PERSPECTIVES

Bone Sarcomas: Pathogenesis and New Therapeutic Approaches

Dominique Heymann[1,2,3] and Françoise Rédini[1,2]

[1]INSERM, UMR 957, Nantes, France
[2]Université de Nantes, Nantes Atlantique Universités, Laboratoire de Physiopathologie de la Résorption Osseuse et Thérapie des Tumeurs Osseuses Primitives, Faculté de Médecine, Nantes, France
[3]University Hospital, Hôtel Dieu, CHU de Nantes, France

Abstract

Bone sarcomas include a very large number of tumor types belonging to the family of primary malignant bone tumors and originate from bone. Osteosarcoma, Ewing's sarcoma and chondrosarcoma are the three main sarcomas diagnosed in humans and despite their scarcity, sarcomas are characterized by a relatively high morbidity and mortality, especially in children and adolescents. Bone sarcomas exhibit highly heterogeneous histologic and molecular profiles; their morphology is one of the key aspects of their identification. With the exception of Ewing's sarcoma, which is related to a chromosomal translocation between the *EWS* gene and a gene from the *ETS* family, or to specific inherited syndromes (*e.g.*, *p53*, *Rb*), the causes of most bone sarcomas are not fully understood. Recent published work demonstrates the key relationship between sarcoma cells and their microenvironment, opening a new era for understanding the pathogenesis of and advancing therapy for bone sarcomas, which are the focus of this *Perspective*. *IBMS BoneKEy*. 2011 September;8(9):402-414.
©2011 International Bone & Mineral Society

Introduction

Bone tumors include a wide variety of cellular entities originating from bone cells or their precursors, termed primary bone tumors, as well as originating from non-osseous origins, known as bone metastases that are common complications of several cancers (*e.g.*, breast, prostate, kidney). Although bone metastases are composed mainly of epithelial-derived cells (carcinoma cells), primary bone tumors are non-epithelial tumors with varying evolutions: benign or malignant (bone sarcomas), associated or not associated with metastases (1). Of all of these tumors, some are purely osteolytic, such as giant cell tumors (benign entity), while specific subtypes lead to a new calcified matrix, as in the case of osteosarcoma, to a cartilaginous extracellular matrix (*e.g.*, chondroma, chondrosarcoma) or induce mixed osteolytic/osteoblastic lesions. This high histological heterogeneity is one of the main features of bone sarcomas and makes their classification very complicated.

The three main bone sarcomas include osteosarcoma, Ewing's sarcoma and chondrosarcoma. Osteosarcomas represent half of these sarcomas and affect a young population with a first peak in incidence around 18 years of age and a second peak occurring around 50 years of age; this second peak corresponds to osteosarcoma occurring in those with Paget's disease or with irradiated bone. The incidence of osteosarcoma is estimated at approximately 4.8 new cases per year per million children under 20 years of age (2;3). Ewing's sarcoma is the second malignant primary bone tumor in children with a peak incidence around 15 years of age and with an incidence of about 2.9 new cases per year per million children under 15 years of age (2;3). For both entities, forms that are metastatic to bone or to the lungs have a poor prognosis. In contrast to osteosarcoma and Ewing's sarcoma, chondrosarcomas are

402

diagnosed mainly in the third to the sixth decade, with an incidence of about 1.8 new cases per million people per year. Chondrosarcomas are highly resistant to both chemotherapy and radiotherapy and current treatment is based essentially on surgical approaches showing high levels of local recurrence (4;5). This *Perspective* focuses on the pathogenesis of the three main bone sarcomas; describes pre-clinical models of these conditions; and discusses new therapeutic approaches.

Bone Sarcomas Are Not Homogeneous Entities and Exhibit Very High Histological and Molecular Diversity

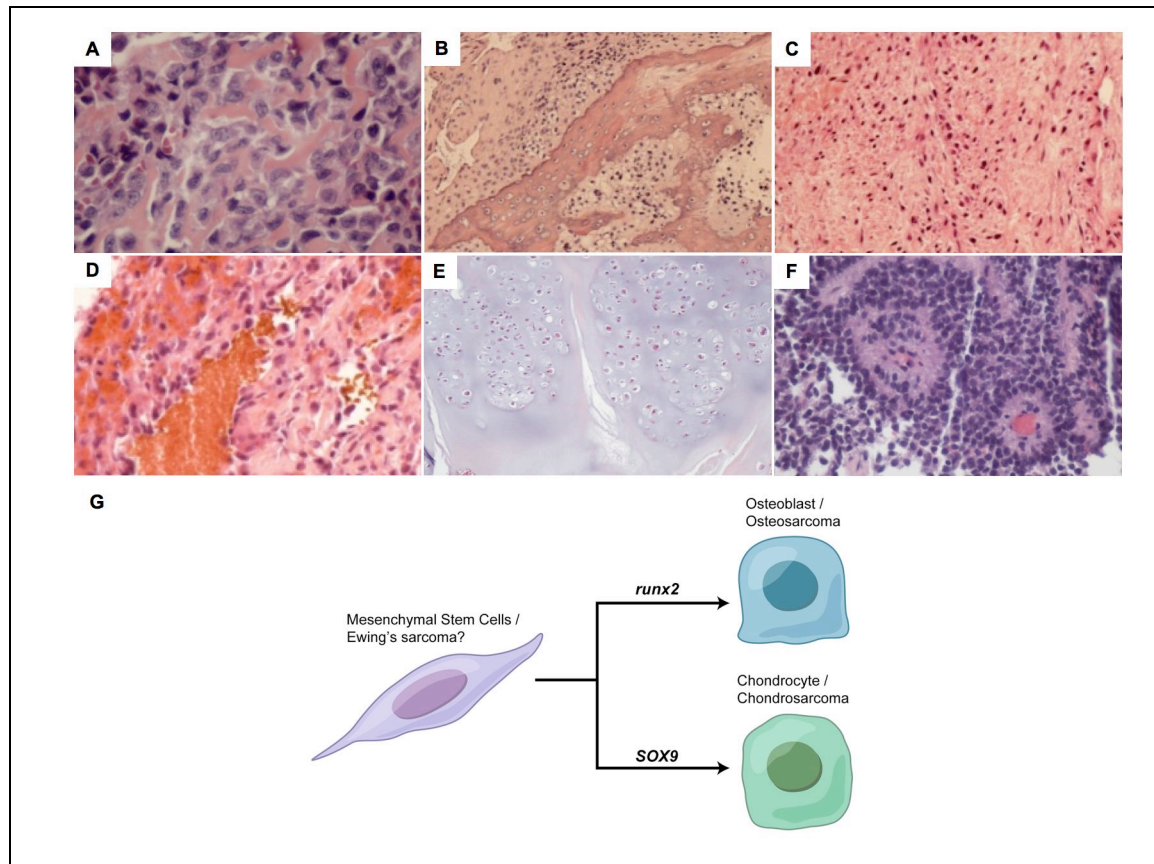Sarcoma cells are non-epithelial cells with a mesenchymal morphology (Fig. 1).

Fig. 1. Bone sarcomas are highly heterogeneous entities at the histological and molecular levels. All osteosarcomas are characterized by at least one focus of osteoid tissue. A) Osteoblastic osteosarcoma; B) Chondroblastic osteosarcoma characterized by a mixed cartilaginous-like and osteoid extracellular matrix, with corresponding cells; C) Fibroblastic osteosarcoma characterized by osteosarcoma cells with a fibroblastic morphology; D) Telangiectasic osteosarcoma: a rare form of osteosarcoma, with a highly vascularized appearance; E) Chondrosarcoma; F) Typical Ewing's sarcoma with CD99^{+} small round cells. Original magnification: x 200. G) The origin of bone sarcoma cells.

Indeed, osteosarcomas, like chondrosarcomas, originate from connective tissues and thus derive from the mesoderm. In this context, it has been suggested that both of these entities derive from multipotent cells called mesenchymal stem cells that are able to differentiate into various cell types like fibroblasts, osteoblasts, chondrocytes, or adipocytes (6;7). During development, some transcription factors generate a hierarchical regulation of expression to drive cell differentiation into specific lineages. For instance, Sry-related high-mobility group box (Sox) transcription factor 9 (Sox9) is clearly important to chondrogenesis (8), and runt-related transcription factor 2 (Runx2) to osteoblastogenesis (9). Similar to osteoblasts and chondrocytes,

osteosarcoma and chondrosarcoma cells express Sox9 (10) and Runx2 (11), consolidating their mesenchymal origin and their commitment to bone and cartilage lineages, respectively. In addition, osteoblast/osteosarcoma cells express specific markers such as alkaline phosphatase, osteocalcin or bone sialoprotein and chondro-cytes/chondrosarcoma cells express aggrecan, type II and type X collagen (Fig. 1G). Finally, osteosarcomas and chondrosarcomas may result from dysregulation of the differentiation program of mesenchymal stem cells (7).

In contrast, the origin of Ewing's sarcoma is uncertain. These tumors were initially included in the primitive neuroectodermal family of tumors (PNET) sharing a chromosomal translocation that involves the *EWS* gene on chromosome 22 with a gene of the erythroblast transformation sequence (*ETS*) family (12). This specific phenotypic implication of *EWS* genes suggests that Ewing's sarcoma may have a neuroectodermal origin and arise from the neural crest. However, the bone localization and recent experiments based on *EWS-FLI1* silencing ascribed the origin of Ewing's cells to mesenchymal stem cell progenitors (13). Indeed, Tirode *et al*. have demonstrated that, upon *EWS-FLI1* silencing, different Ewing cell lines differentiate along the adipogenic lineage and also along the osteogenic lineage upon long-term inhibition of *EWS-FLI1* (13). These observations have been corroborated by Riggi *et al*. who have shown that *EWS-FLI-1* modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming towards Ewing's sarcoma cancer stem cells (14). Despite these observations, the debate over the origin of Ewing's sarcoma continues. Indeed, Rocchi *et al*. (15) have provided evidence of a new role for CD99, a membrane protein expressed in most cases of Ewing's sarcomas, in preventing neural differentiation of Ewing's sarcoma cells and suggest that blockade of CD99 or its downstream molecular pathway may represent a new therapeutic approach for Ewing's sarcoma. Furthermore, in a panel of human Ewing's sarcoma cell lines, these authors demonstrated an inverse correlation

between CD99 and H-neurofilament expression, as well as an inverse correlation between neural differentiation and oncogenic transformation (15). Whether Ewing's sarcoma is of mesodermal or neuroectodermal origin remains a completely open question.

Osteosarcoma is defined by the presence of tumor cells producing an osteoid matrix. According to the degree of differentiation displayed by osteosarcoma cells and the nature of the extracellular matrix produced, three main subtypes have been identified but all of them exhibit at least one focus of osteoid tissue. The most frequent subtype is osteoblastic osteosarcoma (Fig. 1A) with tumor osteoblast-like cells producing osteoid matrix organized in a complex trabecular network that corresponds to the typical 'sunburst' pattern observed with radiography. Consistent with endochondral ossification, the second osteosarcoma subtype, chondroblastic osteosarcoma, is characterized by foci of low differentiated cells producing cartilaginous matrix (Fig. 1B). The third subtype is composed of undifferentiated cells that look like fibroblasts and is termed fibroblastic osteosarcoma (Fig. 1C). Related to this classification, there is a correlation between the degree of osteoblastic differentiation and the prognosis. Additional rare subtypes can also be diagnosed as telangiectasic forms of osteosarcoma with large vessel lakes penetrating the tumor mass (Fig. 1D). Similarly, chondrosarcomas are in fact composed of numerous subtypes displaying varying degrees of differentiation (5;16;17) but are all characterized by the presence of tumor chondrocytes drawn into a hyaline-like extracellular matrix constituting cartilaginous lobules separated by low vascularized fibrous tissue (Fig. 1E). Ewing's sarcomas are the most homogeneous bone sarcomas and are composed of CD99^{+} and undifferentiated small round cell tumors with restricted stroma (Fig. 1F). This lack of differentiation has led to difficulty in understanding their embryologic origin.

The exact causes of osteosarcomas remain unknown, except in inherited syndromes that put patients at very high risk for osteosarcoma such as Li-Fraumeni

syndrome related to *p53* mutation, Rothmund-Thompson, Wermer or Bloom syndromes linked to mutations of helicase genes, or familial cancers involving *Rb* mutations (18;19). Overall, osteosarcomas are sporadic and characterized by complex karyotypes non-associated to recurrent translocations, amplifications or deletions that appear to be more the consequence of a lengthy oncogenic process than the cause of this disease (1;19). Nevertheless, genomic approaches have identified a variety of oncogenes linked to the pathogenesis of osteosarcomas such as *c-Myc*, which encodes a transcription factor that regulates both cell proliferation and growth, *MDM2*, an important negative regulator of *p53*; and *CDK4*, an oncogene associated with the regulation of cell cycle progression (1;19). Although the high complexity of the osteosarcoma genome has complicated the understanding of the etiology of osteosarcoma, the immunodetection of such oncogenes has generated strong interest from pathologists. This is the case, for instance, with *MDM2* and *CDK4*; immunohistochemical expression of these oncogenes provides sensitive tools as markers for the diagnosis of low-grade osteosarcomas, helping to differentiate them from benign fibrous and fibro-osseous lesions, particularly in cases with atypical radio-clinical presentation and/or limited biopsy samples (20).

Similarly to osteosarcomas, chondrosarcomas are associated with heterogeneous and complex cytogenetic factors (1;16;19). However, the progression from benign lesions to malignant tumors is likely better understood for chondrosarcomas than it is for osteosarcomas. Indeed, cartilaginous tumors range from enchondromas to osteochondromas that are benign lesions capable of evolving into malignant chondrosarcomas. The benign lesions result from the de-regulation of components of the Hedgehog signaling pathway, such as *EXT* genes involved in the normal epiphyseal growth plate and in endochondral ossification (16;17). From these benign lesions, the oncogenic process involves numerous extra- or intracellular mediators, such as IGF, CDK4, HIF, MMP, SRC and

AKT, already identified in other cancer cell types (16;17). Although these mediators appear as potential therapeutic targets, benign and malignant cartilaginous lesions are considered resistant to most drugs. However, such markers can be very useful for diagnosis as shown by the immunolocalization of galactin-1, which helps distinguish chondroblastic osteosarcomas from conventional chondrosarcomas (20). In contrast to osteosarcoma and chondrosarcoma, the etiology of Ewing's sarcoma is linked mainly to the translocation t(11;22)(q24;q12) in 85% of cases leading to the *EWS-FLI1* fusion gene, which behaves as an oncogene (12-15). The expression of *EWS-FLI1* associated with the morphological features of the tumors then confirms the diagnosis.

Pathogenesis of Bone Sarcomas: The 'Seed and Soil' Theory

Except for Ewing's sarcoma, which involves a fusion protein playing an oncogenic role, or specific inherited syndromes (*e.g.*, *p53*, *Rb*), the causes of most bone sarcomas are unknown. Most people with osteosarcoma do not have any specific risk factors and the etiology of osteosarcoma is now based mainly on the 'seed and soil' theory proposed by Stephen Paget at the end of the 19th century. This theory has led to the concept of a 'niche,' which is a specialized microenvironment promoting the emergence of tumor stem cells and providing all the required factors for their development. A vicious cycle is established between the bone microenvironment and cancer cells during the progression of bone metastases and primary bone tumors as well (5; 21-23). This concept was initially described for normal hemopoiesis that is sustained by the local osteoblastic niche (24). Indeed, it was shown that bone morphogenetic protein (BMP) signaling through BMP IA receptors controls the number of hemopoietic stem cells by regulating the size of the niche. Similarly, cancer cells growing in bone foci dysregulate bone cell (osteoclast and osteoblast) activities, modify vascularization and alter local immunity, thus contributing to their survival, growth and dissemination. Sarcoma cells secrete osteoclast-activating factors including cytokines (*e.g.*, IL-1, IL-6,

TNF-α), hormones (PTHrP), glycosaminoglycans and/or pro-osteoblastic factors such as BMPs, IGF or Wnts that induce an imbalance in bone remodeling. In turn, osteoblasts and osteoclasts release pro-tumor factors as soluble forms or trapped in the extracellular matrix (5;21-24)

(Fig. 2). Although the bone niche plays a key function in tumor growth, the bone microenvironment contributes to the enhanced resistance of tumors to therapy (5;25) and can sustain cancer cells in a quiescent state (26).
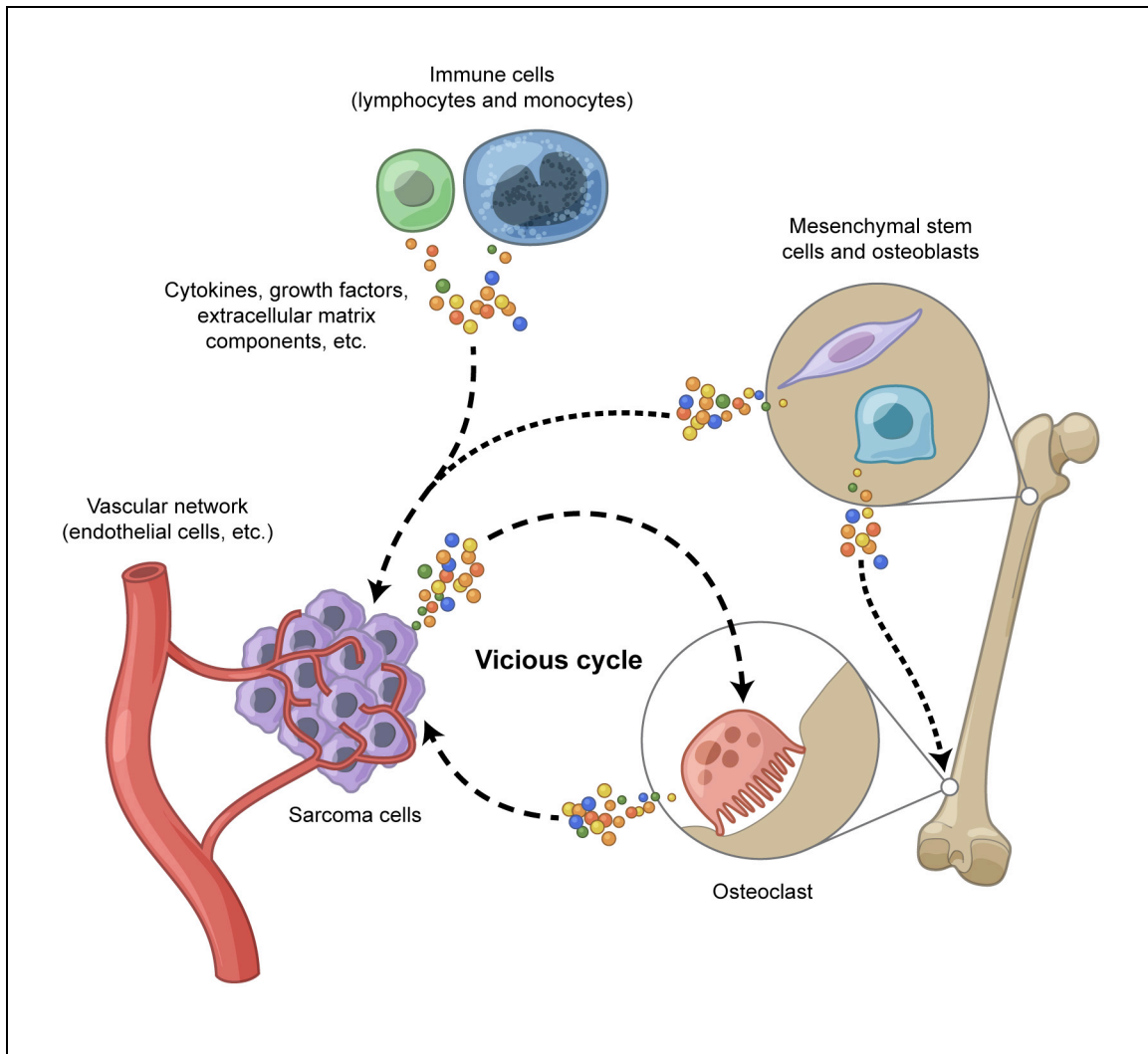

Fig. 2. Bone sarcomas and the 'seed and soil' theory. The bone niche is a specialized microenvironment providing all the required factors for the development of sarcoma cells. A vicious cycle is established between the bone microenvironment and bone sarcoma cells. Indeed, bone sarcoma cells dysregulate bone cell (osteoclast and osteoblast) activities, modify vascularization and alter local immunity, thus contributing to their survival, growth and dissemination.

Animal Models of Bone Sarcomas

Improving the survival of bone tumor patients with metastatic or recurrent disease requires novel therapeutic strategies. For this purpose, a number of murine models of osteosarcoma, Ewing's sarcoma and chondrosarcoma have been developed that permit genetic and pharmacologic manipulations of both tumor and host. To accurately assess the efficacy of intervention in these models requires the ability to quantify tumor burden in bone and in metastases developed in the lungs or

406

bone, with the worst prognostic factors for osteosarcoma and Ewing's sarcoma, respectively. *In vivo* models of bone tumors must first be reliable and reproducible, and also should mimic all aspects of the human condition at the temporal, physiological and histological levels, hence validating the accurate testing of therapeutic strategies. For example, ideally a mouse model of osteosarcoma should demonstrate the expression of several osteoblastic markers (such as alkaline phosphatase, pro-α1 collagen, osteopontin, and osteocalcin) that are distinctive of this tumor type. To be clinically relevant, such models should be developed in an immunocompetent host, display the characteristic production of an osteoid matrix, and be able to spontaneously develop pulmonary metastases.

Animal models reproducing the main primary bone tumors are numerous and can be generated by various approaches: via transplantation or endogenously, orthotopically or heterotopically, and experimentally or via spontaneous metastases. The most relevant models for each tumor type are presented in Table 1 and are summarized below:

--Syngeneic models of osteosarcoma induced (in mice or rats) by the orthotopic injection or transplantation of osteosarcoma murine/rat cells or tumor fragments, respectively. These cell lines present with more or less osteoblastic/osteolytic characteristics, thus reproducing the variability of clinical events observed in patients. Some of these models also develop spontaneous lung metastases. The more relevant models exhibit osteoid matrix production by tumor cells (*e.g.*, OSRGa in rats, MOS-J in mice) (27;28).

--Transgenic models of osteosarcoma induced by deletion of tumor suppressor genes (*p53* and *Rb*) under the control of an osteogenic promoter (osterix1) (29). All of these models present a high percentage of penetrance, with typical bone lesions and spontaneous pulmonary metastases.

--Xenogeneic models of Ewing's sarcoma or osteosarcoma induced in immune-

deficient mice following the orthotopic injection or transplantation of human cells/tumor fragments. All of these models induce the development of primary bone tumors associated with intense bone remodeling and highly osteolytic lesions and spontaneous lung metastases (30). They allow for the study of models induced by cells derived from patients, but do not take the immune system into account.

--Syngeneic and xenogeneic models of chondrosarcoma: very few of these models are available. A rat swarm chondrosarcoma model in immunocompetent animals is often used in preclinical therapeutic and imaging testing (31). Human chondrosarcoma cell lines have also been used to induce xenogeneic models in nude mice (32).

Beyond this non-exhaustive list, there is a lack of availability of syngeneic models of Ewing's sarcoma. Some attempts have been made to modify human or mouse mesenchymal stem cells to overexpress the fusion gene *EWS-FLI1*, and then to inject these modified cells into immunodeficient or immunocompetent mice of the same strain (33). Unfortunately, the models failed in their reproducibility. A recent study from von Levetzow *et al.* describes the consequence of expressing *EWS-FLI1* in human embryonic neural crest stem cells (NCSCs) (34). The authors demonstrate that NCSCs tolerate expression of *EWS-FLI1* and ectopic expression of the oncogene initiates transition to a Ewing sarcoma family tumor (ESFT)-like state. Therefore, there is real hope for the availability of such a syngeneic model in the near future. Beyond these relevant animal models, pre-clinical research over the past few years has also focused on specific imaging of primary bone tumors in order to have an early response to therapeutic strategies, and to further transfer these new imaging techniques to the clinic for early and specific diagnosis. For example, quaternary ammonium (QA) specifically targets the proteoglycan content of the abundant cartilaginous matrix specific to chondrosarcoma (31). This molecular characteristic has been used not only for imaging (technecium linked to QA) but also to vectorize the therapy agent (35).

Table 1. Characteristics of osteosarcoma, Ewing's sarcoma and chondrosarcoma models used in preclinical studies.

OSTEOSARCOMA MODELS					
CELL SPECIES	HOST	INDUCTION APPROACH	BONE LESIONS	PULMONARY METASTASES	REFERENCE
Human					
HOS	immunodeficient	para- or intra-osseous injection	osteolytic	yes (spontaneous)	ATCC CRL 1543
U2OS	immunodeficient	para- or intra-osseous injection	osteolytic	negative	ATCC HTB96
Mouse					
POS-1	C3H/HeJ	transplantation	osteolytic (+++)	yes (spontaneous)	(54)
POS-1	C3H/HeJ	i.v.	na	yes (experimental)	(54)
MOS-J	C57BL/6J	para-osseous injection	osteoblastic (+++)	yes (spontaneous)	(28)
K7M2	BALB/c	transplantation, para-osseous injection	osteolytic (++)	yes (spontaneous)	(55)
Rat					
OSRGa	Sprague Dawley	transplantation	osteolytic and osteoblastic (++)	yes (spontaneous)	(56)
OSRGa	Sprague Dawley	intra-osseous injection	osteoblastic (++)	yes (spontaneous)	(27)

EWING'S SARCOMA MODELS					
CELL SPECIES	HOST	INDUCTION APPROACH	BONE LESIONS	METASTASES	REFERENCE
Human					
BRZ, TC-71, A-673, TC-32, EW7, EW24, RDES, STA-ET1, SK-NMC, SK-ES1	immunodeficient	para- or intra-osseous injection, transplantation	osteolytic (+++)	yes (spontaneous in lungs)	(30)
Mouse					
MSC - EWS-FLI1	C57BL/6J	sub-cutaneous	?	?	(33)

CHONDROSARCOMA MODELS					
CELL SPECIES/NAME	HOST	INDUCTION APPROACH	BONE LESIONS	METASTASES	REFERENCE
Rat/Swarm	Sprague Dawley	para- or intra-osseous transplantation	+	0	(57)
Human CAL78	immunodeficient mice	para-osseous injection	?	?	(32)

New Therapeutic Approaches for Bone Sarcomas

As stated previously, there is an urgent need to develop new therapeutic options for patients in high risk groups, for those who present with pulmonary or bone metastases (for osteosarcoma or Ewing's sarcoma, respectively), for poor responders to chemotherapy, or for patients in relapse. Innovative therapeutic strategies target not only the tumor cells by combinatory approaches, but also the bone microenvironment; innovation also resides in the methodology used (gene therapy, or cell and immune therapies):

--**Combination with chemotherapy.** This approach consists of combining therapies that focus on different molecules in different transduction pathways (a horizontal combinatory strategy), or different targets in the same pathway (a vertical combinatory strategy) or a single target by different mechanisms. All of these innovative strategies aim to overcome resistance phenomena that can occur in a distinct signaling pathway or with chemotherapy. Among these strategies, the combination of RAD001, an inhibitor of the mTOR pathway, with zoledronic acid, which inhibits the enzyme farnesyl diphosphate synthase in the mevalonate pathway, has proven efficacy and synergy in preclinical models of osteosarcoma (36). Another promising approach is the use of tyrosine kinase inhibitors, such as imatinib mesylate (Gleevec), which has demonstrated great efficacy in other sarcomas, such as gastrointestinal stromal tumors (GISTs), from soft tissues and this could be extended to bone sarcomas (37).

--**Targeting the bone microenvironment.** Research has advanced in the field of bone biology and bone-tumor cell interactions, demonstrating the fine regulation that takes place between various protagonists during tumor development in bone. Therefore, several strategies have been proposed that target the osteoclast, the cell responsible for bone resorption, or that target the molecular protagonists involved in the regulation of bone resorption, primarily receptor activator of NF-κB ligand (RANKL). Preclinical studies using bisphosphonates such as zoledronic acid have demonstrated proof-of-concept of such an anti-bone resorption strategy (38). Zoledronic acid not only induces osteoclast apoptosis but also inhibits osteosarcoma, Ewing's sarcoma and chondrosarcoma cell proliferation *in vitro* and tumor progression *in vivo* (39-41). Results regarding the benefit of combining bisphosphonates with chemotherapy in osteosarcoma preclinical models serve as the rationale for the French phase III clinical trial OS2006 for children, adolescents and adults with osteosarcoma. Beyond bisphosphonates, which target osteoclasts, new therapeutic strategies have been developed to target RANKL. Preclinical studies have described the inhibitory effect of osteoprotegerin, the decoy receptor of RANKL, or RANK-Fc on osteosarcoma progression (42;43). Additional studies have shown that RANKL expression can also be downregulated by an RNA interference strategy, leading to the prevention of tumor relapse when associated with chemotherapy treatment (44). Such therapeutic molecules (OPG, RANK-Fc) can be administered as recombinant molecules or by gene transfer. The availability of synthetic vectors such as amphiphilic polymers or cationic glycolipids has dramatically improved the rate of protein expression and production with minimal side effects (45;46).

--**Gene therapy.** This approach offers promising results; for example, the effectiveness of *p53* gene therapy via a transferring-liposome-*p53* complex administered in animal models has been reported (47). Gene transfer is also widely used to overexpress genes of therapeutic interest in the microenvironment, as described above for RANK-Fc or OPG, or for immune-modulatory cytokines (such as IL-12; see below).

--**Cell and immune therapies.** The only cell therapy protocol developed for primary bone tumors is currently focused on Ewing's sarcoma as this tumor can express tumor antigens that can be recognized by T cells, making allogeneic stem cell transplantation a potential option for those patients with refractory disease (48). An update of the results from the Pediatric Stem-Cell Transplant Centers of Düsseldorf and

409

Vienna evaluated the possible therapeutic benefit after allogeneic stem cell transplantation in patients with advanced Ewing's sarcoma. The results showed that event-free survival in advanced Ewing's sarcoma patients was not improved by this technique due to a higher complication rate (49). Concerning osteosarcoma, along with the initial development of chemotherapy were early attempts with immunotherapy, which met with little success. Subsequent approaches to harnessing the immune system have yielded more encouraging results. Liposomal muramyl tripeptide phosphatidylethanolamine (MTP-PE), an activator of monocytes and macrophages inducing the secretion of cytokines such as IL-1, IL-6, and TNF-α, has been tested in clinical studies of osteosarcoma patients, with promising results (50). Moreover, an immunomodulator of interferon for use as maintenance therapy has also been tested (51). Several preclinical studies using IL-12 delivered as aerosol therapy have shown beneficial effects on the inhibition of pulmonary metastases development in osteosarcoma models (52). Moreover, IL-18 associated with IL-12 enhances its therapeutic potential (53).

Conclusion

Although bone sarcomas are oncologic diseases with complex genetics, their pathogenesis is related mainly to bi-directional interactions established between cancer cells and the bone microenvironment. This bone niche now represents a large area of active investigation, whose goal is to overcome cancer cell resistance and to develop new combined therapies.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

References

1. Heymann D, ed. *Bone Cancer: Progression and Therapeutic Approaches*. San Diego: Academic Press;2010.

2. Desandes E, Clavel J, Berger C, Bernard JL, Blouin P, de Lumley L, Demeocq F, Freycon F, Gembara P, Goubin A, Le Gall E, Pillon P, Sommelet D, Tron I, Lacour B. Cancer incidence among children in France, 1990-1999. *Pediatr Blood Cancer*. 2004 Dec;43(7):749-57.

3. Ries LG, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, Bunin GR, eds. *Cancer Incidence and Survival Among Children and Adolescents: United States SEER Program 1975-1995*, National Cancer Institute, SEER Program. NIH Pub. No. 99-4649. Bethesda, MD, 1999.

4. Lee FY, Mankin HJ, Fondren G, Gebhardt MC, Springfield DS, Rosenberg AE, Jennings LC. Chondrosarcoma of bone: an assessment of outcome. *J Bone Joint Surg Am*. 1999 Mar;81(3):326-38.

5. David E, Blanchard F, Heymann MF, De Pinieux G, Gouin F, Rédini F, Heymann D. The bone niche of chondrosarcoma: a sanctuary for drug resistance, tumour growth and also a source of new therapeutic targets. *Sarcoma*. 2011;2011:932451.

6. Deschaseaux F, Sensébé L, Heymann D. Mechanisms of bone repair and regeneration. *Trends Mol Med*. 2009 Sep;15(9):417-29.

7. Mohseny AB, Hogendoorn PC. Concise review: mesenchymal tumors: when stem cells go mad. *Stem Cells*. 2011 Mar;29(3):397-403.

8. Akiyama H. Control of chondrogenesis by the transcription factor Sox9. *Mod Rheumatol*. 2008;18(3):213-9.

9. Karsenty G. Transcriptional control of skeletogenesis. *Annu Rev Genomics Hum Genet*. 2008;9:183-96.

10. Tang X, Lu X, Guo W, Ren T, Zhao H, Zhao F, Tang G. Different expression of Sox9 and Runx2 between chondrosarcoma and dedifferentiated chondrosarcoma cell line. *Eur J Cancer Prev*. 2010 Nov;19(6):466-71.

11. Wagner ER, Luther G, Zhu G, Luo Q, Shi Q, Kim SH, Gao JL, Huang E, Gao Y, Yang K, Wang L, Teven C, Luo X, Liu X, Li M, Hu N, Su Y, Bi Y, He BC, Tang N, Luo J, Chen L, Zuo G, Rames R, Haydon RC, Luu HH, He TC. Defective osteogenic differentiation in the development of osteosarcoma. *Sarcoma*. 2011;2011:325238.

12. Delattre O, Zucman J, Melot T, Garau XS, Zucker JM, Lenoir GM, Ambros PF, Sheer D, Turc-Carel C, Triche TJ, et al. The Ewing family of tumors--a subgroup of small-round-cell tumors defined by specific chimeric transcripts. *N Engl J Med*. 1994 Aug 4;331(5):294-9.

13. Tirode F, Laud-Duval K, Prieur A, Delorme B, Charbord P, Delattre O. Mesenchymal stem cell features of Ewing tumors. *Cancer Cell*. 2007 May;11(5):421-9.

14. Riggi N, Suvà ML, De Vito C, Provero P, Stehle JC, Baumer K, Cironi L, Janiszewska M, Petricevic T, Suvà D, Tercier S, Joseph JM, Guillou L, Stamenkovic I. EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells. *Genes Dev*. 2010 May;24(9):916-32.

15. Rocchi A, Manara MC, Sciandra M, Zambelli D, Nardi F, Nicoletti G, Garofalo C, Meschini S, Astolfi A, Colombo MP, Lessnick SL, Picci P, Scotlandi K. CD99 inhibits neural differentiation of human Ewing sarcoma cells and thereby contributes to oncogenesis. *J Clin Invest*. 2010 Mar 1;120(3):668-80.

16. Bovée JV, Cleton-Jansen AM, Taminiau AH, Hogendoorn PC. Emerging pathways in the development of chondrosarcoma of bone and implications for targeted treatment. *Lancet Oncol*. 2005 Aug;6(8):599-607.

17. Bovée JV, Hogendoorn PC, Wunder JS, Alman BA. Cartilage tumours and bone development: molecular pathology and possible therapeutic targets. *Nat Rev Cancer*. 2010 Jul;10(7):481-8.

18. Ottaviani G, Jaffe N. The etiology of osteosarcoma. C*ancer Treat Res*. 2009;152:15-32.

19. Janeway KA, Walkley CR. Modeling human osteosarcoma in the mouse: From bedside to bench. *Bone*. 2010 Nov;47(5):859-65.

20. Dujardin F, Binh MB, Bouvier C, Gomez-Brouchet A, Larousserie F, Muret A, Louis-Brennetot C, Aurias A, Coindre JM, Guillou L, Pedeutour F, Duval H, Collin C, de Pinieux G. MDM2 and CDK4 immunohistochemistry is a valuable tool in the differential diagnosis of low-grade osteosarcomas and other primary fibro-osseous lesions of the bone. *Mod Pathol*. 2011 May;24(5):624-37.

21. Gomez-Brouchet A, Mourcin F, Gourraud PA, Bouvier C, De Pinieux G, Le Guelec S, Brousset P, Delisle MB, Schiff C. Galectin-1 is a powerful marker to distinguish chondroblastic osteosarcoma and conventional chondrosarcoma. *Hum Pathol.* 2010 Sep;41(9):1220-30.

22. Wittrant Y, Théoleyre S, Chipoy C, Padrines M, Blanchard F, Heymann D, Rédini F. RANKL/RANK/OPG: new therapeutic targets in bone tumours and associated osteolysis. *Biochim Biophys Acta*. 2004 Sep 20;1704(2):49-57.

23. Lamoureux F, Moriceau G, Picarda G, Rousseau J, Trichet V, Rédini F. Regulation of osteoprotegerin pro- or anti-tumoral activity by bone tumor microenvironment. *Biochim Biophys Acta*. 2010 Jan;1805(1):17-24.

24. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003 Oct 23;425(6960):836-41.

25. Borovski T, De Sousa E Melo F, Vermeulen L, Medema JP. Cancer stem cell niche: the place to be. *Cancer Res*. 2011 Feb 1;71(3):634-9.

26. Perrot P, Rousseau J, Bouffaut AL, Rédini F, Cassagnau E, Deschaseaux F, Heymann MF, Heymann D, Duteille F, Trichet V, Gouin F. Safety concern between autologous fat graft, mesenchymal stem cell and osteosarcoma recurrence. *PLoS One*. 2010 Jun 8;5(6):e10999.

27. Cherrier B, Gouin F, Heymann MF, Thiéry JP, Rédini F, Heymann D, Duteille F. A new experimental rat model of osteosarcoma established by intrafemoral tumor cell inoculation, useful for biology and therapy investigations. *Tumour Biol.* 2005 May-Jun;26(3):121-30.

28. Joliat MJ, Umeda S, Lyons BL, Lynes MA, Shultz LD. Establishment and characterization of a new osteogenic cell line (MOS-J) from a spontaneous C57BL/6J mouse osteosarcoma. *In Vivo*. 2002 Jul-Aug;16(4):223-8.

29. Walkley CR, Qudsi R, Sankaran VG, Perry JA, Gostissa M, Roth SI, Rodda SJ, Snay E, Dunning P, Fahey FH, Alt FW, McMahon AP, Orkin SH. Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease. *Genes Dev*. 2008 Jun 15;22(12):1662-76.

30. Teicher BA, Bagley RG, Rouleau C, Kruger A, Ren Y, Kurtzberg L. Characteristics of human Ewing/PNET sarcoma models. *Ann Saudi Med*. 2011 Mar-Apr;31(2):174-82.

31. Miot-Noirault E, Gouin F, Vidal A, Rapp M, Maublant J, Askienazy S, Chezal JM, Heymann D, Redini F, Moins N. First preclinical imaging of primary cartilage neoplasm and its local recurrence using 99mTc-NTP 15-5 radiotracer. *J Nucl Med*. 2009 Sep;50(9):1541-7.

32. Farges M, Mazeau C, Gioanni J, Ettore F, Denovion H, Schneider M. Establishment and characterization of a new cell line derived from a human chondrosarcoma. *Oncol Rep*. 1997 Jul-Aug;4(4):697-700.

33. Riggi N, Cironi L, Provero P, Suvà ML, Kaloulis K, Garcia-Echeverria C, Hoffmann F, Trumpp A, Stamenkovic I. Development of Ewing's sarcoma from primary bone marrow-derived mesenchymal progenitor cells. *Cancer Res*. 2005 Dec 15;65(24):11459-68.

34. von Levetzow C, Jiang X, Gwye Y, von Levetzow G, Hung L, Cooper A, Hsu JH, Lawlor ER. Modeling initiation of Ewing sarcoma in human neural crest cells. *PLoS One*. 2011 Apr 29;6(4):e19305.

35. Peyrode C, Weber V, David E, Vidal A, Auzeloux P, Communal Y, Dauplat MM, Besse S, Gouin F, Heymann D, Chezal JM, Rédini F, Miot-Noirault E. Quaternary ammonium-melphalan conjugate for anticancer therapy of chondrosarcoma: in vitro and in vivo preclinical studies. *Invest New Drugs*. 2011 Apr 16. [Epub ahead of print]

36. Moriceau G, Ory B, Mitrofan L, Riganti C, Blanchard F, Brion R, Charrier C, Battaglia S, Pilet P, Denis MG, Shultz LD, Mönkkönen J, Rédini F, Heymann D. Zoledronic acid potentiates mTOR inhibition and abolishes the resistance of osteosarcoma cells to RAD001 (Everolimus): pivotal role of the prenylation process. *Cancer Res*. 2010 Dec 15;70(24):10329-39.

37. Essat M, Cooper K. Imatinib as adjuvant therapy for gastrointestinal stromal tumors: a systematic review. *Int J Cancer*. 2011 May 1;128(9):2202-14.

38. Heymann D, Ory B, Blanchard F, Heymann MF, Coipeau P, Charrier C, Couillaud S, Thiery JP, Gouin F, Rédini F. Enhanced tumor regression and tissue repair when zoledronic acid is combined with ifosfamide in rat osteosarcoma. *Bone*. 2005 Jul;37(1):74-86.

39. Ory B, Blanchard F, Battaglia S, Gouin F, Rédini F, Heymann D. Zoledronic acid activates the DNA S-phase checkpoint and induces osteosarcoma cell death characterized by apoptosis-inducing factor and endonuclease-G translocation independently of p53 and retinoblastoma status. *Mol Pharmacol*. 2007 Jan;71(1):333-43.

40. Gouin F, Ory B, Rédini F, Heymann D. Zoledronic acid slows down rat primary chondrosarcoma development, recurrent tumor progression after intralesional curretage and increases overall survival. *Int J Cancer*. 2006 Sep 1;119(5):980-4.

41. Odri GA, Dumoucel S, Picarda G, Battaglia S, Lamoureux F, Corradini N, Rousseau J, Tirode F, Laud K, Delattre O, Gouin F, Heymann D, Rédini F. Zoledronic acid as a new adjuvant therapeutic strategy for Ewing's sarcoma patients. *Cancer Res*. 2010 Oct 1;70(19):7610-9.

42. Lamoureux F, Richard P, Wittrant Y, Battaglia S, Pilet P, Trichet V, Blanchard F, Gouin F, Pitard B, Heymann D, Rédini F. Therapeutic relevance of osteoprotegerin gene therapy in osteosarcoma: blockade of the vicious cycle between tumor cell proliferation and bone resorption. *Cancer Res*. 2007 Aug 1;67(15):7308-18.

43. Lamoureux F, Picarda G, Rousseau J, Gourden C, Battaglia S, Charrier C, Pitard B, Heymann D, Rédini F. Therapeutic efficacy of soluble receptor activator of nuclear factor-kappa B-Fc delivered by nonviral gene transfer in a mouse model of osteolytic osteosarcoma. *Mol Cancer Ther*. 2008 Oct;7(10):3389-98.

44. Rousseau J, Escriou V, Lamoureux F, Brion R, Chesneau J, Battaglia S, Amiaud J, Scherman D, Heymann D, Rédini F, Trichet V. Formulated siRNAs targeting Rankl prevent osteolysis and enhance chemotherapeutic response in osteosarcoma models. *J Bone Miner Res*. 2011 Jun 28. [Epub ahead of print]

45. Carrière M, Escriou V, Jollet A, Scherman D, Azoulay M, Monneret C. New synthetic glycolipids for targeted gene transfer: synthesis, formulation in lipoplexes and specific interaction with lectin. *Drug Deliv*. 2004 Nov-Dec;11(6):351-63.

46. Barteau B, Chèvre R, Letrou-Bonneval E, Labas R, Lambert O, Pitard B. Physicochemical parameters of non-viral vectors that govern transfection efficiency. *Curr Gene Ther*. 2008 Oct;8(5):313-23.

47. Nakase M, Inui M, Okumura K, Kamei T, Nakamura S, Tagawa T. p53 gene therapy of human osteosarcoma using a transferrin-modified cationic liposome. *Mol Cancer Ther*. 2005 Apr;4(4):625-31.

48. Lucas KG, Schwartz C, Kaplan J. Allogeneic stem cell transplantation in a patient with relapsed Ewing sarcoma. *Pediatr Blood Cancer*. 2008 Jul;51(1):142-4.

49. Burdach S, van Kaick B, Laws HJ, Ahrens S, Haase R, Körholz D, Pape H, Dunst J, Kahn T, Willers R, Engel B, Dirksen U, Kramm C, Nürnberger W, Heyll A, Ladenstein R, Gadner H, Jürgens H, Göbel U. Allogeneic and autologous stem-cell transplantation in advanced Ewing tumors. An update after long-term follow-up from two centers of the European Intergroup study EICESS. Stem-Cell Transplant Programs at Düsseldorf University Medical Center, Germany and St. Anna Kinderspital, Vienna, Austria. *Ann Oncol*. 2000 Nov;11(11):1451-62.

50. Meyers PA, Schwartz CL, Krailo M, Kleinerman ES, Betcher D, Bernstein ML, Conrad E, Ferguson W, Gebhardt M, Goorin AM, Harris MB, Healey J, Huvos A, Link M, Montebello J, Nadel H, Nieder M, Sato J, Siegal G, Weiner M, Wells R, Wold L, Womer R, Grier H. Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose

methotrexate. *J Clin Oncol*. 2005 Mar 20;23(9):2004-11.

51. Smeland S, Müller C, Alvegard TA, Wiklund T, Wiebe T, Björk O, Stenwig AE, Willén H, Holmström T, Follerås G, Brosjö O, Kivioja A, Jonsson K, Monge O, Saeter G. Scandinavian Sarcoma Group Osteosarcoma Study SSG VIII: prognostic factors for outcome and the role of replacement salvage chemotherapy for poor histological responders. *Eur J Cancer*. 2003 Mar;39(4):488-94.

52. Duan X, Jia SF, Koshkina N, Kleinerman ES. Intranasal interleukin-12 gene therapy enhanced the activity of ifosfamide against osteosarcoma lung metastases. *Cancer*. 2006 Mar 15;106(6):1382-8.

53. Liebau C, Roesel C, Schmidt S, Karreman C, Prisack JB, Bojar H, Merk H, Wolfram N, Baltzer AW. Immunotherapy by gene transfer with plasmids encoding IL-12/IL-18 is superior to IL-23/IL-18 gene transfer in a rat osteosarcoma model. *Anticancer Res*. 2004 Sep-Oct;24(5A):2861-7.

54. Kamijo A, Koshino T, Uesugi M, Nitto H, Saito T. Inhibition of lung metastasis of osteosarcoma cell line POS-1 transplanted into mice by thigh ligation. *Cancer Lett*. 2002 Dec 15;188(1-2):213-9.

55. Khanna C, Prehn J, Yeung C, Caylor J, Tsokos M, Helman L. An orthotopic model of murine osteosarcoma with clonally related variants differing in pulmonary metastatic potential. *Clin Exp Metastasis*. 2000;18(3):261-71.

56. Klein B, Pals S, Masse R, Lafuma J, Morin M, Binart N, Jasmin JR, Jasmin C. Studies of bone and soft-tissue tumours induced in rats with radioactive cerium chloride. *Int J Cancer*. 1977 Jul 15;20(1):112-9.

57. Grimaud E, Damiens C, Rousselle AV, Passuti N, Heymann D, Gouin F. Bone remodelling and tumour grade modifications induced by interactions between bone and swarm rat chondrosarcoma. *Histol Histopathol*. 2002 Oct;17(4):1103-11.