1 Introduction

Tumors of bone are among the most uncommon of all types of neoplasms. 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. OS is a malignant bone tumor characterized by spindle cells producing osteoid. This article will provide a compre- hensive review from the biology of OS and current treatments to new potential therapies for this disease.

2 Demographics

OS is the most common primary solid tumor of bone, comprising about 20% of primary bone sarcomas. Classi- fication of tumors found in bone is summarized in Table 1 based on Mayo Clinic patients (USA) until the end of 1993. 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 is expressed as comparative rates in Table 2. OS is the most common primary bone tumor in childhood and adolescence.

before 5 years of age and infrequent up to the age of 10 years, the extraordinary peak in incidence occurring in the early teenage years is quickly followed by a rapid decline. According to data from the Mayo Clinic, 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. It also represents the second highest cause of

cancer-related death in this age group. In America, approximate 400 new OS cases in children younger than 20 years are reported per year. In Australia, an average of nine to ten cases of OS is diagnosed each year in children under the age of 15. 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. 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. 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.

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. In fact, the peak of incidence of OS is during puberty when the growth spurt is highest. 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. Two recent studies showed that young OS patients were taller than the normal population of the same age group. Exposure to radiation is the only proven exogenous risk factor but with a long interval-10-20 years. Thus, radiation-induced OS is typical of adult age and is rare. Radiation is implicated in approximately 2% of OSs. Paget's disease is known to be associated with a higher incidence of OS. It is also suggested that metallic ions may predispose a person to develop OS. Preceding trauma in the involved bone was found in some OS cases; however, no etiologic relationship with trauma has been found. Numerous recent studies described cytogenetic abnor- malities which are both numerical and structural in OS. Common numerical abnormalities include: gain of chromo- some 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 syn- drome were reported relating to some cases of OS as well. In 1966, Finkel, Biskis, and Jinkins (FBJ) suggested that virus could induce bone sarcomas in animals. 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, which has been found to be associated with a poor response to chemotherapy in patients with

OS. 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. However, long- term follow-up studies have not revealed recipients of SV40- contaminated poliovirus vaccines to be at an increased risk of cancer. 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. 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. 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-ß 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, 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. Pain usually arises after strenuous exercise or a trauma and usually appearing 2-4 months before diagnosis 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. 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. 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. These densities often extend into the contiguous soft tissues. The soft tissue extension may show cloudlike (shadow) radio- densities 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. Some tumors spread in the marrow cavity for surpris- ingly great distance, but most tumors do not spread in the marrow beyond their gross extraosseous limits. 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. Mild anemia may also be present at diagnosis. Furthermore, the erythrocyte sedimentation rate is often high and increases in the presence of relapse. In the absence of metastases, abnormal AP values are correlated with tumor volume and prognosis. Poor prognosis is associated with high AP values. A normal pretreatment serum AP level resulted in a significantly higher 5-year disease free survival (67%) than in patients with higher levels (54%). Patients with normal serum AP also had a significantly longer time to recurrence (25 versus 18 months). Sedimentation rate, C-reactive protein, and lactate dehy- drogenase (LDH) levels may also be elevated. LDH, when elevated, confers a worse prognosis, presumably by indicating a more biologically aggressive tumor. An isotope scan with technetium or thallium 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. 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. 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. Improperly performed biopsies are a frequent cause of misdiagnosis, amputation, and local recurrence, and they may have a negative effect on survival. 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. Currently, WHO recognizes three major histologic subtypes of OS: osteoblastic, fibroblastic, and chondroblastic, reflecting the predominant type of matrix (osteoid, fibrous, or chondroid matrix). It was found that patients with the osteoblastic and chondroblastic histologic patterns tend to have a worse prognosis.

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. 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. 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. In the group of axial location of OS, pelvic OSs account for approximately 7- 9% of all OSs, and spine OSs occur at 0.85-3%. OS occasionally arises in soft tissue, thyroid gland, heart, kidney, uterus, or lung. 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%. 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.

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. 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. Tumors with ATV of less than 150 cm3 exhibits a 92% 5-year metastasis-free survival compared with 58% in tumors with an ATV greater than 150 cm3.

7 Tumor stage

Staging is performed based on the aggressive grade and the extensive and the spread levels of OS. According to the Musculoskeletal Tumor Society Staging System and Enneking System, 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). The staging system is reported in Table 3. Most conven- tional

OSs present as stage IIB tumors which is non- metastatic tumor with an associated soft tissue mass. Stage I-A exhibits nearly 100% 5-year survival rate. 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%. 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. 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 metasta- ses.

8 Metastases and local recurrence

OS usually metastasizes to the lungs or other bones. 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. 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". Skip metastases and regional lymph node metastases are rare with less than 10% each. Distant bone metastases represent the latest stage of disease and are rarely associated with the poorest prognosis. Other metastatic sites at diagnosis are very uncommon. 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. 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). Eighty percent of patients died from metastatic diseases, most commonly in the lungs. Pulmonary metastases which are found at initial diagnosis are generally thought to be associated with a poor outcome. 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. It was found that all patients with local recurrence (LR) also developed lung metatasis at some stage in the course of disease. 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. 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. The survival rate was improved by postoperative radiotherapy in addition to surgery with long-term survival of approximately 50%. The rate dramatically increased to approximately 60- 70% once systemic multiagent chemotherapy followed by surgery has been introduced. 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. Currently, although chemotherapy is undoubtedly the method that is likely to cure the greatest proportion of patients, surgery remains an essential part of the manage- ment 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. 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. 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. 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. Amputa tion is the only safe way of surgery especially in patients with extensive soft tissue components. However, because most tumors arise around the knee joint, amputa tion 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 effective ness of chemotherapy. Currently, amputation has become a more infrequent choice of surgery due to improved adjuvant therapies, operative techniques, and diagnostic methods. 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 r 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. However, a previous study found no differences in survival rate between patients receiving radiotherapy prior to biopsy and historic controls, which discouraged additional inves tigation

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 252 Cancer Metastasis Rev (2009) 28:247–263 reduce tumor viability before surgery, increase the proba bility of performing limb-sparing surgery, and reduce the risk of local recurrence. High-dose irr 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%). 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 theoret ical advantages compared to limb-sparing methods. A major advantage is the precise anatomic fit of the reimplanted bone segment. It avoids the growth discrepan cy 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. Araki et al. reported the use of extracorporeal irradiation (50 Gy) to treat patients with OS. There was no evidence benefit of prethoracotomy or postthoracotomy irradiation was seen. 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. However, a study suggested that successful metastasectomy was possible more often after previous prophylactic lung irradiation than after adjuvant chemotherapy

9.3 Chemotherapy

9.3.1 Prerelapse treatment

OS is one of the first solid tumors for which adjuvant chemotherapy proved to be beneficial. Advances in chemotherapy over the past 30 years have improved limb

salvage and led to higher survival rates. 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 b tive 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. In pulmonary metastasec tomies, 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%. 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, cyclophospha mide, and actinomycin D showed a 55% 2-year disease-free survival rate while patients who received no adjuvant chemotherapy had a 20% survival rate. Link et al. report of chemotherapy. In T10 protocol, the selection of postop erative adjuvant chemotherapy was based on the response of the primary tumor to preoperative therapy. These patients received intensified preoperative intraarterial cisplatin. Post operatively, good responders received adriamycin and cisplatin (or dacarbazine), and poor responders received methotrexate, adriamycin or dacarbazine, bleomycin, and cyclophosphamide or actinomycin. The Children Can cer Group (CCG) in Indianapolis (USA) confirmed the preliminary good results of the T10 protocol. 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 N 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%, respec tively. 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%). Despite previous attempts to improve the outcome of poor responders by modifying the postoperative chemo therapy, 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 trexate, doxorubicin, and cisplatin. Twenty-nine percent of patients in both regimens had 90% tumor necrosis n 254 Cancer Metastasis Rev (2009) 28:247–263 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. Recently, most chemotherapy regimens applied for OS have been based on methotrexate, cisplatin, doxorubicin, and ifosfamide. The mechanisms of action of these chemotherapeutic agents are summarized in Table 4. A study from The German–Austria-Swiss Cooperative Oste osarcoma Study Group (COSS) showed that patients treated with these four drugs presented the best results with a 10-year survival rate of 71%. The Italian and Scandinavian Sarcoma Group attempted to im 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. 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. This conclusion is supported by the results of the European Osteosarcoma Intergroup's and the Italian and Scandinavian Sarcoma Group's studies. 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 chemotherapeut 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. 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). 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 survived for 5 years; however, it must be taken into account that these patients had bigger and inoperable disease. Patients who are not eligible for metastasectomy are candidates for palliative chemotherapy. 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 rapamy cin (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. Generally, patients who relapse following the use of modern treatment approaches including against murine osteosarcoma were also reported. Zoledronic inhibited the growth of OS cells and chemo sentisize these cells to cisplatin. 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. Bisphosphonates induce apoptosis by caspase-3-like protease activation and significantly reduce cell invasion through zinc chelation of metalloproteinase enzymes. A new interest is growing around the field of immuno modulation and its applicability to OS. Interferon (IFN) has been employed in some studies since 1980s and showed promising results. It was administered alone or in combination with chemotherapy. IFNs are pro duced by the cells of the immune system of most vertebrates in response to challenges by foreign agents such as viruses, parasites, and tumor cells. IFNs belong to the large class of glycoprote caspase-8. 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 immuno therapy 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. In a treatment program including pre- and postoper ative IL-2 and chemotherapy for childhood OS, NK counts and activity significantly correlated with clinical outcome. 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. 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. There are studies utilizing antiangiogenic agents such as angiostatin, avastin, and endostatin 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 found that high VEGF levels correlated with increased MVD, increased frequency of metastases, and reduced ove normally involved in the breakdown of the extracellular matrix within the context of physiological tissue remodel ing and angiogenesis. 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. 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, nitric oxide, and reversion inducing cystein-rich protein with Kazal motifs (RECK). RECK RECK is a membrane-bound protein which was first discovered and identified by Takahashi et al. and reported in 1998. It is able to inhibit MMP-9, MMP-2, and MT1-MMP. RECK also has an important role in controlling angiogenesis, which has bee led to a synergistic effect in an animal model of OS. P-glycoprotein P-glycoprotein (P-gp) is a protein responsi ble for energy-dependent drug efflux and encoded by the multiple drug-resistant-1 gene. 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. However, P-gp does not correlate with the level of post-chemotherapy tumor necrosis, 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 corre sponding ligand, stromal cell-derived factor 1 (SDF-1), play a major role in the metastatic process. It has been found that CXCR4/SDF-1 system significantly c therapy in a human OS cell line using a transferring modified cationic liposome, which resulted in a significant inhibition of tumor growth. Another study of gene therapy with polyethyleneimine-p53 complexes showed significant growth suppression of established human OS lung metastases in mice. ErbB-2 ErbB-2 or Her-2/neu is a transmembrane glycopro tein 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. Some studies found that the presence of ErbB-2 protein in OSs significantly correlate with reduced survival, increased metastases, and poor outcome. However, another study concluded conversely. Her ceptin, a drug blocking ErbB-2, has been used successfully in breast cancer clinically and in other cancers in vitro. Herceptin is a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor, which targets the epider mal growth factor 2. A clinical tria of PTHrP in a rat OS cell line (osteoblastic) was found to reduce cell proliferation by 80%. However, over expression of PTHR1 in the HOS OS cell line resulted in the increased proliferation, motility, and invasion of cells through Matrigel. c-Jun c-Jun is an oncogene encoding a basic region-leucine zipper protein which, in combination with c-Fos protein, forms the activator protein-1 early response transcription factor. 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) encapsulated in a cationic multilamellar vesicle liposome. 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. It regressed the growth and metastasis of preest consists of various combinations of surgery and chemo therapy. Cisplatin, doxorubicin, ifosfamine, and metho trexate 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 knowl edge. 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.