# Clinical trials of systemic therapy in osteosarcoma and Ewing's sarcoma: past, present and future

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Due to multiple factors, including their low incidence, heterogeneity and span of ages of affected patients, both osteosarcoma and Ewing's sarcoma pose significant challenges to oncologists and patients. Despite these obstacles, significant progress has been made in the last 40 years in improving the survival of patients with localized osteosarcoma and Ewing's sarcoma through multidisciplinary management. However, patients with primary refractory disease or disseminated disease fare poorly, emphasizing the need for novel therapies. Unfortunately, given their rarity, novel therapies for these tumors are difficult to rigorously trial. Current investigation is focused on identification of active targeted therapies in trials in patients with relapsed or refractory disease. Here we review the past, present and potential future clinical trials of systemic therapy in osteosarcoma and Ewing's sarcoma.

Keywords: adjuvant chemotherapy • clinical trials • event-free survival • Ewing's sarcoma • neoadjuvant (induction) chemotherapy • osteosarcoma • overall survival • relapse • remission • response

Osteosarcoma and Ewing's sarcoma (EWS) represent the two most common primary bone malignancies in children and young adults [1]. These tumors have a peak incidence in the second decade of life; however, they can occur infrequently in very young or older patients. In children and adolescents, osteosarcoma most commonly arises in an extremity while EWS most commonly involves the extremities, pelvis and chest wall. Until the middle of the 20th century, local control by radical surgical resection (i.e., amputation) and radiotherapy were the only treatments available for these tumors. Although remission could occasionally be induced with surgery alone, relapse was nearly inevitable and overall prognosis was dismal, with survival rates of less than 20% for both tumors [2-4]. However, with the advent of cytotoxic chemotherapy in the 1950s and 1960s, rapid progress was made in prolonging survival in osteosarcoma and EWS patients by combining multi-agent chemotherapy with local control modalities. Currently, 5-year overall survival (OS) rates for patients with nonmetastatic disease exceed 60-70% in both osteosarcoma and EWS [5,6]. Despite these dramatic improvements, many patients still fare poorly due to the rare occurrence of primary refractory disease, relapse or the presence of distant metastases at diagnosis. Moreover, survival rates have remained largely unchanged over the past two decades despite active investigation [7]. Current clinical trials are mainly focused on identifying targeted therapies active against osteosarcoma and EWS.

#### Osteosarcoma

The annual incidence of osteosarcoma is approximately 5.4 and 4.0 per million for males and females, respectively [8]. It is the eighth most common pediatric malignancy in the USA [8]. Most cases are sporadic and occur in the second decade of life – correlating with the pubertal growth spurt. However, osteosarcoma can arise

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later in life in conditions characterized by chronic bone remodeling, including Paget disease of bone, hereditary multiple exostoses, enchondromatosis and fibrous dysplasia [8,9]. Osteosarcoma typically presents with pain, with imaging revealing a bone lesion or occasionally a pathologic fracture. Definitive diagnosis relies on tissue biopsy with histopathologic examination. At diagnosis, several clinicopathologic findings have been correlated with long-term survival. Factors negatively affecting prognosis at initial diagnosis include age greater than 12 years, the presence of distant metastases, elevated serum alkaline phosphatase and lactate dehydrogenase, osteoblastic histology, and tumor volume greater than 150 ml [10-12].

#### The past: development of modern methotrexate, doxorubicin and cisplatin chemotherapy for nonmetastatic osteosarcoma

Early studies investigating the role of chemotherapy in treatment of osteosarcoma were performed in patients with metastatic disease, which identified several chemotherapeutic agents capable of inducing tumor regression when used as monotherapy [13,14]. In an effort to decrease rates of relapse in patients with localized disease, investigation in the 1970s next examined the role of postoperative 'adjuvant' chemotherapy following curative surgery. Three independent, single-arm, prospective studies using adjuvant chemotherapy (single agents or combination agents) for patients with localized disease markedly extended the 2-year disease-free survival (DFS) to 45-55% compared with 20% historically (Table 1) [15-17]. To definitively address the role of adjuvant chemotherapy in osteosarcoma, two randomized trials were performed comparing surgery with adjuvant chemotherapy versus surgery alone, which both independently verified the benefit of multi-agent chemotherapy in both disease-free and OS in patients with nonmetastatic osteosarcoma [2,18].

Current osteosarcoma chemotherapy protocols developed from studies of pre-operative 'neoadjuvant' (also known as 'induction') chemotherapy combined with the postoperative adjuvant 'consolidation' chemotherapy reported in the 1980s. Neoadjuvant chemotherapy is given with the dual aims of eradicating undetectable micrometastatic disease present at diagnosis (thought to be present in 80% of cases of osteosarcoma [19]) and decreasing the size of the primary tumor for improved local control to allow limb-sparing surgery. Neoadjuvant therapy also allows the option of tailoring postoperative treatment based on histologic response.

Despite data suggesting that combined neoadjuvant and adjuvant chemotherapy may not improve survival compared with adjuvant chemotherapy alone [20-22], neoadjuvant chemotherapy has become the standard of care. Implementation of neoadjuvant chemotherapy protocols correlated with a marked increase in the proportion of patients with localized osteosarcoma undergoing limb salvage surgery without a concomitant compromise in survival [20,23]. Indeed, with modern combined neoadjuvant/adjuvant chemotherapy, limbsparing surgeries are performed in over 90% of cases [24] compared with only approximately 25% of cases with adjuvant chemotherapy alone [20]. Although improvements in staging and surgical technique likely contribute to these findings, these data indicate that when adequate surgical margins can be achieved, use of limbsparing surgeries following neoadjuvant chemotherapy does not seem to compromise survival compared with more aggressive surgery.

Modern first-line chemotherapy for localized osteosarcoma consists of methotrexate, adriamycin (doxorubicin) and cisplatin given pre- and postoperatively, a regimen termed methotrexate, doxorubicin and cisplatin (MAP). Early studies identified all three of these agents as possessing therapeutic activity against osteosarcoma [15-17,25,26]. Active drugs in osteosarcoma were subsequently studied in various combinations, culminating in the T-10 protocol utilized at Memorial Hospital (NY, USA). T-10 utilized high-dose methotrexate with leucovorin rescue (HDMTX) together with doxorubicin and the combination of bleomycin, cyclophosphamide and actinomycin D (BCD), with cisplatin added postoperatively for poorly responsive patients, reporting a 5-year DFS of 76% [3,21]. Subsequently, the German Cooperative Osteosarkomstudiengruppe (COSS)-80 and COSS-82 trials together demonstrated the essential roles of both doxorubicin and cisplatin as first-line agents in all patients with localized disease, while questioning the relative efficacy of the combination of BCD used in place of these drugs [27,28].

While studies have confirmed the central roles of both doxorubicin and cisplatin in treatment of osteosarcoma [21], the role of methotrexate has been controversial [19]. Although known to possess activity against osteosarcoma as adjuvant monotherapy [16], the inclusion of methotrexate in multidrug protocols was called into question by the European Osteosarcoma Intergroup (EOI) in 1992. In this study, the combination of doxorubicin and cisplatin was reported to be equivalent to a similar regimen containing methotrexate in OS; however, patients in the methotrexate arm received lower cumulative doses of doxorubicin and cisplatin [29]. Following this trial, the EOI conducted two separate randomized trials utilizing the same doxorubicin and cisplatin regimen as a control arm compared with either a HDMTX-containing multi drug T-10-like regimen or dose intensification and compression of doxorubicin and cisplatin [30,31]. While

	Ref.	[15]	[16]	[17]	[2]	[3]	[28]	[18]	[27]	[33]	[51]	[54]	[41]	[5]	[55]	σ
	Results	71% EFS at 18 months	19/20 patients alive at 2–23 months	55% 2-year EFS	2-year EFS in control 17% versus 66% with adjuvant chemotherapy	Possible salvage of poor responders to neoadjuvant chemotherapy	Equal DFS in BCD and P arms and arms with and without IFN	80% of A+BCD patients alive at median of 2 years, 48% in observation group	4-year EFS 49% in study arm versus 68% in control arm	5-year EFS 44%, OS 55% in both arms	5-year EFS: 63%; salvage of poor responders with IE	3-year EFS for local resectable: 76.4%	59% response rate	6-year OS with MTP-PE: 78% versus 70% without MTP-PE	5-year EFS: 66.7%	EFS: Event-free survival; I: Ifosfamide; IE: Ifosfamide an ianolamine; N/A: Not available; OS: Overall survival;
	Intervention arm(s)	A	Σ	C+V+Mel+A	M+A+BCD+P	Multiple	M+A+P, M+A+P+IFN, M+A+BCD+IFN	M+A+BCD	M+BCD; switch to A+P if poor response	A+P	M+A,+P+ postoperative I+E for poor responders	I+C+A+M	I+E	MAP+I, MAP+MTP-PE, MAP+I+MTP-PE	I+Ca+A	Disease-free survival; E: Etoposide; Auramyl tripeptide phosphatidyleth
	Control arm	None	None	None	Observation	None	M+A+BCD	Observation	M+A+P; switch to BCD+I+P if poor response	M+V+A+P+BCD	None	None	None	M+A+P	None	osphamidę; Ca: Carboplatin; DFS: I isplatin; Mel: Melphalan; MTP-PE: N
	Evaluated patients	21	20	18	36	185	158	59	141	391	164	69	41	662	66	in D; C: Cycloph oxorubicin and c
trials.	Stage: localized or metastatic	Localized	Localized	Localized	Localized	Both	Localized	Localized	Localized	Localized	Localized	Both	Metastatic	Localized	Localized	bhamide and actinomyc MAP: Methotrexate, dc
sarcoma	Phase	п	п	п	Π	п	Ш	Ш	Ш	III	п	П	III/II	Ш	п	cyclophosp ethotrexate;
selected osteo	Name/ protocol	N/A	N/A	CONPADRI-I	MIOS	ТЗ, Т7, Т10	COSS-80	T-10B	COSS-82	EOI-2	IOR/OS-2	OS-91	POG 9450	IS-0133	0S-99	cin; BCD: Bleomycin, <sup>1</sup> N: Interferon; M: M. V: Vincristine.
Table 1. S	Year reported	1974	1974	1975	1986	1983	1984	1987	1988	1997	2000	2001	2002	2008	2011	A: Doxorubic etoposide; IF P: Cisplatin; <sup>v</sup>

neither of the investigational arms improved outcomes compared with the control cisplatin and doxorubicin arms, the EOI investigators noted that the doxorubicin and cisplatin regimen utilized in these three trials has consistently yielded lower survival rates than those achieved in contemporary MAP-based trials [30-32]. The inclusion of HDMTX in first-line chemotherapy is further supported by the report that cumulative methotrexate dose correlates positively with prognosis, when combined with doxorubicin and cisplatin [33]. Several studies have investigated methotrexate dosing and pharmacokinetics as related to outcome, concluding that HDMTX is equivalent to moderate-dose methotrexate in terms of survival, but HDMTX is currently favored as it can be administered over a shorter time and with less overall toxicity when leucovorin rescue is effectively used [21,34,35]. The efforts of these many studies have culminated in the standard MAP regimen presented in Figure 1 [36].

#### The present: redrawing the MAP

Trials performed in the past two decades have focused on addition of other active drugs to the core MAP regimen, intensification of front-line therapy, or replacement of the most toxic drugs with those associated with less acute and long-term toxicity. The activity of ifosfamide and etoposide (IE) together or as single agents and in addition to MAP has been investigated. Small studies have demonstrated that these agents possess activity against metastatic, relapsed and refractory osteosarcoma [37-40]. However, despite their known activity in the metastatic setting, the addition of ifosfamide alone or together with etoposide to front-line AP or MAP in Phase II and III trials has failed to demonstrate a clear survival benefit, while inducing high rates of severe hematologic toxicity [5,41-44]. Thus, IE are not routinely utilized as standard first-line chemotherapy for nonmetastatic osteosarcoma.

IE may prove to be cornerstones of strategies to tailor chemotherapy to the tumor response. Analysis of trials utilizing neoadjuvant chemo-therapy have consistently

observed that patients with a 'good' histologic response to chemotherapy (usually defined as tumor necrosis greater than 90% at resection) have superior survival outcomes compared with those with 'poor' responses [3,21,27,31,45,46]. These observations led to the use of alternative chemotherapy drugs postoperatively in tumors that showed a poor histologic response at resection, with the aim of increasing necrosis of any remaining viable tumor cells resistant to the pre-operative regimen [3]. An early study of this strategy at Memorial Hospital suggested that poor responders could be effectively salvaged using this approach by adding cisplatin postoperatively [3]. However, independent studies using similar strategies failed to reproduce this effect [20,23,27,46,47] and longer-term follow-up with a larger cohort of patients on similar protocols at Memorial Hospital found that the benefit observed by altering postoperative chemotherapy was lost over time [21]. However, following the identification of the activity of IE against osteosarcoma, these drugs have emerged as promising potential salvage agents for poor histologic responders. The OS-2 and OS-3 prospective Phase II studies performed at the Rizzoli Institute demonstrated that addition of postoperative IE following MAP induction in patients with tumors showing poor histological response might improve survival in these patients [42,48,49]. Responsebased therapy using IE is currently under investigation as part of the ongoing randomized, Phase III EURAMOS-1 trial (Figure 2) [201].

Addition of a biologic agent to MAP has recently been explored. A multicenter, randomized Phase III trial has investigated the role of liposomal muramyl tripeptide phosphatidylethanolamine (MTP-PE) combined with front-line MAP in localized osteosarcoma (the Children's Cancer Group [CCG] and the Pediatric Oncology Group [POG] Intergroup Study 0133 [IS-0133]) [5.32]. MTP-PE is designed to mimic the inflammatory response associated with deep tissue infections that have been associated positively with long-term survival in osteosarcoma [7]. MTP-PE is derived from a peptidyl glycan present in both Gram-

> positive and Gram-negative cell walls, which activates the cytotoxic activity of monocytes and macrophages to induce these cells to target osteosarcoma cells [9]. In the IS-0133 trial, following MAP induction patients were randomized to receive MTP-PE or no MTP-PE in addition to standard postoperative chemotherapy. This trial also examined the addition of ifosfamide to MAP with or without MTP-PE in a 2 × 2 factorial design. Comparison of the

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		Sur	gery													

**Figure 1. Representative scheme for methotrexate, doxorubicin and cisplatin regimen.** Doses are expressed as cumulative dose per cycle.

A: Doxorubicin 75 mg/m<sup>2</sup>; M: methotrexate 12 g/m<sup>2</sup> (maximum 20 g/m<sup>2</sup>) for one dose; P: Cisplatin 120 mg/m<sup>2</sup>.

Adapted from [36].

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treatment arms found that patients who received MAP with ifosfamide and MTP-PE had 72% 5-year eventfree survival (EFS), compared with 64% in those patients only receiving MAP, suggesting a benefit of the two additional agents together. However, a positive interaction between ifosfamide and MTP-PE was postulated, since those patients who received MAP with ifosfamide had 56% 5-year EFS, suggesting that ifosfamide alone worsens 5 year EFS when added to MAP, while ifosfamide with MTP-PE added to MAP is beneficial [7,32]. At longer follow-up, by examining only the patients with localized, resectable osteosarcoma, a statistically significant interaction of ifosfamide and MTP-PE was not observed, and a statistically significant benefit of MTP-PE in OS was found (70% 6-year OS in patients not receiving MTP-PE compared with 78% in those receiving MTP-PE, p = 0.03) [5]. Thus, MTP-PE may provide a benefit as an adjuvant agent in nonmetastatic, resectable osteosarcoma, although this conclusion remains controversial partially due to the design of the study itself.

Further studies are required to confirm the clinical utility of this agent. If incremental benefit is confirmed, it will be critical to elucidate the population most likely to benefit, given the range and frequency of side effects as well as the potential costs on the global healthcare system. [50,51]. At present, MTP-PE is available in Europe for treatment of osteosarcoma; however, this agent is not available in the USA, having not been approved by the US FDA.

Intensification of neoadjuvant chemotherapy has been investigated in several trials, including a randomized study comparing the T10 with the T12 protocol, which included additional courses of doxorubicin and cisplatin, where no advantage of the intensified T12 protocol was observed [52]. The EOI performed a randomized, Phase III trial comparing standard-dose doxorubicin and cisplatin to dose compressed and intensified doxorubicin and cisplatin with granulocyte colony-stimulating factor support. Although an increased rate of good histologic response was observed with the intensified regimen, both 5-year OS and PFS did not differ between the arms [30].



**Figure 2. EURAMOS-1 trial.** Patients with resectable osteosarcoma (localized or metastatic) uniformly undergo induction therapy with MAP. Following surgery, histologic response to MAP induction is assessed. Good responders are randomized to either continue MAP or continue MAP followed by a 74-week maintenance period with IFN- $\alpha$ 2b. Poor responders either continue MAP, or continue MAP with the addition of IE.

IE: Ifosfamide and etoposide; MAP: Methotrexate, doxorubicin and cisplatin. Adapted from [217].

Other studies, performed largely in pediatric patients, have examined substitution of MAP components with the aim of reducing both short- and longterm toxicities. The Phase II OS-91 and OS-99 trial performed at St Jude Children's Research Hospital (TN, USA) demonstrated that substituting components of MAP therapy with carboplatin and ifosfamide could decrease toxicity without compromising outcome [53,54]. The French SFOP OS94 study was a randomized trial that attempted to reserve the most toxic chemotherapy agents for poor histologic responders. The trial compared HDMTX and doxorubicin (with IE added postoperatively for poor histologic responders) to HDMTX with IE (with doxorubicin and cisplatin for poor responders) in localized disease, with similar outcomes reported for both regimens [55]. Taken together, these studies provide evidence that long-term toxicity of osteosarcoma chemotherapy could be potentially minimized. However, the validity of these protocols in supplanting current front-line strategies will likely require additional randomized trials comparing these regimens directly to MAP.

#### Metastatic, refractory & relapsed osteosarcoma

Although significant progress has been made in treatment of localized osteosarcoma, survival rates for metastatic disease remain poor [11,52]. The mainstay of treatment for metastatic disease is MAP. If resectable lung metastases are identified at diagnosis, first remission may be achieved by surgical removal of these foci at time of resection of the primary lesion [56]. As part of the IS-0133 trial, MTP-PE was investigated as an adjuvant agent in metastatic osteosarcoma, finding a nonsignificant trend toward benefit of MTP-PE in survival [11].

It has been estimated that 94% of osteosarcoma relapses occur within 5 years of diagnosis [57], with first remissions lasting longer than 2 years correlating with improved survival [58]. Surgery serves an essential role in relapse, as metastectomy of sites of pulmonary relapse alone can induce second and subsequent remissions, with an unclear benefit of adjuvant chemotherapy in such patients [58,59]. If second or subsequent remission cannot be achieved surgically, use of chemotherapy has been suggested to improve survival following first and subsequent relapses [58]. Phase II studies have reported that relapsed patients respond to the combination of IE either alone (48% response rate) or together with HDMTX (62% response rate) [37,60]. The regimen of ifosfamide, carboplatin and etoposide (ICE) was shown to have a response rate of 16% in recurrent and refractory osteosarcoma in a small Phase I/II trial of 34 patients [61]. The combination of gemcitabine with oxaliplatin or docetaxel has also been reported to induce responses at low frequency [62,63].

#### The future: targeted therapies on the MAP

Current osteosarcoma trials are based on targeting the biochemical and genetic circuitry thought to regulate osteosarcoma pathogenesis and progression. Investigational agents include monoclonal antibodies targeting cell surface receptors expressed on osteosarcoma cells and secreted cytokines, small molecules inhibiting key intracellular signal transduction proteins that control cell proliferation, survival, angiogenesis and bone turnover, and an exogenous cytokine that regulates osteosarcoma proliferation and differentiation (Figure 3) [64].

Expression of VEGF in osteosarcoma correlates with increased microvessel density in tumors, and is associated with poor response to neoadjuvant chemotherapy, increased rates of pulmonary metastases, and poor DFS and OS [65,66]. Targeting this soluble growth factor has improved outcomes in other malignancies [67]. St Jude Children's Research Hospital is leading a Phase III trial designed to assess the safety and efficacy of bevacizumab, a monoclonal antibody

that directly binds VEGF, combined with first-line chemotherapy agents (MAP for localized disease, with IE added for patients with metastases) for newly diagnosed localized and metastatic osteosarcoma [202] (Table 2). This strategy of the addition of anti-VEGF therapy to standard chemotherapy may be a promising avenue based on preclinical rationale. Expression of the IGF-1 receptor (IGF-1R) is associated with a poorly differentiated, highly proliferative phenotype in osteosarcoma cell lines [68]. A recently completed Phase II trial investigated the safety and activity of the anti-IGF-1R monoclonal antibody SCH 717454 as monotherapy in relapsed, resectable osteosarcoma [203]. Cixutumumab, another monoclonal antibody targeting IGF-1R, is under investigation in children with relapsed solid tumors, enrolling patients with osteosarcoma [204]. The activity of a third anti-IGF-1R monoclonal antibody, R1507, was investigated in a completed Phase II trial in recurrent or refractory sarcoma, including osteosarcoma [205]. While preliminary results have shown only limited activity of anti-IGFR therapy thus far in osteosarcoma patients, combination strategies with other targeted agents or cytotoxics have shown promise preclinically and may be a promising path for further study [69–71].

Expression of the HEGF-receptor 2 (HER2) in osteosarcoma is associated with lower EFS compared with tumors that do not express HER2 [72]. The results are pending of a Phase II trial of methotrexate, doxorubicin, cisplatin, IE with or without trastuzumab, a monoclonal antibody against HER2, for patients with metastatic osteosarcoma [206]. A recently completed Phase II trial assessed the activity of trastuzumab monotherapy in patients with recurrent osteosarcoma [207]. As the importance of HER2 in osteosarcoma has been quite controversial preclinically, these studies may definitively determine whether this strategy is worthy of further study.

Among small molecules undergoing trials for osteosarcoma, considerable interest has focused on the bisphosphonates, a class of drugs widely used for treatment of osteoporosis due to their activity in preventing bone resorption [73]. Extensive preclinical investigation has demonstrated antitumor activity of these agents against osteosarcoma cells in vitro and in vivo, with apparent effects on both osteosarcoma cell growth directly as well as bone catabolism by inhibiting the melavonate pathway and prenylation of small G-proteins [74,75]. This activity seems to synergize with first-line chemotherapy agents [74]. Bisphosphonates also possess antiangiogenic and antitumor immunomodulatory properties through stimulation of  $\gamma\delta$  T cells [76,77]. A recent single-arm, prospective Phase II study examined combining the bisphosphonate pamidronate with



**Figure 3. Mechanism of action of selected anti-osteosarcoma therapies in Phase II and III trials.** HER2: HEGF receptor 2; IFN: Interferon; IGF-1R: IGF-1 receptor; PDGFR: PDGF receptor.

MAP, reporting 5-year EFS of 72% and OS of 93% for patients with localized disease, and 5-year EFS of 45% and OS of 64% in patients with metastatic disease [78]. These promising results should encourage further investigation of the role of bisphosphonates in osteosarcoma. Multiple Phase II and III trials investigating the activity of bisphosphonates in both newly diagnosed and relapsed osteosarcoma are ongoing [208,209].

Trabectedin (ecteinascidin 743, ET-743) is a tetrahydroisoquinoline alkaloid isolated from the marine tunicate *Ecteinascidia turbinata* (a sea squirt) proposed to exert cytostatic and cytotoxic effects through alkylation of guanine residues [79]. Preclinical investigation has demonstrated synergy between trabectedin and first-line chemotherapy agents in induction of osteosarcoma cell cytotoxicity. Moreover, trabectedin is active in osteosarcoma cells resistant to methotrexate and cisplatin [80]. Trabectedin possesses limited activity against relapsed osteosarcoma when used as monotherapy, inducing minor responses in three out of 23 patients in a small, Phase II study [79]. A recently completed Phase II trial assessed the activity of trabectedin in metastatic osteosarcoma following conventional treatment [210].

Preclinical studies have identified the nonreceptor tyrosine kinase as a potential therapeutic target in osteosarcoma. Inhibition of Src activity in vitro inhibits osteosarcoma cell viability, and slows growth of osteosarcoma cell xenografts in immunodeficient mice in vivo [81]. The activity of the Src family kinase inhibitor dasatinib against osteosarcoma cells has recently been confirmed using *in vitro* cell culture systems [82]. A trial has recently been completed assessing the safety and efficacy of dasatinib as a single agent in a cohort of patients with osteosarcoma [83] and in combination with ICE in recurrent or metastatic solid tumors in pediatric patients [211]. The final results of the single agent trial are pending, but preliminarily limited activity was seen. Another placebo-controlled, Phase II trial is testing the efficacy of the oral Src kinase inhibitor AZD0530 in preventing osteosarcoma recurrence following surgical removal of relapsed lung lesions [212].

A second signaling molecule, mTOR, is a target of investigational therapies in sarcomas [84]. mTOR regulates cell response to growth factors and nutrient availability, and participates in intracellular signaling networks interacting with the protein products of several

Table 2. Selected ongoing or recently completed Phase II and III	trials for osteosarcoma.				
Trial	Clinicaltrials.gov identifier	Phase	Drug Class	Target	Ref.
A study of bevacizumab in combination with chemotherapy for treatment of osteosarcoma	NCT00667342	Ш	Antibody	VEGF	[202]
A study to determine the activity of SCH 717454 in subjects with relapsed osteosarcoma or Ewing's sarcoma (study P04720AM3)	NCT00617890	п	Antibody	IGF-1R	[203]
Cixutumumab in treating patients with relapsed or refractory solid tumors	NCT00831844	п	Antibody	IGF-1R	[204]
A study of R1507 in patients with recurrent or refractory sarcoma	NCT00642941	п	Antibody	IGF-1R	[205]
Chemotherapy with or without trastuzumab in treating patients with metastatic osteosarcoma	NCT00023998	п	Antibody	HER2	[206]
Trastuzumab in treating patients with recurrent osteosarcoma	NCT00005033	п	Antibody	HER2	[207]
Evaluation of zoledronic acid as a single agent or as an adjuvant to chemotherapy in high-grade osteosarcoma	NCT00691236	III/II	Small molecule	Osteoclast/melavonate	[208]
Combination chemotherapy with or without zoledronic acid in treating patients with osteosarcoma	NCT00470223	II	Cytotoxic chemotherapy plus small molecule	Osteoclast/melavonate	[209]
Ecteinascidin 743 in treating patients with previously treated metastatic osteosarcoma	NCT00005625	Π	Small molecule/cytotoxic chemotherapy	DNA alkylation	[210]
Dasatinib, ifosfamide, carboplatin and etoposide in treating young patients with metastatic or recurrent malignant solid tumors	NCT00788125	1/П	Cytotoxic chemotherapy plus small molecule	Src family kinases	[211]
A placebo-controlled study of saracatinib (AZD0530) in patients with recurrent osteosarcoma localized to the lung	NCT00752206	П	Small molecule	Src kinase	[212]
Study of AP23573, an mTOR inhibitor, in patients with advanced sarcoma	NCT00093080	П	Small molecule	mTOR	[213]
Safety and efficacy study of torisel and liposomal doxorubicin for patients with recurrent sarcoma	NCT00949325	1/П	Cytotoxic chemotherapy plus small molecule	mTOR	[214]
Temsirolimus and cixutumumab in treating patients with locally advanced, metastatic or recurrent soft tissue sarcoma or bone sarcoma	NCT01016015	п	Antibody plus small molecule	mTOR IGF-1R	[215]
Sorafenib in relapsed high-grade osteosarcoma	NCT00889057	п	Small molecule	Multiple kinases	[216]
Combination chemotherapy, PEG-IFN- $\alpha 2b$ and surgery in treating patients with osteosarcoma	NCT00134030	II	Cytotoxic chemotherapy plus biologic	Multiple	[218]
HER2: HEGF receptor 2; IGF-1R: IGF-1 receptor.					

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oncogenes [85]. Inhibition of mTOR signaling with rapamycin decreases osteosarcoma lung metastases in mice [86]. Interestingly, bisphosphonates appear to sensitize osteosarcoma cells to the effects of mTOR inhibition [87]. Rapamycin analogs are currently being tested in clinical trials to assess their activity in osteosarcoma. A Phase II study showed some activity of AP23573, an mTOR inhibitor, in advanced sarcomas, but full results are pending [213]. A Phase I/II trial is testing the safety and efficacy of the rapamycin analog temsirolimus combined with liposomal doxorubicin in recurrent sarcomas [214]. A third Phase II trial is assessing the efficacy of targeting both mTOR and IGF-1R through use of temsirolimus with cixutumumab in advanced sarcomas, including osteosarcoma [215].

Recent evidence suggests that the Notch signaling pathway is implicated in osteosarcoma metastases and might provide a novel target in osteosarcoma [88]. Studies in other cancers have suggested that targeting this pathway may be a valid therapeutic approach and Phase I studies of multiple agents are underway [89].

The multikinase inhibitor sorafenib (BAY 43–9006) is under clinical investigation in osteosarcoma. Inhibiting the Raf kinases, KIT, FGF receptor-1, RET and PDGF receptor  $\beta$  (PDGFR $\beta$ ), sorafenib abrogates osteosarcoma cell proliferation and survival [90]. Sorafenib also acts as an antiangiogenic agent via its inhibition of VEGF receptor kinases [67]. A Phase II trial is currently recruiting to test the efficacy of sorafenib in preventing progression of osteosarcoma following relapse [216].

The endogenous IFN cytokines have been investigated as therapeutic agents in osteosarcoma for several decades. IFNs modulate tumor progression both directly by inhibiting cell growth and differentiation and indirectly by regulating angiogenesis and the antitumor immune response [91]. Preclinical studies have shown that IFNs inhibits osteosarcoma xenograft growth in nude mice [92]. Beginning in 1971, investigators at the Karolinska Hospital in Stockholm used adjuvant IFN- $\alpha$  for long-term maintenance therapy (duration of treatment ranging from 17 months to 5 years) following osteosarcoma resection without adjuvant chemotherapy, reporting 10-year sarcoma-specific survival of 43% at long-term follow-up, suggesting that IFN- $\alpha$ may be highly effective in maintaining remission [93]. In contrast, COSS-80 (Table 1) tested the addition of adjuvant IFN-β to postoperative chemotherapy versus chemotherapy without IFN in a randomized trial, and did not observe improvement in outcome with use of IFN-B [28]. However, as noted by the Karolinska investigators, both the dose used and the duration of treatment with IFN- $\beta$  in the COSS-80 trial were much reduced than used in the Swedish series [93]. The potential benefits of IFN in combination with first-line chemotherapy are currently under further investigation in the EURAMOS-1 trial sponsored by the Children's Oncology Group (COG) in the USA (Figure 2) [217]. In this Phase III trial, following MAP induction, good histologic responders are randomized to receive or not receive PEGylated IFN-α2b as long-term (74-week) maintenance following adjuvant chemotherapy [218]. Since patients who relapse within 2 years of entering first remission fare poorly compared with patients with longer remissions [58], maintenance strategies that prolong the first remission hold great potential in improving survival rates. Since the EURAMOS-1 trial anticipates accruing 2300 patients, significant insight into the activity of IFN- $\alpha$ 2b in prolonging remission should be gained.

#### EWS

EWS is the second most common primary tumor of bone in young adults, diagnosed at an incidence of three per 1 million per year in white Caucasians, and occurring very rarely in individuals of African and Asian descent [94,95]. EWS affects approximately 560 people in the USA per year [94] and accounts for approximately 40% of bone tumors in children and adolescents [96]. At diagnosis, the median age is 15 years, and there is a 1.5:1 male: female ratio [95]. These statistics include the histologically and genetically similar Askin tumor of the chest wall and peripheral primitive neuroectodermal tumors of soft tissues, which together with EWS comprise the Ewing's family of tumors [97]. The malignant phenotype of these tumors is driven by proteins resulting from a stereotypical set of chromosomal translocations, most commonly the t(11;22)(q24;q12) generating the EWS-FLI1 fusion gene present in approximately 85% of EWS cases, although other fusion genes (usually involving EWS) can be present [98,99].

With modern multidisciplinary management, including chemotherapy, surgery and radiotherapy, patients with localized EWS can expect 5-year OS of approximately 70%, although this declines to 20-30% in metastatic disease [100]. Multiple reports have suggested that age over 15 years, size greater than 8 cm or 200 ml, and the presence of distant metastases correlates negatively with survival [100-107]. Other putative negative prognostic factors include male sex, elevated LDH at diagnosis, first remission less than 2 years, poor histologic response to neoadjuvant chemotherapy (defined as macroscopic viable tumor nodules remaining after induction [108]), and axial primary tumor location [100-102,104,108]. Site of metastasis is also associated with prognosis, with the presence of extrapulmonary metastases associated with lower EFS and OS compared with isolated lung lesions [100,107]. Based on several prognostic factors, risk-stratification schemes have been used in several trials [109,110].

#### VACA for localized EWS

Prior to the introduction of systemic chemotherapy to EWS therapy, treatment relied almost exclusively on local control modalities [4,111]. Since patients usually relapsed despite radical surgery, it was proposed that micrometastases were present in the majority of patients with grossly localized disease at diagnosis, and so adjuvant therapy was investigated [112]. In early trials of monotherapy with various chemotherapy agents, vincristine, cyclophosphamide and actinomycin D were observed to possess activity against EWS [113-116]. At the NCI, progressive combination of these agents together with radiotherapy was trialed in patient series, observing marked improvement in outcomes [112]. In this study, the third combination of drugs used was vincristine, actinomycin D and cyclophosphamide – a regimen that has come to be known as VAC (Tables 3 & 4) [112].

In 1973, doxorubicin was shown to be an active agent against EWS [117], providing a fourth therapeutic drug. At Memorial Hospital, this was combined with VAC (the VACA regimen) and local radiotherapy in a small series of 12 patients, ten of whom had localized disease. At follow-up ranging from 10-37 months, all patients were disease-free [118]. The Intergroup Ewing's Sarcoma Study (IESS)-1, commencing in the USA in 1973, was designed to assess the benefit of doxorubicin as part of adjuvant therapy in EWS. This randomized trial was comprised of three arms: VAC, VACA and VAC with bilateral pulmonary irradiation [119]. This trial reported superior DFS in the VACA arm compared with both of the other arms [120,121], confirming the importance of doxorubicin as an adjuvant drug and installing VACA as standard therapy for EWS.

Table 3. Che	emotherapy regimens in Ewing's sarcoma.
Regimen	Components
VAC	Vincristine, actinomycin D and cyclophosphamide
VACA	Vincristine, actinomycin D, cyclophosphamide and doxorubicin
VAIA	Vincristine, actinomycin D, ifosfamide and doxorubicin
IE	Ifosfamide and etoposide
VDC	Vincristine, doxorubicin and cyclophosphamide
EVAIA	Etoposide, vincristine, actinomycin D, ifosfamide and doxorubicin
VIDE	Vincristine, ifosfamide, doxorubicin and etoposide
VAI	Vincristine, actinomycin D and ifosfamide
Bu-Mel	Busulfan and melphalan

Neoadjuvant chemotherapy was proposed by Rosen and colleagues in 1978 with their T-6 protocol, which utilized an induction regimen of seven drugs (HDMTX, actinomycin D, cyclophosphamide, doxorubicin, bleomycin, 1,3-bis [2-chloroethyl]-1-nitrosurea and vincristine) followed by surgery, radiation or both for local control, and then consolidation with the T-2 protocol [122]. Of 28 patients with localized EWS treated with T-6, 82% were reported disease free at 12-46 months [123]. The neoadjuvant approach was also examined in the Cooperative Ewing's Sarcoma Study (CESS)-81, where 93 patients with localized EWS received two cycles of VACA as induction, followed by local control, and then two more cycles of VACA consolidation therapy. 6-year DFS in this trial was 55% [124]. The strategy of using VACA induction and consolidation has been further validated in the First Ewing's Tumour Study (ET-1) trial from the Children's Cancer Study Group (UKCCSG) and the EW88 trial from France [104,125].

#### Current investigation: modern VACA-IE, risk stratification & dose intensification in localized EWS

Although the development of neoadjuvant VACA combined with multidisciplinary local control resulted in a marked improvement in outcomes in EWS, many patients with localized disease still relapsed. Early trials reported that ifosfamide with or without etoposide could induce responses in EWS patients [40,126]. This led to the addition of IE to EWS treatment regimens. The REN-2 study performed at the Rizzoli institute did not show a benefit of adding IE to VACA (VACA-IE) as compared with historical controls who received VACA alone in patients with localized disease [127]. In contrast, the British ET-2 trial and studies at Memorial Hospital and the NCI all supported the addition of ifosfamide in patients with localized disease, warranting further investigation in front-line therapy [125,128–130].

In 1988, the CCG and POG in the USA opened protocol INT-0091, a Phase III, randomized trial comparing VACA or VACA alternating cycles with IE in EWS [131]. A total of 398 patients with localized EWS were enrolled. 5-year EFS was 54% with VACA and 69% with VACA-IE (p = 0.005), indicating a significant improvement in outcome with the inclusion of IE in front-line therapy [131]. As a result of this study, VACA-IE-based protocols have become the standard of care for localized EWS in the USA (Figure 4).

Multiple studies have attempted to risk-stratify patients based on established prognostic factors in EWS, with more aggressive treatment reserved for 'high-risk' patients. One trial utilizing such a strategy was CESS-86, which recruited patients with localized EWS in Western Europe from 1986 to 1991. Patients

	Ref.	[107]	[117]	[120]	[115]	[124]	S [128]	9% [127]	R [104] n,	[134]	VS: Not
	Results	Improved EFS in intervention versus control arm	75% 5-year DFS	55% 6-year DFS	5-year RFS of 24% in control arm, 60% in arm 1, 44% in arm 2	62% RFS for localized disease	51% 10-year EFS for HR, 52% for SR; $p = N$ .	5-year EFS: localized: 54% in control arm, 6 in intervention arm. Metastasis: 22% in both arms	3-year EFS 74% in SR control arm, 73% in S intervention arm; 47% in the HR control aru 52% in the HR intervention arm	5-year EFS: 72% in standard, 70% in intensified arm	itrexate: F. Etonoside: HR. High-risk: N/A. Not available: J
	Intervention arm(s)	C, C+V, C+V+A+ITM+TBI	V+A+C+D	V+A+C+D	1. V+A+C+D, 2. V+A+C+WLI	V+D+I+A	V+D+C+A for SR; V+A+I+D for HR	V+D+C+A+I+E	V+D+I+C+A for SR; E+V+D+I+A for HR	Intensified V+D+C+I+E	I: Ifosfamide' ITM: Intrathecal metho
	Control arm	No adjuvant therapy	None	None	V+A+C	None	None	V+D+C+A	V+D+I+A for SR and HR	Standard V+D+C+I+E	Event-free survival
	Evaluated patients	23	28	93	342	243	301	518	647	478	Per survival. FFS.
a trials.	Stage: localized or metastatic	Localized	Both	Localized	Localized	Both	Localized	Both	Both	Localized	ovorubicio: DES: Disease-fr
sarcoma	Phase	N/A	II	П	Ш	П	п	Ħ	Ħ	Ξ	mide. D. Dr
selected Ewing's	Name/protocol	N/A	T-2	CESS 81	IESS-1	ET-2	CESS-86	INT-0091	EICESS-92	INT-0154	rin D: C. Cyclophospha
Table 4. S	Year reported	1972	1978	1988	1990	1998	2001	2003	2008	2009	4. Artinom

with tumors larger than 100 ml or located at axial sites were classified as 'high-risk' and received VAIA, and patients with smaller, peripheral tumors were classified as 'standard risk' and received VACA. At a median study period of 133 months, 10-year EFS did not differ between the two strata [105], suggesting that high-risk patients benefited from incorporation of ifosfamide in place of cyclophosphamide, supporting the concept of risk-stratified therapy.

Clinical trials of systemic therapy in osteosarcoma and Ewing's sarcoma Review: Clinical Trial Outcomes

The CESS and UKCCSG study groups merged as the Intergroup Cooperative Ewing's Sarcoma Studies (EICESS). The Phase III EICESS-92 randomized trial aimed to determine if survival would be adversely affected by substituting the presumably more toxic ifosfamide for cyclophosphamide. Standard-risk patients (defined as localized tumors smaller than 100 ml) were randomized to either VAIA or VACA consolidation following VAIA induction and local control. High-risk patients (tumor larger than 100 ml or the presence of metastases) were randomized to either VAIA or VAIA with etoposide (EVAIA) for both induction and consolidation [109]. Among standard-risk patients, 3-year EFS rates were nearly identical between the arms; however, unexpectedly, VACA was associated with higher rates of toxicity compared with VAIA [109], supporting the use of ifosfamide in localized EWS [132]. Among high-risk patients, differences in EFS between the VAIA and EVAIA consolidation arms were nonsignificant, although interpretation of these data is challenging due to the heterogeneity of the patients included in the high-risk stratum. Among high-risk patients without metastases, there seemed to be a trend toward a benefit of EVAIA (3-year EFS HR: 0.80; 95% CI: 0.58-1.09; p = 0.18) [109], potentially supporting a benefit of etoposide.

### Review: Clinical Trial Outcomes Rowe & Chugh

We	eek															
0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48
V D C	I E	V D C	I E	V D C	l E	V D C										
									Loc	al co	ntrol					

**Figure 4. Example VDCA-IE schema (also known as VACA-IE schema).** Therapy begins with VDC, alternating with IE, with cycles every 3 weeks. Local therapy typically occurs after week 12. After cumulative doxorubicin

dose reaches 375 mg/m<sup>2</sup>, VDC cycles are replaced with VAC.

Doses are expressed as total dose per cycle.

A: Actinomycin D 1.25 mg/m<sup>2</sup>; C: Cyclophosphamide 1200 mg/m<sup>2</sup>; D: Doxorubicin 75 mg/m<sup>2</sup>; E: Etoposide 500 mg/m<sup>2</sup>; I: Ifosfamide 9 g/m<sup>2</sup>; V: Vincristine 1 mg/m<sup>2</sup>.

> Risk-stratified therapy for EWS was also investigated in the European Ewing tumor Working Initiative of National Groups Ewing Tumor Studies (EURO-EWING)-99 trial (Figure 5) [219]. Three distinct risk strata have been defined based on histologic response to induction chemotherapy, the presence of metastases and the site of metastases [133,134,219]. Following induction with vincristine, ifosfamide, doxorubicin and etoposide (VIDE), patients with localized disease with a good histologic response were randomized to complete eight cycles of vincristine, actinomycin D and ifosfamide (VAI), or one cycle of VAI followed by seven cycles of VAC, while patients with a poor histologic response were randomized to seven cycles of VAI or one cycle of VAI followed by high-dose therapy with busulfan and melphalan (Bu-Mel) with autologous stem cell support [133]. Results from the arms of the trial including patients with localized EWS are not yet published.

> Intensification of front-line regimens has been investigated in the preceding decades. Intensification of VACA in localized, nonpelvic EWS was tested in the IESS-2 trial where these drugs were administered either as a 'moderate-dose continuous method' or a 'high-dose intermittent method'. Significantly improved 5-year DFS was reported in the high-dose intermittent arm (68 vs 48%; p = 0.02) [135]. The REN-3 prospective, single-arm trial of 157 patients at the Rizzoli Institute utilized intensified induction chemotherapy compared with the regimen used in the REN-2 trial, reporting a 5-year EFS of 71% compared with 54% in REN-2 [127,136]. Five-drug induction analogous to that utilized in REN-3 has become the standard in modern EWS chemotherapy. The POG-CCG designed protocol INT-0154, a randomized Phase III trial comparing a standard-dose schedule VDC-IE with the same drugs given in a dose-intensified schedule. No statistically

significant difference in 5-year EFS was observed between the treatment arms, although the intensified arm was associated with higher rates of toxicity and secondary solid tumors [137]. On the other hand, preliminary results of the COG AEWS0031 study, a Phase III, randomized study wherein patients receive cycles of VDC-IE every 2 or 3 weeks, suggest that intensification of this regimen through interval compression improves 3-year EFS, especially for certain subsets [138]. Therefore, future therapy for EWS may employ intensified, compressed regimens.

#### Metastatic & relapsed EWS

Despite the progress made by several intergroups working in parallel to improve survival rates in localized EWS during the latter half of the 20th century, this progress did not translate to significant improvement in the prognosis of primary metastatic EWS, with 5-year OS remaining approximately 30% [139,140]. The two large, Phase III, randomized trials performed during the 1990s - EICESS-92 and INT-0091 - included patients with metastatic EWS. The EICESS-92 trial reported no difference in EFS between the VAIA or EVAIA arms among patients with metastases within the highrisk stratum (3-year EFS HR: 0.96; 95% CI: 0.67-1.39; p = 0.84), suggesting no benefit of etoposide in metastatic EWS [109]. In the INT-0091 trial, among 120 patients with metastases, no benefit was observed when comparing outcomes of patients with metastases who received VACA-IE compared with VACA (8-year EFS was 20% for both arms) [141]. The POG/CCG investigated intensification of VDC-IE in 110 patients with metastatic EWS in a Phase II study (9457), and reported 24% 2-year EFS, concluding that the intensified regimen provided no overall benefit compared with INT-0091 [142]. Based on the results of the INT-0091 and 9457, VACA is a commonly used front-line regimen for metastatic EWS in the USA.

Another area of study in metastatic EWS has been high-dose therapy [143-145]. Recent trials utilizing this strategy have reported mixed results. The CCG reported a Phase II trial of induction with VDC-IE and consolidation with melphalan, etoposide, total body irradiation with autologous stem cell support in 32 patients with EWS metastatic to bone and/or bone marrow. The investigators reported 2-year EFS of 20% [146]. Oberlin et al. investigated consolidation with high-dose Bu-Mel with autologous stem cell support in 97 patients with primary metastatic EWS. This approach achieved 52% 5-year EFS in patients with lung metastases only, 36% in patients with bone metastases only, and 4% in patients with bone marrow involvement [147]. The EURO-EWING-99 trial (Figure 5) utilized consolidation with Bu-Mel in 281 patients with



**Figure 5. EURO-EWING-99 schema.** EURO-EWING-99 uses a risk-stratification scheme where patients with localized disease or lung metastases are assigned to R1 and R2, which both undergo induction with VIAE. Following local therapy, good histologic responders are randomized to receive either one cycle of VAI and seven cycles of VAC or eight cycles of VAI (R1). Poor histologic responders or patients with lung metastases are randomized to seven cycles of VAI or one cycle of VAI followed by Bu-Mel with stem cell support. Patients with nonlung metastases or multifocal disease undergo induction with VIAE, local therapy, one cycle of VDI, followed by high-dose therapy.

A: Actinomycin D; Bu: Busulfan; C: Cyclophosphamide; D: Doxorubicin; E: Etoposide; I: Ifosfamide; Mel: Melphalan; V: Vincristine. Adapted from [133].

primary disseminated multifocal EWS in the highestrisk arm. 3-year EFS with this regimen was reported as 27%. However, by identifying negative prognostic factors in this large patient cohort (tumor larger than 200 ml, age over 14 years, greater than one focus of bone metastasis, bone marrow involvement, and lung metastases), the investigators reported that patients with three or fewer of these high-risk factors had a 50% 3-year EFS [134]. Therefore, these recent studies indicate that high-dose consolidation strategies may benefit patients with metastases only to lung and the subset of patients with disseminated EWS with few 'high-risk' features. It should also be noted that local control via surgery or radiotherapy or a combination of these modalities with chemotherapy provides benefit even in patients with primary, disseminated, multifocal EWS [148,149].

Relapse of EWS following remission occurs in 30–40% of patients and is associated with a poor prognosis, with 5-year postrecurrence survival estimated to be less than 20%, with fewer than 15% of patients achieving a second remission [150–152]. Factors negatively impacting postrecurrence prognosis include

recurrence less than 2 years after initial diagnosis, elevated LDH at initial diagnosis, and local and metastatic disease at first recurrence [153]. Several secondline therapies for relapsed or primary refractory EWS have been investigated, although few have been tested in Phase II or III trials. A therapeutic window was designed in the POG/CCG 9457 Phase II study to examine the response to topotecan alone or combined with cyclophosphamide in primary metastatic disease, with 21/37 patients having a partial response to the combination [142]. In a separate report of 49 evaluable patients with relapsed or refractory EWS, 16 showed a partial response to topotecan and cyclophosphamide [154]. The combination of carboplatin, etoposide and cyclophosphamide was shown to induce response in 26% of patients with relapsed and refractory EWS in a small trial of 39 patients [155]. A Phase II study using high-dose ifosfamide (15 g/m<sup>2</sup>) as salvage therapy in previously treated EWS patients reported 12 out of 35 patients with response, with two patients having a complete response [156]. Temozolomide plus irinotecan has induced responses in four out of 16 and 12 out of 19 evaluable patients in two retrospective series [157,158]. The combination of gemcitabine and docetaxel was reported to induce three complete responses and one partial response in a series of six pediatric patients with relapsed or refractory EWS [159]. Taken together, these studies have identified several active salvage regimens; however, response rates are variable and are not consistently observed.

#### Current investigational therapies in EWS

Several studies are currently investigating novel therapies for EWS based both on prior preclinical research and clinical trials (Table 5) [64,110]. In vitro studies have shown that targeting EWS-FLI1 by antisense strategies can abrogate the undifferentiated malignant phenotype of EWS cell lines [160], demonstrating that EWS-FLI1 is a potential therapeutic target. However, this targeting technique is not readily translatable to the clinical setting. Moreover, EWS/FLI1 acts as an intracellular transcription factor, which would likely prove to be an elusive target for therapies due to its lack of catalytic activity [161]. Thus, direct EWS/FLI1 transcriptional target genes, proteins that collaborate with EWS/FLI1 to drive the malignant phenotype, or proteins that regulate EWS/FLI1 levels or activity may prove to be optimal targets for novel therapies. Although efforts employing this strategy have thus far yielded little success [161,162], other therapeutic strategies targeting proteins driving the EWS malignant phenotype are under active investigation.

Expression of IGF-1R is required for transformation of fibroblasts by EWS/FLI1, and IGF-1R signaling induces growth of EWS cell lines [163,164]. Therapy directed against IGF-1R can inhibit EWS cell growth in vitro and in xenografts [165-167]. Promising responses were observed using an anti-IGF-1R antibody (AMG 479) in patients with previously treated EWS in a Phase I study [168]. A Phase I study of the anti-IGF-1R antibody figitumumab treated 16 patients with EWS in an expansion cohort. Two patients had an objective response and six patients had stable disease [169]. Additional Phase II studies have been completed and have been reported in abstract form with variable response rates, but approximating 10% [220,221]. There is a clear signal that targeting this pathway is clinically important for a subset of EWS patients. Unfortunately, we have yet to identify the patients who may benefit, the optimal drug and schedule, and ways to avert drug resistance. Both the biological rationale and the clinical experiences of targeting IGF-1R in EWS and other sarcomas are reviewed extensively elsewhere [170,171].

Inhibition of mTOR activity in EWS cells inhibits cell motility downstream of IGF-1R signaling [172]. Treatment of EWS cell lines with rapamycin downregulates EWS/FLI1 protein, induces an EWS/FLI1 'off' gene signature, and inhibits EWS cell proliferation [161,173,174]. In a Phase I trial of the mTOR inhibitor deforolimus, a patient with advanced, refractory EWS exhibited a partial response [175]. A Phase I/II trial of temsirolimus with liposomal adriamycin in recurrent sarcoma is currently recruiting patients [222]. A Phase II study testing the combination of IGF-1R and mTOR inhibition in relapsed or refractory sarcoma is also ongoing [223].

Intracellular signaling cascades regulating EWS cell function can be targeted through the use of kinase inhibitors. The Src kinase inhibitor dasatinib inhibits EWS cell growth [176], and is currently being used in a Phase I/II trial in combination with ifosfamide, carboplatin and etoposide in advanced pediatric cancers, including EWS [219]. A Phase II trial is assessing the efficacy of dasatinib as monotherapy in advanced sarcomas [224]. However, preliminary results of this trial showed no benefit in advanced EWS [83]. The kinase inhibitor sunitinib inhibits the PGDF and VEGF receptors, FLT3 and KIT, and inhibits EWS cell line growth as xenografts. A Phase II trial was recently completed assessing the effect of sunitinib in advanced and recurrent sarcomas, including EWS [225]. Results of this trial are pending.

An ongoing trial is assessing antiangiogenic therapies in EWS. Expression of EWS/FLI1 in fibroblasts induces expression of VEGF [177,178], and VEGF depletion inhibits EWS tumor growth *in vivo* [179-181]. Moreover, patients with EWS have elevated serum levels of VEGF [182,183]. The activity of bevacizumab was recently tested in recurrent or refractory EWS in a Phase II trial coordinated by COG. Patients were randomized to receive either chemotherapy with vincristine, topotecan and cyclophosphamide or these agents together with bevacizumab [226]. Results of this trial have not yet been published.

Expression of EWS/FLI1 induces gene expression programs that markedly shift cellular phenotype from a normal, highly differentiated cell to that of a proliferative, invasive, poorly differentiated cell [160]. Immune therapies are based on the premise that these deranged tumor cells could be recognized as abnormal, foreign cells by naive leukocytes from healthy donors. Multiple Phase II trials are testing the validity of transplanting allogeneic stem cells or specific leukocyte subpopulations from healthy donors in advanced solid tumors, including EWS [227–229].

Both front-line and salvage cytotoxic chemotherapy regimens are also under active investigation. Given its activity against EWS [142,154], COG is coordinating a multicenter, Phase III trial assessing the role of topotecan in front-line therapy regimens for EWS. Patients are randomized to receive either VAC-IE or VAC-IE

Table 5. Selected ongoing or recently completed Phase II and III tri	als for Ewing's sarcoma.				
Trial	Clinicaltrials.gov identifier	Phase	Drug class	Target	Ref.
A study to determine the activity of SCH 717454 in subjects with relapsed osteosarcoma or Ewing's sarcoma (Study P04720AM3)	NCT00617890	п	Antibody	IGF-1R	[220]
A five-tier, open-label study of IMC-A12 in advanced sarcoma	NCT00668148	п	Antibody	IGF-1R	[221]
Safety and efficacy study of torisel and liposomal doxorubicin for patients with recurrent sarcoma	NCT00949325	I/I	Cytotoxic chemotherapy plus small molecule	mTOR	[214]
Temsirolimus and cixutumumab in treating patients with locally advanced, metastatic or recurrent soft tissue sarcoma	NCT01016015	п	Antibody and small molecule	IGF-1R mTOR	[215]
Dasatinib, ifosfamide, carboplatin and etoposide in treating young patients with metastatic or recurrent malignant solid tumors	NCT00788125	I/II	Cytotoxic chemotherapy plus small molecule	Src family kinases	[211]
Trial of dasatinib in advanced sarcomas	NCT00464620	п	Small molecule	Src family kinases	[222]
Vincristine, topotecan and cyclophosphamide with or without bevacizumab in treating young patients with refractory or first recurrent extracranial Ewing's sarcoma	NCT00516295	п	Cytotoxic chemotherapy plus antibody	VEGF	[225]
Stem cell transplantation in patients with high-risk and recurrent pediatric sarcomas	NCT00043979	п	High-dose chemotherapy/immune	N/A	[226]
Hematopoietic stem cell transplantation from human leukocyte antigen compatible donor in Ewing's sarcomas and soft tissue sarcomas	NCT00998361	п	High-dose chemotherapy	N/A	[227]
Irradiated donor lymphocyte infusion in treating patients with relapsed or refractory hematologic cancer or solid tumor	NCT00161187	п	Immune	N/A	[228]
Combination chemotherapy in treating patients with nonmetastatic extracranial Ewing's sarcoma	NCT01231906	Η	Chemotherapy	N/A	[229]
Study in localized and disseminated Ewing's sarcoma (EWING 2008)	NCT00987636	III	High-dose chemotherapy	N/A	[230]
Tandem peripheral blood stem cell rescue for high-risk solid tumors	NCT00179816	II/I	High-dose chemotherapy	N/A	[219]
IGF-IR: Insulin-like growth factor 1 receptor.					

incorporating cycles of vincristine, topotecan and cyclophosphamide in place of some VAC cycles [230]. EURO-EWING-99 examined two different consolidation regimens in patients with localized EWS and good histologic response to VIDE induction (Figure 5).

Given the poor prognosis of metastatic EWS, several trials are addressing front-line induction regimens and consolidation with high-dose therapies. EWING 2008 is a randomized trial that uses a similar risk stratification scheme to EURO-EWING-99 designed to assess the role of high-dose therapy in conjunction with autologous stem cell reinfusion in patients with high-risk disease. Other arms of the study examine the use of fenretinide and/or zoledronic acid to chemotherapy in standard risk patients [219].

#### **Future perspective**

With the advent of chemotherapy in the middle of the 20th century, great progress was made in prolonging the previously dismal long-term survival of patients with osteosarcoma or EWS. Modern multidrug neoadjuvant chemotherapy regimens evolved from innovative, complementary clinical trials that progressively prolonged OS in nonmetastatic osteosarcoma and EWS to nearly 70% by the turn of the millennium. However, this progress has impacted survival in metastatic and relapsed disease to a much lesser extent. Due to the rarity of these tumors, it is difficult to efficiently accrue patients for rigorous, randomized trials testing novel therapies, and so trials are often small, not randomized, and require decades to complete.

Despite the relatively stable survival rates in osteosarcoma and EWS in the past decade, preclinical research on the biology of these tumors has begun to elucidate the signaling networks that drive tumor progression, presenting novel candidates for therapeutic intervention. Many early trials using highly targeted therapies against these molecules have produced

promising results. Several ongoing trials are testing the activity of small molecules and antibodies inhibiting signaling pathways crucial for tumor cell proliferation, survival, and metastasis that, when used therapeutically, should cause reduced systemic toxicity compared with conventional cytotoxic chemotherapy. In the study of these novel targets and agents originally identified in the laboratory (e.g., anti-IGF-1R antibodies, mTOR inhibitors and bisphosphonates), it will be critical to incorporate well-designed correlates in order to identify the patients most likely to benefit. It is clear that targeted therapy is only of benefit in a relatively small subset of patients treated in the relapsed, refractory setting. In order to progress, we must understand when (front-line vs relapsed), why (biologic basis for the target), what (most effective agent in class) and how (optimal drug schedule) to use a novel agent in the patient population most likely to benefit. This will only come with collaborative translational and clinical efforts. Current agents being evaluated include anti-IGF-1R therapy and mTOR inhibition in EWS, and bisphosphonates and antivascular agents in osteosarcoma, while further investigation into potential agents such as PARP inhibitors and notch inhibitors are warranted. With further study of these agents in combinations as well as the identification of new agents, it is hopeful that the current plateau in outcomes for osteosarcoma and EWS patients will rise again.

#### Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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#### **Executive summary**

- Front-line therapy of osteosarcoma and Ewing's sarcoma (EWS) requires multidisciplinary treatment modalities, including surgery, neoadjuvant and adjuvant combination chemotherapy, and radiotherapy in select cases.
- Significant progress has been made in improving survival in localized osteosarcoma and EWS; however, the prognosis for primary
  metastatic and relapsed disease remains poor.
- Front-line chemotherapy for localized and metastatic osteosarcoma includes methotrexate, doxorubicin and cisplatin.
- Front-line chemotherapy for localized EWS includes vincristine, doxorubicin, cyclophosphamide, actinomycin D, ifosfamide and etoposide.
- Patients with metastatic EWS are typically treated with vincristine, doxorubicin, cyclophosphamide and actinomycin D. High-dose chemotherapy with stem cell support as consolidation continues to be under investigation and may prove to be beneficial for patients with widely disseminated EWS.
- The EURAMOS-1, EURO-EWING-99 and EWING 2008 trials should provide significant insight into front-line therapeutic strategies for osteosarcoma and EWS.
- Current trials are investigating several targeted therapies for osteosarcoma and EWS in relapsed or refractory disease. The most promising agents may be trialed in combination with front-line therapies in future clinical trials.

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