New Medical/Biologic Paradigms in the Treatment of Bone Tumors

Peter F. M. Choong

Published online: 9 May 2014
© Springer Science + Business Media New York 2014

Abstract Primary bone tumors are rare, but their treatments have enjoyed considerable improvements in life expectancy because of chemotherapy. When combined with advances in surgery, patients can now expect lives and limbs to behave with greater longevity and normality. The challenge now is how to raise the plateau of survival, and that means finding a successful way to treat metastatic or unsalvageable local disease. It is clear that conventional chemotherapy has limitations and that the answer may lie in a better understanding of the intricate molecular workings of cancer and the pathways that lead to the latter's growth, invasion, and dissemination. There have been great strides made in dissecting out the metastatic cascade with numerous important pathways and molecular switches identified. This has led to the introduction of a number of new drugs and molecules aimed at targeting the molecular vulnerabilities of cancer, allowing greater control of growth, invasion, and metastasis. Some of these have had a large impact on musculoskeletal malignancies. Future successes in treatment will require multimodal targeting of tumors whose signatures will be a composite of histologic

and molecular markers that will pave the way for personalization of treatment strategies.

Keywords Bone tumors · Medical paradigm · Biologic paradigm · Surgery · Sarcoma · Resection · Reconstruction · Extracorporeal irradiation · Chemotherapy · VEGF · PEDF · Matrix modulation · Immune modulation · Gene therapy

Introduction

A primary bone tumor's local extent or its predilection for systemic spread determines how its treatment is addressed. The challenge of treating bone tumors is the removal of the tumor in its entirety, which has implications for local and systemic disease. For local tumors, this will require resection with as low a risk of recurrence as is possible, and the aggressiveness of the approach will be dependent on the tumor's benign or malignant status. For tumors with systemic spread, this almost always implies malignancy, and the foundation of treatment is chemotherapy. Osteosarcoma is the archetypal primary malignant bone tumor (sarcoma) around which treatment strategies have been developed and applied to other histological types. This review examines the development of multimodality care and novel strategies based on limb sparing techniques and targeted molecular processes for the management of malignant tumors of bone.

Limb-Sparing Surgery

Considerable advances have occurred in sarcoma surgery over the last three decades [1]. The key to curing bone sarcoma is

This article is part of the Topical Collection on *Ortho-oncology*.

P. F. M. Choong (✉)
Department of Surgery, St. Vincent's Hospital Melbourne,
University of Melbourne, Clinical Sciences Building, Level 2, 29
Regent Street, Fitzroy, VIC 3065, Australia
e-mail: pchoong@unimelb.edu.au

P. F. M. Choong
Department of Orthopaedics, St. Vincent's Hospital, Melbourne,
VIC, Australia

P. F. M. Choong
Bone and Soft Tissue Sarcoma Service, Peter MacCallum
Cancer Centre, East Melbourne, VIC, Australia

total removal of the tumor. Surgery, as applied to the primary tumor, is a critical component of treatment. Prior to the 1970s, the sole surgical treatment was amputation of the affected limb. Despite this, however, the survival rate for osteosarcoma was dismal, with the majority of patients succumbing to distant metastases despite ablative surgery. This led to the “Cade” protocol, whereby patients with osteosarcoma were treated with local radiotherapy and those who survived 12 months were then subjected to amputation [2]. The advent of chemotherapy (see below) gave rise to the era of limb-sparing surgery, led historically by allograft arthrodesis after resection in the skeletally mature and rotationplasty in the lower limb for tumors about the knee in the pediatric age group. Later advances in joint arthroplasty gave rise to more complex reconstructions including mega-prosthetic replacements.

Principles

With the birth of limb-sparing surgery, came the establishment of the principles of sarcoma surgery. These included

- (1) Resection with oncologic margins
- (2) Reconstruction should be durable and allow a functional limb
- (3) Closure of the soft tissue defect must be possible

Margins

Initially, the accuracy of imaging techniques limited surgeons’ abilities to accurately assess (1) the soft tissue component and its relationship to vital neurovascular and surrounding soft tissue structures, (2) the intraosseous extent of the tumor, and (3) the amount of bone destruction. Because of this, early attempts at limb-sparing surgery were associated with high recurrence rates underscoring the importance of oncologically sound surgical margins. With a classification of gross surgical margins available [3], surgeons were soon able to start planning tumor resections and the necessary reconstructions required to preserve a functional limb. Typically, the margins were widely clear of the tumor, but, as improvements in diagnostic imaging and adjuvant therapy took place, surgeons were able to achieve good local control of disease with margins that were considerably narrower than first recommended. With greater experience and the maturation of the concept of limb-sparing surgery, newer classification systems highlighted the relationship between tissue type and the quality of surgical margins [4]. More recently, optimal adjuvant therapy, such as with ionizing radiation or chemotherapy, has led to the concept of microscopic margins [5].

Reconstructions

Over the last 30 years, reconstructions after sarcoma resection have been prosthetic and biologic, or the combination of these [6]. With increasing experience, the success of these reconstructive techniques has increased, although advances as such have been more in terms of the complexity of the cases to which these techniques have been applied rather than significant paradigm changes in prostheses (Fig. 1). Notwithstanding this, the pursuit of limb-sparing surgery also meant a conscious move to preserve bone stock as surgeons realized that patient survival placed greater demands on the limb, and preserving the limb either through a durable reconstruction or maintaining the opportunity for further procedures usually mandated good bone stock. There has been a move to preserve bone or minimize surgery by the use of non-conventional adjuvant therapies as discussed below.

Extracorporeal Irradiation

Conventional photon therapy is limited to specific histotypes such as Ewing’s sarcoma. Osteosarcoma and chondrosarcoma are essentially radioresistant, and the use of radiotherapy in these tumor types is often restricted to palliation. Extracorporeal irradiation (ECI) is a technique whereby resected bone is subjected *ex vivo* to lethal doses of ionizing irradiation before being replanted. First described in the late 1960s, ECI has seen a resurgence in popularity. This technique calls for resection with oncologic margins, removal of soft tissue and tumor, and then submission to the radiation oncologists for treatment by a single high dose fraction of irradiation (average 50 cGy) over 20 min (Fig. 2). The equivalent dose is almost 20 times higher than would be subjected to the limb in a preoperative or postoperative setting [7]. Replantation of bone irradiated via this manner then allows intercalary [8] or joint reconstruction [9]. The advantages of ECI include near-perfect anatomic matching of bone with the defect, a low infection rate, and minimal immune-rejection. The results of extracorporeal irradiation have been highly promising with low recurrence rates [9]. Non-union and dissolution are complications of radiation-induced osteonecrosis but seem to be well controlled by rigid and robust internal fixation. More recently, some authors have drawn caution to local recurrence using this method because of the potential for radio-resistance and the survival of more hardy clones of cells, particularly cartilaginous tumors [10].

Liquid Nitrogen

Immersing the tumor-bearing bone in liquid nitrogen while still maintaining the attachment of the bone to the body is a

Fig. 1 **a** Sagittal MRI of a 42-year-old male with sacral chordoma involving majority of sacrum. **b** Resection specimen from total sacrectomy including en bloc with rectum. **c** Reconstruction of defect

after total sacrectomy with bilateral A-frame fibular autografts, transverse humeral allograft and rod and pedicle screw fixation

Fig. 2 **a** Coronal MRI of a 49-year-old man with right periacetabular chondrosarcoma. **b** Internal hemipelvectomy specimen. **c** Curettage of chondrosarcoma from periacetabulum prior to extracorporeal irradiation. **d** Resection specimen within specimen container receiving

external beam radiation. **e** Plain antero-posterior radiograph showing replantation of autograft periacetabular bone internally fixed with plate and screw fixation, and total joint replacement

recent advance [11, 12] of the well-established technique of cryotherapy [13–15]. The extent to which neoplastic tissue is destroyed is similar to extracorporeal irradiation, but has the advantage that the tumorous bone is not truly resected from the body but rather left attached to that part of the limb in which the tumor has arisen. This technique is more suited to tumors adjacent to joints, allowing joint replacement to follow the cryotherapy procedure [12]. Reports of this technique have highlighted a low recurrence rate and an acceptable fracture rate through bone treated via cryotherapy. The use of stemmed implants to reduce the risk of pathologic fracture has been advantageous. A recent finding is that cryotherapy may induce an immunological reaction in tumor tissue that is synergistic with conventional chemotherapy [11, 16].

Heavy Ion Irradiation

Limited sites around the world offer heavy ion (carbon ions or protons) radiotherapy to sarcoma, which is usually reserved for unresectable disease or after incomplete resection [17]. The efficacy of this technique has been attributed to the higher physical selectivity of heavy ions as compared to photons [18, 19]. When used as a sole modality in unresectable tumors such as chordoma, or in combination with conventional chemotherapy for pelvic or vertebral osteosarcoma, respectable local control rates around 70 % at 3 years and 60 % at 5 years were reported [17, 20, 21••].

Chemotherapy

The efficacy of chemotherapy was first reported in patients with metastatic osteosarcoma [22] and was subsequently applied to other chemo-sensitive bone sarcomas. Translation of this experience to patients with non-metastatic disease strongly suggested superiority of combined surgery and chemotherapy over surgery alone [23]. Doxorubicin and methotrexate were the first drugs successfully employed for this therapy, and these were later joined by cisplatin and ifosfamide [24]. With the advent of chemotherapy for osteosarcoma, what was previously a fatal disease now saw a profound improvement in survival with the high likelihood for cure in many patients [24].

The rationale for the move from post-operative chemotherapy (adjuvant) to a pre-operative setting (neoadjuvant) was based on the goal of eradicating micrometastases, destruction of the primary tumor with a reduction in tumor size, and the opportunity to assess the post-surgical specimen for effectiveness of chemotherapy regimes

[25]. Neoadjuvant chemotherapy had a significant impact on the modern management of sarcoma. First, the local effect on the primary tumor was to reduce peritumoral edema, incite a fibrous capsule around the extraosseous component, and reduce the size of the tumor in many cases. For tumors such as Ewing's sarcoma, this latter effect may be profound. With advances in imaging, these chemotherapy-induced changes led to a change in the philosophy of tumor surgery from amputation to limb-sparing techniques.

Challenge Facing Chemotherapy

Despite the efficacy of chemotherapy, an average of 30 % of patients with extremity osteosarcoma continued to succumb to distant metastases [25–27]. Predicting who would develop metastases after chemotherapy then became a critical step in the patient's journey. The ability to accurately determine the response of the tumor to the chemotherapy by its percentage necrosis was a significant achievement [28], and when it was subsequently shown that patients with tumor necrosis greater than 90 % had a better prognosis than patients with less than 90 % tumor necrosis [29], many believed that intensifying therapy (higher doses, prolonged chemotherapy) in the latter high-risk group would improve cure rates. Disappointingly, no studies to date have concluded any significant benefit from intensifying or altering chemotherapeutic agents [27]. This failure is the impetus behind the global drive to develop newer more targeted therapies for osteosarcoma [30].

Understanding the behavior of tumors as they grow, progress, and metastasize has provided key information for the development of anti-tumoral strategies. The metastatic cascade [31], including tumor cell proliferation, cell-cell interactions, tumor-induced angiogenesis, tumor cell chemotaxis, invasion, entry into and emergence from the vascular system, and distant replantation and growth, is highly active in most cancers and has also been well reported in sarcoma. The mechanisms that drive these steps in the cascade are being deciphered and many appear common to a variety of tumor types. The molecular vulnerabilities that these mechanisms pose provide potential targets for novel therapies that may answer the call to improve the survival of sarcoma patients. This journey is not without its pitfalls and, while the elucidation of mechanisms shows promise towards finding solutions to better survival, the myriad of adaptive steps that cancers take and the complex signaling pathway means that no single step in the pathway is likely to be pivotal [32••].

Targeting the Malignant Phenotype

Angioactive Factors

Vascular Endothelial Growth Factor (VEGF)

VEGF is a nascent protein that stimulates microvascular beds to develop. It plays a significant role in the progression of many cancers by increasing their blood supply bringing nourishment and also a means by which the leading edge of tumor may extend further [33]. A number of antiangiogenic agents exist, such as angiostatin [34], avastin, and endostatin [35, 36], which can inhibit the VEGF signaling pathway and abrogate cancer growth. However, the prognostic significance of VEGF and microvascular density in osteosarcoma remains controversial [37, 38•]. Some studies [39, 40] found that high VEGF levels correlated with increased risk of metastases, while some reported an absent or even converse relationship [41, 42].

Pigment Epithelium Derived Factor (PEDF)

PEDF is a 50-kDa multifunctional glycoprotein, which inhibits angiogenesis [33], and in osteosarcoma does this via inhibition of VEGF and induced apoptosis of endothelial cells through the Fas/FasL death pathway [43]. It has an important cell-cycle regulatory role in cell differentiation, proliferation, and apoptosis [44••]. PEDF is more potent than any of the other known angiogenesis inhibitors being twice as potent as angiostatin and more than seven times as potent as endostatin [45]. In vivo models of PEDF overexpression demonstrated suppression of cancer growth, invasion, and metastases [46].

A study showed that osteoclast differentiation, receptor activator of N kappa B ligand (RANKL)-mediated survival, and bone resorption activity was inhibited by PEDF in a dose-dependent manner [47]. PEDF upregulated osteoprotegerin (OPG), which naturally blocks OCL maturation in primary osteoblasts and OCL precursor cells [47]. These results suggest that PEDF inhibits OCL function via regulating OPG expression, and thereby contributes to the maintenance of bone homeostasis. This may be another pathway by which PEDF exerts anti-osteosarcoma activity.

Matrix Modulation

Matrix Metalloproteinases (MMPs)

MMPs are physiologic enzymes involved in the breakdown of the extracellular matrix and are important for tissue remodeling and angiogenesis [48]. Excessive production of certain MMPs has been associated with cancer invasion and metastasis [49••], including osteosarcoma [50]. For

example, osteosarcoma 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 [51]. Sulfated glucosamine, histone deacetylases [120], nitric oxide [121], and reversion-inducing cysteine-rich protein with Kazal motifs (RECK) [52] are substances that can inhibit MMP-9 production and, therefore, reduce invasion and metastasis in cultured cells and animal cancer models.

Reck

RECK is a membrane-bound protein [53], which is able to inhibit MMP-9, MMP-2, and MT1-MMP [54]. By inhibiting MMPs and possibly VEGF, RECK also has an important regulatory role in angiogenesis [54]. Downregulation of RECK in many common tumors [33] and a number of osteosarcoma cell lines [55] is associated with poor outcome. Kang et al. [55] 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.

Urokinase Plasminogen Activator (uPA) and Receptor (uPAR)

uPA is a serine protease inhibitor which upregulates MMPs and promotes the invasion of tumor [56]. When bound to it, receptor uPA becomes active and is upregulated in many common tumors and linked to poor outcome [57••, 58, 59]. In osteosarcoma, an inverse relationship exists between uPA levels and survival time [60] while downregulation of uPAR using antisense clones in an in vivo osteosarcoma model reduced primary growth and inhibition of pulmonary metastases [61]. A combination exposure of uPAR downregulation and PEDF treatment led to a synergistic effect in an animal model of osteosarcoma [62]. uPAR is thought to mediate the action of PEDF [62].

Immune Modulation

Interferon (IFN)

IFN is a cytokine with antiviral activity that can inhibit viral replication within host cells, activate natural killer cells and macrophages, enhance antigen presentation to lymphocytes, and induce resistance by host cells to viral infection. Because of the theory that some osteosarcoma may be associated with virus induction and that IFN levels are noted to be elevated in peripheral blood of patients with high grade osteosarcoma [63••, 64], IFN has been deployed

in trial settings both alone or in combination with chemotherapy [65, 66].

Studies of IFN- α in combination with etoposide [67] and doxorubicin [68] demonstrated enhancement of p53-dependent apoptosis when deployed in a strategy that targeted osteosarcomas with a functioning p53. Others also showed that IFN- α enhanced Fas-induced apoptosis in sensitized cells by upregulating Fas receptors and caspase-8 [69, 70]. This work underpinned the strategy of combined immunotherapy with IFN- γ and anti-Fas monoclonal antibodies or cytotoxic T cells that bore the Fas ligand.

Interleukins (ILs)

ILs are cell signaling cytokines within the immune system. IL-2 is known to facilitate immunoglobulin production by B cells and induce natural killer (NK) proliferation and differentiation [71•]. Combined therapy with IL-2 and chemotherapy delivered in a neoadjuvant and adjuvant setting led to increased counts of NK, which correlated with clinical outcome [72]. IL-2 may have a role in guarding against surgery-induced metastases because it facilitated the strategic localization of NK cells around a tumor.

Established tumors are complex structures, which consist of neoplastic and non-transformed cells. These latter cells include stromal cells, and vascular tree and immune cells. The feature that characterizes the immune cells in tumors is their dysregulation and functional impairment. Strategies that aim to reprogram this aberrant microenvironment might dramatically augment cancer therapies [73]. A better understanding of the cellular constituents of tumors and the mechanisms involved in immune evasion may help guide the next generation of innovative cancer immunotherapies.

GM-CSF

GM-CSF enhances antibody-dependent cellular toxicity, and, when deployed in various tumor models, enhanced the immune response to tumor vaccines as well as NK cell function [74, 75••]. When used together with chemotherapy in soft tissue sarcoma, GM-CSF improved survival in some early studies of adult patients [76]. This finding, however, should be accepted cautiously as a more recent systematic review found that, on the basis of the available evidence, high-dose chemotherapy with growth factor or autologous bone marrow/stem cell transplantation demonstrated no meaningful improvement, such that authors recommended against routine treatment of patients with inoperable, locally advanced, or metastatic soft tissue sarcoma with high-dose chemotherapy with growth factor [75].

The role of aerosolized GM-CSF in metastatic disease is not new. Work with pulmonary metastases from a range of cancers, including renal carcinoma, melanoma, and Ewing's sarcoma, showed partial efficacy [77, 78], and a strategy that delivers aerosolized GM-CSF to the lungs in an experimental animal model demonstrated an inhibitory effect on pulmonary metastases of osteosarcoma [79]. A Children's Oncology Group (COG) trial is currently in progress in pediatric patients.

MTP-PE

MTP-PE is a synthetic lipophilic analog of muramyl dipeptide, a cell wall component of mycobacterium, which is able to stimulate monocytes and macrophages [80]. When encapsulated in liposomes, MTP-PE is preferentially delivered to pulmonary macrophages. Its role as an adjuvant strategy for osteosarcoma has been previously demonstrated [80]. Recently, COG carried out long-term follow-up of the key trial of chemotherapy with or without mifamurtide (liposomal muramyl tripeptide phosphatidyl ethanolamine; L-MTP-PE) [81]. This group demonstrated that addition of L-MTP-PE to chemotherapy significantly improved overall survival at 6 years from 70 % with chemotherapy alone to 78 % with chemotherapy and L-MTP-PE ($p = 0.03$). L-MTP-PE has also been utilized in high-risk, metastatic, or recurrent osteosarcoma. When the metastatic cohort was considered in isolation, the addition of liposomal MTP-PE to chemotherapy did not achieve a statistically significant improvement in outcome [82]. However, the pattern of outcome was similar to the pattern in non-metastatic patients.

Tumor Suppressor Gene Therapy

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 [83]. The tumor suppressor genes, p53 [84] and retinoblastoma (Rb) [85], are pivotal in preventing tumorigenesis, and loss of p53 and Rb functionality can be detected in many sarcomas including osteosarcoma. The inactivation of this gene results in the loss of cell cycle and repair mechanism, and a loss of antiangiogenesis. A number of functional p53 mutations have been identified [86]. Although sporadic, germline p53 mutations do occur rarely and the Li-Fraumeni syndrome is well described [87••].

The efficacy of p53 gene therapy in a human OS cell line in vivo model using a transferring-modified cationic liposome or polyethyleneimine-p53 complexes, resulted in

a significant inhibition of tumor growth [88] and suppression of established human osteosarcoma lung metastases [89]. Recent work with murine models reported the anti-tumor effect of adenoviral vector-mediated p53 gene transfer on the growth of canine osteosarcoma [90].

Genetic techniques have also been useful for reverting chemoresistance [91, 92], acting synergistically with chemotherapy [93], improving delivery [94], and increasing sensitivity of tumor cells to chemotherapy [95–97]. While these and other studies are highlighting the benefits for gene therapy as a future modality, overcoming potentially the most important barrier, poor transfection efficiency, needs to occur for gene therapy to be adopted into practice.

Suicide Gene Therapy

Suicide gene therapy was shown to be effective when the adenoviral vector Ad-osteocalcin (OC)-E1a (OCap1), which contains a murine OC promoter, facilitated viral replication and subsequent tumor lysis in vitro [98]. Since that time, advancement of suicide gene therapy has advanced significantly, and a recent study reported that Rexin-G, a nonreplicative pathology-targeted retroviral vector bearing a cytotoxic cyclin G1 construct, was tested in a Phase I/II study for gemcitabine-resistant pancreatic cancer. This demonstrated a dose-dependent efficacy [99]. Work with neuroendocrine tumors, gliomas, and osteosarcoma show similar efficacy [100–102]. Although there is a growing interest in the use of suicide gene therapy for osteosarcoma, what continues to be highlighted are the use of replication-incompetent viruses in achieving complete tumor kill in vivo, viral-induced toxicity in the host, and vector delivery issues [103].

Cell Signaling

ErbB-2

ErbB-2 or Her-2/neu is a transmembrane glycoprotein produced by the c-erbB-2 gene and plays a significant role in the pathogenesis of breast cancer, but its role in OS is still controversial [33]. Recent studies suggest that this signaling pathway does regulate invasion and migration in vitro [104]. Some studies found that the presence of ErbB-2 protein in OSs significantly correlate with reduced survival, increased metastases, and poor outcome [105, 106]. 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 osteosarcoma is in progress [139].

Ezrin

Ezrin is a protein having roles in cell–cell interactions, signal transduction, and linkage between actin filament and the cells membrane [107]. Upon upregulation, it leads to metastasis. In pediatric osteosarcoma patients, increased ezrin expression is associated with reduced disease-free intervals [108]. 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 [108]. Wan et al. [109] have used rapamycin to inhibit ezrin-mediated pathways, leading to reduced lung metastasis in a mouse model of osteosarcoma.

Parathyroid Hormone-Related Peptide (PTHrP)

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 % [110]. However, over-expression of PTHR1 in the HOS OS cell line resulted in the increased proliferation, motility, and invasion of cells through Matrigel [111].

c-Jun

c-Jun is an oncogene encoding a basic region-leucine zipper protein [112] which, in combination with c-Fos protein, forms the activator protein-1 early response transcription factor [113]. c-Jun DNA enzyme (DNAzyme) encapsulated in a cationic multilamellar vesicle liposome inhibited the growth and metastasis of osteosarcoma in an orthotopic spontaneously metastasizing model of the disease [114, 115]. c-Jun DNAzyme nanoparticle formulated from chitosan was also found to be more active against osteosarcoma cells, inducing apoptotic cell death in these cells [116]. When delivered in combination with doxorubicin, c-jun DNAzyme inhibited the growth and metastasis of pre-established tumors, [117]. c-Jun knockdown chemosensitized these cells to doxorubicin treatment.

Insulin-Like Growth Factor (IGF)-1

IGF-1 signaling is a known mediator of bone growth [118] and is one of most potent natural activators of the AKT and MAPK signaling pathways relevant in the development, growth, survival, and progression of cancer [119, 120]. Its receptor is present on clonal osteosarcoma and Ewing's cells [121, 122], and IGF-1 has been implicated in the

growth and/or metastasis of osteosarcoma in vitro and in vivo [123]. These characteristics, together with the association between the period of rapid growth during adolescence and the occurrence of osteosarcoma, implicate IGF-1 and its receptor as a potential target for inhibition. Several Phase II studies have demonstrated the safety and efficacy of a strategy to inhibit IGF-1 receptor with monoclonal antibodies in solid tumors including bone and soft tissue sarcomas [124, 125].

P-Glycoprotein

P-glycoprotein (P-gp) is a protein responsible for energy-dependent drug efflux and is encoded by the multiple drug-resistant-1 (MDR-1) gene [126]. P-gp is known to have a critical role in the multi-resistance of human osteosarcoma [127]. 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 [128]. This appeared to conflict with a study that showed that MDR-1 levels and not P-gp correlated with prognosis with Ewing's sarcoma [129]. A prospective study examining outcomes of patients with localized high-risk soft tissue sarcoma of limbs and trunk wall reported that it was the relative level of MDR-1/P-gp that was prognostic [130]. A recent study showed that siRNA downregulated MDR1 mRNA expression by 50 % in breast carcinoma and osteosarcoma cell lines and significantly inhibited tumor cell proliferation up to 90 % ($p < 0.01$) when co-administered with doxorubicin or methotrexate, despite the known chemoresistance of the cell lines [131]. These results suggest the potential clinical application of anti-MDR1 siRNA to restore chemosensitivity and possibly improve the therapeutic ratio of these cytotoxic drugs.

Chemokine Receptor CXCR4

CXCR4 and its corresponding ligand, stromal cell-derived factor 1 (SDF-1), play a major role in the metastatic process [132]. It has been found that the CXCR4/SDF-1 system significantly correlates with the presence of metastases in a number of tumors. In osteosarcoma, the increase of CXCR4 mRNA expression leads to reduced overall survival and correlates with the presence of metastases at diagnosis [133]. Therefore, CXCR4 is a potential target for chemotherapy. Perissinotto et al. [134] used T134 peptide to inhibit the CXCR4 site in a mouse model, resulting in the elimination of lung metastases in all the tested mice.

mTOR Inhibition

Another promising approach is the use of mammalian target of rapamycin, (mTOR). 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 [135]. mTOR inhibitors, derived from rapamycin, are active against tumor cells by blocking mTOR activity leading to inhibition of cell growth and have shown activity against osteosarcoma [136]. A preclinical trial study showed activity of mTOR inhibitors against sarcoma cell lines [109]. These studies have led to Phase II studies of the mTOR inhibitor ridaforolimus in patients with advanced bone and soft tissue sarcomas [137•].

Heat-Shock Protein (HSP90)

HSP 90 is a chaperone protein involved in many cellular survival pathways including regulating the stability, activity and intracellular sorting of its client proteins, which are involved in multiple oncogenic processes [138]. These HSP90 dependent proteins maintain the malignant phenotype through specific functions including signal transduction (i.e., mutant epidermal growth factor receptor), angiogenesis (i.e., vascular endothelial growth factor), anti-apoptosis (i.e., AKT), and metastasis (i.e., matrix metalloproteinase 2 and CD91) [139]. Because of these specific functions, HSP90 has emerged as a viable target for antitumor drug development [139]. Development of HSP90 inhibitors has led to preclinical and clinical data with HSP90 inhibitors in various cancer models. These studies appear promising, and hint at the potential for tumor-selective cytotoxicity as well as enhanced sensitization to chemo- and radiotherapy [140•].

Anti-osteoclastic Agents

Bisphosphonates

Osteoclasts have drawn attention as a therapeutic target in various bone disorders including osteosarcoma. The dynamic relationship between osteoblasts and osteoclasts, mediated by the RANK/RANKL/OPG system, is thought to be central in the recruitment of osteoclast and osteoclast action by osteosarcoma. This tight relationship makes osteosarcoma an ideal candidate for osteoclast-targeted therapy [141•].

Bisphosphonate, a potent anti-osteoclastic agent, was shown to have in vitro anti-tumor activity in a rat clonal osteosarcoma cell line [142]. Minodronate, incadronate [143], risedronate [144], and zoledronic acid [145] are a class of new nitrogen-containing bisphosphonates, which have demonstrated in vitro inhibitory activity on human OS cell growth. Bisphosphonates action is now thought to be through the induction of apoptosis [146, 147]. Bisphosphonates such as risedronate and zoledronic acid have also

Fig. 3 a CT scan of pelvis showing giant cell tumor of posterior ilium. Co-registered PET scan showing increased metabolic activity of giant cell tumor. Photomicrograph ($\times 60$) of biopsy specimen showing typical features of giant cell tumor with abundant giant cells (*black arrow*), and stromal mononuclear cells (*white arrow*). **b** CT scan of pelvis 3 months after commencement of denosumab therapy showing ossification of giant cell tumor. Co-registered PET scan now showing minimal metabolic activity after denosumab treatment. Photomicrograph (low power $\times 10$, high power $\times 60$) showing ossification of tumor (*black arrow*), and complete absence of giant cells stromal cells (*white arrow*)

been shown to have a synergistic effect when delivered in combination with carboplatin, doxorubicin, vincristine, or etoposide in vitro. [144, 148, 149]. The bisphosphonate drug alendronate was able to suppress bone remodeling and tumor osteolysis in canines with osteosarcoma [150]. In vivo studies using a mouse osteosarcoma model have also demonstrated growth suppression of the primary tumor and a reduction in the development and burden of pulmonary disease following treatment with zoledronate [151]. However, others using the same system noted only

an anti-osteolytic effect on osteosarcoma but not an anti-metastatic effect [152]. These in vitro data have raised great interest in the adjuvant role of bisphosphonates in the management of malignant bone tumors [153, 154].

RANK-Fc

RANK-Fc is a recombinant RANK-L antagonist that is formed by fusing the extracellular domain of RANK to the Fc portion of human immunoglobulin G(1), [hIgG(1)]. It

has shown a variety of anti-neoplastic activities targeting tumors that are osteolytic such as myeloma [155]. More recently, in an orthotopic mouse model of osteosarcoma, subcutaneous delivery of RANK-Fc reduced lung metastases, preserved bone structure, and reduced osteoclast formation, bone resorption, and RANKL-induced anti-apoptosis [156]. The mechanism of action was thought to be through inhibiting ERK activation and evoking caspase-3-mediated anoikis in human osteosarcoma cells [157].

Denosumab

Denosumab is a fully humanized antibody with a strong affinity for RANKL. Its inhibitory effect has been utilized for the management of postmenopausal osteoporosis and the bone destruction from metastatic disease [158]. Interest in bone tumors has been not only for metastatic disease [159], for which it has been shown to be very effective, but also for its impact on quality of life and pain control, which it has been shown, in three large series, to be superior to zoledronic acid [160]. Denosumab has recently been used in giant cell tumor of bone and shown to be associated with tumor responses characterized by ossification, reduction in the size of the soft tissue component, and almost complete absence of giant cells and the mononuclear stromal cell (Fig. 3). As a result of the use of denosumab, this study reported a reduction in the need for morbid surgery in patients with giant cell tumor of bone [161].

Conclusions

Treatment of primary musculoskeletal malignancies has enjoyed considerable success since the advent of chemotherapy with survival improving from a universally fatal condition to one where the 5-year survival approximates 75 %. Advancements in imaging and surgery have altered the surgical paradigm such that patients may rightfully expect limb preservation as a primary outcome. This has led to the establishment of a multidisciplinary approach that is now *de rigueur*, and which strives for greater longevity and normality from life and limb. The biggest challenges now are the management of locally unresectable lesions and the advent of metastases.

Coincident with these improvements has been a better understanding of the nature of cancer as researchers move from defining the gross to the molecular behavior of these tumors. The world of molecular targeted therapy has highlighted the complex pathways, which malignancies take to abrogate the body's defenses. Discoveries of important molecular switches that once triggered may result in profound tumor inhibition has led to some

optimism about a "magic bullet" for cancer. This is a long way off, and most likely will rely on a combination of bullets.

Compliance with Ethics Guidelines

Conflict of Interest Peter F. M. Choong has received grant support from the Australian Orthopaedic Association and the Cancer Council of Victoria.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

References

Recently published papers of particular interest have been highlighted as:

- Of importance
- Of major importance

1. Choong P, Sim F. Limb-sparing surgery for bone tumors: new developments. *Semin Surg Oncol*. 1997;13:64–9.
2. Cade S. Osteogenic sarcoma. A study based on 133 patients. *J R Coll Surg Edinb*. 1955;1:4–9.
3. Enneking W, Spanier S, Goodman M. A system for the surgical staging of musculoskeletal sarcoma. *Clin Orthop Relat Res*. 1980;153:106–20.
4. Kawaguchi N, Ahmed A, Matsumoto S, et al. The concept of curative margin in surgery for bone and soft tissue sarcoma. *Clin Orthop Relat Res*. 2004;419:165–72.
5. Stoeckle E, Coindre J, Kind M, et al. Evaluating surgery quality in soft tissue sarcoma. *Recent Res Cancer Res*. 2009;179:229–42.
6. Yasko A. Surgical management of primary osteosarcoma. *Cancer Treat Res*. 2009;152:125–45.
7. Poffyn B, Sys G, Mulliez A, et al. Extracorporeally irradiated autografts for the treatment of bone tumours: tips and tricks. *Int Orthop*. 2011;35:889–95.
8. Puri AGA, Jambhekar N, Laskar S. The outcome of the treatment of diaphyseal primary bone sarcoma by resection, irradiation and re-implantation of the host bone: extracorporeal irradiation as an option for reconstruction in diaphyseal bone sarcomas. *J Bone Joint Surg Br*. 2012;94:982–8.
9. Hong A, Millington S, Ahern V, et al. Limb preservation surgery with extracorporeal irradiation in the management of malignant bone tumor: the oncological outcomes of 101 patients. *Ann Oncol*. 2013;24:2676–80.
10. Hatano H, Ogose A, Hotta T, et al. Extracorporeal irradiated autogenous osteochondral graft: a histological study. *J Bone Joint Surg Br*. 2005;87:1006–11.
11. Nishida H, Yamamoto N, Tanzawa Y, et al. Cryoimmunology for malignant bone and soft-tissue tumors. *Int J Clin Oncol*. 2011;16:109–17.
12. Tsuchiya H, Nishida H, Srisawat P, et al. Pedicle frozen autograft reconstruction in malignant bone tumors. *J Orthop Sci*. 2010;15:340–9.
13. Jacobs P, Clemency RJ. The closed cryosurgical treatment of giant cell tumor. *Clin Orthop Relat Res*. 1985;192:149–58.

14. Malawer M, Bickels J, Meller I, et al. Cryosurgery in the treatment of giant cell tumor. A long-term followup study. *Clin Orthop Relat Res*. 1999;359:176–88.
15. Robinson D, Yassin M, Nevo Z. Cryotherapy of musculoskeletal tumors—from basic science to clinical results. *Technol Cancer Res Treat*. 2004;3:371–5.
16. Kawano M, Nishida H, Nakamoto Y, et al. Cryoimmunologic antitumor effects enhanced by dendritic cells in osteosarcoma. *Clin Orthop Relat Res*. 2010;468:1373–83.
17. Matsumoto K, Imai R, Kamada T, et al. Impact of carbon ion radiotherapy for primary spinal sarcoma. *Cancer Treat Res*. 2013;119:3496–503.
18. Wakatsuki M, Magpayo N, Kawamura H, et al. Differential bystander signaling between radioresistant chondrosarcoma cells and fibroblasts after X-ray, proton, iron ion and carbon ion exposures. *Int J Radiat Oncol Biol Phys*. 2012;84:E103–8.
19. Blakely E, Kronenberg A. Heavy-ion radiobiology: New approaches to delineate mechanisms underlying enhanced biological effectiveness. *Radiat Res*. 1998;150:126–45.
20. Kamada T, Tsujii H, Tsuji H, et al. Efficacy and safety of carbon ion radiotherapy in bone and soft tissue sarcomas. *J Clin Oncol*. 2002;20:4466–71.
21. •• Ciernik IF, Niemierko A, Harmon DC, et al. Proton-based radiotherapy for unresectable or incompletely resected osteosarcoma. *Cancer*. 2011;117:4522–30. *An important and informative study regarding the efficacy of proton-based radiotherapy in unsalvageable tumors.*
22. Jaffe N, Frei ER, Traggis D, et al. Adjuvant methotrexate and citrovorum-factor treatment of osteogenic sarcoma. *N Engl J Med*. 1974;291:994–7.
23. Sinks L, Mindell E. Chemotherapy of osteosarcoma. *Clin Orthop Relat Res*. 1975;111:101–4.
24. Hattinger C, Pasello M, Ferrari S, et al. Emerging drugs for high-grade osteosarcoma. *Expert Opin Emerg Drugs*. 2010;15:615–34.
25. Ferrari S, Palmerini E, Staals E, et al. The treatment of non-metastatic high grade osteosarcoma of the extremity: review of the Italian Rizzoli experience. Impact on the future. *Cancer Treat Res*. 2009;152:275–87.
26. Briccoli A, Rocca M, Salone M, et al. High grade osteosarcoma of the extremities metastatic to the lung: long-term results in 323 patients treated combining surgery and chemotherapy, 1985–2005. *Surg Oncol*. 2010;19:193–9.
27. Ferrari S, Palmerini E. Adjuvant and neoadjuvant combination chemotherapy for osteogenic sarcoma. *Curr Opin Oncol*. 2007;19:341–6.
28. Bacci G, Mercuri M, Longhi A, et al. Grade of chemotherapy-induced necrosis as a predictor of local and systemic control in 881 patients with non-metastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy in a single institution. *Eur J Cancer*. 2005;41:2079–85.
29. Raymond A, Chawla S, Carrasco C et al. Osteosarcoma chemotherapy effect: a prognostic factor. *Semin Diagn Pathol*. 1987;4:212–36.
30. Ta H, Dass C, Choong P, et al. Osteosarcoma treatment: state of the art. *Cancer Metastasis Rev*. 2009;28:247–63.
31. Pantel K, Brakenhoff R. Dissecting the metastatic cascade. *Nat Rev Cancer*. 2004;4:448–56.
32. •• Bidard F, Pierga J, Soria J, et al. Translating metastasis-related biomarkers to the clinic—progress and pitfalls. *Nat Rev Clin Oncol*. 2013;10:169–79. *An excellent review of the state-of-the-art on using metastasis-related biomarkers to drive personalized medicine in cancer. It highlights the potential advantages of this technology but also clearly points out that success is not easily obtained because of the complexity and redundancy of cancer systems.*
33. Clark JCM, Dass CR, Choong PFM. A review of clinical and molecular prognostic factors in osteosarcoma. *J Cancer Res Clin Oncol*. 2007;134:281–97.
34. Quan GMY, Choong PFM. Anti-angiogenic therapy for osteosarcoma. *Cancer Metastasis Rev*. 2006;25:707–13.
35. Folkman J. Endogenous angiogenesis inhibitors. *Acta Pathol Microbiol Immunol Scand*. 2004;112:496–507.
36. Sjin RMTT, Naspinski J, Birsner AE, et al. Endostatin therapy reveals a U-shaped curve for antitumor activity. *Cancer Gene Ther*. 2006;13:619–27.
37. Qu J, Wang M, He H, et al. The prognostic value of elevated vascular endothelial growth factor in patients with osteosarcoma: a meta-analysis and systemic review. *Cancer Res Clin Oncol*. 2012;138:819–25.
38. • Chen D, Zhang Y, Zhu K, et al. A systematic review of vascular endothelial growth factor expression as a biomarker of prognosis in patients with osteosarcoma. *Tumour Biol*. 2013;34:1895–99. *A good review of vascular endothelial growth factor and its pathophysiologic significance in osteosarcoma.*
39. Kaya M, Wada T, Akatsuka T, et al. Vascular endothelial growth factor expression in untreated osteosarcoma is predictive of pulmonary metastasis and poor prognosis. *Clin Cancer Res*. 2000;6:572–7.
40. Kaya M, Wada T, Nagoya S, et al. The level of vascular endothelial growth factor as a predictor of a poor prognosis in osteosarcoma. *J Bone Joint Surg Br*. 2009;91:784–8.
41. Ek ETH, Ojaimi J, Kitagawa Y, et al. Does the degree of intratumoral microvessel density and VEGF expression have prognostic significance in osteosarcoma? *Oncol Rep*. 2006;16:17–23.
42. Sorensen F, Jensen K, Vaeth M, et al. Immunohistochemical estimates of angiogenesis, proliferative activity, p53 expression, and multiple drug resistance have no prognostic impact in osteosarcoma: a comparative clinicopathological investigation. *Sarcoma*. 2008;2008:874075. doi:10.1155/2008/874075.
43. Volpert O, Zaichuk T, Zhou W, et al. Inducer-stimulated Fas targets activated endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor. *Nat Med*. 2002;8:349–57.
44. •• Crawford S, Fitch P, Veliceasa D, et al. The many facets of PEDF in drug discovery and disease: a diamond in the rough or split personality disorder? *Expert Opin Drug Discov*. 2013;8:769–92. *Good review article on the multifunctional activity of PEDF.*
45. Dawson DW, Volpert OV, Gillis P, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science*. 1999;285:245–8.
46. Ek E, Dass C, Contreras K, et al. Pigment epithelium-derived factor overexpression inhibits orthotopic osteosarcoma growth, angiogenesis and metastasis. *Cancer Gene Ther*. 2007;14:616–26.
47. Akiyama T, Dass CR, Shinoda Y, et al. PEDF regulates osteoclasts via osteoprotegerin and RANKL. *Biochem Biophys Res Commun*. 2010;391:789–94.
48. Chen Q, Jin M, Yang F, et al. Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. *Mediat Inflamm*. 2013;2013:928315. doi:10.1155/2013/928315.
49. •• Husmann K, Arlt M, Muff R, et al. Matrix metalloproteinase 1 promotes tumor formation and lung metastasis in an intratibial injection osteosarcoma mouse model. *Biochim Biophys Acta*. 2013;1832:347–54. *A compact study using a mouse model on the important role of MMP-1 in the development of primary tumor growth and lung metastases in osteosarcoma.*
50. Korpi J, Hagström J, Lehtonen N, et al. Expression of matrix metalloproteinases-2,-8,-13,-26, and tissue inhibitors of

- metalloproteinase-1 in human osteosarcoma. *Surg Oncol*. 2011;20:18–22.
51. Foukas AF, Deshmukh NS, Grimer RJ, et al. Stage-IIB osteosarcoma around the knee. A study of MMP-9 in surviving tumour cells. *J Bone Joint Surg Br*. 2002;84:706–11.
 52. Nagini S. RECKing MMP: relevance of reversion-inducing cysteine-rich protein with kazal motifs as a prognostic marker and therapeutic target for cancer (a review). *Anticancer Agents Med Chem*. 2012;12:718–25.
 53. Meng N, Li Y, Zhang H, et al. RECK, a novel matrix metalloproteinase regulator. *Histol Histopathol*. 2008;23:1003–10.
 54. Clark J, Thomas D, Choong P, et al. RECK—a newly discovered inhibitor of metastasis with prognostic significance in multiple forms of cancer. *Cancer Metastasis Rev*. 2007;26:675–83.
 55. Kang HG, Kim HS, Kim KJ, et al. RECK expression in osteosarcoma: correlation with matrix metalloproteinases activation and tumor invasiveness. *J Orthop Res*. 2007;25:696–702.
 56. Choong PFM, Nadesapillai APW. Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. *Clin Orthop Rel Res*. 2003;415:S46–58.
 57. •• Mason S, Joyce J. Proteolytic networks in cancer. *Trends Cell Biol*. 2011;21:228–37. *This is a good review about how proteases interact with each other, and with endogenous inhibitors and other signaling molecules, in a coordinated fashion to regulate tumor progression.*
 58. Hildenbrand R, Allgayer H, Marx A, et al. Modulators of the urokinase-type plasminogen activation system for cancer. *Expert Opin Investig Drugs*. 2010;19:641–52.
 59. Harbeck N, Schmitt M, Meisner C, et al. Ten-year analysis of the prospective multicentre Chemo-N0 trial validates American Society of Clinical Oncology (ASCO)-recommended biomarkers uPA and PAI-1 for therapy decision making in node-negative breast cancer patients. *Eur J Cancer*. 2013;49:1825–35.
 60. Taubert H, Magdolen V, Kotzsch M. Impact of expression of the uPA system in sarcomas. *Biomark Med*. 2013;7:473–80.
 61. Dass CR, Nadesapillai APW, Robin D, et al. Downregulation of uPAR confirms link in growth and metastasis of osteosarcoma. *Clin Exp Metastasis*. 2005;22:643–52.
 62. Dass C, Choong P. uPAR mediates anticancer activity of PEDF. *Cancer Biol Ther*. 2008;7:1262–70.
 63. •• Buddingh E, Ruslan S, Berghuis D, et al. Intact interferon signaling in peripheral blood leukocytes of high-grade osteosarcoma patients. *Cancer Immunol Immunother*. 2012;62:941–47. *Interesting article on interferon signaling in the peripheral blood of patients with osteosarcoma. It draws links between the mechanism of interferon anti-tumor action and the presence of interferon receptors on, and therefore vulnerability of osteosarcoma cells to, interferon treatment.*
 64. Whelan J, Patterson D, Perisoglou M, et al. The role of interferons in the treatment of osteosarcoma. *Pediatr Blood Cancer*. 2010;54:350–4.
 65. Strander H. Interferons and osteosarcoma. *Cytokine Growth Factor Rev*. 2007;8:373–80.
 66. Luetke A, Meyers P, Lewis I, et al. Osteosarcoma treatment—where do we stand? A state of the art review. *Cancer Treat Rev*. 2013; doi:10.1016/j.ctrv.2013.11.006.
 67. Yuan X, Zhu X, Liang S, et al. Interferon- α enhances etoposide-induced apoptosis in human osteosarcoma U2OS cells by a p53-dependent pathway. *Life Sci*. 2008;82:393–401.
 68. Yuan X-W, Zhu X-F, Huang X-F, et al. Interferon- α enhances sensitivity of human osteosarcoma U2OS cells to doxorubicin by p53 dependent apoptosis. *Acta Pharmacol Sin*. 2007;28:1835–41.
 69. Inaba H, Glibetic M, Buck S, et al. Interferon-gamma sensitizes osteosarcoma cells to Fas-induced apoptosis by upregulating Fas receptors and caspase-8. *Pediatr Blood Cancer*. 2004;43:729–36.
 70. Li Z, Xu Q, Peng H, et al. IFN- γ enhances HOS and U2OS cell lines susceptibility to $\gamma\delta$ T cell-mediated killing through the Fas/Fas ligand pathway. *Int Immunopharmacol*. 2011;11:496–503.
 71. • Monjazeb A, Hsiao H, Sckisel G, et al. The role of antigen-specific and non-specific immunotherapy in the treatment of cancer. *J Immunotoxicol*. 2012;9:248–58. *A review of immunotherapy covering the basic theories and giving examples of current and future directions.*
 72. Antony G, Dudek A. Interleukin 2 in cancer therapy. *Curr Med Chem*. 2010;17:3297–302.
 73. Kerkar S, Restifo N. Cellular constituents of immune escape within the tumor microenvironment. *Cancer Res*. 2012;72:3125–30.
 74. Vacchelli E, Eggermont A, Fridman W, et al. Trial watch: immunostimulatory cytokines. *Oncoimmunology*. 2013;2:e24850.
 75. •• Kozłowska A, Mackiewicz J, Mackiewicz A. Therapeutic gene modified cell based cancer vaccines. *Gene*. 2013;525:200–07. *This is a good review of the field of gene-based cancer vaccines. It covers the theory of cancer vaccines, the strengths and weaknesses, and what is needed to enhance this technology.*
 76. Edmonson J, Long H, Kvols L, et al. Can molgramostim enhance the antitumor effects of cytotoxic drugs in patients with advanced sarcomas? *Ann Oncol*. 1997;8:637–41.
 77. Rao R, Anderson P, Arndt C, et al. Aerosolized granulocyte macrophage colony-stimulating factor (GM-CSF) therapy in metastatic cancer. *Am J Clin Oncol*. 2003;26:493–8.
 78. Anderson P, Markovic S, Sloan J, et al. Aerosol granulocyte macrophage-colony stimulating factor: a low toxicity, lung-specific biological therapy in patients with lung metastases. *Clin Cancer Res*. 1999;5:2316–23.
 79. Anderson P, Kopp L, Anderson N, et al. Novel bone cancer drugs: investigational agents and control paradigms for primary bone sarcomas (Ewing's sarcoma and osteosarcoma). *Expert Opin Investig Drugs*. 2008;17:1703–15.
 80. Nardin A, Lefebvre M, Labroquère K, et al. Liposomal muramyl tripeptide phosphatidylethanolamine: targeting and activating macrophages for adjuvant treatment of osteosarcoma. *Curr Cancer Drug Targets*. 2006;6:123–33.
 81. Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein MI, Betcher D, Ferguson WS, Gebhardt MC, Goorin AM, Harris M, Kleinerman E, Link MP, Nadel H, Nieder M, Siegal GP, Weiner MA, Wells RJ, Womer RB, Grier HE, Children's Oncology Group. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group. *J Clin Oncol*. 2008;26:633–8.
 82. Chou AJ, Kleinerman ES, Krailo MD, Chen Z, Betcher DL, Healey JH, Conrad EU 3rd, Nieder ML, Weiner MA, Wells RJ, Womer RB, Meyers PA, Children's Oncology Group. Addition of muramyl tripeptide to chemotherapy for patients with newly diagnosed metastatic osteosarcoma: a report from the Children's Oncology Group. *Cancer*. 2009;115:5339–48.
 83. Teodoro JG, Evans SK, Green MR. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *J Mol Med*. 2007;85:1175–86.
 84. Yang JZW. New molecular insights into osteosarcoma targeted therapy. *Curr Opin Oncol*. 2013;25:398–406.
 85. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. *J Clin Invest*. 2012;122:1951–7.
 86. Miller CW, Aslo A, Won A, et al. Alterations of the p53, Rb and MDM2 genes in osteosarcoma. *J Cancer Res Clin Oncol*. 1996;122:559–65.
 87. •• Ognjanovic S, Olivier M, Bergemann TL, Hainaut P. Sarcomas in TP53 germline mutation carriers: a review of the IARC

- TP53 database. *Cancer* 2012;118:1387–96. *Excellent review of TP53 germline mutations in sarcoma.*
88. Nakase M, Inui M, Okumura K, et al. p53 gene therapy of human osteosarcoma using a transferrin-modified cationic liposome. *Mol Cancer Ther.* 2005;4:625–31.
 89. Densmore CL, Kleinerman ES, Gautam A, et al. Growth suppression of established human osteosarcoma lung metastases in mice by aerosol gene therapy with PEI–p53 complexes. *Cancer Gene Ther.* 2001;8:619–27.
 90. Kanaya N, Yazawa M, Goto-Koshino Y, et al. Anti-tumor effect of adenoviral vector-mediated p53 gene transfer on the growth of canine osteosarcoma xenografts in nude mice. *J Vet Med Sci.* 2011;73:877–83.
 91. Liang Z, Li Y, Huang K, et al. Regulation of miR-19 to breast cancer chemoresistance through targeting PTEN. *Pharm Res.* 2011;28:3091–100.
 92. Bhatla T, Wang J, Morrison D, et al. Epigenetic reprogramming reverses the relapse-specific gene expression signature and restores chemosensitivity in childhood B-lymphoblastic leukemia. *Blood.* 2012;119:5201–10.
 93. Grohar P, Segars L, Yeung C, et al. Dual targeting of EWS-FLI1 activity and the associated DNA damage response with Trabectedin and SN38 synergistically inhibits Ewing sarcoma cell growth. *Clin Cancer Res.* 2013; doi:10.1158/1078-0432.CCR-13-0901.
 94. Zhao F, Yin H, Li J. Supramolecular self-assembly forming a multifunctional synergistic system for targeted co-delivery of gene and drug. *Biomaterials.* 2014;35:1050–62.
 95. Zhou Z, Jia S, Hung M, et al. E1A sensitizes HER2/neu-over-expressing Ewing's sarcoma cells to topoisomerase II-targeting anticancer drugs. *Cancer Res.* 2001;61:3394–8.
 96. Zhou Z, Guan H, Kleinerman E. E1A specifically enhances sensitivity to topoisomerase II α targeting anticancer drug by upregulating the promoter activity. *Mol Cancer Res.* 2005;3:271–5.
 97. Lin G, Chen Q, Yu S, et al. Overexpression of human telomerase reverse transcriptase C-terminal polypeptide sensitizes HeLa cells to 5-fluorouracil-induced growth inhibition and apoptosis. *Mol Med Rep.* 2014;9:279–84.
 98. Cheon J, Ko S, Gardner T, et al. Chemogene therapy: osteocalcin promoter-based suicide gene therapy in combination with methotrexate in a murine osteosarcoma model. *Cancer Gene Ther.* 1997;4:359–65.
 99. Chawla S, Chua V, Fernandez L, et al. Advanced phase I/II studies of targeted gene delivery in vivo: intravenous Rexin-G for gemcitabine-resistant metastatic pancreatic cancer. *Mol Ther.* 2010;18:435–41.
 100. Akerstrom V, Chen C, Lan M, et al. Adenoviral insulinoma-associated protein 1 promoter-driven suicide gene therapy with enhanced selectivity for treatment of neuroendocrine cancers. *Ochsner J.* 2013;13:91–9.
 101. Yawata T, Maeda Y, Okiku M, et al. Identification and functional characterization of glioma-specific promoters and their application in suicide gene therapy. *J Neurooncol.* 2011;104:497–507.
 102. Finocchiaro L, Villaverde M, Gil-Cardesa M, et al. Cytokine-enhanced vaccine and interferon- β plus suicide gene as combined therapy for spontaneous canine sarcomas. *Res Vet Sci.* 2011;91:230–4.
 103. Witlox M, Lamfers M, Wuisman P, et al. Evolving gene therapy approaches for osteosarcoma using viral vectors: review. *Bone.* 2007;40:797–812.
 104. Wang TFWH, Wang H, Peng AF, Luo QF, Liu ZL, Zhou RP, Gao S, Zhou Y, Chen WZ. Inhibition of fatty acid synthase suppresses U-2 OS cell invasion and migration via downregulating the activity of HER2/PI3K/AKT signaling pathway in vitro. *Biochem Biophys Res Commun.* 2013;440:229–34.
 105. Onda M, Matsuda S, Higaki S, et al. ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. *Cancer.* 1996;71:71–8.
 106. Zhou H, Randall RL, Brothman AR, et al. Her-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification. *Oncology.* 2003;25:27–32.
 107. Hunter KW. Ezrin, a key component in tumor metastasis. *Trends Mol Med.* 2004;10:201–4.
 108. Khanna C, Wan X, Bose S, et al. The membrane-cyto-skeleton linker ezrin is necessary for osteosarcoma metastasis. *Nat Med.* 2004;10:182–6.
 109. Wan X, Mendoza A, Khanna C, et al. Rapamycin inhibits ezrin-mediated metastatic behaviour in a murine model of osteosarcoma. *Cancer Res.* 2005;65:2406–11.
 110. Pasquini GM, Davey RA, Ho PW, et al. Local secretion of parathyroid hormone-related protein by an osteoblastic osteosarcoma (UMR 106-01) cell line results in growth inhibition. *Bone.* 2002;31:598–605.
 111. Yang R, Hoang BH, Kubo T, et al. Over-expression of parathyroid hormone Type 1 receptor confers an aggressive phenotype in osteosarcoma. *Int J Cancer.* 2007;121:943–54.
 112. Dass CR, Choong PF. C-jun: pharmaceutical target for DNAzyme therapy of multiple pathologies. *Pharmazie.* 2008;63:411–4.
 113. Bohmann D, Bos TJ, Admon A, et al. Human proto-oncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science.* 1987;238:1386–92.
 114. Dass CR, Khachigian LM, Choong PFM. c-Jun is critical for the progression of osteosarcoma: proof in an orthotopic spontaneously metastasizing model. *Mol Cancer Res.* 2008;6:1289–92.
 115. Dass CR, Friedhuber AM, Khachigian LM, et al. Downregulation of c-jun results in apoptosis-mediated anti-osteosarcoma activity in an orthotopic model. *Cancer Biol Ther.* 2008;7:1033–6.
 116. Dass CR, Friedhuber AM, Khachigian LM, et al. Biocompatible chitosan-DNAzyme nanoparticle exhibits enhanced biological activity. *J Microencapsul.* 2008;25:421–5.
 117. Dass CR, Khachigian LM, Choong PFM. c-Jun knockdown sensitizes osteosarcoma to doxorubicin. *Mol Cancer Ther.* 2008;7:1909–12.
 118. Mohan S, Kesavan C. Role of insulin-like growth factor-1 in the regulation of skeletal growth. *Curr Osteoporos Rep.* 2012;10:178–86.
 119. Grimberg A. Mechanisms by which IGF-I may promote cancer. *Cancer Biol Ther.* 2003;2:630–5.
 120. Zhu C, Qi X, Chen Y, et al. PI3K/Akt and MAPK/ERK1/2 signaling pathways are involved in IGF-1-induced VEGF-C upregulation in breast cancer. *J Cancer Res Clin Oncol.* 2011;137(11):1587–9, 137:1587–89.
 121. Philippou A, Armakolas A, Panteleakou Z, et al. IGF1Ec expression in MG-63 human osteoblast-like osteosarcoma cells. *Anticancer Res.* 2011;31:4259–65.
 122. Mckinsey E, Parrish J, Irwin A, et al. A novel oncogenic mechanism in Ewing sarcoma involving IGF pathway targeting by EWS/Flt1regulated microRNAs. *Oncogene.* 2011;30:4910–20.
 123. Macewen EG, Pastor J, Kutzke J, et al. IGF-1 receptor contributes to the malignant phenotype in human and canine osteosarcoma. *J Cell Biochem.* 2004;92:77–91.
 124. Tap W, Demetri G, Barnette P, et al. Phase II study of ganitumab, a fully human anti-type-1 insulin-like growth factor receptor antibody, in patients with metastatic Ewing family tumors or desmoplastic small round cell tumors. *J Clin Oncol.* 2012;30:1849–56.
 125. Weigel B, Malempati S, Reid J, et al. Phase 2 trial of cixutumumab in children, adolescents, and young adults with refractory solid tumors: a report from the Children's Oncology Group. *Pediatr Blood Cancer.* 2013; doi:10.1002/pbc.24605.

126. Clarke R, Leonessa F, Trock B. Multidrug resistance/P-glycoprotein and breast cancer: review and meta-analysis. *Semin Oncol.* 2005;32:S9–15.
127. Brambilla D, Zamboni S, Federici C, et al. P-glycoprotein binds to ezrin at amino acid residues 149–242 in the FERM domain and plays a key role in the multidrug resistance of human osteosarcoma. *Int J Cancer.* 2012;130:2824–34.
128. Baldini N, Scotlandi K, Serra M, et al. P-glycoprotein expression in osteosarcoma: a basic for risk-adapted adjuvant chemotherapy. *J Orthop Res.* 1999;17:629–32.
129. Roundhill E, Burchill S. Membrane expression of MRP-1, but not MRP-1 splicing or Pgp expression, predicts survival in patients with ESFT. *Br J Cancer.* 2013;109:195–206.
130. Martin-Broto J, Gutierrez A, Ramos R, et al. MRP1 overexpression determines poor prognosis in prospectively treated patients with localized high-risk soft tissue sarcoma of limbs and trunk wall: an ISG/GEIS study. *Mol Cancer Ther.* 2013; doi:10.1158/1535-7163.MCT-13-0406.
131. Perez J, Bardin C, Rigal C, et al. Anti-MDR1 siRNA restores chemosensitivity in chemoresistant breast carcinoma and osteosarcoma cell lines. *Anticancer Res.* 2011;31:2813–20.
132. Cojoc M, Peitzsch C, Trautmann F, et al. Emerging targets in cancer management: role of the CXCL12/CXCR4 axis. *Onco Targets Ther.* 2013;6:1347–61.
133. Laverdiere C, Hoang BH, Yang R, et al. Messenger RNA expression levels of CXCR4 correlate with metastatic behavior and outcome in patients with osteosarcoma. *Clin Cancer Res.* 2005;11:2561–7.
134. Perissinotto E, Cavalloni G, Leone F, et al. Involvement of chemokine receptor 4/stromal cell-derived factor 1 system during osteosarcoma tumor progression. *Clin Cancer Res.* 2005;11:490–7.
135. Vilella-Bach M, Nuzzi P, Fang Y, et al. The fkbp12-rapamycin-binding domain is required for fkbp12-rapamycin-associated protein kinase activity and g1 progression. *J Biol Chem.* 1999;274:4266–72.
136. Ory B, Moriceau G, Redini F, et al. Mtor inhibitors (rapamycin and its derivatives) and nitrogen containing bisphosphonates: Bi-functional compounds for the treatment of bone tumours. *Curr Med Chem.* 2007;14:1381–7.
137. •• Chawla SP, Staddon AP, Baker LH, et al. Phase II study of the mammalian target of rapamycin inhibitor ridaforolimus in patients with advanced bone and soft tissue sarcomas. *J Clin Oncol.* 2012;30:78–84. *Important study showing the efficacy of rapamycin inhibitor in patients with sarcoma. However, an important finding was that clinical response did not seem to correlate with outcome.*
138. Maloney A, Workman P. HSP90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin Biol Ther.* 2002;2:3–24.
139. Hwang M, Moretti L, Lu B. HSP90 inhibitors: multi-targeted antitumor effects and novel combinatorial therapeutic approaches in cancer therapy. *Curr Med Chem.* 2009;16:3081–92.
140. •• Hong D, Banerji U, Tavana B, et al. Targeting the molecular chaperone heat shock protein 90 (HSP90): lessons learned and future directions. *Cancer Treat Rev.* 2013;39:375–387. *Good review of heatshock protein in cancer. Targeting particular isoforms and the mechanisms that influence HSP90's function may have therapeutic benefit.*
141. •• Akiyama T, Dass CR, Choong PF. Novel therapeutic strategy for osteosarcoma targeting osteoclast differentiation, bone-resorbing activity, and apoptosis pathway. *Mol Cancer Ther.* 2008;7:3461–69. *Novel targeting mechanism of osteosarcoma through osteoclast inhibition.*
142. Mackie PS, Fisher JL, Zhou H, et al. Bisphosphonates regulate cell growth and gene expression in the UMR 106-01 clonal rat osteosarcoma cell line. *Br J Cancer.* 2001;84:951–8.
143. Kubo T, Shimose S, Matsuo T, et al. Inhibitory effects of a new bisphosphonate, minodronate, on proliferation and invasion of a variety of malignant bone tumor cells. *J Orthop Res.* 2006;24:1138–44.
144. Murayama T, Kawasoe Y, Yamashita Y, et al. Efficacy of the third-generation bisphosphonate risedronate alone and in combination with anticancer drugs against osteosarcoma cell lines. *Anticancer Res.* 2008;28:2147–54.
145. Kubista B, Trieb K, Sevela F, et al. Anticancer effects of zoledronic acid against human osteosarcoma cells. *J Orthop Res.* 2006;24:1145–52.
146. Thaler R, Spitzer S, Karlic H, et al. Ibandronate increases the expression of the pro-apoptotic gene FAS by epigenetic mechanisms in tumor cells. *Biochem Pharmacol.* 2013;85:173–85.
147. Wang H, Liu Y, Fan L, et al. A new bisphosphonate derivative, CP, induces gastric cancer cell apoptosis via activation of the ERK1/2 signaling pathway. *Acta Pharmacol Sin.* 2013;34:1535–44.
148. Horie N, Murata H, Kimura S, et al. Combined effects of a third-generation bisphosphonate, zoledronic acid with other anticancer agents against murine osteosarcoma. *Br J Cancer.* 2007;96:255–61.
149. Benassi M, Chiechi A, Ponticelli F, et al. Growth inhibition and sensitization to cisplatin by zoledronic acid in osteosarcoma cells. *Cancer Lett.* 2006;250:194–205.
150. Tomlin JL, Pead MJ, Muir P. Use of the bisphosphonate drug alendronate for palliative management of osteosarcoma in two dogs. *Vet Rec.* 2000;147:129–32.
151. Dass CR, Choong PF. Zoledronic acid inhibits osteosarcoma growth in an orthotopic model. *Mol Cancer Ther.* 2007;6:3263–70.
152. Labrinidis A, Hay S, Liapis V, et al. Zoledronic acid protects against osteosarcoma-induced bone destruction but lacks efficacy against pulmonary metastases in a syngeneic rat model. *Int J Cancer.* 2010;127:345–54.
153. Subbiah V, Ludwig J. Review: ewing sarcoma treatment: a role for bisphosphonates? *Clin Adv Hematol Oncol.* 2010;8:503–4.
154. Moriceau G, Ory B, Gobin B, et al. Therapeutic approach of primary bone tumours by bisphosphonates. *Curr Pharm Des.* 2010;16:2981–7.
155. Sordillo E, Pearse R. RANK-Fc: a therapeutic antagonist for RANK-L in myeloma. *Cancer.* 2003;97:802–12.
156. Akiyama T, Dass CR, Shinoda Y, et al. Systemic RANK-Fc protein therapy is efficacious against primary osteosarcoma growth in a murine model via activity against osteoclasts. *J Pharm Pharmacol.* 2010;62:470–6.
157. Akiyama T, Choong P, Dass C. RANK-Fc inhibits malignancy via inhibiting ERK activation and evoking caspase-3-mediated anoikis in human osteosarcoma cells. *Clin Exp Metastasis.* 2010;27:207–15.
158. Pageau S. Denosumab. *Mabs.* 2009;1:210–5.
159. Rolfo C, Racz L, Russo A, et al. Molecular target therapy for bone metastasis: starting a new era with denosumab, a RANKL inhibitor. *Expert Opin Biol Ther.* 2014;14:15–26.
160. Von Moos RBJ, Egerdie B, Stopeck a, Brown Je, Damyanov D, Fallowfield Lj, Marx G, Cleeland Cs, Patrick DI, Palazzo Fg, Qian Y, Braun a, Chung K. Pain and health-related quality of life in patients with advanced solid tumours and bone metastases: integrated results from three randomized, double-blind studies of denosumab and zoledronic acid. *Support Care Cancer.* 2013;21:3497–507.
161. Chawla S, Henshaw R, Seeger L, et al. Safety and efficacy of denosumab for adults and skeletally mature adolescents with giant cell tumour of bone: interim analysis of an open-label, parallel-group, phase 2 study. *Lancet Oncol.* 2013;14:901–8.