Expert Opinion

Strategies for the targeted delivery of therapeutics for osteosarcoma

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Background: Conventional therapy for osteosarcoma has reached a plateau of 60 – 70%, a 5-year survival rate that has changed little in two decades, highlighting the need for new approaches. *Objective*: To review the alternative means of delivering effective therapy for osteosarcoma that reach beyond the central venous catheter. *Methods*: Drawing on the author's own experiences providing care to high-risk osteosarcoma patients and reviewing the last two decades of literature describing sarcoma therapy, available information is summarized about potential osteosarcoma treatments that deliver therapy by a less conventional route. *Results/conclusions*: Intra-arterial chemotherapy has a limited impact on survival, but may help to achieve a better limb salvage. Intrapleural chemotherapy is important for managing malignant effusions. The development of inhalation therapies, treatments that target new bone formation such as bisphosphonates, chemically targeted radiation and antibody-based therapies all have potential to improve osteosarcoma therapy.

Keywords: bisphosphonates, inhalation chemotherapy, intrapleural chemotherapy, osteosarcoma, samarium

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1. Introduction

1.1 Osteosarcoma: the 'growing pain' that slays youth

Osteosarcoma is the most common bone cancer and a frequent cause of both morbidity and mortality in pediatric oncology [1,2]. In contrast to carcinomas that strike adults, it is relatively rare, affecting < 1000 people in the US each year [3]. However, for the individuals affected, osteosarcoma is particularly cruel: it has a peak incidence in adolescents, with a predilection for larger, more athletic or active young people. Often the diagnosis comes during the adolescent growth spurt, and the tumor arises most often at the ends of long bones, with distal femur, proximal tibia and proximal humerus being the most common sites identified [4]. The first symptoms usually are dismissed by parents and physicians as growing pains, and patients may be treated with pain killers or physical therapy for weeks or even months before an X-ray is obtained that shows the abnormality. Radiographs may show either a lytic lesion of bone or, more commonly, a bone mass with an adjacent soft tissue mass containing calcification [4]. The name of the disease, which is in fact a contraction of the phrase 'osteogenic sarcoma', is a description of the tumor's behavior: osteosarcoma is the tumor that makes bone, and by definition a sarcoma must make at least some osteoid to be called an osteosarcoma. The diagnosis remains a clinical pathology diagnosis, as there is no characteristic mutation or immunohistochemical marker that defines the disease.

Standard therapy for osteosarcoma consists of chemotherapy given in the neoadjuvant and adjuvant settings [5-7], with surgery as the preferred means of local control [5,8]. Whereas amputations were the usual means of achieving local control historically [9], advances in surgical technique and improvements made in the technology of artificial joint and bone replacements have allowed most patients to

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have some form of limb salvage (reviewed in [10]). Although orthopedists continue to recommend a rotation-plasty as the most functional type of limb salvage [11,12], the more 'normal' appearance of a fully salvaged leg contributes to the much greater popularity of this operation in the US and much of the rest of the world, despite the reduced functionality. The adoption in the 1980s of doxorubicin and cisplatinum-based chemotherapy (AP), now usually given together with high-dose methotrexate (MAP), improved survival from ~ 20% of patients with localized tumors treated with surgery alone to ~ 70% for non-metastatic patients given MAP [2,5,7]. The role of ifosfamide in the newly diagnosed patient remains controversial [7], and a worldwide clinical trial is underway at present to address this question.

Unfortunately, a long series of clinical trials in Europe and the US, trying different chemotherapy agents, combinations and schedules, has not improved the survival of osteosarcoma patients significantly beyond the 60 – 70% achieved in the 1980s. Although there does appear to be a dose–response curve for some chemotherapy agents in this disease – ifosfamide, for example, gives better response rates when 14 g or more per square meter rather than 9 g or less per cycle are given [13-17] – the impact of conventional cytotoxic agents given systemically by means of the central venous line clearly has reached a plateau. Immune approaches that promote phagocytosis, such as mifuramide [7], may improve that survival by as much as 8%, but the bulk of recurrent osteosarcoma patients go on to die from disease [8]. If survival is to be improved, new approaches are needed. Rather than continuing to identify different poisons to inject into central venous catheters, it is time to 'think outside the lines'.

2. Intra-arterial chemotherapy

The first approach attempted to targeted drug delivery for osteosarcoma was intra-arterial chemotherapy administration, usually with cisplatin [18]. Methotrexate also has been given via this route [19]. This technique was a logical extension of the intra-arterial chemotherapy and embolization techniques used with great success to treat liver tumors and other amenable lesions. The approach relied on the laboratory-based identification of a dose–response curve for osteosarcoma to the conventional agents (cisplatinum in particular) above the concentrations that could be administered safely in venous infusions.

Technically, the approach is relatively straightforward. For a distal femur tumor, the arterial circuit is accessed from the contralateral femoral artery and a contrast-filled catheter is passed to a site just proximal to the site of the tumor. Fluoroscopy is used to identify the optimal location from which the most contrast material enters the tumor with the minimum of contrast reaching normal tissues (**Figure 1**). The catheter is then secured into place and chemotherapy, most often cisplatinum, is given through this catheter over 4 h. The patient receives the same chemotherapy dose that would have been given intravenously, and systemic toxicities are similar. However, the effective concentration at the site of the tumor is much higher, leading to better tumor necrosis [20].

Unfortunately, this treatment is not completely benign. The best catheter placement almost always also gives a high dose of cisplatinum to the skin overlying the tumor, and severe, painful chemical skin burns can result. Furthermore, giving intra-arterial chemotherapy requires patients to receive general anesthesia for each cycle of chemotherapy given, and patients must spend an uncomfortable 12 h with a heavy sandbag over the site of catheter placement once the catheter is removed. These costs would be tolerable, though, if the approach improved outcomes.

Unfortunately, it has been shown clearly that giving intra-arterial cisplatin does not improve survival [13,21,22], although some orthopedic oncology surgeons indicate that limb salvage procedures are much easier to perform when the patient has been given intra-arterial chemotherapy. Given the increased cost, complexity and potential for increased morbidity associated with this technique, it cannot be recommended routinely. However, for selected patients where this technique may allow for a better or easier reconstruction (when the tumor mass abuts the neurovascular bundle, as in **Figure 1**, for example), it should be considered in cancer centers that have the appropriate technology.

3. Intrapleural chemotherapy

The lungs are the primary site of metastasis and recurrence for osteosarcoma, and there is a tendency for metastatic lesions to appear in the distal portions of the pulmonary tree, near the pleural surface. Although this location can make the tumors easier to resect and with minimal loss of lung tissue, it does mean that pulmonary metastases of osteosarcoma often break through the pulmonary surface, conferring a worse prognosis [23]. Pleural disease often results in a malignant effusion, heralded by chest pain, shortness of breath and decreased breath sounds on the involved side. Standard management for this condition involves placement of a chest tube for fluid drainage.

The chest tube in patients with malignant effusion represents an opportunity to provide effective therapy directly to the site of progression [24]. Once the bulk of the fluid is drained, cisplatin can be infused into the pleural space at a dose of 60 mg/m^2 in 100 cm^3 normal saline. The chest tube is then clamped for 4 h, after which residual fluid is removed. The volume returned may be substantially more or less than the infused volume, and the patient does receive some systemic delivery of cisplatin, although less than if the same dose were given intravenously. Chest tube drainage usually declines to minimal amounts within 2 days, and the chemotherapy also acts as a sclerosing agent, preventing future lung collapse.

The use of chest tubes for malignant effusion is mercifully infrequent, so only selected patients have the opportunity and need for intrapleural cisplatin. However, ~ 35% of

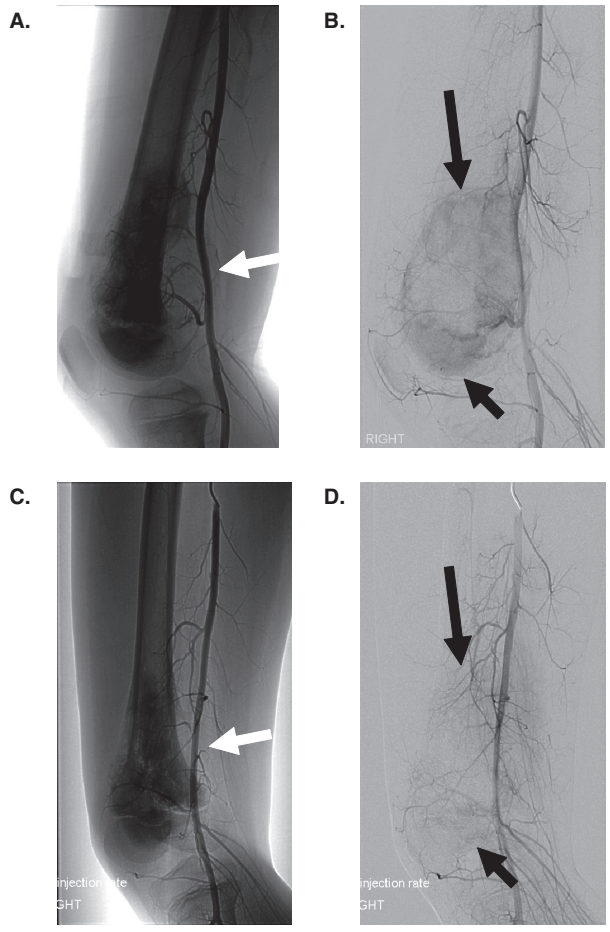

Figure 1. Arteriograms from an osteosarcoma patient before and after receiving intra-arterial cisplatin. A patient with right distal femur osteosarcoma was treated with four courses of intra-arterial cisplatin (120 mg/m^2 infused over 4 h per cycle) together with systemic doxorubicin (90 mg/m^2 intravenously). **A** and **B** show the pretreatment arteriograms, whereas **C** and **D** show the arteriograms obtained after three courses of therapy, concurrent with placement of the intra-arterial catheter for the fourth cycle. In **A**, a soft tissue mass (white arrow) is clearly visible, deforming the femoral artery. In **B**, with the bone image digitally subtracted, the vascular blush of abnormal tumor blood supply is clearly seen (black arrows). In **C** and **D**, the deformation of the femoral artery from the soft tissue mass is completely resolved (white arrow), and the abnormal tumor blood vessels that cause the vascular blush are now eliminated.

osteosarcoma patients eventually receive a thoracotomy, and all of these patients need a chest tube after surgery. Consideration should be given to testing potential intrapleural therapies, which could be done initially using the companion canine spontaneous osteosarcoma models [25,26].

4. Inhalation chemotherapy

It is no surprise that intra-arterial chemotherapy did not improve survival, as control of the primary tumor is not the main cause of mortality. With osteosarcoma, the lungs are the primary site of relapse, and progressive pulmonary disease is the most common cause of death from osteosarcoma. For this reason, the author's institution and others have taken a leading role in developing inhaled forms of chemotherapy.

The topic of inhaled chemotherapy has been reviewed recently [27]. For osteosarcoma specifically, Dr Kleinerman's group at the Children's Cancer Hospital at MD Anderson has done some of the most important preclinical work evaluating inhaled chemotherapy for osteosarcoma. They have shown that aerosolized gemcitabine is highly effective at reducing the size and number of pulmonary metastases of osteosarcoma [28,29]. More interestingly, the primary tumor in experimental mice bearing osteosarcoma was also reduced in size when pulmonary metastatic lesions were treated with inhaled gemcitabine [29]. Treatment of mice with inhaled gemcitabine also resulted in a significant upregulation of the death molecule Fas on the surface of osteosarcoma cells. This is highly important, as Kleinerman's group has also shown that Fas downregulation is necessary for the survival of osteosarcoma pulmonary metastases. As the lung is rich in expression of Fas ligand, upregulation of Fas by inhaled chemotherapy may result in increased apoptosis in metastatic cancer cells in the lungs. Thus, inhaled gemcitabine may also have an indirect, apoptosis-inducing effect as well as its direct cytotoxic effect on osteosarcoma lung metastases.

The principle has also been proven using large animal models. Carlos Rodriguez and co-workers at the University of California in Davis have treated dogs with spontaneous osteosarcoma with inhaled gemcitabine [30]. When dogs with osteosarcoma lung metastases were treated with a dilute aerosol gemcitabine solution twice a week, they tolerated the therapy for many weeks without any identifiable lung toxicity. All treated animals had an increase in the necrosis of lung metastases. Similar to the observations that Kleinerman made in mouse models, the dog lungs also showed an increase in Fas expression on the osteosarcoma metastases with aerosolized gemcitabine. Aerosolized gemcitabine has also been given safely to baboons without causing identifiable lung toxicity [31].

The author's institution has completed a Phase I study of a related compound, 9-nitro-camptothecin, for inhalation [32]. In this adult study, 13.3 μg/(kg day) via inhalation was the recommended Phase II dose. This treatment was provided by 2 consecutive 30 min inhalations from a nebulizer given Monday to Friday for 8 weeks of every 10 weeks. With higher doses, the dose-limiting toxicities included chemical pharyngitis and fatigue. A parallel study in pediatric patients has been completed, but has yet to be reported. Richard Gorlick has also completed a study using aerosolized liposomal cisplatin, but has yet to report the results of this study.

For acute lymphoblastic leukemia in children, an improvement in survival has come from using a prolonged period of maintenance chemotherapy after the intensive chemotherapy is completed. With the advent of inhaled chemotherapy for

osteosarcoma, a similar concept of maintenance chemotherapy may be applied to this disease, hopefully with similar improvements in survival.

5. Antibodies and targeting modalities

In one sense, the ultimate 'targeted therapy' is the delivery of monoclonal antibodies with specificities defined to inhibit key signals of tumor growth or survival. One of the first classes of these to be developed were antibodies targeting the epidermal growth factor receptor (EGFR) [33,34] and other members of the ERBB family, such as Her-2 [35]. The success of these agents in improving outcomes for patients with high-risk carcinomas [35] helped usher in a wave of many tumor-targeting antibodies into the clinic, including anti-GD2 for neuroblastoma [36,37], another solid tumor of childhood.

Some interesting early observations were nearly forgotten in the enthusiasm that surrounded the development of antibodies for clinical use. First, much of the early work examining the biology of EGFR signaling and trafficking used osteosarcoma cell lines [38-40]. Then, beginning a decade ago, several groups identified expression of Her-2 in osteosarcoma as an adverse prognostic factor associated with increased metastasis [41-44]. For several years there was controversy about these observations, with multiple conflicting reports being published. However, most of the dissenting reports used methods designed to detect the Her-2 overexpression observed in breast cancer, in which gene amplification and overexpression (1 million – 2 million molecules per cell), compared with normal levels of expression (30,000 – 100,000 molecules per cell), is associated with worse outcome in that disease [45-47]. In osteosarcoma, the relevant comparison is between modest expression (20,000 – 50,000 molecules per cell) compared with absent expression, and more sensitive methods are required. Since those early reports, it has been confirmed with modern methods that osteosarcoma cell lines do express EGFR, Her-2 and Her-4 [48], and these receptors are constitutively phosphorylated [49], suggesting that they participate meaningfully in tumor pathogenesis. EGFR has already been used for targeted therapy of osteosarcoma using adenoviral vectors in experimental models [50]. Overall, at least 80% of osteosarcoma tumors are expected to express EGFR, although much of this expression may be cytoplasmic [48], and only about half will demonstrate dense membranous expression by immunohistochemistry [51].

Based on the correlative studies associating Her-2 expression with higher metastasis, a clinical trial using trastuzumab (anti-Her-2 MAb) in combination with standard chemotherapy was started in the Children's Oncology Group for children with high-risk metastatic osteosarcoma at diagnosis, which has completed accrual. Although the outcomes from this trial have yet to be reported, the fact that the trial was able to be completed without reports of unexpected adverse events suggests that treating children with anti-Her-2 monoclonal antibodies is safe, even in combination with traditional chemotherapy. Anti-EGFR medications also have been given to children safely. As conjugated antibody medications have proved effective for some leukemias and other cancers, it seems likely that antibodies directed against the ERBB family would be effective carriers of selective antitumor drugs, providing a targeted therapy for osteosarcoma patients.

Nanoparticles may also be an effective way of delivering targeted therapy for osteosarcoma. A full discussion of the most common category of nanoparticles used for cancer, liposomal chemotherapy, is beyond the scope of this article. There are other sorts of nanoparticle, however, that may be an outstanding solution to an important technical problem.

In the laboratory setting, various forms of nucleic acids, including siRNA, shRNA and catalytic nucleic acids such as DNAzymes, have all been used with great efficacy specifically to downregulate particular genes within cancer cells. Unfortunately, there has been a large technical barrier to adapting this genetic innovation for clinical use. Of particular difficulty is the problem of targeting genetic material to the cells of interest. In this regard, two particular approaches deserve special mention. The first is Rexin-G, a pathotropic nanoparticle that has proved effective in delivering genetic material encoding a dominant negative cyclin G1 construct [52]. This drug first showed efficacy for stage for stage IV pancreatic cancer and was given accelerated approval and orphan drug status in the US on that basis [53]. It was then found to have broader clinical benefit [54]. On the basis of widespread antitumor activity found in preclinical studies, a Phase I and Phase II study of Rexin-G in osteosarcoma was undertaken [55]. Very few treatment-related adverse events were noted. Of the 17 evaluable patients treated in the Phase II osteosarcoma study, 3 showed a partial response by Choi criteria and 12 achieved stable disease by Choi criteria. In this population of patients with highly resistant refractory metastatic osteosarcoma, the median progression-free survival exceeded 3 months and the median overall survival was nearly 7 months [55].

Rexin-G is a very specific compound, delivering a dominant negative cyclin G1. A more broadly applicable technology would be the use of chitosan nanoparticles to deliver specific gene therapy [56]. Chitosan, which is made from chemical modification of chitin, can be made into an adaptable nanoparticle that efficiently carries genetic material into tumors. The development and use of chitosan have been reviewed recently [57,58]. Crispin Dass and colleagues have shown that chitosan nanoparticles bearing DNAzymes specific for c-Jun can sensitize resistant osteosarcoma to doxorubicin [59]. Although a great deal of preclinical and clinical work remains, these promising early studies show that the technology can be an effective means of delivering specific gene therapy.

6. Samarium and bisphosphonates

Another means of providing targeted therapy to the bone is to exploit the unique affinity of bone for phosphates and phosphonates. This chemical affinity has long been

exploited diagnostically in bone scans, in which radioactive technetium 99 (^{99}Tc) is conjugated to a phosphate or phosphonate [60-62]. A radio-sensitive camera then detects the emissions from the ^{99}Tc, which has been incorporated into newly made bone. This same type of chemical conjugation can be used to deliver treatment doses of radiation to sites of bone metastasis and other sites of bone turnover. This has been done effectively with the agent samarium-153 ethylene diamine tetramethylene phosphonate (Samarium, ^{153}Sm, or Quadramet). As ^{153}Sm is taken up in essentially the same distribution as a bone scan, a bone scan can be used to predict the distribution of radiation achieved with ^{153}Sm (**Figure 2**). This agent was initially developed to treat painful bone metastasis, usually in the setting of palliation for diseases such as prostate and breast cancer [63]. Bruland and colleagues proved the principle that this agent could be used for osteosarcoma using the companion canine model, an observation confirmed by other studies [64-66]. Subsequently, Pete Anderson and others have shown the utility of this agent in treating human osteosarcoma [67-69]. For patients with bone metastases from osteosarcoma, the radiation will be confined almost exclusively to the metastases. For this reason, radio-sensitizing agents such as gemcitabine, which normally cannot be used with radiation, are in fact highly effective [67,70]. In the author's clinic gemcitabine is given 24 h after the infusion of samarium. Although Pete Anderson has shown that ultrahigh doses of samarium can be used in the setting of autologous stem cell rescue [67,68], the remissions achieved with this technique have not been durable, and samarium is predominantly being used in a more conventional dosing scheme [69]. Myelotoxicity is the predominant dose-limiting toxicity, although this usually is manageable even in combination with external beam radiotherapy [71].

The same chemical affinity of phosphonates for newly formed bone provides the basis for the effectiveness of bisphosphonates in limiting osteoclastic bone resorption in osteoporosis [72]. By their chemical structure, bisphosphonates are taken up in newly formed bone in just the same way that ^{99}Tc is taken up in a bone scan, or ^{153}Sm is taken up in therapeutic radiation for bone metastasis. In the case of bisphosphonates, however, the effect is less immediate. Normal bone is continuously remodeled, and bisphosphonates such as zoledronic acid are taken up in this newly synthesized bone. As osteoclasts later resorb bisphosphonate-containing bone, the bisphosphonate is released at very high concentrations at the bone–osteoclast interface (reviewed in [72]). Nitrogen-containing bisphosphonates such as zoledronic acid inhibit the mevalonic acid synthesis pathway, which is essential for synthesizing the prenyl adjuncts farnesyl pyrophosphate (a 15-carbon chain) and geranylgeranyl pyrophosphate (a 20-carbon chain) [73,74]. Prenylation provides an essential lipid anchor to many signaling molecules, including Ras, and inhibition of prenylation usually induces cell death. The net effect is reduced osteoclast function and reduced bone resorption.

Bisphosphonates are effective in reducing the progression of bone metastases in several carcinomas, and can provide symptomatic pain relief [75-77]. This effect led to their approval by the FDA for treating bone metastasis in cancer, an indication that is independent of the histologic type of cancer. As such, bisphosphonates are approved in the US for treating osteosarcoma patients with bone metastasis. Bisphosphonates have yielded promising results from *in vitro* testing [74,78,79], as well as in murine [80] and canine [81] systems of osteosarcoma.

When osteosarcoma patients develop bone metastases, the normal bone is lysed, sometimes by the direct action of osteosarcoma cells but more commonly by recruitment of normal osteoclasts. The effect of zoledronic acid against osteosarcoma cell lines *in vitro* has been evaluated, and tumorcidal activity has not been observed at concentrations achievable in serum [82]. However, tumor cell killing is readily observed at the concentrations expected near the lytic bone interface (the author's own unpublished observations). Thus, it could be predicted that bisphosphonate infusions would have little impact on established tumors in osteosarcoma patients, but may be helpful in preventing the development of new metastatic lesions.

This effect is exactly what has been observed in our use of zoledronic acid in patients with advanced osteosarcoma. Both Pete Anderson and I have given zoledronic acid concurrently with several chemotherapy agents (including liposomal doxorubicin, ifosphamide, cisplatin, methotrexate, bevacizumab and sirolimus) to osteosarcoma patients under our care. Calcium supplementation is required, and no severe toxicities have been encountered. In these patients, a characteristic pattern has emerged: the pain from established bone metastases is diminished, although it is difficult to determine whether this is due to the bisphosphonates, the chemotherapy, or concurrent radiotherapy. More importantly, the patients essentially do not develop any new lytic bone lesions. As lytic bone lesions are the cause of severe pain in patients dying from osteosarcoma, effective use of bisphosphonates is transforming the course of palliation for this disease. Patients still succumb to refractory osteosarcoma (often in the lungs and soft tissues), but they have fewer bone lesions and require less opiate-based pain relief, providing better quality of life during palliation. A clinical trial within the Children's Oncology Group is assessing the feasibility of incorporating bisphosphonate therapy with conventional MAP chemotherapy in patients newly diagnosed with high-risk osteosarcoma.

It is important to remember that osteosarcoma, by definition, creates new bone within tumors. As the chemical structure of bisphosphonates and tetraphosphonates targets these compounds to newly formed bone, this targeting effect could be harnessed to deliver new therapeutics at higher concentration within the growing tumors themselves, provided the conjugates are not toxic to normal osteoclasts and marrow components.

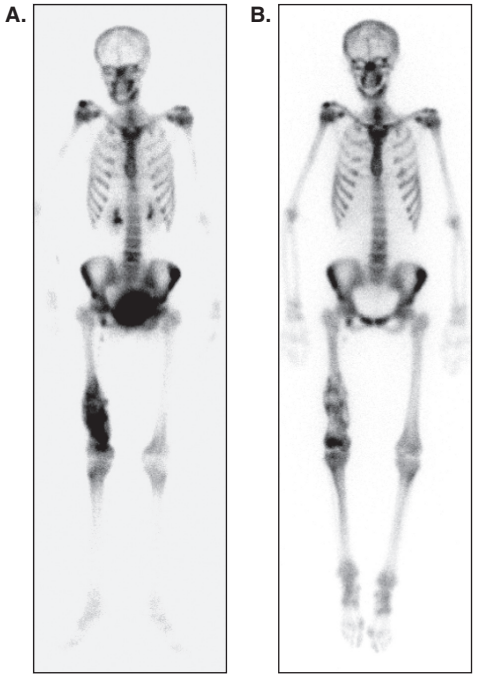

Figure 2. Bone scan with ^{99}Tc and scan of therapeutic ^{153}Sm from the same patient. A. A teenager with small-cell osteosarcoma of the right distal femur and widely metastatic disease had a bone scan after receiving four courses of doxorubicin (90 mg/m^2) and ifosfamide (9 g/m^2). The radiotracer filling the bladder is clearly seen, as is the abnormal uptake in the right distal femur. **B.** Following 6 weeks of external beam radiotherapy (60 g total dose), augmented with cisplatin (60 mg/m^2 per dose on 2 consecutive weeks) and high-dose methotrexate (12 g/m^2 given in week 4), the patient was treated with samarium-153 ethylene diamine tetramethylene phosphonate (1 mCi/kg) on the penultimate day of external beam radiotherapy. The image shown was taken 24 h after samarium infusion. Tracer signal is decreased in the primary tumor, consistent with a treatment effect during external beam radiotherapy. Gemcitabine was administered after the final dose of external beam radiation was delivered. The visibility of normal bones on the samarium scan provides an explanation for the sometimes prolonged marrow suppression observed following samarium treatment.

7. Conclusion

New therapies will be essential for improving survival in osteosarcoma, especially for patients with initially metastatic disease and multifocal relapse. Inhalation therapy is a much more promising approach than intra-arterial treatments, which are technically cumbersome, and expensive and uncomfortable. By contrast, inhaled therapies can be administered at home, are low-cost and 'low-tech', and provide treatment to the most critical organ preferentially. Intracavitary cytotoxic treatments, especially intrapleural cisplatin, should be considered whenever a malignant effusion develops that requires placement of a drain. Bisphosphonates such as zoledronic acid should be considered for any patients considered at high risk for future development of bone metastasis, and new therapies should be developed that exploit the unique affinity of phosphonates for newly synthesized bone.

8. Expert opinion

It is clear that standard chemotherapeutics, given by means of standard routes of administration, have reached their limits for improving outcomes in osteosarcoma. Over the last two decades, multiple clinical trials have evaluated more intensive chemotherapy protocols and the addition of different or extra agents, with only modest improvements in event-free and overall survival. Unfortunately, > 30% of patients diagnosed with localized osteosarcoma this year are still expected to have a recurrence, and most of these will recur with tumor that is refractory to most conventional osteosarcoma treatments. The doses required to achieve a significant response for most chemotherapies following relapse rapidly become too high for the bone marrow to tolerate repeatedly. Clearly, new approaches are needed, in terms of both agents and modes of delivery.

The key target organ in osteosarcoma is the lung, as this is the site where most first relapses occur. With better therapies targeted directly to the lung, micrometastatic and grossly metastatic disease in the lung can be subjected to therapeutic levels of cytotoxic agents without causing dose-limiting damage to the marrow. Important initial steps have been taken in developing inhalation agents for clinical use, and preliminary results from clinical trials show minimal side effects. Hard work still remains: building on the success of the early studies using inhaled chemotherapy, the agents and combinations that will maximize this approach need to be found. It is likely that both systemic and inhaled agents will be required. The schedule, timing and location of drug delivery will all be important in developing treatments that provide the greatest improvement in 'good quality-of-life' time. By promoting home nebulization of chemotherapy together with remote spirometry, clinical trial designers can develop effective and safe treatments that offer better quality of life and may give greater efficacy.

Although the lungs are usually the first site of relapse in osteosarcoma patients, the metastatic site that often accounts for the most severe symptoms, especially pain, is bone. Whether identified at the time of initial diagnosis or during subsequent relapse, bone metastasis in osteosarcoma portends a poor outcome, and few patients survive 5 years or more after extrapulmonary or osseous recurrence. For this reason, agents that target the biology of the bone will be important in moving the survival curve and controlling symptoms for osteosarcoma patients. Samarium-153 ethylene diamine tetramethylene phosphonate (^{153}Sm-EDTMP) is the hallmark agent for directing radiation to bone metastases. For selected patients, ^{153}Sm-EDTMP can provide durable disease control and effective symptom relief. The tetraphosphonate chelate group attached to the radioactive ^{153}Sm could be considered for developing even better targeted therapies. As newer agents are developed, however, it will be important to remember the

limitations already seen from therapy with ^{153}Sm-EDTMP: only those tumors that make new bone will be treated effectively, and the toxicity on the bone marrow can be quite severe, at least for ^{153}Sm-EDTMP.

Another way to exploit bone biology to provide effective antitumor therapy may be to use nitrogen-containing bisphosphonates such as zoledronic acid. Although these agents are unlikely to cause direct killing of existing tumors to any great degree, bisphosphonates are incorporated into new bone as it is remodeled. It is hypothesized that, given sufficient treatment in advance with zoledronic acid or similar agents, the normal bones become effectively shielded against future bone metastases developing, because new lesions would be releasing extremely high concentrations of drug at the tumor–bone interface, effectively inhibiting mevalonate pathway synthesis and stopping the signals of all prenylated proteins. Expert biochemists should explore whether toxins or biologic modifiers could be covalently bonded to nitrogen-containing bisphosphonates to provide further protection of bones against future relapse.

One unexplored opportunity for targeted drug delivery occurs at the time of biopsy itself. Although laboratory-based investigators such as the author and clinical pathologists often express a fondness for the large amounts of tumor that can be obtained from open biopsy, the vast majority of bone cancer patients now are diagnosed from biopsies obtained with a core needle. A similar needle is used when thermal ablation (radiofrequency ablation or cryoablation) is used to palliate metastatic bone sarcoma lesions. The placing of a large needle into the center of a tumor is a therapeutic opportunity that often is missed. For example, one could envisage coupling thermal ablation with immune modulation: a tumor could be heated to induce heat-shock proteins and/or frozen to create necrosis, then a gel containing IL-2, IL-12 and/or IL-18 could be injected into the cavity created by the core needle. These cytokines could recruit phagocytes, precursors to dentritic cells, and naive T cells to the tumor and nearby lymph nodes, where the adaptive immune system could mount responses against tumor-specific antigens, providing patients with protective immunity against their own tumors. The recent approval of mifuramide (L-MTP-PE) in Europe demonstrates a growing awareness that immunologic approaches to osteosarcoma can lead to improved survival.

The old paradigm of providing cytotoxic systemic therapy through a central venous line and local control surgery as the only means of treating bone sarcomas needs to be modified. Certainly, chemotherapy has been important in improving survival of patients with clinically localized disease from ~ 20 to ~ 70%, but more needs to be done. Inhalation agents, targeted small molecules that exploit tumor cell biology, and creative biochemically targeted agents such as bisphosphonates and tetraphosphonates, will be needed to move the survival curve and cure more of the young people stricken by bone sarcomas each year. The time has come to treat 'outside the lines'.

Declaration of interest

The author declare no conflicts of interest and has received no payment for the preparation of this manuscript.

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