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Anti-Tumour Treatment

Osteosarcoma treatment – Where do we stand? A state of the art review

Anja Luetke [a,1], Paul A. Meyers [b], Ian Lewis [c], Heribert Juergens [a,*]

[a] Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany
[b] Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
[c] Alder Hey Children's NHS FT, Liverpool, United Kingdom

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ABSTRACT

Long-term outcome for patients with high-grade osteosarcoma has improved with the addition of systemic chemotherapy, but subsequent progress has been less marked. Modern, multiagent, dose-intensive chemotherapy in conjunction with surgery achieves a 5-year event-free survival of 60–70% in extremity localized, non-metastatic disease. A major, as yet unsolved, problem is the poor prognosis for metastatic relapse or recurrence, and for patients with axial disease. This article reviews the current state of the art of systemic osteosarcoma therapy by focusing on the experiences of cooperative osteosarcoma groups. Also, we shed light on questions and challenges posed by the aggressiveness of the tumor, and we consider potential future directions that may be critical to progress in the prognosis of high-grade osteosarcoma.

Introduction

Following the implementation of chemotherapy in the 1970s, the treatment of high-grade malignant osteosarcoma (OS) has made important progress. However, survival rates continue to be unsatisfactory in the metastatic and relapse setting. Understanding OS biology still remains a complex challenge. An unknown etiology, high genetic instability of OS cells, a wide histological heterogeneity, lack of biomarkers, high local aggressiveness, and a rapid metastasizing potential create pivotal questions to be answered. The purpose of this paper is to outline recent developments in the field of osteosarcoma therapies.

Search strategy and selection criteria

We searched PubMed for the past 12 years (January 2001–October 2013) with the terms "osteosarcoma" and "treatment". The abstracts were screened to identify those research studies and review articles we judged relevant to our objectives. This procedure identified 166 potentially eligible publications which were studied in detail. A particular relevance was given to reports on systemic therapy. References from these articles were also obtained, and review articles are cited to provide readers with more details than this review has room for. The date of the last search was October 8, 2013.

What do we know about OS?

Background

Osteosarcoma (OS) defines neoplasms that share the histological finding of osteoid production in association with malignant mesenchymal cells. These tumors are generally locally aggressive and tend to produce early systemic metastases [1]. A distinction is generally drawn between different histologic types of OS (conventional, teleangiectatic, parosteal, periosteal, low-grade central, small cell, not otherwise specified). The conventional type is

Abbreviations: OS, osteosarcoma; SEER, Surveillance Epidemiology and End-Results Program of the US National Cancer Institute; MSKCC, Memorial Sloan-Kettering Cancer Center; COG, Children's Oncology Group; OAS, overall survival; EFS, event-free survival; HDMTX, high-dose methotrexate; (SSG), Scandinavian Sarcoma Group; EURAMOS-1, European and American Osteosarcoma Study Group 1 trial; MAP, high-dose methotrexate and doxorubicin and cisplatin; COSS, Cooperative Osteosarcoma Study Group; EOI, European Osteosarcoma Intergroup; SMNs, second malignant neoplasms; HER2/neu, human epidermal growth factor receptor 2; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; MTP-PE, muramyl tripeptide phosphatidylethanolamine = mifamurtide; G-CSF, granulocyte-colony stimulating factor; IOR, Istituto Ortopedico Rizzoli; IFN-α, interferon alpha; POG, Pediatric Oncology Group; ISG, Italian Sarcoma Group; SFOP, Société Française D'oncologie Pédiatrique.

* Corresponding author. Address: Pediatric Hematology and Oncology, University Children's Hospital Münster, Albert-Schweitzer-Campus 1, Gebäude A1, 48149 Münster, Germany. Tel.: +49 251 834 7742; fax: +49 251 834 7828.
E-mail address: jurgh@uni-muenster.de (H. Juergens).

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the most common, and has been subdivided based on the predominant features of the cells (osteoblastic, chondroblastic, fibroblastic), although without clear significant differences of clinical outcome [2]. This article addresses high-grade osteosarcoma, which accounts for 80–90% of all OS [3]. In the majority of primary OS, the etiology is unknown. Cytogenetic studies have shown various complex changes involving some chromosomes but without any specific pattern [4]. Two genes – a hereditary mutation of retinoblastoma, and an autosomic recessive mutation of p53 in the Li-Fraumeni syndrome – localized in 13q14 and 17p13, respectively, are currently proposed to be involved in a stepwise accumulation of genomic defects [4].

Epidemiology

OS is classified as an orphan disease with an overall incidence of 0.2–3/100 000 per year (0.8–11/100,000 per year in the age group 15–19 years) in the EU [3]. Despite its rarity, it has been reported to be the third most common cancer in adolescence, occurring less frequently than only lymphomas and brain tumours in this age group [5]. An association between rapid bone growth and osteosarcoma has been postulated, given the tumor's typical metaphyseal location and its peak incidence during adolescence and early adulthood as well as the male predominance of 60% [6]. OS is extremely rare in children before the age of 5 years [7].

Tumor sites

The most common primary sites of OS are the distal femur, the proximal tibia, and the proximal humerus, with more than half originating around the knee [8,9]. About 10% develop in the axial skeleton, most commonly the pelvis [10,11]. An analysis of the SEER database revealed a higher percentage of axial tumors in patients aged 60 and above (39.7%) when compared to patients aged $\leqslant$25 (12.2%) or 25–59 years (35.3%) [12]. It is well established that axial locations result in a considerably worse outcome than primary disease location within the appendicular skeleton [10,12]. The 5 year survival of OS in the pelvis ranges from 27% to 47% [13]. OS in the spine has been linked with median survival times of 10–38 months [14,15]. A recently published report from the Children's Oncology Group (COG) found that survival with metastatic disease in the absence of a pelvic primary tumor was similar to that for localized or metastatic pelvic OS [16].

Metastatic disease and local recurrence

At the time of OS diagnosis, about 10–20% of patients present with macroscopic evidence of metastatic disease, most commonly (90%) in the lungs, but metastases can also develop in bone (8–10%) and rarely in lymph nodes [8,17–19]. However, 80–90% of patients are assumed to have micrometastatic disease, which is subclinical or undetectable using current diagnostic modalities [6]. Regarding lung metastases, thoracic CT-scanning is considered gold standard and remains the most reliable imaging tool [20]. In OS patients with radiographic pulmonary metastases, CT, however, has two limits: not all lung nodules found during surgery are evident on the CT scan, and not all nodules seen on the CT scan are true metastatic lesions, in particular in lesions smaller than 5 mm [21].

A total of 30–40% of patients with localized OS will develop a local or distant recurrence [22]. Approximately 90% of relapses are lung metastases, which usually occur in the first 2–3 years [14,23–25]. Relapse 5 years after initial treatment of OS is uncommon, arising in between 1% and 2% of all osteosarcoma patients [26]. Hauben et al. found a trend for late relapse to arise more commonly in chondroblastic subtypes [26]. Osteosarcoma recurrences are associated with a rather poor prognosis [22,27]. Five-year overall survival (OAS) for recurrent OS has been reported to be 23–29% (pulmonary metastases only: 28–33%) [20]. In one series of patients who relapsed, 31% of those with local recurrence alone were cured by further treatment, as compared with only 10% of those with metastases [28]. The outlook is considered to be extremely poor for patients who present with synchronous regional bone metastases (skip metastases), either in the primary bone site or transarticular [29]. Aggressive multimodal therapy holds the promise to achieve prolonged survival, especially in patients in whom these metastases occur within the same bone as the primary lesion and whose tumors respond well to chemotherapy [30]. Bielack et al. reported survival estimates with second and subsequent osteosarcoma recurrences. Five-year OAS and event-free survival (EFS) rates were 16% and 9% for second, 14% and 0% for third, 13% and 6% for fourth, and 18% and 0% for fifth recurrences, respectively [31]. The median interval from first to second recurrence was found to be nine months, and the median interval between subsequent recurrences remained quite constant at approximately 6 months [31].

Current therapeutic strategies

Current management comprises preoperative (neoadjuvant) chemotherapy followed by surgical removal of all detectable disease (including metastases), and postoperative (adjuvant) chemotherapy, preferably within the setting of clinical trials [17]. OS is considered resistant to applicable doses of radiation [23,32]. Supplemental therapeutic approaches such as chemoembolization or angio-embolization, thermal ablation, radiofrequency ablation, and cryotherapy are experimental [23].

Surgery

Complete surgical resection, if feasible, remains essential for cure [23]. Current surgical strategies focus on refining the nature and scope of resection to preserve uninvolved tissues, and on the adoption of novel biological and nonbiological skeletal and soft-tissue reconstruction methods to optimize function [33]. Advances in imaging techniques and positive effects of preoperative chemotherapy have led to a major shift away from amputation towards limb-salvage (conservative) surgery, with the latter being expanded to around 80% of patients [9,34]. Local recurrence rates of 2–3% after amputation and 5–7% after conservative surgery have been reported, with no significant differences in survival [23,35]. The incidence of local recurrence has been closely related to the achieved surgical margins (intralesional – within lesion, marginal – within reactive zone, wide – through normal tissue and beyond reactive zone, radical – extracompartmental), with only a wide margin being considered appropriate [23,28]. Even so, no general definition exists on the adequate thickness of the normal cuff, also as this varies depending on layers of reactive tissue surrounding the tumor and the responsiveness to preoperative chemotherapy. In OS patients who achieved complete surgical remission with adequate margins, surgical margin width in bone did not correlate with the local recurrence rate [36].

Thoracotomy with metastasectomy remains an essential and effective adjunct to multiagent chemotherapy in the treatment of pulmonary metastases. Surgical resection is considered if all lung nodules can be removed and a sufficient amount of pulmonary tissue can be saved to maintain adequate pulmonary function [23].

Chemotherapy

Recently, most chemotherapy regimens applied for OS have been based around 4 drugs; high-dose methotrexate (HDMTX)

with leucovorin rescue, doxorubicin (adriamycin), cisplatin, and ifosfamide [14]. These agents were integrated into various chemotherapy protocols. The range of dosages most commonly used are as follows: doxorubicin (cumulative dose from 240 to 480 mg/m [2]; dose per cycle from 60 to 90 mg/ [2]), methotrexate (cumulative dose from 48 to 168 g/m^2; dose per cycle 12 g/m^2), cisplatin (cumulative dose from 480 to 600 mg/m^2; dose per cycle from 100 to 120 mg/m^2), and ifosfamide (cumulative dose from 30 to 69 g/m^2; dose per cycle from 6 to 14 g/m^2) [37].

Preoperative 'neoadjuvant' chemotherapy is generally administered for a period of about 8–10 weeks prior to surgery. Following surgical resection and a brief lapse to allow for wound healing, postoperative adjuvant chemotherapy is continued for a period of another 12–29 weeks [6,38,39]. The preoperative chemotherapeutic treatment offers an opportunity to allow time for planning limb salvage surgery and reconstructive procedures, to study the histological effect of preoperative chemotherapy on the primary tumor – better response is strongly correlated with better outcome – and also potentially to modify postoperative chemotherapy accordingly [39].

Radiotherapy

Though OS is considered a radioresistant tumor, radiotherapy can be an option as local treatment of unresectable tumors, following intralesional resection, or as palliation of symptomatic metastases [32,40]. Some chemotherapeutic agents (e.g. ifosfamide, cisplatin, HDMTX) seem to markedly improve the effectiveness of local control radiotherapy [23]. For some patients, the combined approach of irradiation with chemotherapy may produce long-term remission [10]. Mahajan et al. analyzed their radiation experience in 39 high-risk, metastatic, and/or recurrent patients during a consecutive period of 20 months. The median radiation dose and number of fractions of radiation was 30 Gy in 10 fractions. Chemotherapy was used in 80% radiotherapy courses. The early results confirmed that external beam radiotherapy with systemic treatment may provide a successful multimodality approach to local control and symptom relief [41]. Machak et al. used radiation after effective induction chemotherapy for nonmetastatic osteosarcoma of the extremities and found that it can be a reliable modality to control local disease and preserve limb function [42]. Ciernik et al. demonstrated that proton therapy to deliver high radiotherapy doses allowed locally curative treatment for some patients with unresectable or incompletely resected OS [43]. Reports with the use of samarium bone-seeking radioisotope therapy as a method to provide palliation for patients with bone metastases indicate feasibility, but so far the role of this treatment modality is not well defined [44,45].

Chemotherapy – Where do we stand?

In the prechemotherapy era, which means before 1970, OS was a disease with a very poor outcome (survival rate less than 20%) [34,45,46]. Clinically detectable pulmonary metastases usually evolved within the first 12 months following amputation. This has been used to support the concept that microscopic involvement of the lung was already present at the time of operation [47]. The chemotherapy regimens that pioneered in the 1970s and early 1980s markedly improved survival rates, [17,34] and during the last three decades various chemotherapy protocols have been investigated. The initial move for neoadjuvant treatment was made by a study group at the Memorial Sloan-Kettering Cancer Center (MSKCC), who published several consecutive series using increasingly complex chemotherapy regimens, such as the T-10 protocol [48]. The latter was adopted by multi-institutional American and European groups who carried out confirmatory trials. At the same time, a series of studies using other multiagent regimens were performed [45]. All these efforts yielded the combined approach of multi-agent neoadjuvant with adjuvant chemotherapy. The pivotal importance of this treatment approach has been proven by the Multi-Institutional Osteosarcoma Study (MIOS) [49]. By contrast, in a prospective trial conducted by the Pediatric Oncology Group (POG) there was no advantage in EFS for nonmetastatic OS patients given presurgical chemotherapy [50].

Since the 1980s, many OS treatment and research protocols have included HDMTX with leucovorin rescue, doxorubicin, and cisplatin, a regimen often referred to as MAP, but there is still no consensus on their optimal combination [51]. The SSG XIV protocol and the standard group of the EURAMOS-1 trial are representative of recently applied MAP regimens [38,52]. The role of HDMTX has not yet been fully clarified [53]. Daw et al. conducted a multi-institutional trial (OS99) that evaluated the efficacy of carboplatin, ifosfamide, and doxorubicin without HDMTX in 72 patients with newly diagnosed, localized, resectable OS. The regimen used was found to produce outcomes comparable to those of cisplatin-containing or HDMTX-containing regimens. Carboplatin, ifosfamide, and doxorubicin given without HDMTX resulted in 5-year EFS and survival estimates of 66.7% and 78.9%, respectively [53]. Ifosfamide both alone and in combination with etoposide also has been controversial and remains under investigation [6]. Cisplatin had been delivered intra-arterially in an attempt to increase its local efficacy. However, it is clear now that administration of intra-arterial cisplatin in the context of multiagent chemotherapy does not translate into a better survival [37,54–57]. Other chemotherapeutic agents such as bleomycin, cyclophosphamide, and dactinomycin (actinomycin D) were mainly abandoned, as they have not proved to be as efficient as the aforementioned drugs [6,37].

The prognostic relevance of dose intensity in the treatment of OS is still under discussion. Meyers et al. retrospectively analyzed 279 patients treated at MSKCC. A delay of more than 24 days in the resumption of chemotherapy for patients with lower degrees of necrosis was associated with an increased risk of recurrence and death [48]. Imran et al. retrospectively assessed the prognostic significance of the time to resumption of chemotherapeutic treatment after surgery in 703 patients with localized OS in an extremity. The results of this study demonstrated that a delay of more than 21 days was associated with an increased risk of death, although no association with EFS was found [58]. A Cooperative Osteosarcoma Study Group (COSS) analysis included 917 consecutive patients aged below 40 years with high-grade, nonmetastatic OS of the extremities. In the overall setting of intensive multidrug treatment (HDMTX, doxorubicin, cisplatin, and ifosfamide), there was no detectable correlation between higher dose intensities and better outcomes [59]. This conclusion is also supported by the results of the European Osteosarcoma Intergroup's (EOI) and the Italian (ISG) and Scandinavian (SSG) Sarcoma Group's studies [14]. The EURAMOS-1 Intergroup, a collaboration of COG, COSS, EOI and SSG designed a prospective randomized study to see (a) whether the addition of interferon improved outcome in good responders and (b) whether the addition of 2 additional agents increased disease-free survival or OAS in patients with poor histological response [52].

By now modern multiagent, dose-intensive chemotherapy (in conjunction with surgery) achieves a 5-year EFS of about 60–70% in extremity localized, non-metastatic disease [14]. Nagarajan et al. reported the very long-term outcomes of 5-year survivors of childhood OS diagnosed from 1970 to 1986. Among the 733 patients, subsequent survival at 10, 15, and 20 years since diagnosis was 93.5%, 90.4%, and 88.6%, respectively [60]. However, reported survival estimates in OS may range widely, due to heterogeneous selection criteria and varying trial designs [34]. In the metastatic

Table 1
Selected clinical trials of neoadjuvant/adjuvant therapy in osteosarcoma.

Study	N	Drugs	5-Year survival	Comments
MSKCC T-10 *single center* [48]	279	M (preop) GR: M + A + BCD or PR: A + P + BCD (postop)	76% (EFS) *for patients aged ⩽21 years*	Most of the current treatment strategies have evolved from the lessons learned from the T-10 protocol
1975–1984				
SSG-II *multicenter* [75]	97	M (preop) GR: M + A + BCD or PR: A + P + BCD (postop)	54% (EFS) 64% (OAS)	Results of the T-10 protocol could not be confirmed
1982–1989				
EOI-1 *multicenter, RCT* [83]	198	A + P ± M (preop/postop)	A + P: 57% (EFS) A + P: 64% (OAS) A + P + M: 41% (EFS) A + P + M: 50% (OAS)	A brief intensive chemotherapy regimen of A + P has produced good results
1983–86				
COSS-86 *multicenter* [56]	171	*Low risk patients:*	10-Year survival	Use of isfosfamide for high-risk patients; intra-arterial vs. intravenous administration of cisplatin
1986–1988		M + A + P (preop/postop) *High risk patients*:* M + A + P + I (preop/postop)	66% (EFS) 72% (OAS)	
IOR/OS-2 single center [78]	164	M + A + P (preop) GR: M + A + P or PR: M + A + P + I/E (postop)	65% (EFS)	I/E provided good salvage for PR
1986–1989				
EOI-2 *multicenter, RCT* [84]	391	A + P or M + A + VCR (preop) A + P or M + A + VCR + BCD (postop)	44% (EFS) 55% (OAS)	No difference in survival between the two-drug and multi-drug regimen
1986–1991				
POG-8651 *multicenter* [50]	100	None or M + A + P (preop)	Immediate surgery: 69% (EFS)	No advantage of preoperative chemotherapy
1986–1993		M + A + P + BCD (postop)	Neoadjuvant chemo: 61% (EFS)	
SSG-VIII *multicenter* [76]	113	M + A + P (preop) GR: M + A + P or PR: M + A + P + I/E (postop)	63% (EFS) 74% (OAS)	Lack of benefit of modifying postoperative therapy for PR
1990–1997				
IOR/OS-4 *single center* [79]	133	Preop/postop: M + A + P + I	56% (EFS) 71% (OAS)	No benefit of neoadjuvant ifosfamide
1993–1995				
COG (INT- 0133) *multicenter, RCT* [72]	662	[*Regimen a*] preop: M + A + P	6-Year survival without MTP: 61% (EFS), 70% (OAS) with MTP: 67% (EFS) 78% (OAS)	Possible effects between ifosfamide and mifamurtide
1993–1997		postop: M + A + P vs. M + A + P + MTP [*regimen b*] preop: M + A + I postop: M + A + P + I vs. M + A + P + I + MTP		
EOI-3 *multicenter, RCT* [85]	497	Preop/postop: A + P vs. A + P + G–CSF	40% (EFS) 56% (OAS)	Histologic response as the key treatment-related predictive factor has been challenged
1993–2002				
SFOP-OS94 *multicenter, RCT* [82]	234	Preop: M + I/E [*regimen a*] vs. M + A [*regimen b*] Postop [*regimen a*]: GR: M + I/E PR: A + P postop *[regimen b]*: GR: M + A PR: I/E	62% (EFS) 76% (OAS)	A preoperative chemotherapy regimen combining high-dose M + I/E improved the proportion of good histologic response compared to a regimen based on M + A
1994–2001				
ISG/SSG-I *multicenter* [80]	182	Preop/postop: M + A + P + high-dose I	64% (EFS) 77% (OAS)	No advantage of neoadjuvant high-dose ifosfamide
1997–2000				
SSG-XIV *multicenter* [39]	63	Preop: M + A + P postop: GR: M + A + P PR: M + A + P + I	70% (EFS) 76% (OAS)	Salvage therapy given to PR did not improve outcome to a similar degree as for GR
2001–2005				
EURAMOS-1 *multicenter, RCT* [52,86]	2260	Preop: M + A + P postop: GR: M + A + P vs. M + A + P + INF-α PR: M + A + P vs. M + A + P + I/E	First results announced for 2013	Includes axial as well as extremity tumors and patients with metastatic as well as nonmetastatic disease, as long as all sites are deemed resectable
2005–2011				

N, patient number; A, doxorubicin; P, cisplatin; M, high-dose methotrexate; I, ifosfamide; E, etoposide; BCD, bleomycin-cyclophosphamide-dactinomycin; VCR, vincristine; MTP, muramyl tripeptide phosphatidylethanolamine (MTP-PE, mifamurtide); G-CSF, granulocyte-colony stimulating factor, preop, preoperatively; postop, postoperatively; chemo, chemotherapy; GR, good responder; PR, poor responder; RCT, randomized controlled trial.
* Tumour length ⩾1 of 3 of the involved bone, and/or ⩾20% chondroid ground substance in the biopsy specimen, and/or reduction of early and/or late phase activity in sequential ^{99}Tc-MDP bone scans.

relapse or recurrent conditions, EFS of OS patients at 3–5 years has remained at 10–30% since the early 1980s [8]. 5-year OAS in patients with pulmonary dissemination as only metastatic site turned out to be 18–33% [20].

The role of second-line chemotherapy for resectable recurrences is controversial, since prospective, randomized studies in this setting are lacking [61]. In a retrospective analysis of 60 patients, a favorable role for chemotherapy was demonstrated, [62]

while other larger retrospective studies only show OAS benefit in patients who could not have a complete surgical resection [25]. Based on the database of COSS, Kempf-Bielack et al. analyzed 576 patients with recurrent OS and reported that second-line chemotherapy, especially with more than one agent, seems to contribute to limited improvements in EFS outcome: The use of second-line chemotherapy correlated with good response to first-line chemotherapy, multiple lesions at relapse, and bilateral pulmonary involvement, but not time to relapse [22].

Chemotherapy toxicity

The chemotherapy treatment of OS is associated with important short- and long-term collateral toxic effects [37]. Acute toxicities such as alopecia, myelosuppression, mucositis, and nausea and vomiting are common complications of most cytotoxic chemotherapy regimens [51]. The major causes of rare cases of toxic deaths have been early or late cardiac failure due to doxorubicin toxicity and sepsis following febrile neutropenia [63].

The risk of cardiac toxicity was related to both dose intensity and total cumulative dose of doxorubicin, with a significant increase in the incidence of heart failure occurring after the administration of 550 mg/m [2][51]. Phase 3 osteosarcoma cooperative group trials report an incidence of clinically apparent cardiac toxicity of 0–4%. In most of the cases, cardiotoxicity was noted 1–12 weeks after the completion of therapy [51]. Cisplatin may cause high-frequency hearing loss, which has been reported in as many as 11% of patients [45]. The risk of ototoxicity increases with higher cumulative doses, higher individual doses, and younger age [51]. The risk of cisplatin-associated nephrotoxcitiy and gonadal dysfunction was associated with higher dose rates and greater dose intensity as well [51]. Data on female infertility following OS therapy is limited. In one study, 6% of female patients treated with MAP plus ifosfamide experienced early menopause [51]. HDMTX in MAP chemotherapy regimens is typically given at a dose of 12 g/m [2], with hydration and alkalinisation to promote methotrexate excretion and leucovorin rescue to protect normal cells from the effects of folate depletion. Despite appropriate precautions, HDMTX-induced renal dysfunction continues to occur in approximately 1.8% of patients who are treated on clinical protocols with optimal supportive care, and the mortality rate among those patients has been shown to be 4.4% [45,64]. The risk of ifosfamide-associated nephrotoxicity was associated with higher cumulative doses, and younger age at the time of administration [51]. Ferrari et al. evaluated the influence of age and sex on chemotherapy-related toxicity in a MAP plus high-dose ifosfamide regimen. They found a higher incidence of grade four neutropenia and thrombocytopenia in children (4–14 years) and females. Delayed methotrexate excretion was higher in adults (>20–40 years) than in adolescents and children [65].

Second malignant neoplasms (SMNs) have been reported to develop for up to 25 years after osteosarcoma therapy [51]. The SMNs for which this population is at increased risk include leukaemia, myelodysplastic syndrome, breast cancer, CNS tumours, and soft-tissue and bone sarcomas [51]. The majority (about 86%) of SMNs occur >10 years from diagnosis, and the cumulative incidence of SMNs in 5-year survivors at 25 years was 5.4% [60]. In retrospective analyses, SMNs were more common in female survivors, [66,67] in survivors who had metastatic disease at presentation, [68] and in survivors with uncommon histological subtypes [67].

Chemotherapy drug resistance

OS tumors may be inherently resistant to chemotherapy agents or may become unresponsive to these drugs during the chemotherapeutic treatment, which occurs in 35–45% of patients [37,69]. The question of when chemotherapy resistance emerges is still unanswered. Proposed mechanisms imply pertubations in signal transduction pathways (e.g. activation and overexpression of HER2/neu, MAPK, and PI3K), increased drug efflux, increased intracellular detoxification, alterations of topoisomerase II, increased DNA damage repair, impaired transport into the cell, increased levels of dihydrofolate reductase and polyglutamylation (methotrexate), mutations in dihydrofolate reductase (decreased affinity for methotrexate), increased glutathione detoxification, increased cellular thiol levels, and increased aldehyde dehydrogenase activities [37,69]. Recently, Huang et al. implicated the DNA binding protein high mobility group box 1 (HMGB1), which is also involved in several inflammatory diseases, in the development of drug resistance in OS. Anti-cancer agents including doxorubicin, cisplatin, and methotrexate each induced HMGB1 upregulation in human OS cells. The authors demonstrated that chemotherapy-induced HMGB1 expression promoted autophagy, which inhibited apoptosis and increased drug resistance [70].

Experiences of Osteosarcoma Collaborative Groups (examples)

The low incidence of OS is a strong argument for international collaboration and led to the establishment of several multi-institutional cooperative groups in the U.S. and in Europe (Table 1).

Cooperative Osteosarcoma Study Group (COSS)

COSS was founded in 1977 and has since registered some 3500 bone sarcoma patients from over 200 institutions [9]. In COSS-86, chemotherapy was intensified by adding ifosfamide to an already aggressive regimen of MAP for pathologic poor responders. With a long term EFS of 66%, these results were the best published so far by COSS [55,56]. Bielack et al. reviewed 2464 consecutive patients with high-grade OS, who had been diagnosed between 1980 and 2005. Intended treatment included surgery and multidrug chemotherapy, with HDMTX, doxorubicin, cisplatin, and ifosfamide being used in most protocols. While survival expectancies improved from the first to the second half of the recruitment period, no further improvement was evident within the latter period. The survival probability at 10 years approached 70% for patients with localized extremity osteosarcomas, but ranged below one-third for patients with axial or primary metastatic tumours, despite identical treatment guidelines [9].

Pediatric Oncology Group (POG) and Children's Oncology Group (COG)

The advantages of presurgical chemotherapy include early administration of systemic chemotherapy, shrinkage of primary tumor, and pathologic identification of risk groups. The theoretic disadvantage is that it exposes a large tumor burden to marginally effective chemotherapy [50]. A randomized study of the Pediatric Oncology Group (POG 8651) compared immediate surgery with delayed surgery after induction of chemotherapy. Outcome was not significantly different between both arms, which means that there was no advantage in EFS for patients given presurgical chemotherapy [50].

In a randomized 2 × 2 factorial study (INT0133), the children's oncology group compared three-drug chemotherapy with cisplatin, doxorubicin, and HDMTX with four-drug chemotherapy with cisplatin, doxorubicin, HDMTX and ifosfamide for the treatment of osteosarcoma. The study also investigated the addition of an immunomodulatory treatment with mifamurtide (muramyl tripeptide phosphatidylethanolamine, MTP-PE). The addition of mifamurtide significantly improved the patient survival with

6-year OAS of 78% versus 70% in chemotherapy alone ($p = 0.03$).There was no benefit to the addition of ifosfamide to MAP [71]. This trial has been accompanied by some controversy. Whilst the initial report raised the possibility of interaction between the two interventions and resulted in some doubt and discussion about the applicability of the reported benefit of MTP-PE [72,73], the conclusive trial publication [71] refuted the suggestion of any interaction. The European Medicinies Agency (EMA) examined the trial results in detail prior to granting a license for MTP-PE (Mepact) for use as first line treatment in combination with chemotherapy in patients under 30 years old with non-metastatic osteosarcoma. Subsequently in the UK, the National Institute for Clinical Excellence (NICE) has assessed the cost benefit of MTP-PE in this therapeutic setting and approved its use as first line therapy.

Scandinavian Sarcoma Group (SSG)

Since 1982, members of the SSG have enrolled 330 OS patients into four consecutive trials. In all the studies, chemotherapy was based on MAP, and for the latter three trials, ifosfamide was also used. Postoperative chemotherapy was stratified by histological response to neoadjuvant chemotherapy. While the treatment regimen in the SSG-II trial was adapted from the Memorial Sloan-Kettering's T-10 protocol, [74] the study SSG-VIII used a more aggressive preoperative MAP based combination therapy, with change to ifosfamide plus etoposide to salvage pathologic poor responders. The response rate increased, but EFS for pathologic poor responders were not different compared to a previous SSG trial, indicating that a better response rate was not translated into a survival advantage [75]. The most recent SSG XIV study is based on the SSG-VIII protocol with some modifications. It was activated in 2001 as an interim protocol before the start of EURAMOS-1, with the rationale to keep a maximum dose-intensity of all three proven active drugs [76]. The 5-year EFS of poor histological responders receiving add-on treatment with ifosfamide was 47%, as compared to 89% for good histological responders [39].

Italian Sarcoma Group/Scandinavian Sarcoma Group (ISG/SSG)

A previous single center trial (IOR/OS-2) at the Istituto Ortopedico Rizzoli (IOR) resulted in a significant better EFS by more intensive preoperative chemotherapy and the addition of ifosfamide and etoposide for pathologic poor responders, respectively [77]. The next trial, IOR/OS-4, added ifosfamide to preoperative chemotherapy, but the results did not differ from those achieved by using ifosfamide only in the adjuvant regimen [78].

From 1997 to 2000 a total of 182 patients were included in the ISG/SSG I trial, which was undertaken to explore the effect of adding high-dose ifosfamide (15 g/m^2) to MAP also in the preoperative phase. Granulocyte colony-stimulating factor (G-CSF) support was mandatory after the high-dose 4-drug combination. The results show that addition of high-dose ifosfamide to HDMTX, cisplatin, and doxorubicin in the preoperative phase is feasible, but with major renal and hematologic toxicities, and survival rates similar to those obtained with four-drug regimens using standard-dose ifosfamide [79].

A further analysis from the IOR evaluated improvements in OAS over 21 years (1982–2002). Within 1458 included patients, survival has statistically improved from 51% in 1982 to 68% in 2002. Patients who benefited most were those who relapsed or presented with metastatic disease at diagnosis or had axial tumors [80].

Société Française d'oncologie Pédiatrique (SFOP)

Between June 1994 and June 2001, 239 patients were included in the SFOP-OS94 randomized multicenter trial. This study was designed to determine whether preoperative chemotherapy regimen combining high-dose methotrexate courses and etoposide-ifosfamide could improve the proportion of good histologic response compared to a regimen based on HDMTX and doxorubicin, in children/adolescents with localized high-grade limb osteosarcoma. Postoperative chemotherapy was adapted to the histologic response. In conclusion, this trial provided good evidence that the combination of etoposide-ifosfamide with HDMTX led to a higher rate of good responses than doxorubicin plus HDMTX [81].

European Osteosarcoma Intergroup (EOI)

The common trend from the American, German, and Italian groups was the progressive use of more drugs in prolonged schedules to try to increase cure rates. An alternative approach was that of the EOI, which sought to use shorter dose-intense regimens in a series of prospective studies [45]. Between 1983 and 2002, the EOI recruited over one thousand patients with localized extremity osteosarcoma to three randomized controlled trials. Each of the trials used a standard treatment arm of perioperative doxorubicin and cisplatin chemotherapy. Comparators were addition of HDMTX (EOI-1), a multidrug regimen (EOI-2), and a dose-intense schedule (EOI-3) [55]. In the first study, a two drug combination of doxorubicin and cisplatin turned out to be superior to a less intense MAP regimen [82] In the EOI-2 trial, outcome was similar in the doxorubicin plus cisplatin and multi-drug arm [83]. In the third trial, it was possible to increase the dose intensity by shortening the interval between subsequent cycles of chemotherapy, using G-CSF, by 30%. This resulted in a significant higher proportion of pathologic good responders. However, outcome was similar in both arms, suggesting that the increased histological response rate was reflecting the given pre-operative dose and not translated into better survival [84].

European and American Osteosarcoma Study Group (EURAMOS)

EURAMOS was founded in 2001. Four trial groups (COSS, COG, EOI, and SSG) joined together to undertake the first clinical trial of EURAMOS, EURAMOS-1, which opened in 2005. The study design includes a standard preoperative therapy using MAP. Following surgery, patients were stratified according to histological response. Patients classified as good responders ($\geqslant$90% necrosis) were randomized to continue MAP or to receive MAP followed by maintenance pegylated interferon alpha (IFN-α), while poor responders were randomized to either continue MAP or to receive MAP plus ifosfamide and etoposide [52]. Compared to the SSG XIV protocol, ifosfamide was introduced earlier in the postoperative regimen of EURAMOS-1, and in combination rather than as a single agent [39]. In contrast with previous studies, the trial also included axial as well as extremity tumors and patients with metastatic as well as nonmetastatic disease, as long as all sites are deemed resectable. Assessment of quality of life and parallel biologic studies are included [52]. EURAMOS-1 closed to registration on 30 June 2011. In the whole trial, 2260 patients have been registered [85].

In summary, it is universally accepted to use a combination therapy with at least three drugs in the treatment for localized osteosarcoma [55]. Drug regimens including methotrexate plus doxorubicin plus cisplatin (MAP) are most widely applied [55], whereas evidence for adding further agents, e.g. ifosfamide, remains controversial.

Table 2
Novel and emerging strategies for the treatment of osteosarcoma [6,17,37,94,95].

Strategy	Drugs/compounds
Novel delivery mechanisms	→ SLIT™ cisplatin (aerosolized liposomal formulation)
Overcoming drug resistance	→ Gemcitabine
• Inhibition of cellular DNA synthesis and cell growth	→ Doxetacel
• Induction of apoptosis and cell cycle arrest	→ Trimetrexate (does not require RCF for transport into cell)
• Novel antifolates	→ Curcumin
• Inhibition of drug efflux	
Inhibition of signaling receptors and transduction	→ Robatumumab, Figitumumab, Cixutumumab
• IGF/IGF-1R pathway	→ Ridaforolimus, Everolimus
• mTOR pathway	→ Sorafenib,[95] Dasatinib, Saracatinib
• Src pathway	→ Trastuzumab
• HER2-overexpression	
Altering the tumor microenvironment	→ Zoledronic acid, Pamidronate
Inhibition of osteoclast-mediated bone destruction	→ Denosumab
• Bisphosphonates	→ Bevacizumab
• RANKL inhibitors	→ Endostar
Inhibition of angiogenesis	
• VEGF inhibitors	
• Collagen XVIII-α 1	

SLIT, sustain release lipid inhalation targeting; RCF, reduced folate carrier; IGF-1R, insulin-like growth factor 1; mTOR, Mammalian target of rapamycin; Src, src is a membrane-associated tyrosine kinase; HER2, human epidermal growth factor receptor 2; RANKL, receptor activator of nuclear factor-κB ligand.

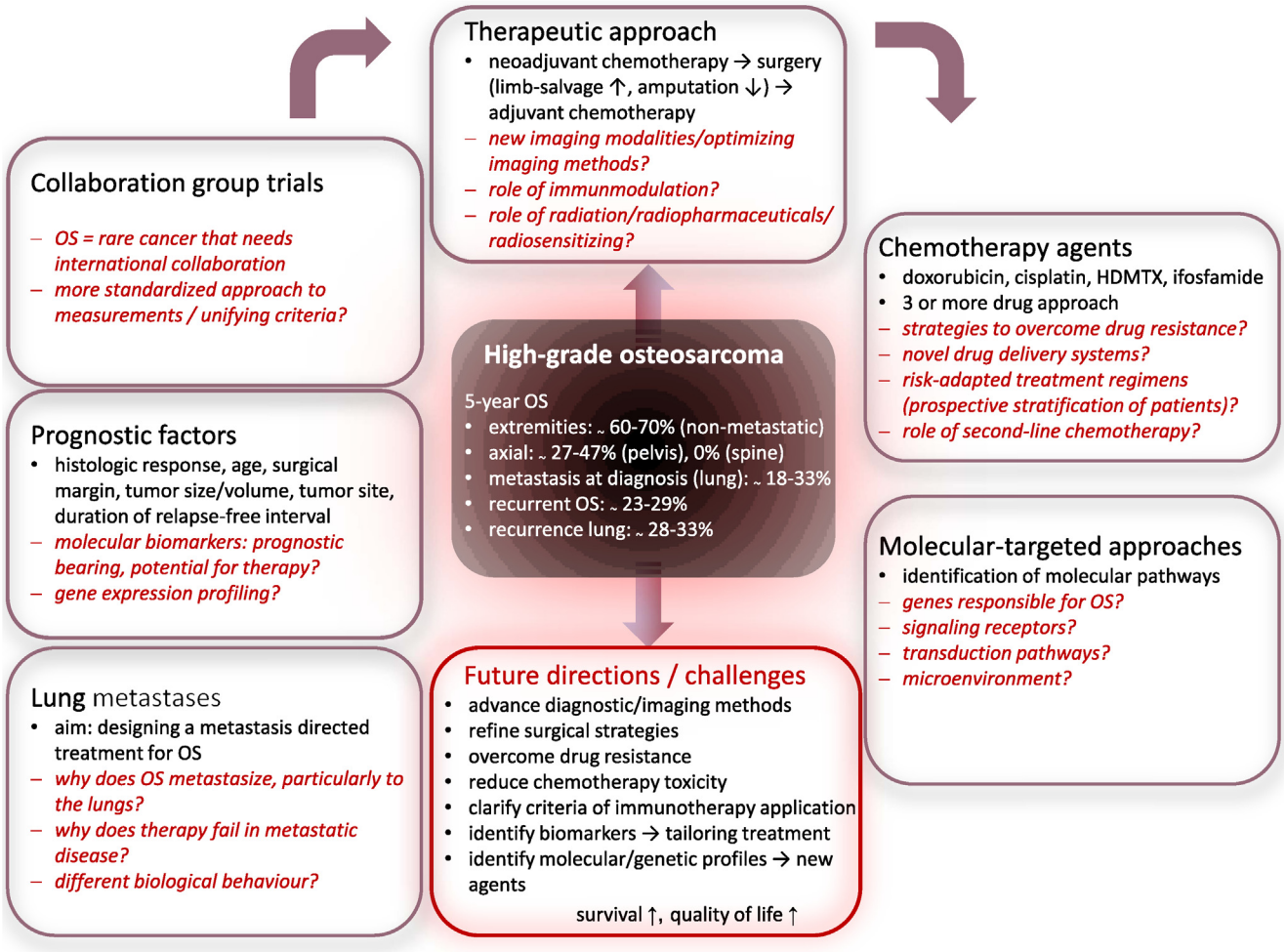

Fig. 1. High-grade osteosarcoma: questions to be answered and future challenges – a synopsis model.

Prognostic factors and biomarker research

Traditional prognostic factors for localized OS include age, tumor volume and location (axial vs. appendicular sites), surgical resection margin, histologic response to preoperative chemotherapy, and duration of relapse-free intervals (<2 years vs. >2 years). [11,31,61,86–88]. Mortality risk increased with age when evaluated by decade [9,12,87,89]. The poor prognosis in patients over 40 has been linked to a higher rate of axial tumor, more frequent metastases at presentation, and decreased tolerance of high-dose chemotherapy [8,11,90].

Histological response of the resected tumor to preoperative chemotherapy represents the most important prognostic factor to date, with patients who achieve a good histological response (usually defined as ⩾90% necrosis) having a better prognosis than those who do not [12]. Studies have consistently demonstrated 5-year EFS rates of 35–45% for poor responders and 70–80% for good responders [45]. However, some findings suggest that although intensified chemotherapeutic regimens increased tumor necrosis, the overall survival remained unchanged [88,91]. More recently, there has been some interest in using PET technology and dynamic MRIs to assess histologic response to chemotherapy and/or to predict EFS [6,17].

The adequacy of surgical resection margin is closely related to local recurrence in OS. However, the best margin width still remains controversial. In a recent study, Li et al. investigated the impact of a close margin (<5 mm) on local recurrence and OAS for patients treated with neoadjuvant chemotherapy. The average follow up was 87.6 months. Compared with wide margins, close margins did not lead to increased local recurrence [92]. These results are in line with a study by Andreou et al. who found no differences in the local recurrence rate for varying surgical margin widths in patients who achieved complete surgical remission [36].

At the molecular level, a plethora of potential molecular markers have been associated with prognosis in OS. Among them are alkaline phosphatase (AP), P-glycoprotein (likelihood of doxorubicin resistance), ErbB-2, p53, survivin (reduced survival prediction), ezrin, and CXCR4 (association with micrometastases) [11]. However, published evidence is limited by contradictory results, and no reliable molecular prognostic markers are available so far [11].

Current attempts are being made to predict the patient response to preoperative chemotherapy based on their genetic profiles. It has been described that osteosarcomas have numerous chromosomal aberrations and are characterized by complex karyotypes. The identification of gene signatures is assumed to be crucial to developing a better understanding of the molecular pathogenesis and to discovering new targets for OS treatment [4,93].

Strategies beyond current treatment regimens

Currently there are a variety of agents that have appeared to be of potential clinical interest for high-grade OS, with an emphasis on novel drug delivery systems, immunotherapy, molecular targeted approaches of signaling pathways, and manipulation of the tumor environment [17,37,94,95]. Basic research identified specific targets, and this was accompanied by wide screens of available drugs [37]. Table 2 focuses on some emerging strategies for OS tumor inhibition and candidate drugs/compounds being considered for use.

One of the focal questions is treatment of recurrent OS. A non-randomized, patient-access protocol assessed efficacy outcomes following the immunomodulator mifamurtide. First results have been published, suggesting an impact of mifamurtide on patient outcomes [96]. In a phase II trial, the multikinase inhibitor sorafenib was explored in relapsed and unresectable high-grade OS after failure of standard multimodal therapy. Sorafenib demonstrated activity in terms of progression-free survival at 4 months with some unprecedented long-lasting responses [95].

Implications for future research

Conventional chemotherapy has been essential to improve the survival of high-grade OS patients, but has reached a plateau phase since the 1980s. Efforts to approach a more effective chemotherapeutic regimen have failed to further improve patient outcome. Continuing research into novel therapeutic modalities and more target-selective treatment is urgently needed (Fig. 1).

Conflict of interest

None declared.

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