Osteosarcoma – An Evaluation of Current Diagnosis, Treatment and Chemotherapy

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Abstract

Osteosarcoma is a bone tumour that most often affects children and young adults. Although a combination of surgery to remove the primary tumour and chemotherapy prior to and after the surgery has led to an improved survival rate, local recurrence and metastases still develop in two-fifths of patients. A definitive therapy is yet to be determined for this deadly disease. This article discusses the current status on diagnosis and treatment, with an emphasis on developing new molecularly targeted therapies.

Keywords

Bone tumour, chemotherapy, osteosarcoma, sarcoma, tumour surgery, 2-methoxyestradiol

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Osteosarcoma is the most common malignant primary bone tumour in children and adolescents. It is the sixth leading cancer in children >15 years of age. There are approximately 400 new cases each year in the US.[1,2] Osteosarcoma affects males more frequently than females, with a ratio of 1.6:1. It occurs early in females due to the earlier onset of growth spurt.[3–5] The second peak for osteosarcoma is between the sixth and eighth decades. The pathogenesis is still unclear, although several factors have been proposed. The standard clinical treatment consists of a combination of surgery and chemotherapy. There is limited knowledge, however, on how different types of surgery (limb salvage versus amputation), chemotherapy (pre- and post-operative) or other treatments (e.g. radiotherapy) influence the outcome.[1]

Osteosarcoma has been reported to occur in all bones of the body. It has an affinity with the metaphyseal portions of the long bones. It is commonly localised in the distal femur and proximal tibia region, which was observed in 973 out of 1,649 cases in a study carried out at the Mayo Clinic in Rochester.[6] These sites generally contain growth plates with higher proliferative activity and turnover of bone.[7] The next most common location is the proximal humerus. It also rarely affects the bones of the hands and wrists.

In the group of axial locations of osteosarcoma, pelvic osteosarcomas account for approximately 7–9% of all osteosarcomas.[3] Spine osteosarcomas occur in 0.85–3% of cases.[9] Osteosarcoma occasionally arises in the soft tissue, thyroid gland, heart, kidney, uterus and lung.[3,10]

A staging system described by Enneking, which has been further accepted by the Musculoskeletal Tumor Society, divides all bone tumours including osteosarcoma into three stages based on grade, as described in *Table 1*.[11] Another staging system described by the American Joint Committee on Cancer is based on tumour grade, size and the presence and location of metastases (see *Table 1*).[12]

Aetiology

Although it is likely that osteosarcoma arises from the osteoprogenitor cells, given its ability to form bone it is unclear at present what the cell of origin is for osteosarcoma. Recent findings have implicated mesenchymal stem cells as possible progenitors.[13] These data are far from definitive, however, and additional work will be needed to further confirm and define these results.

Transforming growth factor-β, a known inhibitor of the Rb gene, is elevated in high-grade osteosarcoma compared with low-grade osteosarcoma.[3,14] It may contribute to the aggressive behaviour of these tumours.

Many studies have shown a correlation between the faster rate of bone growth in puberty and occurrence of osteosarcoma.[13] Additional studies point out that young osteosarcoma patients are taller than the normal population of the same age group.[15,16] Preceding trauma has been proposed as a causative factor in some osteosarcoma cases; however, no evidence-based aetiological relationship with trauma has been established.[3,17]

Other predisposing factors include exposure to ionising radiation and a history of metabolic bone diseases (i.e. Paget's disease).[1,12] Radiation is the proven risk factor in osteosarcoma, but it is rare and occurs after a long period of time has passed.[18] Paget's disease is known to be associated with a higher incidence of adult osteosarcoma.[6]

Co-existence of osteosarcoma with a number of rare inherited syndromes – such as Bloom syndrome, Rothmund-Thomson syndrome

Table 1: Surgical Staging of Bone Sarcomas

Enneking Staging System				American Joint Committee on Cancer Staging System			
Stage	Grade	Site	Metastasis	Size and Site	Lymph Node	Metastasis	Grade
IA	Low	Intracompartmental	No	≤8 cm	No	No	Low
IB	Low	Extracompartmental	No	>8 cm	No	No	Low
IIA	High	Intracompartmental	No	≤8 cm	No	No	High
IIB	High	Extracompartmental	No	>8 cm	No	No	High
III	Any	Any	Regional or distant	Skip	No	No	Any
IVA	-	-	-	Any	No	Lung	Any
IVB	-	-	-	Any	Yes	Any	Any
IVB	-	-	-	Any	Any	Other	Any

Sources: Enneking et al., Clin Orthop Relat Res*, 1980:106–20.*[11]

and Li-Fraumeni syndrome – suggests that gene mutations may play a role in its pathogenesis.[3,6,19,20]

Characteristics

Despite success with a combination of surgery and chemotherapy, local recurrence and metastases (usually at the lungs) develop in approximately 30–40% of all patients and appear to be the major cause of death.[3,21,22] Bone metastases usually only become established after pulmonary metastases have occurred. Following metasteses in the lungs, bone appears to be the second major site of metastases.[17] Other metastatic sites at diagnosis are very uncommon.[17] Tumour nodules growing outside the reactive zone, but within the same bone or across the neighbouring joint, are termed 'skip lesions'.[23]

Diagnosis

Symptoms and Signs

Pain and swelling are the major symptoms of osteosarcoma.[6] Pain usually arises after exercise or a trauma and progresses over time. Swelling appears later, with a hard painful mass in the affected region. Osteosarcoma is also rarely associated with anorexia, weight loss, fever and fatigue.[3,24]

Laboratories

Laboratory findings may show an increase in alkaline phosphatase activity and in 30% of cases an increase in lactic dehydrogenase level in the serum. Mild anaemia may also be present at diagnosis. Furthermore, the erythrocyte sedimentation rate is often high and increases with relapse.[25] In the absence of metastases, abnormal alkaline phosphatase values are correlated with tumour volume and prognosis.[3,26–28]

Imaging

Imaging techniques play an important role in osteosarcoma diagnosis and treatment.[29] Direct communication between the radiologist and orthopaedic surgeon would therefore be helpful in determining the diagnosis. Once osteosarcoma is suspected, the patient should undergo an advanced imaging procedure for confirmation. Advanced imaging is performed to more precisely define the extent of the primary tumour and assess its location relative to the adjacent bones, muscles, joints, blood vessels and nerves.[29]

Visualisation of the tumour on plain X-rays can help in diagnosis. Typically, patients with extremity osteosarcoma have poorly defined lytic and sclerotic lesions involving the metaphyseal part of the bone. Cortical destruction with lifting of the periosteum – 'Codman's triangle' – is often present. Some cases display the 'sunburst' pattern, with suggestion of bone spicule formation within the tumour.[1] Computed tomography (CT) and magnetic resonance imaging (MRI) have been used to investigate the extension of tumours and the involvement of surrounding structures, such as vessels, nerves and soft tissues.[3,30]

CT of the lung is part of the basic staging of osteosarcoma. High-resolution CT scans and spiral technique can be performed with 5mm or less collimation and is preferably imaged during a single breath-hold.[29,31] In children with osteosarcoma, it is recommended that single photon emission CT be performed in conjunction with planar whole-body scintigraphy to characterise uptake at the primary tumour site.[32] It is also recommended in suspected lung metastases.[29]

MRI has the ability to predict tumour necrosis before surgical resection and remains an exciting prospect for osteosarcoma.[32] The MRI should include long-axis imaging with T1 and/or short tau inversion recovery (STIR) sequences performed through the entire bone involved to assess the extent of the tumour and the presence of 'skip' metastases. Axial imaging with fast-spin echo T2 weighted sequences with fat saturation or STIR sequences through the tumour are best to determine the relationship of the tumour to the adjacent soft tissues and vascular structures. Gradient echo sequences can be added to confirm flow within the blood vessels. Post-gadolinium T1 weighted fat-saturated sequences are helpful in determining areas of tumour necrosis. Relatively small field of view imaging in two planes is recommended to assess joint involvement.[29,32,33] An isotope scan with technetium or thallium can show the intense hotspot of the tumour and any skip or distant bone metastases.[3,23,30] Positron emission tomography (PET) scans are not routinely performed in osteosarcoma patients yet. Despite this, there are limited studies that show PET scans may be useful in predicting response to chemotherapy and in differentiating post-operative changes from recurrent tumour.[1,34,35]

Biopsy

Biopsy is a main diagnostic method for osteosarcoma. The biopsy should be carefully planned, with a multidisciplinary approach involving musculoskeletal radiologist, pathologist and orthopaedic or surgical oncologist. This will be essential to ensure the feasibility of the procedure, the adequacy of the specimen and above all to maintain the viability of definitive surgery with a possibility of limb salvage.[36] Incorrectly performed biopsies are a cause of misdiagnosis, amputation and local tumour recurrence.[3,23] They may also have a negative effect on survival.[3,23]

Table 2: Chemotherapeutic Agents Commonly Used in Osteosarcoma

Agent	Mechanism of Action
Doxorubicin	Intercalates at point of local uncoiling of the DNA double helix and inhibits the synthesis of DNA and RNA
Cisplatin	Binds directly to tumour DNA and inhibits the synthesis of DNA through the formation of DNA cross-links
Ifosfamide	Causes cross-linking of DNA strands, which inhibits the synthesis of DNA and protein
Methotrexate	Is a folate antimetabolite and inhibits the synthesis of purine and thymidylic acid by binding to dihydrofolate reductase

Source: Wittig JC et al., Am Fam Physician*, 2002;65:1123–32.*[23]

Histology

Histologically, osteosarcoma is characterised by a proliferation of malignant spindle cells. Osteosarcoma produces osteoid substance. It is the bone matrix that distinguishes an osteosarcoma from other tumours having similar stromal structures. Several histological subtypes of osteosarcoma exist. These include osteoblastic (the most common), chondroblastic, fibroblastic, telangiectatic, small cell, parosteal, periosteal, high-grade surface and secondary osteosarcoma.[37]

Differential Diagnosis

Osteosarcoma should be differentiated from malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, lymphoma, aneurismal bone cyst, giant cell tumour and fibrous dysplasia.

Treatment

Multimodality therapy has become the standard approach to the treatment of patients with osteosarcoma. Patients with suspected or confirmed osteosarcoma should be evaluated and treated at a tertiary cancer centre within a multidisciplinary sarcoma programme. This programme should include paediatric, medical, radiation, orthopaedic and surgical oncologists, musculoskeletal pathologists and radiologists. Successful treatment involves proper diagnosis, neoadjuvant and adjuvant multiagent chemotherapy, and aggressive surgery. There should be an emphasis in the direction of limb-sparing procedures and even newer therapies aimed at the tumour microenvironment.[36,38,39]

Chemotherapy

Advances in chemotherapy over the past 30 years have improved limb salvage and led to higher survival rates.[23] Chemotherapy has also been shown to reduce the number of pulmonary metastases or to delay their appearance, which facilitates surgical removal. The drugs used are: cyclophosphamide, vincristine, melphalan, adriamycin (doxorubicin), methotrexate, cisplatin, decarbazine, bleomycin, dactinomycin, actinomycin and leucovorin rescue.[3] The most commonly used chemotherapy is a combination of three of the following: methotrexate, cisplatin, doxorubicin and ifosfamide (see *Table 2*).

Non-metastatic osteosarcoma treatment regimens include pre-operative and post-operative chemotherapy. Pre-operative chemotherapy induces necrosis in the primary tumour, which facilitates surgical resection and provides early treatment of micrometastatic diseases.[23] Patients with good histological response to pre-operative chemotherapy (>90% tumour necrosis) at the time of surgical resection show optimum survival.[40,41] The degree of tumour necrosis when used as a marker of chemosensitivity has proven an important factor predictive of survival. Response to chemotherapy is also predictive of the need for further resections.[3,9] Regimens using the classical three-drug combination have achieved a five-year event-free survival of >70% in most reports.[38]

In the US and Europe, the most widely used chemotherapy combination is cisplatin, doxorubicin and high-dose methotrexate in the pre-operative 'induction' setting.[1,42]

Another study, which involved the addition of muramyl tripeptide ethanolamine (MTP-PE) to the three-drug regimen, showed that MTP-PE significantly improves overall survival, but routine use of this agent has not been adopted.[43]

Recent therapeutic advances have focused on circumventing chemotherapy resistance mechanisms, incorporation of non-classic agents into upfront therapy, targeting of the tumour microenvironment and investigating the role of novel delivery mechanisms.[1,44]

Patients with unresectable disease may gain some survival benefit with additional chemotherapy. The Memorial Sloan-Kettering Cancer Center reported that patients who had unresectable disease treated with chemotherapy alone had an average survival of almost 15 months after first relapse.[1,45] The use of chemotherapy after relapse is still under discussion and contradictory results have been reported.[46] At present, no evidence on the best second-line chemotherapy treatment for relapse patients is available.

The variety of relapse patterns makes it almost impossible to perform a randomised study to investigate the role of chemotherapy after relapse. At the Rizzoli Institute, the first treatment choice for patients with osteosarcoma relapse diseases is surgery (43%). Other choices include surgery combined with chemotherapy (42%), chemotherapy alone (14%) and no specific treatment at all (0.2%). The five-year disease-free survival rates according to type of treatment were 22.4% for patients receiving surgery and 17.8% for those treated with surgery and chemotherapy.[3,40]

Surgery

Advances in chemotherapy, imaging, surgical technique and biomaterial engineering have led to a new era of surgical management for osteosarcoma.[36,47] While historically most patients were treated with amputation, advances over the past three decades have made limb-salvage surgery a viable option, with similar survival outcomes when properly performed.[1,48] Surgical treatment of extremity osteosarcoma should be, by definition, a wide excision, followed by bone and soft-tissue reconstruction.[49] Reconstructive options include limb-salvage surgery with autogenous bone grafts (vascularised or devascularised), structural bone grafts (osteoarticular and intercalary), distraction osteogenesis and metallic endoprosthetics.[36]

Since osteosarcoma most commonly affects the metaphyseal region, surgical resection is often intra-articular or through the joint. For intra-articular resections, reconstruction may be achieved with osteoarticular allograft obtained from a bone bank and size-matched to the patient's anatomy.

Endoprosthetic joint replacements are metal implants, which historically were custom-built for individual patients. Currently,

endoprosthetic systems are modular and allow for customisation to be achieved in the operating room by mixing and combining standard components. These systems offer immediate joint stability and early weight-bearing. For children, expandable prostheses have recently become available, allowing limited compensatory growth.[1,50,51]

Allograft-prosthetic composites combine the two previously described procedures by fixing a prosthesis within the allograft bone and attaching the composite to the host bone. This combination takes advantage of the allograft's biological ingrowth potential and its bone restoration, while benefiting from joint replacement stability, modularity, reliability and longevity.[52]

The Van Ness rotationplasty is a compromise between an amputation and a limb-salvage procedure, sometimes employed for large tumours of the distal femur. It serves to functionally convert an above-knee amputation into a below-knee amputation. It has been characterised as an intercalary amputation with salvaging of the nerves and vessels.[53,54]

Another technique that has become popular is the intercalar bone transport method described by Ilizarov. It is preferred because it is a biological technique, has relatively few complications compared to other alternatives, has the ability to adapt to defects of any diameter and length and allows early weight-bearing and motion of the extremity.[28,55,56]

Surgery for metastatic disease also plays an important role in treating osteosarcoma. Some patients have a primary tumour along with limited pulmonary involvement at diagnosis. In other patients, metastases can occur (mostly in the lungs and bones) during relapse. In these cases, in addition to management of the primary tumour, surgical resection of pulmonary nodules or metastatic bones appears to significantly increase the survival of patients or results in a prolonged disease-free interval.[3]

Radiation Therapy

Osteosarcoma is a relatively radio-resistant malignancy. For this reason, chemotherapy and surgery have been the primary treatment options. Radiation therapy in the primary local control setting should be considered on a case-by-case basis for patients with unresectable tumours and where margins of resection are positive.[36,57]

Prognosis

Currently, few prognostic factors exist for osteosarcoma. While survival for patients without metastatic disease is approximately 70%, with metastatic disease at diagnosis it is >20%. Similarly, patients with recurrent or progressive disease have a long-term survival of <20%.[38,58] Other prognostic factors include tumour grade, patient age, tumour size, site of primary disease and serum markers, such as lactate dehydrogenase and alkaline phosphatase.[1,49,59,60]

Generally, a relapse following the use of modern treatment approaches including chemotherapy and surgery leads to a significantly lower probability of survival. Pulmonary metastases that are found at initial diagnosis are associated with a poor outcome. Poor prognosis was also found in patients with:[3,9,24,42]

- a large number of metastases;
- bilateral disease;
- a short time interval between local treatment and the development of metastatic disease; and
- a poor response to pre-operative chemotherapy.

Table 3: Emerging Agents of Interest in Osteosarcoma

Agent	Mechanism of Agent Action	Stage in Study
Deforolimus (AP-23573)/temsirolimus (CCI-779)/rapamycin	mTOR inhibition	Phase III trials
Pamidronic acid/ zoledronic acid	Bisphosphonate	Phase II trial
Cediranib (AZD-2171)	Tyrosine kinase inhibitor of VEGF-receptor pathway	Pre-clinical trial
Muramyl tripeptide phosphatidylethano-lamine	Stimulates monocytes and macrophages	Phase III trial
2 ME	Inhibition of angiogenesis, induction of apoptosis	Phase II trial
Octreotide	IGF-1 reseptor inhibition	Phase I and II trial
IFN	Antiangiogenic activity antiviral activity	EURAMOS
IL	Inductor for differentiation and proliferation of natural killer cells	Pre-clinical trial
Angiostatin, avastin, and endostatin	Inhibit the VEGF signaling pathway	Phase I clinical trial

2 ME = 2-methoxyestradiol; EURAMOS = European American Oncology Study Group; IFN = interferon; IGF-1 = insulin-like growth factor-1; IL = interleukin; mTOR = mammalian target of rapamycin; VEGF = vascular endothelial growth factor.

Research and Emerging Treatments

Future treatment of osteosarcoma may involve the application of agents that are currently being investigated in pre-clinical models for other cancers (see *Table 3*). Over the past few decades, new chemotherapeutic agents have been added to the family of anticancer drugs. For example, combination therapy with gemcitabine and docetaxel in refractory bone sarcomas was well tolerated and demonstrated antitumour activity.[36,61] Trimetrexate and other novel antifolates have been shown to have activity in a wide range of solid tumours *in vitro*. Desite this, however, myelosuppression (grades 3 and 4) was observed in most cases[1] and few new agents have shown activity or clinical benefit in osteosarcoma.

In vitro studies have demonstrated the inhibitory activity of new nitrogen-containing bisphosphonates, such as minodronate, incadronate, risedronate and zoledronic acid on human osteosarcoma cell growth.[3,62–64] The antitumour activities of risedronate in combination with carboplatin, doxorubicin, vincristine or etoposide were synergistically augmented on several osteosarcoma cell lines.[63]

Tumour suppressor pathways governed by p53 and Rb genes have been implicated in the pathogenesis of osteosarcoma.[1,65,66] Interleukins, a group of cytokine signalling molecules, have been studied as immunotherapy for osteosarcoma. Interleukin-2 is able to facilitate the production of immunoglobulin made by B cells and induce the differentiation and proliferation of natural killer (NK) cells. In a treatment programme including pre- and post-operative

interleukin-2 and chemotherapy for childhood osteosarcoma, NK cell counts and activity significantly correlated with clinical outcome.[3,67,68]

The function of type-I IFN in counteracting osteosarcoma growth has been investigated by several groups.[69–71] To achieve success, the IFN had to be given early during tumour development. This finding and other clinical results indicate that IFNs may exert their effects through anti-angiogenic effects.[70] The effect of IFN is currently being investigated in the European American Oncology Study Group 1 (EURAMOS-1) trials.

An alternative approach has been to use MTP-PE, which is derived from bacille Calmette-Guérin and is a potent macrophage activator. Recently, the addition of liposomal MTP-PE in combination with adjuvant chemotherapy resulted in a statistically significant increase in overall survival (78%) compared with standard combination chemotherapy (70%). Other immune strategies have focused on generating T-cell responses by vaccination with the anti-idiotypic antibody mimicking CD55, a complement regulatory protein expressed by many solid tumours including osteosarcoma. The use of dendritic cell vaccines to enhance cytotoxic T-cell activation is also being evaluated in xenograft models.[36,43,72–74]

An ongoing clinical trial, the EURAMOS-1, is investigating the addition of ifosfamide and etoposide to post-operative chemotherapy. The aim is to see whether adding these drugs to cisplatin, doxorubicin and methotrexate improves the event-free survival and overall survival for patients with resectable osteosarcoma and a poor histological response to 10 weeks of pre-operative chemotherapy. This study is also investigating the addition of interferon-alpha2b (IFN-α2b), as maintenance therapy after post-operative chemotherapy with triple-drug therapy. It aims to determine whether maintenance therapy improves the event-free survival and overall survival for patients with resectable osteosarcoma and a good histological response to 10 weeks of pre-operative chemotherapy.[1] Another promising approach is the use of mTOR inhibitors in osteosarcoma. A pre-clinical trial study showed that mTOR inhibitors had activity against sarcoma cell lines, including osteosarcoma.[3,46]

Inhibitors of receptor tyrosine kinases including insulin-like growth factor-1 (IGF-1) have been found to be mitogenic in *in vitro* models of osteosarcoma. Several new IGF-1 receptor antagonists are being tested in phase I and II studies for sarcomas.[7]

Vascular endothelial growth factor (VEGF) has been recognised as a potential target of therapy in paediatric osteosarcoma as studies have shown that VEGF expression correlates with metastasis and poor outcome. Cediranib (AZD-2171) is a specific VEGF-receptor inhibitor that has demonstrated growth inhibition in osteosarcoma models.[1] There are many studies utilising anti-angiogenic agents, but the prognostic significance of VEGF in osteosarcoma remains controversial.[7,75–77]

2-methoxyestradiol (2-ME) is a new agent. It is a physiological inhibitor of tumour cell proliferation and acts as a potential therapeutic agent in several types of tumour, including bone tumours.[78–83] 2-ME is highly cytotoxic to osteosarcoma cells, but is harmless to normal bone cells.[79] In addition, it has been shown that 2-ME is well-tolerated in animals. The antitumour mechanism of 2-ME has been studied by several groups. It includes inhibition of angiogenesis, inhibition of the cell cycle, disturbances in mitosis and induction of apoptosis.[78–83] Other agents that have been implicated in the antitumour effects of osteosarcoma include:[3,9,84–87]

- ezrin (also known as cytovillin or villin-2);
- chemokine receptor 4;
- P-glycoprotein;
- human epidermal growth factor receptor-2;
- parathyroid hormone-related protein;
- C-Jun;
- pemetrexed;
- granulocyte-macrophage colony-stimulating factor;
- trastuzumab;
- high-dose methotrexate with leucovorin rescue with or without glucarpidase; and
- dasatinib.

Conclusion

The prognosis of localised osteosarcoma has improved dramatically over the past three decades with multimodal treatment using aggressive surgery and combination chemotherapy. Despite these advances for localised disease and with the development of newer chemotherapeutic agents, the prognosis for metastatic, refractory and recurrent osteosarcoma is still dismal.

Multidisciplinary management within a comprehensive cancer centre is extremely important to the diagnosis, medical, surgical and overall care of patients with osteosarcoma. Continued emphasis should be placed on pre-clinical basic science and translational research aimed at furthering the understanding of osteosarcoma. Such work should have the ultimate goal of providing patients with new, molecularly targeted therapies. ■

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