Cancer Metastasis Rev (2009) 28:247–263
DOI 10.1007/s10555-009-9186-7

NON-THEMATIC REVIEW

Osteosarcoma treatment: state of the art

Hang T. Ta · Crispin R. Dass · Peter F. M. Choong · Dave E. Dunstan

Published online: 12 February 2009

Abstract Osteosarcoma (OS) is a class of cancer originating from bone, mainly afflicting children or young adults. It is the second highest cause of cancer-related death in these age groups, mainly due to development of often fatal metastasis, usually in the lungs. Survival for these patients is poor despite the aggressive use of surgery, chemotherapy, and/or radiotherapy. Thus, new effective drugs and other forms of therapy are needed. This article reviews the biology and the state of the art management of OS. New experimental drugs and potential therapies targeting molecular pathways of OS are also discussed.

Keywords Osteosarcoma · Tumor · Cancer · Bone · Chemotherapy · Radiotherapy

1 Introduction

Tumors of bone are among the most uncommon of all types of neoplasms [1]. Approximately 1,500 new sarcomas of bone are recorded in the USA per year while 93,000 new cases of lung carcinoma and 88,000 new cases of breast carcinoma are diagnosed (data reported in 1996). Therefore, on a global scale, bone tumors are relatively unimportant. However, many of bone tumors affect young children and are associated with radical surgery and expensive and painful chemotherapy. Among various types of bone tumors, osteosarcoma (OS) is the most common primary solid tumor of bone in childhood and adolescence [1]. OS is a malignant bone tumor characterized by spindle cells producing osteoid [2]. This article will provide a comprehensive review from the biology of OS and current treatments to new potential therapies for this disease.

H. T. Ta · D. E. Dunstan
Department of Chemical and Biomolecular Engineering,
University of Melbourne,
Melbourne, VIC 3010, Australia

C. R. Dass (✉) · P. F. M. Choong
Departments of Orthopaedics and Surgery,
St Vincent's Hospital Melbourne,
Melbourne, VIC 3065, Australia
e-mail: Crispin.DASS@svhm.org.au

P. F. M. Choong
Sarcoma Service, Peter MacCallum Cancer Centre,
Melbourne, VIC 3002, Australia

2 Demographics

OS is the most common primary solid tumor of bone, comprising about 20% of primary bone sarcomas. Classification of tumors found in bone is summarized in Table 1 based on Mayo Clinic patients (USA) until the end of 1993 [1]. The classification is based on the cytologic features or the recognizable products of the proliferating cells. In most instances, the tumors are considered to arise from the type of tissue they produce, but this assumption cannot be proven using current methods.

According to Table 1, osteogenic tumors are the third prevalent tumors of bone. Of the 2,136 osteogenic tumors, 1,649 were OSs which appeared as the second common type of malignant bone tumors and as the most common type of malignant solid bone tumors.

A comparison in the occurrence of OS in different states of Australia and in overseas countries [3] is expressed as comparative rates in Table 2.

OS is the most common primary bone tumor in childhood and adolescence [1, 4, 5]. Extremely uncommon before 5 years of age and infrequent up to the age of

Table 1 Classification of tumors of bone in Mayo Clinic patients until the end of 1993 [1]

Histologic type	Total cases		Benign		Malignant	
	No.	Percentage (%)	Tumor	No.	Tumor	No.
Hematopoietic	4,443	40.07			Myeloma	3,749
					Lymphoma	694
Chondrogenic	2,420	21.83	Osteochondroma	872	Chondrosarcoma	775
			Chondroma	355	Secondary chondrosarcoma	120
			Chondroblastoma	119	Dedifferentiated chondrosarcoma	120
			Chondromyxoid fibroma	45	Mesenchymal chondrosarcoma	34
Osteogenic	2,136	19.27	Osteoid osteoma	331	**Osteosarcoma**	**1,694**
			Osteoblastoma	87	Parosteal osteosarcoma	69
Unknown	1,149	10.36	Giant cell tumor	568	Ewing's sarcoma	512
					Malignancy in giant cell tumor	35
					Adamantinoma	34
Histiocytic	92	0.83	Fibrous histiocytoma	9	Malignant fibrous histiocytoma	83
Fibrogenic	268	2.42	Hamartoma	1	Desmoplastic fibroma	12
					Fibrosarcoma	
Notochordal	356	3.21			Chordoma	356
Vascular	201	1.81	Hemangioma	108	Hemangioendothelioma	80
					Hemangiopericytoma	13
Lipogenic	8	0.07	Lipoma	7	Liposarcoma	1
Neurogenic	14	0.13	Neurilemoma	14		
Total	11,087	100.00		2,496		8,591

Table 2 Incidence of osteosarcoma in Australia and overseas countries [3]

Locality	Rate[a]
Melbourne	2.5
Rural and Regional Victoria	3.7
Victoria	2.9
Queensland	4.5
New South Wales	2.9
Australia	2.2
International	
Canada	2.9
USA	3.3
Japan	2.3
Hong Kong	2.6
Denmark	1.8
Finland	3.2
France	2.7
Italy	3.9
Netherlands	2.9
Sweden	2.7
Switzerland	2.5
UK England & Wales	2.6
UK Scotland	1.9

[a] The number of cases per year per million children under age of 15

10 years, the extraordinary peak in incidence occurring in the early teenage years is quickly followed by a rapid decline [6]. According to data from the Mayo Clinic [1], of the 1,649 OSs, 77 cases occurred in children up to 10 years old, 758 cases occurred in teenagers and adolescent from 10 to 20 years old, and 283 cases were young adults from 20 to 30 years of age. In older adults, the OS cases decreased with increasing ages. The peak incidence is in the second decade of life. The median peak age is 16 years. OS is also reported as the sixth most common type of cancer in children and young adults [4, 5]. It also represents the second highest cause of cancer-related death in this age group [7]. In America, approximate 400 new OS cases in children younger than 20 years are reported per year [8]. In Australia, an average of nine to ten cases of OS is diagnosed each year in children under the age of 15 [3]. In Victoria, an average of two to three cases is diagnosed each year. Generally, the pattern of prognosis in different age groups has a tendency towards unfavorable outcomes in patients both younger and older than the adolescent [9]. OS affects males more than females with the ratio of 1.6:1. Females have a peak incidence a little earlier than males due to the earlier onset of their growth spurt [10, 11]. At all ages above 10 years, the tumor is more common in males than females. Before age 10, girls are likely as boys to develop OS [12].

3 Etiology

The causes of OS are not completely known. Many studies have demonstrated a correlation between the faster growing bone rate in puberty and the occurrence of OS [13–15]. In fact, the peak of incidence of OS is during puberty when the growth spurt is highest [16]. Another evidence supporting this relationship is the early peak age in girls as compared with boys, corresponding to the earlier age of their growth spurt [8]. Two recent studies showed that young OS patients were taller than the normal population of the same age group [15, 17].

Exposure to radiation is the only proven exogenous risk factor but with a long interval—10–20 years [18]. Thus, radiation-induced OS is typical of adult age and is rare. Radiation is implicated in approximately 2% of OSs [19]. Paget's disease is known to be associated with a higher incidence of OS [1]. It is also suggested that metallic ions may predispose a person to develop OS [1]. Preceding trauma in the involved bone was found in some OS cases; however, no etiologic relationship with trauma has been found [4].

Numerous recent studies described cytogenetic abnormalities which are both numerical and structural in OS [8]. Common numerical abnormalities include: gain of chromosome 1, losses of chromosomes 9, 10, 13, and/or 17, and partial or complete losses of the long arm chromosome 6. Frequent structural abnormalities include rearrangements of chromosomes 11, 19, and 20. Gene mutation in a number of rare inherited syndromes such as Bloom syndrome, Rothmund–Thomson syndrome, and Li–Fraumeni syndrome were reported relating to some cases of OS as well [1, 20, 21].

In 1966, Finkel, Biskis, and Jinkins (FBJ) suggested that virus could induce bone sarcomas in animals [22]. They found that a virus named FBJ could be a potent inducer of OS in mice. The oncogene in FBJ is related to a naturally occurring proto-oncogene called c-Fos [23], which has been found to be associated with a poor response to chemotherapy in patients with OS [24]. In 1998, Mendoza et al. reported the integration of simian virus 40 (SV40), an accidental contaminant of poliovirus vaccines used widely between 1955 and 1962, in human OS DNA [25]. However, long-term follow-up studies have not revealed recipients of SV40-contaminated poliovirus vaccines to be at an increased risk of cancer [26]. In 1996, a study showed that 11 of 18 OS samples had evidence of incorporated SV40 DNA, and in 1997, another study showed that 50% of OS samples had incorporated SV40 DNA [8]. However, there is no convincing data that viruses are a major etiologic factor in OS.

The p53 and Rb tumor suppressor pathways are proven to be involved in the pathogenesis of OS [8]. It was reported that most tumor samples have some type of combined inactivation of the Rb and p53 tumor suppression pathways. A recent study showed that 22% of OS samples showed p53 mutations. Patients with retinoblastoma in which germline mutations of the Rb gene are common have increased incidence of OS as well. TGF-β is a growth factor found in high levels in high-grade OS than in low-grade lesions and is a known inhibitor of the Rb gene product [27], perhaps contributing to the aggressive behavior of these tumors as well.

4 Diagnosis and destructive process

Pain and swelling are the cardinal symptoms of OS [1]. Pain usually arises after strenuous exercise or a trauma and usually appearing 2–4 months before diagnosis [28] and progressing over time. Swelling appears later with a hard painful mass in the affected region. Pathologic fracture can occur but is distinctly uncommon in physical examination. OS is rarely associated with anorexia, weight loss, fever, and fatigue [29]. Typically, OS starts intramedullary and grows toward the cortex. The destructive process may be limited to the medulla, but it usually involves the cortex as well, and the cortex is nearly always perforated by the growing tumor. An OS grows in a radial manner, forming a ball-like mass [30]. When it penetrates the bony cortex, it compresses the surrounding muscles into a pseudocapsular layer termed "reactive zone."

When the OS produces calcifying and ossifying osteoid substance, radiological examination shows various degrees of density within the affected area of bone [1]. These densities often extend into the contiguous soft tissues. The soft tissue extension may show cloudlike (shadow) radiodensities and/or stripes of increased density perpendicular to the cortex (Fig. 1). When the OS has breached the cortex, the extra-osseous mass may completely encircle the bone. Radiological examination can determine the degree of pathological fracture which contributes to prognosis of the disease. In a retrospective study of two groups of approximately 50 patients, one group with pathological fracture and the other without, the fracture group had a 55% 5-year survival compared with 77% in those without [9].

Some tumors spread in the marrow cavity for surprisingly great distance, but most tumors do not spread in the marrow beyond their gross extraosseous limits [1]. Skip areas of medullary involvement are extremely rare. Once the tumor has destroyed the cortex and formed soft-tissue masses, pulmonary metastasis develop eventually.

The laboratory findings may show an increase in alkaline phosphatase (AP) and in 30% of cases an increase in lactic dehydrogenase in the serum [31]. Mild anemia may also be present at diagnosis. Furthermore, the erythrocyte sedimentation rate is often high and increases

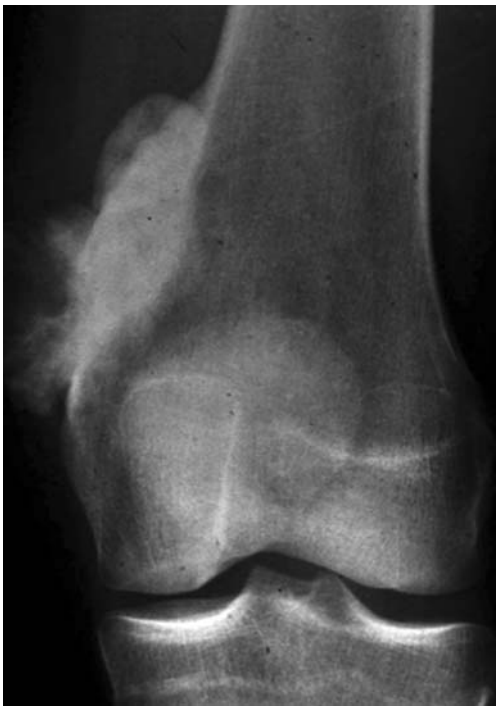

Fig. 1 X-ray of OS lesion. Lesions can be poorly marginated, appear radiolucent, radiodense, or mixed lucent and dense (as depicted here), depending on the degree of osteoid mineralization

in the presence of relapse [32]. In the absence of metastases, abnormal AP values are correlated with tumor volume and prognosis [33]. Poor prognosis is associated with high AP values [1]. A normal pretreatment serum AP level resulted in a significantly higher 5-year disease free survival (67%) than in patients with higher levels (54%) [9]. Patients with normal serum AP also had a significantly longer time to recurrence (25 versus 18 months) [9]. Sedimentation rate, C-reactive protein, and lactate dehydrogenase (LDH) levels may also be elevated [2]. LDH, when elevated, confers a worse prognosis, presumably by indicating a more biologically aggressive tumor [34].

An isotope scan with technetium [35] or thallium [30] can show the intense hotspot of the tumor and any skip or distant bone metastases. Computed tomography (CT) and magnetic resonance imaging of bone lesions have been used to investigate the extension of tumors and the involvement of surrounding structures such as vessels, nerves, and soft tissues [35]. CT of the lung is part of the basal staging. Bone scans (nuclear scintigraphy) and FDG-PET are useful adjuncts but are more pertinent to staging than for evaluation of the primary lesion [2]. The most valuable use of bone scan is the detection of metastatic deposits within skeleton.

Biopsy is a key diagnostic method for an OS and can be carefully planned according to the site and definitive surgery [36]. Improperly performed biopsies are a frequent cause of misdiagnosis, amputation, and local recurrence, and they may have a negative effect on survival [30]. The biopsy can be an incisional (open) or needle (closed) biopsy. OS is histologically characterized by the production of "tumor osteoid" or immature bone directly from a malignant spindle cell stroma [4]. Currently, WHO recognizes three major histologic subtypes of OS: osteoblastic, fibroblastic, and chondroblastic, reflecting the predominant type of matrix (osteoid, fibrous, or chondroid matrix) [4]. It was found that patients with the osteoblastic and chondroblastic histologic patterns tend to have a worse prognosis [1].

5 Tumor site

OS can occur in various types of bone but tends to form in areas of rapid bone growth or turnover, such as in the long bones of a growing adolescent [29]. It frequently localizes in the distal femur and proximal tibia region with 670 and 303 cases, respectively, out of 1,649 total cases at Mayo Clinic until the end of 1993. These sites contain large growth plates with high proliferative activity and turnover of bone [9]. The next common site is the proximal humerus with 155 cases in the same period. OS rarely affected bones of the hands and wrists (only four of the 1,649 OSs). It occurs primarily in the metaphysic or metadiaphysis of long bones but tends to invade the epiphysis even in the presence of a growth plate [37]. In the group of axial location of OS, pelvic OSs account for approximately 7–9% of all OSs [38], and spine OSs occur at 0.85–3% [39]. OS occasionally arises in soft tissue, thyroid gland, heart, kidney, uterus, or lung [40].

OS in the proximal tibia is associated with a 5-year survival rate of 77.5%, which is slightly better than the distal femur at 66% [9]. The 5-year survival of OS in the pelvis ranges from 27% to 47%. OS in the spine has been linked with a very poor prognostic outlook with median survival times of only 10–23 months. The highest OS survival rates have been identified in OS of forearm and hand. High-grade tumors of the distal upper limb had a remarkable 81.3–86.5% 5-year survival rate [9].

6 Tumor size

One of the key measures of prognosis in OS is tumor size. In previous studies, tumor size can be determined based on absolute tumor length, relative tumor length, and less frequently, absolute tumor plane [41].

Absolute tumor volume (ATV), calculated with a specific ellipsoid formula based on absolute length, depth, and width, is the most recent method to evaluate tumor size [9]. Tumors with ATV of less than 150 cm^3 exhibits a 92%

5-year metastasis-free survival compared with 58% in tumors with an ATV greater than 150 cm^3.

7 Tumor stage

Staging is performed based on the aggressive grade and the extensive and the spread levels of OS [2]. According to the Musculoskeletal Tumor Society Staging System [42] and Enneking System [30], tumor stages have been classified based on tumor grade (I, low grade; II, high grade), tumor extension (A, intraosseous involvement; B, extraoesseous extension) and the presence of distant metastases (III) [42]. The staging system is reported in Table 3. Most conventional OSs present as stage IIB tumors which is non-metastatic tumor with an associated soft tissue mass [30].

Stage I-A exhibits nearly 100% 5-year survival rate [9]. This stage is much less common than the aggressive types. Stage II-B presents worse prognosis with a 5-year survival rate of around 40–47%. Stage III holds the 5-year survival close to 0%, but this rate has changed significantly in the last few decades due to a combination of chemotherapy, helical CT for diagnosis of pulmonary metastases and improved surgical techniques. If only pulmonary metastases are found at diagnosis, the current 5-year survival of this stage may be as high as 68% [9].

The American Joint Committee on Cancer Staging System is similar to the Musculoskeletal Tumor Society Staging System, but it classifies stage III as any tumor with skip metastases [43]. In addition, stages I and II are subdivided into A and B categories depending on tumor size being greater or less than 8 cm in any dimension, rather than intra- or extra-compartmental. Moreover, it has the extra stage IV which is divided into IV-A, describing pulmonary metastases, and IV-B, describing other metastases [43].

8 Metastases and local recurrence

OS usually metastasizes to the lungs [4] or other bones [1]. Metastasis to other bones may occur early and widespread, suggesting a multifocal origin of the sarcoma, or it may be delayed and localized, suggesting that a new tumor has developed. At diagnosis, classic OS is localized in one bone site in 80% of cases and presents with metastases in about 20% of patients [44]. The lung is the most common metastatic site, followed by bone. The bone metastases usually establish only after pulmonary metastases have occurred. Tumor nodules growing outside the reactive zone but within the same bone or across the neighboring joint are term “skip lesions” [30]. Skip metastases and regional lymph node metastases are rare with less than 10% each [4]. Distant bone metastases represent the latest stage of disease and are rarely associated with the poorest prognosis [30]. Other metastatic sites at diagnosis are very uncommon [4].

Table 3 Surgical staging of bone sarcomas [42]

Stage	Grade	Site	Metastasis
IA	Low	Intracompartmental	No
IB	Low	Extracompartmental	No
IIA	High	Intracompartmental	No
IIB	High	Extracompartmental	No
III	Any	Any	Regional or distant

In the report of Bacci et al. regarding the 27-year experience with 1,148 patients at Rizzoli Institute, Italy, 0.4% of patients who relapsed had local recurrence, 12% had local recurrence plus metastases, and 88% had metastases only [45]. The rate of local recurrence was 2.8% for patients treated with amputation; 6.2% for patients treated with limb salvage; and 5.3% for patients treated with rotationplasty. However, these differences were not statistically significant. Yet, the rate significantly depended on the surgical margins (inadequate vs. adequate—24% vs. 3.6%). In patients treated with neoadjuvant chemotherapy, the rate dramatically depended on tumor necrosis response to preoperative treatment (good vs. poor—8.4% vs. 3.9%). The first site of metastases was the lung with 89% of cases. Eight percent of patients had metastases in other bones and only 2% had metastases in other sites. The average time to relapse was 21.3 months (ranged from 2 to 204 months) and was significantly longer for patients with normal serum AP values (18 vs. 25 months); in patients treated with neoadjuvant chemotherapy than in those treated with adjuvant chemotherapy (24 vs. 16 months); and in good responders to preoperative treatment in comparison with poor responders (22 vs. 17 months) [45].

Eighty percent of patients died from metastatic diseases, most commonly in the lungs [46]. Pulmonary metastases which are found at initial diagnosis are generally thought to be associated with a poor outcome [9]. There was a survival advantage for patients with no more than three lung nodules and unilateral lung metastases. Patients with skip lesions carry a particularly bad prognosis even in the modern treatment era, with a reported survival average of 27.2 months from diagnosis, which is far worse than patients with only lung metastases [9].

It was found that all patients with local recurrence (LR) also developed lung metatasis at some stage in the course of disease [9]. The combination of LR and metastasis is worse than metastasis alone. There was 96.1% mortality in the LR group, compared with 72.1% in the group of only metastasis. Poor prognosis was found to correlate with LR occurring within the first year after resection.

9 Current treatments

The overall or the 5-year survival rate of patients with OS was 10–20% before the 1970s when treatment was mainly limb amputation [4, 47]. Over the past three decades, the development of surgical techniques and the application of radiotherapy and/or effective systemic chemotherapy has made limb salvage procedures a safe alternative to amputation and led to an increase in disease-free and overall survival rates [30, 48]. The survival rate was improved by postoperative radiotherapy in addition to surgery with long-term survival of approximately 50% [49]. The rate dramatically increased to approximately 60–70% once systemic multiagent chemotherapy followed by surgery has been introduced [50]. Chemotherapy drugs can be administered both before and after surgery. Nowadays, the standard treatment of patients with conventional OS consists of combination of chemotherapy and treatment. Radiotherapy can be also applied in the treatment program along with surgical resection. Despite all efforts in the field, no major changes in treatment and outcome have been achieved in the past few years.

9.1 Surgery

Before 1970, amputation was the sole treatment [46]. Currently, although chemotherapy is undoubtedly the method that is likely to cure the greatest proportion of patients, surgery remains an essential part of the management program of all patients with OS. In the absence of effective chemotherapy, surgery offers the only possible chance of cure. Even with effective chemotherapy, OS is rarely cured without surgical resection [51]. The aim of surgery is to completely resect the tumor to produce the minimum risk of local recurrence and the maximum chance of overall survival [12]. In addition, surgery is required to reconstruct the patient's limb after resection of the tumor, leading to a better quality of life for the patient.

9.1.1 Local disease

The entire tumor mass including the reactive zone must be resected to ensure removal of all gross tumor [30]. Thus, the surgical margin must be wide. Surgery for local disease can be carried out with an amputation or limb salvage depending on location and extent of disease and response of primary tumor to preoperative chemotherapy [8]. Amputation is the only safe way of surgery especially in patients with extensive soft tissue components [12]. However, because most tumors arise around the knee joint, amputation is usually high above the knee or sometimes a disarticulation of the hip. In these cases, limb salvage surgery (bone replacements) can be conducted instead but with increased risks of complications such as local recurrence. The number of patients likely to be at risk of LR depends on the margins of excision and the effectiveness of chemotherapy [12]. Currently, amputation has become a more infrequent choice of surgery due to improved adjuvant therapies, operative techniques, and diagnostic methods [9]. In limb salvage surgery, patients can retain the limb and should, thus, have improved function. The options available for limb salvage include resection of tumor without replacement, endoprosthetic replacement, rotationplasty, allografts, and autografts. Endoprosthetic replacement is the most common option. Another interesting and attractive option is to use the individual's own bone, removing it, sterilizing it, and reimplanting it [12]. Special considerations for the skeletally immature patients need to be taken into account in reconstruction of the defect [8]. In these cases, reconstruction must be dynamic in order to accommodate future growth.

9.1.2 Metastatic disease

Surgery for metastatic disease also plays an important role in treating OS. Some patients have a primary tumor along with the limited pulmonary involvement at diagnosis. In other patients, metastases can occur (mostly in lung and bones) in the relapse of initially localized OS. In these cases, in addition to aggressive multiagent chemotherapy and surgical management of the primary tumor, surgical resection of pulmonary nodules or metastatic bones appears to have significantly increased survival of patients or results in a prolonged disease-free interval.

9.2 Radiotherapy

9.2.1 Prebiopsy

Low-dose irradiation (approximately 10 Gy) can be administered before the initial biopsy in order to reduce the viability of the cancerous cells that can be disseminated into the bloodstream by the biopsy [47]. However, a previous study found no differences in survival rate between patients receiving radiotherapy prior to biopsy and historic controls, which discouraged additional investigation [47].

9.2.2 Local disease

In modern radiotherapy practice, it is rare to be asked to use radiotherapy as the primary local treatment for OS except for lesions in inaccessible sites. In certain situations, the use of radiation can be considered. Preoperative radiotherapy has been given in the context of a research protocol to

reduce tumor viability before surgery, increase the probability of performing limb-sparing surgery, and reduce the risk of local recurrence [52, 53]. High-dose irradiation can be considered for patients with microscopically positive margins following resection or with nonresectable primary tumors such as difficult pelvic bone sites, vertebral column, or base of skull and for patients who refuse definite surgery [8, 47]. In these patients, in lieu of surgery, photon irradiation (50–70 Gy) can be used as primary treatment which may be in conjunction with aggressive chemotherapy [47]. In 2003, among 30 patients with pelvic OS who refused definitive surgery, radiotherapy was delivered to 11 patients [38]. The 5-year survival of these patients (16%) was superior to that of those not irradiated (0%). A study in Russia reported that 31 patients with limb OS who refused definitive amputation were treated with multiagent chemotherapy (cisplatin and doxorubicin) and high-dose irradiation [54]. Local progression-free survival of these patients was 56% (40% for 50 Gy or less, 77% for more than 50 Gy). Postoperative irradiation also increased the survival rate. A previous study reported that seven patients with OS of the spine who received postoperative irradiation had a higher long-term survival (~50%) than those who did not (~10%) [39].

Among the more innovative uses of radiotherapy in OS treatment has been extracorporeal irradiation. Bone is taken out for irradiation and then reimplanted into the body. Reimplantation of irradiated bone provides several theoretical advantages compared to limb-sparing methods. A major advantage is the precise anatomic fit of the reimplanted bone segment. It avoids the growth discrepancy commonly seen in prosthetic replacements, the graft rejection, and the risk of viral transmission. It is also theoretically possible that dead tumor cells in the irradiated bone may stimulate a desirable immunologic response [55, 56]. Araki et al. reported the use of extracorporeal irradiation (50 Gy) to treat patients with OS [57]. There was no evidence of local recurrence or symptoms of graft failure such as severe pain or severe fracture occurring in these patients.

9.2.3 Lung metastasis

The tendency of OS to metastasize to the lungs stimulated interest in the use of prophylactic lung irradiation. In previous studies, prophylactic lung irradiation was conducted along with chemotherapy and showed some positive results. The French Bone tumor Study Group published studies on 41 cases of extremity OS treated with chemotherapy and 20 Gy of prophylactic lung irradiation [47]. The 5-year disease survival was 58% and the overall survival was 66%, which compared well with historic controls. However, there was marked lung toxicity including restrictive ventilatory effects, infections, and *Pheumocystis carinii* pneumonia. A study from the Mayo Clinic showed no benefit of prophylactic pulmonary irradiation in OS [58].

For patients with pulmonary metastatic disease, no benefit of prethoracotomy or postthoracotomy irradiation was seen [59]. An aggressive program for treatment with chemotherapy, whole lung irradiation, and boost irradiation to individual metastases was conducted by Weichselbaum et al. and did not result in better outcomes compared to another program with chemotherapy, thoracotomy, and no whole lung irradiation [60]. However, a study suggested that successful metastasectomy was possible more often after previous prophylactic lung irradiation than after adjuvant chemotherapy [59].

9.3 Chemotherapy

9.3.1 Prerelapse treatment

OS is one of the first solid tumors for which adjuvant chemotherapy proved to be beneficial [47]. Advances in chemotherapy over the past 30 years have improved limb salvage and led to higher survival rates [30]. Chemotherapy has also been shown to reduce the number of pulmonary metastases or to delay their appearance which facilitates surgical removal. Chemotherapy agents are normally administered systemically to the body by intraarterial or intravenous routes [47]. Generally, surgery plus modern multidrug chemotherapy has dramatically increased the 5-year disease-free survival rate of patients to 60–70%. Used drugs are cyclophosphamide, vincristine, melphalan, adriamycin (doxorubicin), methotrexate, cisplatin, decarbazine, bleomycin, dactinomycin, actinomycin, and leucovorin rescue.

Nonmetastatic OS Current standard regimens include preoperative and postoperative chemotherapy. Preoperative chemotherapy induces tumor necrosis in the primary tumor which facilitates surgical resection and provides early treatment of micrometastatic diseases [30]. Optimum survival was normally found in patients with good histologic response of the preoperative chemotherapy (more than 90% tumor necrosis) at the time of surgical resection [45, 47, 61]. The degree of tumor necrosis used as a marker of chemosensitivity has proven an important factor predictive of survival. The survival rate was improved when postoperative combination chemotherapy was chosen based on the degree of the tumor necrosis induced by preoperative therapy. Response to chemotherapy is also predictive of the need for further resections [9]. In pulmonary metastasectomies, all patients requiring more than one operation had less than 80% necrosis post-chemotherapy. The combina-

tion of methotrexate, cisplatin, and adriamycin provided good response rate at 65.7% [9].

The benefit to adjuvant chemotherapy was demonstrated in many studies. In the trial from 1981 to 1984 at the University of California at Los Angeles, all 59 patients received preoperative adriamycin. Thirty-two patients who were randomized to receive adjuvant postoperative high-dose methotrexate, adriamycin, bleomycin, cyclophosphamide, and actinomycin D showed a 55% 2-year disease-free survival rate while patients who received no adjuvant chemotherapy had a 20% survival rate [62]. Link et al. reported a randomized trial which presented a 66% 2-year relapse-free survival rate in patients received adjuvant cyclophosphamide, bleomycin, actinomycin, methotrexate, covorin rescue, doxorubicin, and cisplatin compared to a 17% rate in the control group [63]. Eilber et al. reported similar results, definitely proving that adjuvant chemotherapy produced higher disease-free survival rates for patients with nonmetastatic OS [62].

The selection of postoperative adjuvant chemotherapy based on the degree of the tumor necrosis induced by preoperative therapy improved the patient survival rate. M. D. Anderson Cancer Center in Texas (USA) reported a 54% 5-year disease-free survival for patients treated with a protocol called T7 that consisted of preoperative adriamycin and intraarterial cisplatin followed postoperatively by the same drugs from 1979 to 1982 [64]. Another report from M.D. Anderson group covering the years 1983 to 1988 presented a 69% 3-year disease-free survival of 60 patients with modified T7 protocol called T10, a neoadjuvant regime of chemotherapy. In T10 protocol, the selection of postoperative adjuvant chemotherapy was based on the response of the primary tumor to preoperative therapy. These patients received intensified preoperative intraarterial cisplatin. Postoperatively, good responders received adriamycin and cisplatin (or dacarbazine), and poor responders received methotrexate, adriamycin or dacarbazine, bleomycin, and cyclophosphamide or actinomycin [65]. The Children Cancer Group (CCG) in Indianapolis (USA) confirmed the preliminary good results of the T10 protocol [66]. They used CCG-782 protocol, T10 protocol with modifications in the drug combination, for 268 patients with nonmetastatic OS from 1983 to 1986. The 8-year disease-free survival was 53% and the overall survival rate was 60%. Good histologic responders has a 8-year disease-free survival rate of 81% and a overall survival rate of 87% while poor histologic responders had the rates of 46% and 52%, respectively. The trials conducted at New York's Memorial Sloan–Kettering Cancer Center from 1976 to 1986 also presented very good outcomes with 10-year survival of 279 patients at 73% [47]. Therefore, the histological response of the primary tumor to neoadjuvant chemotherapy was an important predictor of survival.

Bacci et al. reported the long-term results (1972–1999) achieved in a large series of patients at Rizzoli Institute, Italy [45], which again confirmed the benefit of neoadjuvant chemotherapy. One thousand, one hundred forty-eight patients with nonmetastatic OS of the extremity were treated with adjuvant chemotherapy (before 1983) and with neoadjuvant chemotherapy (after 1983). In neoadjuvant chemotherapy, treatment protocols were selected based on the histological response of preoperative-chemotherapy-treated tumor. Statistically, the rate of limb salvage increased from 20% (for patients treated with adjuvant chemotherapy from 1972 to 1982) to 71% (for patients treated with neoadjuvant chemotherapy from 1983 to 1999). In patients who received neoadjuvant treatment, chemotherapy-related tumor necrosis was good in 62% and poor in 38% of patients. The rate of good histologic responses was not related to patients' age, site or size of tumor, and serum AP levels at presentation but was slightly better for female than for male. The 5-year disease-free survival and overall survival were 57% and 66%, respectively. The 10-year disease-free survival and overall survival were 52% and 57%, respectively. These survival rate results significantly correlated with serum AP levels, the type of chemotherapy (neoadjuvant vs. adjuvant—61% vs. 43%), and with histologic response to preoperative treatment (good response vs. poor response—67% vs. 48%) [45].

Despite previous attempts to improve the outcome of poor responders by modifying the postoperative chemotherapy, their prognosis remains poor. Therefore, there is a need to predict responses to preoperative chemotherapy at the time of diagnosis, which will provide the basis for the development of a more effective therapy to those who are likely to have a poor response. Recently, based on microarray technology, a multigene classifier has been developed to predict the response of OS to preoperative chemotherapy at the time of diagnosis [67]. Forty-five genes that could distinguish good and poor responders to primary chemotherapy were identified. Generally, poor responders presented overexpression of most of these genes. Several predictor genes have properties that relate to bone development, cancer biology, and drug resistance.

In spite of the improved outcome obtained with adjuvant chemotherapy, the study of the European Osteosarcoma Intergroup reported in 1997 showed no difference between the two-drug and multidrug regimens in treatments of patients with operable and nonmetastatic OS. These patients were randomized to receive (1) doxorubicin and cisplatinum preoperatively or (2) vincristine, methotrexate, and doxorubicin preoperatively and postoperative bleomycin, cyclophosphamide, actinomycin, vincristine, methotrexate, doxorubicin, and cisplatin. Twenty-nine percent of patients in both regimens had 90% tumor necrosis in

response to preoperative chemotherapy. Overall survival was 65% at 3 years and 55% at 5 years in both groups. Therefore, there was no difference in survival between the two-drug and multidrug regimens. They concluded that the two-drug regimen was shorter in duration and better tolerated and was, therefore, the preferred treatment in operable nonmetastatic OS [68].

Recently, most chemotherapy regimens applied for OS have been based on methotrexate, cisplatin, doxorubicin, and ifosfamide [69]. The mechanisms of action of these chemotherapeutic agents are summarized in Table 4. A study from The German–Austria-Swiss Cooperative Osteosarcoma Study Group (COSS) showed that patients treated with these four drugs presented the best results with a 10-year survival rate of 71% [70]. The Italian and Scandinavian Sarcoma Group attempted to improve prognosis of nonmetastatic OS by increasing the dose of ifosfamide besides standard doses of methotrexate, cisplatin and doxorubicin, however, the outcomes (5-year disease-free survival 63%, 5-year overall survival 75%) were not higher than those with all standard doses [50]. The Brazilian Osteosarcoma Treatment Group investigated chemotherapy regimens with above four drugs with the addition of carboplatin and epirubicin in different combinations in localized OS patients [71]. Yet, the overall results (5-year disease-free survival 45.5%, 5-year overall survival 60.5%) were worse than those receiving the classic four-drug regimens. In 2005, the Children's Cancer Group and Pediatric Oncology Group reported a notable study in which classic cytotoxic chemotherapy was combined to a biologic treatment [72]. In this study, the addition of muramyl tripeptide-phosphatidylethanolamine (MTP-PE) to the classic four-drug regimen did improve the patient outcome with 5-year event-free survival of 72%. MTP-PE is a component of the cell wall of the *bacillus Calmette–Guerin* conjugated to phosphatidyl ethanolamine and encapsulated in liposomes with immunostimulating activity. The study also suggested that better results could be achieved possibly due to the interaction between ifosfamide and MTP-PE.

Table 4 Mechanisms of action of chemotherapeutic agents used in the treatment of OS [30]

Agent	Mechanism of action
Doxorubicin (Adriamycin)	Doxorubicin intercalates at point of local uncoiling of the DNA double helix and inhibits the synthesis of DNA and RNA.
Cisplatin (Patinol)	Cisplatin binds directly to tumor DNA and inhibits the synthesis of DNA through the formation of DNA cross-links.
Ifosfamide	Ifosfamide causes crosslinking of DNA strands, which inhibits the synthesis of DNA and protein.
Methotrexate	Methotrexate is a folate antimetabolite and inhibits the synthesis of purine and thymidylic acid by binding dihydrofolate reductase.

The role of chemotherapy dose intensity in OS has been widely debated. Recently, the Cooperative Osteosarcoma Study Group reported the largest study on dose intensity in OS including 917 patients aged below 40 years, and no relation between dose intensity and prognosis was found [73]. This conclusion is supported by the results of the European Osteosarcoma Intergroup's and the Italian and Scandinavian Sarcoma Group's studies [50, 74]. These findings suggest that approaches other than increasing dose intensity are required to improve the outcome of patients with OS.

Synchronous metastatic OS In spite of an aggressive surgical and chemotherapeutical approach, the prognosis of patients with synchronous metastases is worse than that of patients without metastases at diagnosis [69]. The set of four drugs including cisplatin, methotrxate, ifosfamide, and doxorubicin is again a common regime used in first-line treatment of synchronous metastatic OS. Carboplatin was substituted for cisplatin in a chemotherapy regime, but very poor results were reported [75]. This suggests that in metastatic patients, the use of carboplatin instead of cisplatin fails to improve patient outcome. The outcome of patients with synchronous multifocal OS (synchronous appearance of multiple OS localizations in the skeleton with or without pulmonary metastases) is also poor [76] although better prognosis was reported for patients with skip metastases treated with four-drug regimes [77]; therefore, new therapeutic approaches are needed for these patients.

9.3.2 Post-relapse treatment

Despite the success of aggressive combined treatments, local recurrence and metastases (mostly at lungs) still develop in approximately 30–40% of all patients, which is the major cause of death from this disease [7, 78]. The time to relapse not only depended on serum AP values (normal versus high: 25 versus 18 months) but also significantly depended on the type of chemotherapy (neoadjuvant versus adjuvant: 24 versus 16 months), and on histologic response to preoperative treatment (good response versus poor response: 22 versus 17 months) [45].

The type of treatment performed to manage metastases in relapsed patients was not standardized but performed on an individual basis, which considered the initial therapy, the site, and the number of metastases or recurrent tumors; the length of the disease-free interval; and the type of chemotherapy previously applied to patients. Surgical

resection of all sites of metastases on the best timing was always the key and pivotal treatment for patients with relapse [45]. Chemotherapy after relapse is still under discussion and contradictory results have been reported [69]. At present, no evidence on the best second-line chemotherapy treatment is available. The variety of relapse patterns makes it almost impossible to perform a randomized study to investigate the role of chemotherapy after relapse. Second line chemotherapy with drugs not used in the adjuvant and neoadjuvant treatment or with greater concentrations of previously used drugs was generally given in addition to patients with incomplete surgical removal of metastases [45].

At the Rizzoli Institute, Italy, the first treatment choice of patients with OS relapse diseases is surgery (43%) [45]. Other choices are surgery combined with chemotherapy (42%), only chemotherapy (14%), and no specific treatment at al (0.2%). The 5-year disease-free survival rates according to type of treatment were 22.4% for patients treated by surgery and 17.8% for those treated with surgery and chemotherapy. Patients treated only by chemotherapy survived for 5 years; however, it must be taken into account that these patients had bigger and inoperable disease [45].

Patients who are not eligible for metastasectomy are candidates for palliative chemotherapy [29]. Patients with limb salvage showed responses to ifosfamide and ectoside with higher responses reported for combination than single-agent ifosfamide. High-dose methotrexate has moderate single-agent activity and novel agents such as ecteinascidin 743 (trabectedin) and deforolimus also has limited single-agent activity for OS. Gemcitabine with docetaxel may be useful as well. Recently, the mammalian target of rapamycin (mTOR) inhibitor AP23573 has yielded occasional durable partial responses in patients with metastatic disease, raising the hope that combinations of an mTOR inhibitor and either cytotoxic or other targeted agents can be more effective against recurrent disease [79].

Generally, patients who relapse following the use of modern treatment approaches including chemotherapy and surgery have a significantly lower probability of survival [8]. Poor prognosis was also found in patients with a large number of metastases, bilateral disease, a short time interval between local therapy and the development of metastatic disease, and a poor response to preoperative chemotherapy [29].

10 New drugs

Some promising new drugs have been reported for OS management. *In vitro* studies have demonstrated the inhibitory activity of new nitrogen-containing bisphosphonates such as minodronate, incadronate [80], risedronate [81], and zoledronic acid [82] on human OS cell growth, suggesting a beneficial contribution treatment, including analgesic effects in patients with OS. The antitumor activities of risedronate in combination with carboplatin, doxorubicin, vincristine, or etoposide were synergistically augmented on several OS cell lines [81]. The combined effects of zoledronic acid with other anticancer agents against murine osteosarcoma were also reported [83]. Zoledronic inhibited the growth of OS cells and chemosentisize these cells to cisplatin [84]. The bisphosphonate drug alendronate was used to suppress bone remodeling and tumor osteolysis as a palliative treatment for two dogs with OS in a study of Tomlin et al. showing positive results [85]. Bisphosphonates induce apoptosis by caspase-3-like protease activation and significantly reduce cell invasion through zinc chelation of metalloproteinase enzymes [69].

A new interest is growing around the field of immunomodulation and its applicability to OS. Interferon (IFN) has been employed in some studies since 1980s and showed promising results. It was administered alone [86, 87] or in combination with chemotherapy [88–90]. IFNs are produced by the cells of the immune system of most vertebrates in response to challenges by foreign agents such as viruses, parasites, and tumor cells [91, 92]. IFNs belong to the large class of glycoproteins known as cytokines. IFN was discovered as an antiviral substance which assists the immune response by inhibiting viral replication within host cells, activating natural killer cells and macrophages, increasing antigen presentation to lymphocytes, and inducing the resistance of host cells to viral infection. Subsequently, IFNs have been used for the treatment of many virus-associated tumors [93]. In certain cases, OS is believed to be an example of a tumor disease caused by a virus as mentioned above. Some investigators suggested that IFNs exerted their antitumor effects through their antiangiogenic activity [94, 95]. Recent studies have demonstrated that IFN-α enhanced the sensitivity of human OS cells to chemotherapeutic agents such as etoposide [96] and doxorubicin by p53-dependent apoptosis [96, 97]. Thus, the proper combination with IFNα and conventional chemotherapeutic agents may be a rational strategy for improving the treatment of OS with functional p53. Hiroto et al. also reported that IFN-γ sensitized OS cells to Fas-induced apoptosis by upregulating Fas receptors and caspase-8 [98]. Therefore, combined immunotherapy with IFN-y and either anti-Fas monoclonal antibody or cytotoxic T cells that bear Fas ligand might be a useful adjunctive therapy for patients with OS.

Interleukins (ILs), a group of cytokine immune system signaling molecules, have been also studied as immunotherapy for OS. IL-2 is able to facilitate the production of immunoglobulins made by B cells and induce the differen-

tiation and proliferation of natural killer (NK) cells [99, 100]. In a treatment program including pre- and postoperative IL-2 and chemotherapy for childhood OS, NK counts and activity significantly correlated with clinical outcome [101]. Chemotherapy did not influence the modification of NK cells and NK activities induced by IL-2. In another study, Schwartz et al. reported the antitumor effects of IL-12 based on increased numbers of strategically located NK cells and advocates a prophylactic approach against the potential metastasis-promoting effects of surgery [102]. These results suggested a possible role of the NK cells in the control of OS. Liposomal MTP-PE, an activator of monocytes and macrophages and an inducer of the secretion of different cytokines (IL-1, IL-6), was employed successfully in dogs with OS [103].

Another promising approach is the use of mammalian target of mTOR inhibitors in OS [69]. mTOR, a member of the phosphoinositide-kinase family, is a key component of the phosphoinositide 3-kinase or Akt signaling pathway that mediates cell growth and proliferation. mTOR inhibitors, derived from rapamycin, are active against tumor cells by blocking mTOR activity leading to inhibition of cell growth. Preclinical trial study showed an activity of mTOR inhibitors against sarcoma cell lines including OS [104].

Other potential therapies which target molecular markers or pathways of OS are now being more widely studied, and some preclinical studies seem encouraging. This will be discussed in more details in the next section of this review.

11 Modern molecular markers and potential treatments

There are a number of molecular pathways involved in tumorigenesis being studied, which may be used to predict specific outcomes such as the likelihood of micrometastases at diagnosis and response to chemotherapy. These pathways can be potential targets for new OS therapies.

VEGF Vascular endothelial growth factor (VEGF) is a naturally occurring protein stimulating the development of microvascular beds in tissues and plays a significant role in the progression of many cancers by increasing their blood supply [9]. There are studies utilizing antiangiogenic agents such as angiostatin [105], avastin, and endostatin [106, 107] to inhibit the VEGF signaling pathway in the attempt to inhibit cancer growth. However, the prognostic significance of VEGF and microvascular density (MVD) in OS is still controversial and a definite conclusion has not yet been reached. Some studies [108–110] found that high VEGF levels correlated with increased MVD, increased frequency of metastases, and reduced overall survival, but some [111–113] did not found any correlation or even a converse relationship.

PEDF Pigment epithelium derived factor (PEDF) is a 50-kDa glycoprotein which has a protective role against excessive angiogenesis [9]. PEDF is found to be downregulated in many common cancers [112]. It is a potent inhibitor of angiogenesis in OS via inhibition of VEGF and induced apoptosis of endothelial cells through the Fas/FasL death pathway [114–116]. It also promotes cell differentiation and influences cell proliferation by regulating the cell cycle [117] and inducing apoptosis [118]. It was found that PEDF was more potent than any of the other known angiogenesis inhibitors. It was more than twice as potent as angiostatin and more than seven times as potent as endostatin [114]. *In vivo* models, PEDF overexpression led to suppression of cancer growth, invasion and metastases [78]. However, there has been no definite study correlating PEDF levels and clinical outcomes in OS.

MMPs Matrix metalloproteinases (MMPs) are enzymes normally involved in the breakdown of the extracellular matrix within the context of physiological tissue remodeling and angiogenesis [9]. Excessive production of certain MMPs has been recognized as an important factor in cancer invasion and metastasis. For example, OSs with positive presence of MMP-9 (gelatinase B) was associated with an overall 5-year survival of 28% in comparison to 79% for the negative group [119]. A number of substances have been found to inhibit MMP-9 production and, therefore, reduced invasion and metastasis in cultured cells and animal cancer models. They include sulfated glucosamine, histone deacetylases[120], nitric oxide [121], and reversion-inducing cystein-rich protein with Kazal motifs (RECK) [122].

RECK RECK is a membrane-bound protein which was first discovered and identified by Takahashi et al. and reported in 1998 [123]. It is able to inhibit MMP-9, MMP-2, and MT1-MMP [124]. RECK also has an important role in controlling angiogenesis, which has been attributed to RECK's inhibition of MMPs and possibly its inhibition of VEGF [124]. RECK is downregulated in many common tumors [9] and a number of OS cell lines [122] and related to poor outcome. Kang et al. reported that overexpression of RECK by liposome transfection of SaOS-2 cells (a human OS cell line) have been correlated with reduced cell invasion across a matrigel layer *in vitro* [122].

uPA/uPAR The urokinase activator (uPA) protein upregulates MMPs and promotes the invasion of tumor [125]. uPA becomes active once binding to its receptor (uPAR). The uPA/uPAR system was found to be upregulated in many common tumors and linked to poor outcome [9]. In OS, there is distinct and inverse relationship between uPA levels and survival time [126] downregulation of uPAR using

antisense clones within an *in vivo* OS model which resulted in reduced primary growth in the tibia and inhibition of pulmonary metastases [127]. Recently, a combination exposure of uPAR dowregulation and PEDF treatment has led to a synergistic effect in an animal model of OS [128].

P-glycoprotein P-glycoprotein (P-gp) is a protein responsible for energy-dependent drug efflux and encoded by the multiple drug-resistant-1 gene [9]. It was found that high levels of expressed P-gp in OS were associated with a significant reduction in the disease-free survival time. A study showed that P-gp was responsible for cancer cell resistance to doxorubicin as a single agent post-operatively, leading to an even worse survival time compared to patients with negative P-gp tumors [129]. However, P-gp does not correlate with the level of post-chemotherapy tumor necrosis [130], conflicting with the understanding of P-gp being involved in chemotherapy resistance by means of actively pumping these agents out of cells.

CXCR4 Chemokine receptor (CXCR4) and its corresponding ligand, stromal cell-derived factor 1 (SDF-1), play a major role in the metastatic process [9]. It has been found that CXCR4/SDF-1 system significantly correlates with the presence of metastases in a number of tumors. In OS, the increase of CXCR4 mRNA expression leads to reduced overall survival and correlates with the presence of metastases at diagnosis [131]. Therefore, CXCR4 is a potential target for chemotherapy. Perissinotto et al. tried to use T134 peptide to inhibit the CXCR4 site in a mouse model, resulted in the elimination of lung metastases in all the tested mice [132].

p53 Tumor-suppressor gene p53 and its protein product play an important role in the inhibition of most tumors' formation and growth including suppression of metastasis and inhibition of new blood vessel development [133]. The inactivation of this gene results in the loss of cell cycle and repair mechanism, and a loss of antiangiogenesis. In many OS cell lines, a number of p53 mutations which led to altered protein expression have been identified [134]. It is also noted that a few OS cases were associated with either germline p53 mutations or the Li–Fraumeni syndrome [135]. Nakase et al. examined the efficacy of p53 gene therapy in a human OS cell line using a transferring-modified cationic liposome, which resulted in a significant inhibition of tumor growth [136]. Another study of gene therapy with polyethyleneimine-p53 complexes showed significant growth suppression of established human OS lung metastases in mice [137].

ErbB-2 ErbB-2 or Her-2/neu is a transmembrane glycoprotein produced by c-erbB-2 gene and plays a significant role in the pathogenesis of breast cancer but its role in OS is still controversial [9]. Some studies found that the presence of ErbB-2 protein in OSs significantly correlate with reduced survival, increased metastases, and poor outcome [138, 139]. However, another study concluded conversely. Herceptin, a drug blocking ErbB-2, has been used successfully in breast cancer clinically and in other cancers *in vitro* [9]. Herceptin is a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor, which targets the epidermal growth factor receptor 2. A clinical trial of herceptin in OS is under progress [140].

Ezrin Ezrin is a protein having roles in cell–cell interactions, signal transduction, and linkage between actin filament and the cells membrane [141]. Upon upregulation, it leads to metastasis. In pediatric OS patients, increased ezrin expression is associated with reduced disease-free intervals [142]. It was found that downregulation of ezrin expression in a mouse model of human OS resulted in pulmonary metastasis inhibition through the MAPK signaling pathway [142]. Wan et al. have used rapamycin to inhibit ezrin-mediated pathways, leading to reduced lung metastasis in a mouse model of OS [143].

PTHrP/PTHR1 Parathyroid hormone-related peptide (PTHrP) and its receptor (PTHR1) are known to be involved in tumor progression, bone metastases, and hypercalcemia due to malignancy [9]. The PTHrP/PTHR1 system has diverging actions on tumor progression, involving progression or inhibition depending perhaps on whether ligand or receptor is upregulated. Overexpression of PTHrP in a rat OS cell line (osteoblastic) was found to reduce cell proliferation by 80% [144]. However, overexpression of PTHR1 in the HOS OS cell line resulted in the increased proliferation, motility, and invasion of cells through Matrigel [145].

c-Jun c-Jun is an oncogene encoding a basic region-leucine zipper protein [146] which, in combination with c-Fos protein, forms the activator protein-1 early response transcription factor [147]. It was demonstrated that the growth and metastasis of osteosarcoma in an orthotopic spontaneously metastasizing model of the disease were inhibited by a c-*Jun* DNA enzyme (DNAzyme) [148] encapsulated in a cationic multilamellar vesicle liposome [149]. DNAzymes are oligonucleotides capable of specific catalysis of target mRNA. In another study, a c-Jun DNAzyme nanoparticle formulated from chitosan was also found to be more active against OS cells, inducing apoptotic cell death in these cells [150]. It regressed the growth and metastasis of preestablished tumors, especially in combination with doxorubicin [151]. c-Jun knockdown chemosensitized these cells to doxorubicin treatment.

IGF-1 Insulin-like growth factor-1 (IGF-1) has been implicated in the growth and/or metastasis of OS based on *in vitro* and *in vivo* studies [152–155]. It is one of most potent natural activators of the AKT and MAPK signaling pathways relevant in the development, growth, survival, and progression of cancer [156]. The presence of IGF-1 receptor on OS cells has suggested the use of a growth hormone antagonist such as somatostatin in the treatment of OS patients [157]. However, a pilot study employing a somatostatine analog at different doses on 21 OS patients showed no significant response [158].

12 Conclusion

OS is the most common primary bone tumor in childhood and adolescence. It usually involves long bones and is a highly aggressive tumor that metastasizes primarily to the lungs. Currently, the standard therapy in most countries consists of various combinations of surgery and chemotherapy. Cisplatin, doxorubicin, ifosfamine, and methotrexate are commonly used drugs. Doses of chemotherapy have to be high to affect prognosis but are connected to severe side effects. Irradiation has been used for additional treatment and for palliation of some patients, but most of these tumors are not very radiosensitive. Hence, radical surgery is still compulsory, according to present knowledge. There is a clear need for newer effective agents for patients with OS, especially for patients with metastatic disease or disease recurrence. New drugs such as bisphosphonates, interferon, interleukin, and monoclonal antibodies have been trialed in preclinical and clinical studies, showing encouraging results. Several molecular pathways or markers of OS forming and developing have been revealed, which promises new effective treatments for this disease.

References

1. Unni, K. K. (1996). *Dahlin's bone tumors: General aspects and data on 11,087 cases*. Philadelphia: Lippincott-Raven.
2. Buecker, P. J., Gebhardt, M. C., Weber, K. (2005). Osteosarcoma. Liddy Shriver Sarcoma Initiative. http://sarcomahelp.org/Newsletters/V02N01/Osteosarcoma/osteosarcoma.htm. Accessed 30 September 2008.
3. The-Cancer-Council-Victoria. (2006). Osteosarcoma and flouride. http://www.ada.org.au/app_cmslib/media/lib/0703/m50781_v1_osteosarcomaandfluoride.pdf. Accessed 20 June 2008.
4. Longhi, A., Errani, C., De Paolis, M., Mercuri, M., & Bacci, G. (2006). Primary bone osteosarcoma in the pediatric age: state of the art. *Cancer Treatment Reviews*, *32*, 423–436.
5. Quan, G. M. Y., Ojaimi, J., Nadesapillai, W. A. P., Zhou, H., & Choong, P. F. M. (2002). Resistance of epiphyseal cartilage to invasion by osteosarcoma of antiangiogenic factors. *Pathobiology*, *70*, 361–367.
6. Whelan, J. (2005). Advances in osteosarcoma. In T. O. B. Eden, et al. (Eds.), *Cancer and the adolescent* (pp. 113–120). Malden: Blackwell.
7. Dass, C. R., Ek, E. T., Contreras, K. G., & Choong, P. F. (2006). A novel orthotopic murine model provides insights into cellular and molecular characteristics contributing to human osteosarcoma. *Clinical and Experimental Metastasis*, *23*, 367–380.
8. Marina, N., Gebhardt, M., Teot, L., & Gorlick, R. (2004). Biology and therapeutic advances for pediatric osteosarcoma. *The Oncologist*, *9*, 422–441.
9. Clark, J. C. M., Dass, C. R., & Choong, P. F. M. (2007). A review of clinical and molecular prognostic factors in osteosarcoma. *Journal of Cancer Research Clinical Oncology*, *134*, 281–297.
10. Rytting, M., Pearson, P., & Raymond, A. K. (2000). Osteosarcoma in preadolescent patients. *Clinical Orthopaedics*, *373*, 39–50.
11. Fraumeni, J. F. (1967). Stature and malignant tumors of bones in childhood and adolescence. *Cancer*, *20*, 967–973.
12. Grimer, R. J. (2005). Osteosarcoma and surgery. In T. O. B. Eden, et al. (Eds.), *Cancer and the adolescent* (pp. 121–132). Malden: Blackwell.
13. Withrow, S. J., Powers, B. E., Straw, R. C., & Wilkins, R. M. (1991). Comparative aspects of osteosarcoma. Dog versus man. *Clinical Orthopaedics and Related Research*, *270*, 159–168.
14. Tjalma, R. A. (1966). Canine bone sarcoma: estimation of relative risk as a function of body size. *Journal of the National Cancer Institute*, *36*, 1137–1150.
15. Cotterill, S. J., Wright, C. M., Pearce, M. S., & Craft, A. W. (2004). Stature of young people with malignant bone tumors. *Pediatric Blood and Cancer*, *42*, 59–63.
16. Gelberg, K. H., Fitzgerald, E. F., Hwang, S., & Dubrow, R. (1997). Growth and development and other risk for osteosarcoma in children and young adults. *International Journal of Epidemiology*, *26*, 272–278.
17. Longhi, A., Pasini, A., Cicognani, A., Baronio, F., Pellacani, A., & Baldini, N. (2005). Height as a risk factor for osteosarcoma. *Journal of pediatric hematology/oncology*, *27*, 314–318.
18. Longhi, A., Barbieri, E., & FAbbri, N. (2003). Radiation-induced osteosarcoma arising 20 years after the treatment of Ewing's sarcoma. *Tumorigenesis*, *89*, 569–572.
19. Picci, P. (2007). Osteosarcoma (Osteogenic sarcoma). *Orphanet Journal of Rare Diseases*, *2*, 1–4.
20. Fuschs, B., & Pritchard, D. J. (2002). Etiology of osteosarcoma. *Clinical orthopaedics*, *397*, 40–52.
21. Wang, L. L., Gannavaparu, A., & Kozinets, C. A. (2003). Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. *Journal of the National Cancer Institute*, *95*, 669–674.
22. Finkel, M. P., Biskis, B. O., & Jinkins, P. B. (1966). Virus induction of osteosarcomas in mice. *Science*, *151*, 698–701.
23. Fuchs, B., & Pritchard, D. J. (2002). Etiology of osteosarcoma. *Clinical Orthopaedics*, *397*, 40–52.
24. Kakar, S., Mihalov, M., Chachlani, N. A., Ghosh, L., & Johnstone, H. (2000). Correlation of c-fos, p53, and PCNA expression with treatment outcome in osteosarcoma. *Journal of surgical oncology*, *73*, 125–126.
25. Mendoza, S. M., Konishi, T., & Miller, C. W. (1998). Integration of SV40 in human osteosarcoma DNA. *Oncogene*, *17*, 2457–2462.
26. Engels, E. A. (2005). Cancer risk associated with receipt of vaccines contaminated with simian virus 40: epidemiologic research. *Expert Review of Vaccines*, *4*, 197–206.

27. Franchi, A., Arganini, L., & Baroni, G. (1998). Expression of transforming growth factor beta isoforms in osteosarcoma variants: association of TGF beta 1 with high grade osteosarcomas. *Journal of pathology*, *185*, 284–289.
28. Huvos, A. (1991). *Bone tumors: Diagnosis, treatment and prognosis*. Philadelphia: Saunders.
29. Skubitz, K. M., & D'Adamo, D. R. (2007). Sarcoma. *Mayo Clinic Proceedings*, *82*, 1409–1432.
30. Wittig, J. C., Bickels, J., Priebat, D., Jelinek, J., Kellar-Graney, K., Shmookler, B., et al. (2002). Osteosarcoma: a multidisciplinary approach to diagnosis and treatment. *American Family Physician*, *65*, 1123–1132.
31. Link, M. P., Goorin, A. M., Horowitz, M., Meyer, W. H., Belasco, J., Baker, A., et al. (1991). Adjuvant chemotherapy of high-grade osteosarcoma of the extremity. Updated results of the multi-institutional osteosarcoma study. *Clinical Orthopaedics and Related Research*, *270*, 8–14.
32. Hannisdal, E., Solheim, O. P., Theodorsen, L., & Host, H. (1990). Alterations of blood analyses at relapse of osteosarcoma and Ewing's sarcoma. *Acta oncologica*, *29*, 585–587.
33. Bacci, G., Longhi, A., Ferrari, S., Lari, S., Manfrini, M., Donati, D., et al. (2002). Prognostic significance of serum alkaline phosphatase in osteosarcoma of the extremity treated with neoadjuvant chemotherapy: recent experience at Rizzoli Institute. *Oncology reports*, *9*, 171–175.
34. Bacci, G., Longhi, A., Ferrari, S., Briccoli, A., Donati, D., Paolis, M. D., et al. (2004). Prognostic significance of serum lactate dehydrogenase in osteosarcoma of the extremity: Experience at Rizzoli on 1421. *Tumorigenesis*, *90*, 478–484.
35. Aisen, A. M., Martel, W., Braunstein, E. M., McMillin, K. I., Phillips, W. A., & Kling, T. F. (1986). MRI and CT evaluation of primary bone and soft-tissue tumors. AJR. *American Journal of Roentgenology*, *146*, 749–756.
36. Mankin, H. J., Mankin, C. J., & Simon, M. A. (1996). The hazards of the biopsy, revisited: members of the Musculoskeletal Tumor Society. *The Journal of bone and joint surgery*, *78*, 656–663.
37. Dahlin, D. C. (1978). Osteosarcoma of bone and a consideration of prognostic variables. *Cancer Treatment Reports*, *62*, 189–192.
38. Ozaki, T., Flege, S., Kevric, M., Lindner, N., Masas, R., Delling, G. S. R., et al. (2003). Osteosarcoma of the pelvic: experience of the Cooperative Osteosarcoma Study Group. *Journal of Clinical Oncology*, *21*, 334–341.
39. Ozaki, T., Flege, S., Liljenqvist, U., Hillmann, A., Delling, G. S. R., Salzer-Kuntschik, M., et al. (2002). Osteosarcoma of the spine: experience of the Cooperative Osteosarcoma Study Group. *Cancer*, *94*, 1069–1077.
40. Magishi, K., Yoshida, H., Izumi, Y., Ishikawa, N., & Kubota, H. (2004). Primary osteosarcoma of the lung: report of a case. *Surgery Today*, *34*, 150–152.
41. Bieling, P., Rehan, N., Winkler, P., Helmke, K., Maas, R., Fuchs, N., et al. (1996). Tumor size and prgonosis in sggressively treated osteosarcoma. *Journal of Clinical Oncology*, *14*, 848–858.
42. Wolf, R. E., & Enneking, W. F. (1996). The staging and surgery of musculoskeletal neoplasms. *The Orthopedic clinics of North America*, *27*, 473–481.
43. Greene, F. L., Page, D. L., Fleming, I. D., Fritz, A., Balch, C. M., Haller, D. G., et al. (2002). *AJCC cancer staging manual (6th)*. New York: Springer.
44. Meyers, P. A., & Gorlick, R. (1997). Osteosarcoma. *Pediatric clinics of North America*, *44*, 973–989.
45. Bacci, G., Longhi, A., Fagioli, F., Briccoli, A., Versari, M., & Picci, P. (2005). Adjuvant and neoadjuvant chemotherapy for osteosarcoma of the extremities: 27 years experience at Rizzoli Institute, Italy. *European Journal of Cancer*, *41*, 2836–2845.
46. Patel, S. J., Lynch, J. W. J., Johnson, T., Carroll, R. R., Schumacher, C. R. N., Spanier, S., & Scarborough, M. (2002). Dose-intense ifosfamide/doxorubicin/cisplatin based chemotherapy for osteosarcoma in adults. *American Journal of Clinical Oncology*, *25*, 489–495.
47. Halperin, E. C. (2005). Osteosarcoma. In E. C. Halperin, et al. (Eds.), *Pediatric Radiation Oncology* (pp. 291–318). Philadelphia: Williams & Wilkins.
48. Ferguson, W. S., & Goorin, A. M. (2001). Current treatment of osteosarcoma. *Cancer Investigation*, *19*, 292–315.
49. Ozaki, I., Flege, S., Lijenqvist, U., Hillmann, A., Delling, G., Salzer-Kuntschik, M., et al. (2002). Osteosarcoma of the spine: experience of the Cooperative Osteosarcom Study Group. *Cancer*, *94*, 1069–1077.
50. Ferrari, S., Smeland, S., Mercuri, M., Bertoni, F., Longhi, A., Ruggieri, P., et al. (2005). Neoadjuvant chemotherapy with high-dose ifosfamide, high-dose methotrexate, cisplatin and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. *Journal of Clinical Oncology*, *23*, 8845–8852.
51. Jaff, N., Carrasco, H., Raymond, K., Ayala, A., & Eftekhari, F. (2003). Cancure in patients with osteosarcoma be achieved exclusively with chemotherapy and abrogation of surgery. *Cancer*, *95*, 2202–2210.
52. Lee, E. S. (1971). Treatment of bone sarcoma. *Proceedings of the Royal Society of Medicine*, *64*, 1179–1181.
53. Philips, T. L., & Sheline, G. E. (1969). Radiation therapy of malignant bone tumors. *Radiology*, *92*, 1537–1545.
54. Machak, G. N., Tkachev, S. I., Solovyev, Y. N., Sinyukov, P. A., Ivanov, S. M., Kochergina, N. V., et al. (2003). Neoadjuvant chemotherapy and local radiotherapy for high grade osteosarcoma of the extremities. *Mayo Clinic Proceedings*, *78*, 147–155.
55. Yamamoto, T., Akisue, T., Marui, T., Nagira, K., & Kurosaka, M. (2002). Osteosarcoma of the distal radius treated by intraoperative extracorporeal irradiation. *Journal of the Hand Surgery*, *27*, 160–164.
56. Hong, A., Stevens, G., Stalley, P., Pendlebury, S., Ahern, V., Ralston, A., et al. (2001). Extracorporeal irradiation for malignant bone tumors. *International Journal of Radiation Oncology, Biology, Physics*, *50*(2), 441–447.
57. Araki, N., Myoui, A., Kuratsu, S., Hashimoto, N., Inoue, T., Kudawara, I., et al. (1999). Intraoperative extracorporeal autogenous irradiated bone graft in tumor surgery. *Clinical Orthopaedics*, *368*, 196–206.
58. Gilchrist, G. S., Pritchard, D. J., & Dahlin, D. C. (1981). Management of osteogenic sarcoma: a perspective based on the Mayo Clinic experience. *National Cancer Institute Monograph*, *56*, 193–199.
59. Giritsky, A. S., Etucubanas, E., & Mark, J. B. (1978). Pulmonary resection in children with metastaic osteogenic sarcoma: improved survival with surgery, chemotherapy, and irradiation. *The Journal of Thoracic and Cardiovascular Surgery*, *75*, 354–362.
60. Weichselbaum, R. R., Cassady, J. R., & Jaffe, N. (1977). Preliminary results of aggressive multimodality therapy for metastatic osteosarcoma. *Cancer*, *40*, 78–83.
61. Picci, P., Bacci, G., Campanacci, M., Gasparini, M., Pilotti, S., Cerasoli, S., et al. (1985). Histologic evaluation of necrosis in osteosarcoma induced by chemotherapy. *Cancer*, *56*, 1515–1521.
62. Eilber, F., Giuliano, A., Eckardt, J., Patterson, K., Moseley, S., & Goodnight, J. (1987). Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. *Journal of Cancer and Clinical Oncology*, *5*, 21–26.
63. Link, M. P., Goorin, A. M., Horowitz, M., Meyer, W. H., Belasco, J., Baker, A., et al. (1991). Adjuvant chemotherapy of high-grade osteosarcoma of the extremity. Updated results of the Multi-

Institutional Osteosarcoma Study. *Clinical orthopaedics*, *270*, 8–14.
64. Rosen, G., Caparros, B., Huvos, A. G., Kosloff, C., Nirenberg, A., Cacavio, A., et al. (1982). Preoperative chemotherapy of osteosarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to the preoperative therapy. *Cancer*, *49*, 1221–1230.
65. Hudson, M., Jaffe, M. R., Jaffe, N., Ayala, A., Raymond, A. K., Carrasco, H., et al. (1990). Pediatric osteosarcoma: therapeutic strategies, results, and prognostic factors derived from a 10-year experience. *Journal of Clinical Oncology*, *12*, 1988–1997.
66. Provisor, A. J., Ettinger, L. J., Nachman, J. B., Krailo, M. D., Makley, J. T., Yunis, E. J., et al. (1997). Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group. *Journal of Clinical Oncology*, *15*, 76–84.
67. Man, T.-K., Chintagumpala, M., Visvanathan, J., Shen, J., Perlaky, L., Hicks, J., et al. (2005). Expression Profiles of Osteosarcoma That Can Predict Response to Chemotherapy. *Cancer Research*, *65*, 8142–8150.
68. Souhami, R. L., Craft, A. W., Van der Eijken, J. W., Nooij, M., Spooner, D., Bramwell, V. H., et al. (1997). Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. *Lancet*, *350*, 911–917.
69. Ferrari, S., & Palmerini, E. (2007). Adjuvant and neoadjuvant combination chemotherapy for osteogenic sarcoma. *Current Opinion in Oncology*, *19*, 341–346.
70. Fuchs, N., Bielack, S. S., Epler, D., Biding, P., Delling, G., Körholz, D., et al. (1998). Long-term results of the co-operative German-Austrian-Swiss Osteosarcoma Study Group's protocol COSS-86 of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. *Annals of Oncology*, *9*, 893–899.
71. Petrilli, A. S., de Camargo, B., Filho, V. O., Bruniera, P., Brunetto, A. L., Jesus-Garcia, R., et al. (2006). Results of the Brazilian Osteosarcoma Treatment Group Studies III and IV: prognostic factors and impact on survival. *Journal of Clinical Oncology*, *24*, 1161–1168.
72. Meyers, P. A., Schwartz, C. L., Krailo, M. D., Kleinerman, E. S., Betcher, D., Bernstein, M. L., et al. (2005). Osteosarcoma: a randomized prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin and high-dose methotrexate. *Journal of Clinical Oncology*, *23*, 2004–2011.
73. Eselgrim, M., Grunert, H., Kuhne, T., Zoubek, A., Kevric, M., Bürger, H., et al. (2006). Dose intensity of chemotherapy for osteosarcoma and outcome in the Cooperative Osteosarcoma Study Group (COSS) trial. *Pediatric Blood and Cancer*, *47*, 42–50.
74. Lewis, I. J., Weeden, S., Machin, D., Stark, D., & Craft, A. W. (2000). Received dose and dose intensity of chemotherapy and outcome in nonmetastatic extremity osteosarcoma. European Osteosarcoma Intergroup. *Journal of Clinical Oncology*, *18*, 4028–4037.
75. Daw, N. C., Billups, C. A., Rodriguez-Galindo, C., McCarville, M. B., Rao, B. N., Cain, A. M., et al. (2006). Metastatic osteosarcoma. *Cancer*, *106*, 403–412.
76. Bacci, G., Fabbri, N., Balladelli, A., Forni, C., Palmerini, E., & Picci, P. (2006). Treatment and prognosis for synchronous multifocal osteosarcoma in 42 patients. *Journal of Bone and Joint Surgery American*, *88*, 1071–1075.
77. Kager, L., Zoubek, A., Kastner, U., Kempf-Bielack, B., Potratz, J., Kotz, R., et al. (2006). Skip metastases in osteosarcoma : Experience of The Cooperative Osteosarcoma Study Group. *Journal of Clinical Oncology*, *24*, 1535–1541.
78. Ek, E. T. H., Dass, C. R., Contreras, K. G., & Choong, P. F. M. (2007). Pigment epithelium-derived factor overexpression inhibits orthotopic osteosarcoma growth, angiogenesis and metastasis. *Cancer Gene Therapy*, *14*, 616–626.
79. Okuno, S. (2006). Mammalian target of rapamycin inhibitors in sarcomas. *Current Opinion in Oncology*, *18*, 360–362.
80. Kubo, T., Shimose, S., Matsuo, T., Tanaka, K., Yasunaga, Y., Sakai, A., et al. (2006). Inhibitory effects of a new bisphosphonate, minodronate, on proliferation and invasion of a variety of malignant bone tumor cells. *Journal of Orthopaedic Research*, *24*, 1138–1144.
81. Murayama, T., Kawasoe, Y., Yamashita, Y., Ueno, Y., Minami, S., Yokouchi, M., et al. (2008). Efficacy of the third-generation bisphosphonate risedronate alone and in combination with anticancer drugs against osteosarcoma cell lines. *Anticancer Reseach*, *28*, 2147–2154.
82. Kubista, B., Trieb, K., Sevelda, F., Toma, C., Arrich, F., Heffeter, P., et al. (2006). Anticancer effects of zoledronic acid against human osteosarcoma cells. *Journal of Orthopaedic Research*, *24*, 1145–1152.
83. Horie, N., Murata, H., Kimura, S., Takeshita, H., Sakabe, T., Matsui, T., et al. (2007). Combined effects of a third-generation bisphosphonate, zoledronic acid with other anticancer agents against murine osteosarcoma. *British Journal of Cancer*, *96*, 255–261.
84. Benassi, M., Chiechi, A., Ponticelli, F., Pazzaglia, L., Gamberi, G., Zanella, L., et al. (2006). Growth inhibition and sensitization to cisplatin by zoledronic acid in osteosarcoma cells. *Cancer Letters*, *250*, 194–205.
85. Tomlin, J. L., Pead, M. J., & Muir, P. (2000). Use of the bisphosphonate drug alendronate for palliative management of osteosarcoma in two dogs. *The Veterinary Record*, *147*, 129–132.
86. Kotz, R., Plattner, E., Ramach, W., Flener, R., & Bodo, G. (1982). Interferon/controlled study in 3-year survival of patients with recurrent osteosarcoma. *Arzneimittelforschung*, *32*, 446–448.
87. Müller, C. R., Smeland, S., Bauer, H. C., Saeter, G., & Strander, H. (2005). Interferon-alpha as the only adjuvant treatment in high-grade osteosarcoma: long term results of the Karolinska Hospital series. *Acta Oncologica*, *44*, 475–480.
88. Strander, H., Aparisi, T., Blomgren, H., Broström, L. A., Cantell, K., Einhorn, S., et al. (1982). Adjuvant interferon treatment of human osteosarcoma. *Recent Results Cancer Research*, *80*, 103–107.
89. Strander, H., Bauer, H. C., Brosjö, O., Kreicbergs, A., Lindholm, J., Nilsonne, U., et al. (1993). Adjuvant interferon treatment in human osteosarcoma. *Cancer Treatment and Research*, *62*, 29–32.
90. Winkler, K., Beron, G., Kotz, R., Salzer-Kuntschik, M., Beck, J., Beck, W., et al. (2004). Adjuvant chemotherapy in osteosarcoma—Effects of cisplatinum, BCD, and fibroblast interferon in sequential combination with HD-MTX and adriamycin. *Journal of Cancer Research and Clinical Oncology*, *106*, 1–7.
91. Stanton, G. J., Weigent, D. A., Fleischmann, W. R. J., Dianzani, F., & Baron, S. (1987). Interferon review. *Investigative Radiology*, *22*, 259–273.
92. Jonasch, E., & Haluska, F. G. (2001). Interferon in oncological practice: Review of interferon biology, clinical applications, and toxicities. *Oncologist*, *1*, 34–55.
93. Strander, H. (1989). The action of interferon on virus-associated human neoplasms. *Cancer Surveys*, *8*, 755–792.
94. Glasgow, L. A., Crane, J. L. Jr., Kern, E. R., & Youngner, J. S. (1978). Antitumor activity of interferon angainst murine osteogenic sarcoma *in vitro* and *in vivo*. *Cancer Treatment Reports*, *62*, 1881–1888.
95. Glasgow, L. A., & Kern, E. R. (1981). Effect of interferon administration on pulmonary osteogenic sarcomas in an experimental murine model. *Journal of the National Cancer Institute*, *67*, 207–212.

96. Jia, S. F., An, T., Worth, L., & Kleinerman, E. S. (1999). Interferon-alpha Enhances the Sensitivity of Human Osteosarcoma Cells to Etoposide. *Journal of Interferon and Cytokine Research*, *19*, 617–624.
97. Yuan, X.-w., Zhu, X.-f., Huang, X.-f., Sheng, P.-y., He, A.-s., Yang, Z.-b., et al. (2007). Interferon-a enhances sensitivity of human osteosarcoma U2OS cells to doxorubicin by p53-dependent apoptosis. *Acta Pharmacologica Sinica*, *28*, 1835–1841.
98. Inaba, H., Glibetic, M., Buck, S., Ravindranath, Y., & Kaplan, J. (2004). Interferon-alpha sensitizes osteosarcoma cells to Fas-induced apoptosis by up-regulating Fas receptors and caspase-8. *Pediatrica Blood Cancer*, *43*, 729–736.
99. Waldmann, T. A. (2006). The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nature reviews. Immunology*, *6*, 595–601.
100. Waldmann, T. A., & Tagaya, Y. (1999). The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annual Review of Immunology*, *17*, 19–49.
101. Luksch, R., Perotti, D., Cefalo, G., Gambacorti Passerini, C., Massimino, M., Spreafico, F., et al. (2003). Immunomodulation in a treatment program including pre- and post-operative interleukin-2 and chemotherapy for childhood osteosarcoma. *Tumorigenesis*, *89*, 263–268.
102. Schwartz, Y., Avraham, R., Benish, M., Rosenne, E., & Ben-Eliyahu, S. (2008). Prophylactic IL-12 treatment reduces postoperative metastasis: mediation by increased numbers but not cytotoxicity of NK cells. *Breast Cancer Research and Treatment*, *107*, 211–223.
103. Nardin, A., Lefebvre, M. L., Labroquere, K., Faure, O., & Abastado, J. P. (2006). Liposomal muramyl tripeptide phosphatidylethanolamine: Targeting and activating macrophages for adjuvant treatment of osteosarcoma. *Current Cancer Drug Targets*, *6*, 123–133.
104. Chawla, S. P., Tolcher, A. W., Staddon, A. P., Schuetze, S. M., D'Amato, G. Z., Blay, J. Y., et al. (2006). Updated results of a phase II trial of AP23573, a novel mTOR inhibitor, in patients (pts) with advanced soft tissue or bone sarcomas (Abstract). *Journal of Clinical Oncology, ASCO Annual Meeting Proceedings*, Part I., 24 (June 20 Supplement), Abstract 9505.
105. Quan, G. M. Y., & Choong, P. F. M. (2006). Anti-angiogenic therapy for osteosarcoma. *Cancer Metastasis Review*, *25*, 707–713.
106. Folkman, J. (2004). Endogenous angiogenesis inhibitors. *Acta pathologica, microbiologica, immunologica Scandinavica*, *112*, 496–507.
107. Tjin Tham Sjin, R. M., Naspinski, J., Birsner, A. E., Li, C., Chan, R., Lo, K. M., et al. (2006). Endostatin therapy reveals a U-shaped curve for antitumor activity. *Cancer Gene Therapy*, *13*, 619–627.
108. Mohammed, R. A., Green, A., El-Shikh, S., Paish, E. C., Ellis, I. O., & Martin, S. G. (2007). Prognostic significance of vascular endothelial cell growth factors -A, -C and -D in breast cancer and their relationship with angio- and lymphangiogenesis. *British Journal of Cancer*, *96*, 1092–1100.
109. Kaya, M., Wada, T., Akatsuka, T., Kawaguchi, S., Nagoya, S., Shindoh, M., et al. (2000). Vascular endothelial growth factor expression in untreated osteosarcoma is predictive of pulmonary metastasis and poor prognosis. *Clinical Cancer Research*, *6*, 572–577.
110. Hara, H., Akisue, T., Fujimoto, T., Imabori, M., Kawamoto, T., Kuroda, R., et al. (2006). Expression of VEGF and its receptors and angiogenesis in bone and soft tissue tumors. *Anticancer Research*, *26*, 4307–4311.
111. Mantadakis, E., Kim, G., Reisch, J., McHard, K., Maale, G., Leavey, P. J., et al. (2001). Lack of prognostic significance of intratumoral angiogenesis in non metastatic osteosarcoma. *Journal of Pediatric Hematology/Oncology*, *23*, 286–289.
112. Ek, E. T. H., Ojaimi, J., Kitagawa, Y., & Choong, P. F. M. (2006). Does the degree of intratumoral microvessel density and VEGF expression have prognostic significance in osteosarcoma? *Oncology Reports*, *16*, 17–23.
113. Kreuter, M., Bieker, R., Bielack, S. S., Auras, T., Buerger, H., Gosheger, G., et al. (2004). Prognostic relevance of increased angiogenesis in osteosarcoma. *Clinical Cancer Research*, *10*, 8531–8537.
114. Dawson, D. W., Volpert, O. V., Gillis, P., Crawford, S. E., Xu, H., & Benedict, W. (1999). Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science*, *285*, 245–248.
115. Volpert, O. V., Zaichuk, T., Zhou, W., Reiher, F., Ferguson, T. A., & Stuart, P. M. (2002). Inducer-stimulated Fas targets activates endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor. *Nature Medicine*, *8*, 349–357.
116. Cai, J., Jiang, W. G., Grant, M. B., & Boulton, M. (2006). Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of VEGFR-1. *Journal of biological chemistry*, *281*, 3604–3613.
117. Pignolo, R. J., Francis, M. K., Rotenberg, M. O., & Cristofalo, V. J. (2003). Putative role for EPC-1/PEDF in the growth arrest of human diploid fibroblasts. *Journal of cellular physiology*, *195*, 12–20.
118. Abe, R., Shimizu, T., Yamagishi, S., Shibaki, A., Amano, S., & Inagaki, Y. (2004). Overexpression of pigment epithelium-derived factor decreases angiogenesis and inhibits the growth of human malignant melanoma cells *in vivo*. *The American Journal of Pathology*, *164*, 1225–1232.
119. Foukas, A. F., Deshmukh, N. S., Grimer, R. J., Mangham, D. C., Mangos, E. G., & Taylor, S. (2002). Stage-IIB osteosarcoma around the knee. A study of MMP-9 in surviving tumour cells. *The Journal of Bone and Joint Surgery, British volume*, *84*, 706–711.
120. Vinodhkumar, R., Song, Y. S., Ravikumar, V., Ramakrishnan, G., & Devaki, T. (2007). Depsipeptide a histone deacetylase inhibitor down regulates levels of matrix metalloproteinases 2 and 9 mRNA and protein expressions in lung cancer cells (A549). *Chemico-Biological Interactions*, *165*, 220–229.
121. Shin, C. Y., Lee, W. J., Choi, J. W., Choi, M. S., Ryu, J. R., Oh, S. J., et al. (2007). Down-regulation of matrix metalloproteinase-9 expression by nitric oxide in lipopolysaccharide-stimulated rat primary astrocytes. *Nitric Oxide*, *16*, 425–432.
122. Kang, H. G., Kim, H. S., Kim, K. J., Oh, J. H., Lee, M. R., Seol, S. M., et al. (2007). RECK expression in osteosarcoma: correlation with matrix metalloproteinases activation and tumor invasiveness. *Journal of Orthopaedic Research*, *25*, 696–702.
123. Takahashi, C., Sheng, Z., Horan, T. P., Kitayama, H., Maki, M., Hitomi, K., et al. (1998). Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 13221–13226.
124. Oh, J. H., Takahashi, R., Kondo, S., Mizoguchi, A., Adachi, E., Sasahara, R. M., et al. (2001). The membrane-anchored MMP inhibitor RECK is a key regulator of extracellular matrix integrity and angiogenesis. *Cell*, *107*, 789–800.
125. Choong, P. F. M., & Nadesapillai, A. P. W. (2003). Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. *Clinical orthopaedics and related research*, *415*(Suppl), S46–S58.
126. Choong, P. F. M., Ferno, M., Akerman, M., Willen, H., Langstrom, E., Gustafson, P., et al. (1996). Urokinase-

plasminogen-activator levels and prognosis in 69 soft-tissue sarcomas. *International Journal of Cancer*, *69*, 268–273.
127. Dass, C. R., Nadesapillai, A. P. W., Robin, D., Howard, M. L., Fisher, J. L., Zhou, H., et al. (2005). Downregulation of uPAR cofirms link in growth and metatasis of osteosarcoma. *Clinical and Experimental Metastasis*, *22*, 643–652.
128. Dass, C. R., & Choong, P. F. M. (2008). uPAR mediates anticancer activity of PEDF. *Cancer Biology and Therapy*, *7*, 1262–1270.
129. Baldini, N., Scotlandi, K., Serra, M., Picci, P., Bacci, G., Sottili, S., et al. (1999). P-glycoprotein expression in osteosarcoma: a basic for risk-adapted adjuvant chemotherapy. *Journal of Orthopaedic Research*, *17*, 629–632.
130. Chan, H. S., Grogan, T. M., Haddad, G., DeBoer, G., & Ling, V. (1997). P-glycoprotein expression: critical determinant in the response to osteosarcoma chemotherapy. *Journal of the National Cancer Institute*, *89*, 1706–1715.
131. Laverdiere, C., Hoang, B. H., Yang, R., Sowers, R., Qin, J., Meyers, P. A., et al. (2005). Messenger RNA expression levels of CXCR4 correlate with metastatic behavior and outcome in patients with osteosarcoma. *Clinical Cancer Research*, *11*, 2561–2567.
132. Perissinotto, E., Cavalloni, G., Leone, F., Fonsato, V., Mitola, S., Grignani, G., et al. (2005). Involvement of chemokine receptor 4/stromal cell-derived factor 1 system during osteosarcoma tumor progression. *Clinical Cancer Research*, *11*(2 Pt 1), 490–497.
133. Teodoro, J. G., Evans, S. K., & Green, M. R. (2007). Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *Journal of Molecular Medicine*, *85*, 1175–1186.
134. Miller, C. W., Aslo, A., Won, A., Tan, M., Lampkin, B., & Koeffler, H. P. (1996). Alterations of the p53, Rb and MDM2 genes in osteosarcoma. *Journal of Cancer Research and Clinical Oncology*, *122*, 559–565.
135. McIntyre, J. F., Smith-Sorensen, B., Friend, S. H., Kassel, J., Borresen, A. L., Yan, Y. X., et al. (1994). Germline mutations of the p53 tumor suppressor gene in children with osteosarcoma. *Journal Clinical Oncology*, *12*, 925–930.
136. Nakase, M., Inui, M., Okumura, K., Kamei, T., Nakamura, S., & Tagawa, T. (2005). p53 gene therapy of human osteosarcoma using a transferrin-modified cationic liposome. *Molecular Cancer Therapeutics*, *4*, 625–631.
137. Densmore, C. L., Kleinerman, E. S., Gautam, A., Jia, S.-F., Xu, B., Worth, L. L., et al. (2001). Growth suppression of established human osteosarcoma lung metastases in mice by aerosol gene therapy with PEI-p53 complexes. *Cancer Gene Therapy*, *8*, 619–627.
138. Onda, M., Matsuda, S., Higaki, S., Ijima, T., Fukushima, J., Yokokura, A., et al. (1996). ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. *Cancer*, *77*, 71–78.
139. Zhou, H., Randall, R. L., Brothman, A. R., Maxwell, T., Coffin, C. M., & Goldsby, R. E. (2003). Her-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification. *Journal of Pediatric Hematology/Oncology*, *25*, 27–32.
140. US-National-Cancer-Institute. (2001). Phase II Study of Chemotherapy With or Without Trastuzumab (Herceptin®) in Patients With Metastatic Osteosarcoma. http://www.cancer.gov/clinicaltrials/COG-AOST0121. Accessed 30-09 2008.
141. Hunter, K. W. (2004). Ezrin, a key component in tumor metastasis. *Trends in Molecular Medicine*, *10*, 201–204.
142. Khanna, C., Wan, X., Bose, S., Cassaday, R., Olomu, O., Mendoza, A., et al. (2004). The membrane-cyto-skeleton linker ezrin is necessary for osteosarcoma metastasis. *Nature Medicine*, *10*, 182–186.
143. Wan, X., Mendoza, A., Khanna, C., & Helman, L. J. (2005). Rapamycin inhibits ezrin-mediated metastatic behaviour in a murine model of osteosarcoma. *Cancer Research*, *65*, 2406–2411.
144. Pasquini, G. M., Davey, R. A., Ho, P. W., Michelangeli, V. P., Grill, V., Kaczmarczyk, S. J., & Zajac, J. D. (2002). Local secretion of parathyroid hormone-related protein by an osteoblastic osteosarcoma (UMR 106–01) cell line results in growth inhibition. *Bone*, *31*, 598–605.
145. Yang, R., Hoang, B. H., Kubo, T., Kawano, H., Chou, A., Sowers, R., et al. (2007). Over-expression of parathyroid hormone Type 1 receptor confers an aggressive phenotype in osteosarcoma. *International Journal of Cancer*, *121*, 943–954.
146. Dass, C. R., & Choong, P. F. (2008). C-jun: pharmaceutical target for DNAzyme therapy of multiple pathologies. *Pharmazie*, *63*, 411–414.
147. Bohmann, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K., & Tjian, R. (1987). Human proto-oncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science*, *238*, 1386–1392.
148. Dass, C. R., Khachigian, L. M., & Choong, P. F. M. (2008). c-Jun is critical for the progression of osteosarcoma: proof in an orthotopic spontaneously metastasizing model. *Molecular Cancer Research*, *6*, 1289–1292.
149. Dass, C. R., Friedhuber, A. M., Khachigian, L. M., Dunstan, D. E., & Choong, P. F. (2008). Downregulation of c-jun results in apoptosis-mediated anti-osteosarcoma activity in an orthotopic model. *Cancer biology & therapy*, *7*, 1033–1036.
150. Dass, C. R., Friedhuber, A. M., Khachigian, L. M., Dunstan, D. E., & Choong, P. F. (2008). Biocompatible chitosan-DNAzyme nanoparticle exhibits enhanced biological activity. *Journal of Microencapsulation*, *25*, 421–425.
151. Dass, C. R., Khachigian, L. M., & Choong, P. F. M. (2008). c-Jun knockdown sensitizes osteosarcoma to doxorubicin. *Molecular Cancer Therapeutics*, *7*, 1909–1912.
152. MacEwen, E. G., Pastor, J., Kutzke, J., Tsan, R., Kurzman, I. D., Thamm, D. H., et al. (2004). IGF-1 receptor contributes to the malignant phenotype in human and canine osteosarcoma. *Journal of Cellular Biochemistry*, *92*, 77–91.
153. Sekyi-Otu, A., Bell, R. S., Ohashi, C., Pollak, M., & Andrulis, I. L. (1995). Insulin-like growth factor 1 (IGF-1) receptors, IGF-1, and IGF-2 are expressed in primary human sarcomas. *Cancer Research*, *55*, 129–134.
154. Pollak, M., Sem, A. W., Richard, M., Tetenes, E., & Bell, R. (1992). Inhibition of metastatic behavior of murine osteosarcoma by hypophysectomy. *Journal of the National Cancer Institute*, *84*, 966–971.
155. Burrow, S., Andrulis, I. L., Pollak, M., & Bell, R. S. (1998). Expression of insulin-like growth factor receptor, IGF-1, and IGF-2 in primary and metastatic osteosarcoma. *Journal of Surgical Oncology*, *69*, 21–27.
156. Frincu-Mallos, C. (2007). Novel anti-IGF-1 receptor drug, R1507, shows potential in sarcoma patients. http://www.pslgroup.com/dg/216426.htm. Accessed 30/09 2008.
157. Pollak, M., Sem, A. W., Richard, M., Tetenes, E., & Bell, R. (1992). Inhibition of metastatic behavior of murine osteosarcoma by hypophysectomy. *Journal of the National Cancer Institute*, *84*, 966–971.
158. Mansky, P. J., Liewehr, D. J., Steinberg, S. M., Chrousos, G. P., Avila, N. A., Long, L., et al. (2002). Treatment of metastatic osteosarcoma with the somatostatin analog OncoLar: significant reduction of insulin-like growth factor-1 serum levels. *Journal of Pediatric Hematology/Oncology*, *24*, 440–446.