promote this practice as a standard of care for all patients^[75–82].

The use of extended low-dose maintenance therapy for patients in remission after conventional therapy is of special interest, although the benefits of this approach have not been established^[83]. If a relapse is delayed, retreatment with a previously effective regimen might be useful^[60].

As conventional chemotherapy has modest activity, all patients with recurrent and metastatic disease should receive treatment in a clinical trial when available. New studies on ES explore the efficacy of untested chemotherapy, targeted therapy, immunotherapy, tumour-cell environment modulators, and epigenetic therapeutics.

7. Ongoing clinical trials

7.1. DNA damage

Chemotherapy is still the backbone of treatment of both localized and metastatic disease. Attempts to improve disease control with new chemotherapeutic agents are currently underway.

The interaction of chimeric protein EWSR1-FLI1 with tubulin- α stabilizing microtubules during metaphase lays the foundation for research on microtubule inhibitors in metastatic ES^[85]. Results of preclinical studies with eribulin and nab-paclitaxel^[86, 87] led to independent clinical research with both drugs. Eribulin and nab-paclitaxel as monotherapy or in different combinations are under investigation in Phase I-II clinical trials (Table 2).

Trabectedin is a DNA-minor-groove binder widely used in soft-tissue sarcomas that seems to be especially active in translocation-related sarcomas^[88]. Thus, its use in ES looks appealing. However, monotherapy has not achieved encouraging response rates^[89], and a Phase I trial is investigating its combination with irinotecan in sarcoma patients. Lurbinectedin has similar biological activity to trabectedin^[90]. The slightly different structure (a tetrahydro- β -carboline replacing the tetrahydroisoquinoline of trabectedin), though, might confer higher affinity to transcription factors and explain the better tumour response to this compound, as a Phase II study of lurbinectedin monotherapy in pretreated ES patients showed (4 partial responses out of 28 participants)^[91].

DNA damage can also be exploited by PARP inhibition, as the latter leads to persistent single-strand breaks and eventually to cellular apoptosis. EWS-FLI1 has been found to confer sensitivity in PARP-1 inhibition and to maintain a feed-forward loop that drives PARP-1 expression, whereas PARP-1 inhibition has been found to prevent tumour growth and metastasis in EWS-FLI positive xenografts^[92]. Preclinical data demonstrating sensitivity of ES cell lines to PARP inhibitors^[93] led to a Phase II study of olaparib in patients with relapsed disease, where 4 of 12 enrolled patients had disease stabilization, but no objective responses occurred^[94]. PARP inhibitors have shown encouraging antitumour activity when combined with DNA-damaging chemotherapy in xenografts^[95–97]. Niraparib has shown encouraging results when combined with temozolomide in preclinical studies^[98, 99]. Ongoing Phase I trials of olaparib–temozolomide as well as niraparib–temozolomide with or without irinotecan will determine whether further trials with PARP inhibitors should be conducted. A group of

cyclin-dependent kinases regulates the expression of DNA damage repair genes, including BRCA1 and ATR. A selective covalent inhibitor of CDK12, CDK13, and CDK7 known as THZ531, acts primarily via CDK12 inhibition. Preclinical studies show synergy with PARP inhibitors and antitumour activity in ES mouse models that express EWS-FLI1^[100].

7.2. Blocking DNA transcription

Because the transcription factor EWSR1-FLI1 seems to orchestrate the tumour biology, the inhibition of histone deacetylases to prevent DNA transcription is a plausible strategy. Histone deacetylase inhibitors are epigenetic drugs that regulate gene expression through action on histones and nonhistone proteins. Preclinical studies have proven their ability to inhibit tumour growth mainly by upregulating tumour suppressor genes and downregulating oncogenes, decreasing invasion, metastasis, and angiogenesis, and inducing apoptosis and cycle cell arrest in ES cells^[101]. Studies have shown that they sensitize sarcoma to chemotherapy, reverse multidrug resistance, and have a synergistic effect on pazopanib^[102–112]. Given their acceptable toxicity profile, they seem to be ideal candidates for combination treatment. Along this line, the HDAC-inhibitor vorinostat is currently being evaluated in combination with chemotherapy in relapsed and refractory solid and central nervous system tumours in a Phase I trial.

The bromodomain and extraterminal domain (BET) family of proteins recognise the acetylated lysine residues of histones and transcription factors and are supposed to have a central role in cancer. With this mechanism of action, BET inhibitors merit investigation in ES. Preclinical data^[113, 114] show that inhibition of BET negatively affects gene expression through the EWS fusion protein, and it downregulates the insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3), a member of the IGF2BP family. These proteins bind mRNAs via 6 RNA-binding domains, thus controlling cytoplasmic stability and translation of transcripts. IGF2BP3, but not IGF2BP1 nor IGF2BP2, has been suggested as a high-risk biomarker in ES patients. Altogether, these findings make BET inhibitors an appealing field of clinical research in ES.

On the other hand, histone-demethylases have a known role in tissue differentiation and cancer^[115]. Histone lysine-specific demethylase 1 (LSD-1), the first historically identified histone demethylase, is a highly expressed enzyme in samples and blocks the transcriptional activation of downstream targets of

Table 2: Agents under investigation in Phase I, II clinical trials, against Ewing's sarcoma.

Agent	Target/mechanism of action	Phase	Eligibility	Status
Eribulin	Microtubule inhibitor	II	12 months to 18 years old	Recruiting
Eribulin–irinotecan	Microtubule inhibitor - cytotoxic	I–II	6 months to 17 years old	Recruiting
Nab-paclitaxel–gemcitabine	Microtubule inhibitor - cytotoxic	II	12 to 30 years old	Recruiting
		I	6 months to 30 years old	Recruiting
Nab-paclitaxel	Microtubule inhibitor	II	6 months to 80 years old	Recruiting
Trabectedin–irinotecan	Cytostatic–cytotoxic	I	10 years and older	Not yet recruiting
SM-88–MPS	Protein synthesis, multiagent	II	12 years and older	Recruiting
Ganitumab– chemotherapy	IGF-1R, cytotoxic/ cytostatic	III	Up to 50 years old	Active, not recruiting
Cabozantinib	MET	II	2 to 30 years old	Recruiting
Palbociclib–temozolomide +/- irinotecan	CDK4/6, multiagent	I	2 to 20 years old	Recruiting
Palbociclib–ganitumab	CDK4/6, IGF-1R	II	12 to 50 years old	Recruiting
Pazopanib–irinotecan–temozolomide	Multityrosine kinase inhibitor, multiagent	I	6 to 30 years old	Active, not recruiting
Olaparib–temozolomide +/- irinotecan	PARP, multiagent	I	16 years and older	Recruiting
Niraparib–irinotecan–temozolomide	PARP, multiagent	I	13 years and older	Recruiting
INCB059872	LSD-1	Ib	12 years and older	Recruiting
Seclidemstat	LSD-1	I	12 years and older	Recruiting
Abemaciclib–temozolomide/irinotecan	CDK4/6, multiagent	I	Up to 18 years old	Recruiting
Selinexor–ixazomib	CRM1, PSMB5	I	14 years and older	Not yet recruiting
Vorinostat–chemotherapy	Histone deacetylases, multiagent	I	1 to 30 years old	Recruiting
Ivosidenib	IDH-1	II	1 to 21 years old	Not yet recruiting
Vigil–temozolomide–irinotecan	Biological, multiagent	III	2 years and older	Active, not recruiting
CAR-T cells	Biological	I – II	18 to 65 years old	Recruiting
EGFR806 CAR T cell immunotherapy	Biological	I	1 to 26 years old	Recruiting
C7R-GD2.CART cells	Biological	I	1 to 74 years old	Recruiting
pbi-shRNA™ EWS/FLI1 Type 1 LPX	Biological	I	8 years and older	Active, not recruiting
CLR 131	Radioionated drug	I	2 to 21 years old	Recruiting

Note: IGF-1R = insulin-like growth factor 1 receptor; MET = hepatocyte growth factor receptor; CDK4/6 = cyclin-dependent kinases 4/6; PARP = poly-ADP-ribose polymerase; LSD-1 = lysine-specific histone demethylase 1A; CRM1 = chromosomal maintenance 1; PSMB5 = proteasome 20S subunit beta 5; IDH-1 = isocitrate dehydrogenase 1; CAR-T = chimeric antigen receptor T.

EWS-FLI1, thus leading to tumour growth, possibly by inhibiting p53 activity^[116]. LSD-1 inhibition has induced apoptosis in ES cell lines^[117] and two Phase I trials investigating the LSD-1 inhibitors INCB059872 and seclidemstat are ongoing at present.

Furthermore, there are some EWS-FLI1 target agents that have shown encouraging results in the preclinical setting and could be exploited in further studies. The binding between EWS-FLI1 fusion protein and RNA helicase A has been targeted via TK216, an investigational product that has demonstrated encouraging antitumour activity against ES cells in

preclinical studies^[118]. A Phase II study is currently in the expansion phase (NCT02657005).

8. Drugs with activity on cell cycle

Many anticancer drugs take advantage of the high cell-division rate and exert their activity on the cell cycle. Antimetabolites, widely used in oncology to cause cell death during phase S, are a good example of this. With this same rationale, the dysfunctional tyrosine SM-88 (racemetyrosine) interrupts the protein synthesis in

Figure 1: Current treatment algorithm of localized Ewing's sarcoma. Note: VDC/IE = vincristine-doxorubicin-cyclophosphamide/ifosfamide-etoposide; VAI = vincristine-actinomycin D-ifosfamide; VIDE = vincristine-ifosfamide-doxorubicin-etoposide; RT = radiotherapy; chemo = chemotherapy; BSC = best supportive care; MSI-h = microsatellite instability high; dMMR = deficient mismatch repair; PD = progressive disease.

cancer cells. Tested in a Phase I study of 30 participants with advanced metastatic cancer, it achieved four complete and six partial responses^[119] and is currently being evaluated in combination with rapamycin, phenytoin, and methoxsalen in a Phase II trial.

More recently, interest has shifted to G1-S checkpoint, regulated by a complex intracellular pathway of effectors and inhibitors, where CDK4/6 have a crucial role by phosphorylating the RB protein. Tumour suppressors p16^{INK4a} and p14^{ARF} are both coded by the CDKN2A gene and inhibit the complex CyclinD1-CDK4/6, disrupting the progression of the cell cycle^[120]. CDK4/6 inhibitors led to cytostasis and apoptosis in ES cell lines^[121] and antitumour effects in preclinical ES models^[122]. Palbociclib and abemaciclib are currently being tested in combination with temozolomide-irinotecan in two separate Phase I trials.

Activation of the p53 tumour suppressor gene is another possible target for exploitation. The Mouse double minute 2 homolog (MDM2) oncoprotein downregulates

p53, leading to accelerating tumour formation^[123–126]. Because p53 mutations are rare in ES, the inhibition of MDM2 has been successfully tested in ES cell lines, showing also synergistic action with chemotherapeutic drugs^[126]. Similarly, MDMX or else MDM4 inhibition activates p53 and induces apoptosis^[127]. ALRN-6924, a dual MDM2/MDMX inhibitor, is currently being studied in Phase I trials for pediatric cancer (NCT03654716) and for patients with advanced solid tumours including ES in combination with paclitaxel (NCT03725436).

Targeting of oncogenes is a widely exploited strategy, but boosting normal tumour suppressor proteins would be another interesting approach in ES. Selinexor, a small-molecule inhibitor of the nuclear export protein XPO1 leading to accumulation of tumour suppressor proteins and thus promoting apoptosis, has shown encouraging results in preclinical and Phase I studies^[128–131]. Moreover, combination with proteasome inhibitors might provide synergistic activity^[132, 133]. With this rationale, a Phase I trial investigating the

Figure 2: Current treatment algorithm of metastatic Ewing's sarcoma. Note: VDC/IE = vincristine-doxorubicin-cyclophosphamide/ifosfamide-etoposide; VAI = vincristine-actinomycin D-ifosfamide; VIDE = vincristine-ifosfamide-doxorubicin-etoposide; RT = radiotherapy; chemo = chemotherapy; BSC = best supportive care; MSI-h = microsatellite instability high; dMMR = deficient mismatch repair; WLI = whole lung irradiation; PR = partial response; CR = complete response; PD = progressive disease.

combination of selinexor and ixazomib in advanced sarcoma patients is currently recruiting patients.

9. Drugs targeting intracellular pathways

Because EWS-FLI1 is a clear driver in ES, inhibition of known intracellular pathways related to survival and cell division might not be a strategy on which to rely. The hyperexpression of phosphorylated AKT in ES cells^[134] promoted the research of mTOR inhibitors. However, results of early phase studies with monotherapy are disappointing^[135], and combination of temsirolimus with chemotherapy has only shown modest activity in Phase I and II studies including patients with sarcomas^[136, 137]. This

failure can be explained by the fact that hyperactivation of PI3K/AKT/mTOR and MEK/ERK/MAPK pathways is related to the strong activation of the insulin-growth factor-1 receptor (IGF-1R) due to the pubertal growth spurt^[138]. As a result, most ES tissues express IGF-1R and its inhibition has induced tumour regression in preclinical studies^[139–142]. Ganitumab, an IGF-1R inhibitor, showed encouraging results in both Phase I^[143] and Phase II^[144] clinical trials, with a clinical benefit rate of 36.8% when used as monotherapy. To improve these results, ganitumab is now being tested in combination with palbociclib in a Phase II trial (NCT04129151) and in combination with chemotherapy for newly diagnosed metastatic ES has completed recruitment and results are eagerly awaited (NCT02306161).

10. Antiangiogenics

Angiogenesis plays a significant role in tumour progression and antiangiogenic drugs are used against many types of neoplasms^[145]. It is another intriguing target in ES^[146–149] with a number of promising agents under investigation. Pazopanib mainly targets VEGFR-1 and -2, PDGFR- α and - β , and c-Kit^[150] and it has been tried in ES patients after its approval for soft tissue sarcoma^[151], even if they were not included in this Phase III study. Several case reports demonstrate responses in patients with ES^[152–154], but prolonged use is associated with resistance^[155]. Besides, preclinical studies in xenografts have revealed statistically prolonged EFS, but without any objective responses^[156]. Cabozantinib is a VEGFR2, c-MET, and AXL targeting agent that has shown antitumour activity in osteosarcoma and ES tumour models in preclinical studies^[157]. After encouraging results of a Phase II study in recurrent ES (clinical benefit rate of around 74%, median PFS of 4.4 months, and median OS of 10.2 months)^[158], a Phase II study of cabozantinib in younger patients with sarcomas is ongoing. Regorafenib is a multikinase inhibitor targeting VEGFR-1-3, FGFR1, PDGFR- α and - β , CSFR-1, and c-Kit. It has shown efficacy in pretreated patients with metastatic osteosarcoma^[159, 160]. A Phase I trial assessed its activity in heavily pretreated patients with sarcomas, leading to a 10% response rate with a median PFS of 3.6 months and a median duration of response of 5.5 months^[161]. Anlotinib, an oral VEGFR, FGFR, PDGFR, and c-kit inhibitor, has been studied in combination with irinotecan after encouraging results in advanced soft-tissue sarcoma^[162] and this combination also led to promising results in a Phase Ib/II trial including patients with recurrent and relapsed ES^[163].

11. Genetic therapy

Genetic therapy is very promising in medical oncology, especially in cases of diseases with dismal prognosis, as it combines the advances of molecular biology and genetic engineering^[164]. pbi-shRNA EWS-FLI Type 1 LPX is a functional plasmid DNA construct, designed to transcribe both siRNA and miRNA-like effectors that target the Type 1 translocation junction region of the EWS-FLI1 transcribed mRNA sequence. Preclinical studies have proven the safety and efficiency of this method^[165] and results from a Phase I trial are pending.

12. Immunotherapy

With the advent of ICIs as effective treatment for several carcinomas, many attempts to describe the immunological profile and potential biomarkers and new targets have been made. In this regard, a large genomic survey found that ES has a very low TMB (0.15 mutations/MB)^[166], and gangliosides have been proven to exert an important action in tumourigenesis and metastases, rising as likely targets for next-generation treatments^[167].

Recently, ICIs have been approved for the treatment of a broad range of cancers and other immunotherapeutic strategies, such as vaccines and chimeric antigen receptor (CAR) T-cells are under evaluation in Phase I–III clinical trials. The anti-PD1 pembrolizumab was approved for patients with metastatic MSI-High solid tumours after achieving an objective response rate of 34.3% in a basket study^[168]. Clearer data are needed for sarcoma patients, as its efficacy might be restricted to specific subtypes, as learned by the results of SARC028 study with pembrolizumab^[169]. Low expression of PD-1 and PD-L1 (less than 25%) alongside a low tumour-mutation burden in comparison to other malignant diseases^[170–174] might be the basis of the disappointing outcomes of ICI in ES^[169, 175, 176].

These results drive researchers' attention to other strategies aimed at unmasking tumour cells to the immune system by recognition of cell-surface proteins expressed by the cancer cells and not by the healthy ones, known as tumour-associated antigens (TAA). Cluster of differentiation 99 (CD99) is a characteristic TAA of ES, thought to inhibit neural differentiation through the MAPK pathway, thus assisting in cell proliferation and tumour growth^[177]. A radioiodinated anti-CD99 antibody has been successfully used for image-targeting ES in murine xenografts, which introduces the possibility of creating a CD99-targeted immunotherapy^[178]. Expression of disialogangliosides on the surface of tumours originated from the neural crest is a reactivated field of research in ES after decades of silence^[179–182]. The recognition of ES cells by CAR T-cells against ganglioside-2 (GD2) showed cytotoxic efficacy both in vitro and in vivo^[183] and states the rationale of an ongoing Phase I trial evaluating the use of C7R-GD2 CART cells in GD2-positive cancers.

Vaccination with irradiated autologous ES cells engineered to secrete granulocyte macrophage colony stimulating factor (GM-CSF) and to suppress the expression of immunosuppressors transforming-growth factors β (TGF β) 1 and 2 (Vigil vaccine) has achieved a one-year OS of 73% with a good safety profile in a

Phase I clinical trial^[184]. These results led to the design of a Phase III clinical trial of Vigil vaccine in combination with temozolomide and irinotecan for patients with metastatic ES.

13. Conclusion

Recurrent or relapsed ES is a disease of poor prognosis, as the current second-line chemotherapeutic options have been unsuccessful. Progress in ES is far behind other neoplastic diseases due to the nature of the disease, the relatively low number of patients, and also because of the fact that many ongoing clinical trials are not ES-targeted, but include pediatric and young adult

patients with a variety of recurrent oncologic diseases. Research has proved that monotherapy is ineffective, so combinations with EWS-FLI1 targeted agents and other agents (e.g., antiangiogenics or genetic therapy) would be worth investigating. A possibly exploitable strategy in recurrent or relapsed ES would be extended testing for second-hit mutations in all patients to seek personalized treatment alongside EWS-FLI1-targeted agents.

Author Contribution

All authors contributed equally to the preparation of this article.

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