Pathology of Bone Metastasis

1

Carlo Della Rocca and Claudio Di Cristofano

Abstract

Bone metastases are a frequent complication of advanced cancer. Interactions between cancer cells and marrow stromal cells and bone turnover mechanisms are crucial in metastases growth and the pathogenesis of bone damage. Metastatic tumour cells stimulate the bone remodelling and indirectly induce the osteocytes to release several growth factors that promote the proliferation of stromal, haematopoietic and neoplastic cells in a sort of vicious circle. Histological examination of bone metastasis of known origin is performed usually to define prognostic and/or predictive markers for target cancer therapy; in the 10–30% of patients in which the primary tumour is not identified, the histologic findings derived from bone biopsy could be diagnostic by morphological or immunohistochemical assessment of the neoplastic tissue.

1.1 Introduction

Bone metastases are a frequent complication occurring in patients with advanced cancer, and these are a significant problem in the management of cancer patients. Patients with bone metastases often have a poor prognosis, and it results from the systemic spread of tumour. The skeleton is the third most frequent site for metastatic carcinoma dissemination after the lung and liver, and its colonization causes significant morbidity in patients with solid tumours. The breast and prostate cancer are responsible for more than 80% of cases of bone metastases, but also haematopoietic malignancies, such as multiple myeloma, or sarcomas may develop bone metastases.

Bone metastases are often characterized as osteolytic, as in breast cancer, or osteoblastic, as in prostate cancer. Although each bone segment may be involved, the thoracic spine is the most frequently involved site, followed by the cervical and lumbosacral spine.

Pain is the most common symptom of bone metastases related to pathological fractures, microfractures or interruption of the cortical bone; more rarely it is secondary to mechanical disturbances due to deformities.

C. D. Rocca, M.D. (✉)
Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Azienda Ospedaliera Universitaria Policlinico Umberto I, Rome, Italy
e-mail: carlo.dellarocca@uniroma1.it

C. Di Cristofano, M.D.
Pathology Unit, Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome - Polo Pontino, Latina, Italy

V. Denaro et al. (eds.), *Management of Bone Metastases*, https://doi.org/10.1007/978-3-319-73485-9_1

Pathological fracture occurs in the absence of a mechanical stress sufficient to interrupt the continuity of the bone segment, but it is caused by pre-existing bone structural modifications, such as the presence of metastases.

The interest in the study of bone metastases is not only due to the high prevalence of these lesions in cancer patients but also because it is a model to study the interaction of tumour cells and the associate marrow stromal cells. Indeed, bone metastases represent the first good example of the importance of the microenvironment in metastatic spread.

The biological affinity of cancer cells for the bone is due to the high vascularization of the bone marrow; moreover the bone microenvironment produces factors that promote the survival and the proliferation of cells.

The metastatic cancer cells migrate to the bone marrow across the sinusoidal wall and proliferate and stimulate bone turnover with the development of osteolytic or osteoblastic lesions. These two radiological aspects of metastases represent the two extremes of the abnormal regulation of physiological processes involved in bone remodelling.

Under physiological conditions, the bone homeostasis requires a continuous bone remodelling to adjust its resistance according to the load to which it is subjected and to remodel its form according to mechanical stress, depositing new organic matrix and removing the worn part.

This balance between bone regeneration and degradation is guaranteed by the coupled action of osteoblasts and osteoclasts. Osteoblasts are involved directly in remodelling, secreting organic matrix components and adjusting the deposition of minerals. When the osteoblasts become trapped in the matrix and transform in osteocytes unable to proliferate, the bone formation is interrupted.

Osteoclasts are the major actors in bone resorption; they form an extracellular bone compartment where pouring hydrochloric acid, proteolytic enzymes and other proteins is required for the acidic digestion of the organic and inorganic bone matrix. Osteoblasts secrete lysosomal enzymes that degrade the calcium ions, collagen fibres, glycoproteins and proteoglycans. The action of the osteoblasts and osteoclasts depends on the systemic factors including hormones and cytokines that promote their proliferation and modulate their actions.

All types of bone metastases, characterized as osteolytic, osteoblastic or mixed, are due to osteoclast activation, and this is associated with increased serum biochemical markers of bone remodelling, such as pyridinoline (PYD) that reflects the degradation of mature collagens or bone-specific alkaline phosphatase (BALP) that is associated to bone formation.

Several studies have been performed to assess the utility of markers of bone turnover to evaluate bone metastases [1, 2] or to monitor anticancer treatment response [1, 3, 4], as well as to predict bone complications. However, the clinical practice guidelines do not recommend the use of bone turnover markers to understand the clinical data and the treatment response in metastatic patients [5–7].

1.2 Bone Turnover and Metastasis

The bone remodelling is the process whereby microscopic mature bone tissue is reabsorbed and equivalent new bone tissue is formed. The process takes place at the BMU (bone multicellular units) level. The BMU are temporary microanatomic structures with resorption of old bone by osteoclasts and by a reconstruction phase, with osteoblasts activity. The two phases are in equilibrium, and the more bone is reabsorbed, the more it will be formed. For a long time it has been believed that osteocytes were only viewer of this important process, but now it's well known that they play an important role in bone turnover control and regulation. Dendritic shape is a characteristic of osteocytes, and the dendrites are longer and more abundant in mineralized matrix close to the bone surface; moreover the number of osteocyte dendrites is inversely proportional to the cell size and activity. In the transformation of the active osteoblast in the corresponding osteocyte, dendrite proliferation is directly related to osteocyte maturation.

The network formed by osteocytes with their dendritic extensions allows to control, through chemical mediators, bone formation and resorption and haematopoiesis [8]. In this model, osteocytes "feel" the load variations in the bone and, for a sort of piezoelectric stimulus, begin to produce some factors, such as the sclerostin, that stimulate osteoblasts and osteoclasts to adapt bone microarchitecture to mechanical variations [9, 10].

Sclerostin is a protein expressed mainly by mature osteocytes (Fig. 1.1), but is not expressed in osteoblasts, and it has an inhibitory activity on Wnt-β-catenin pathway [11]. The β-catenin is involved in many processes; it plays a key role in cytoskeleton and intercellular junction stabilization. Moreover, when Wnt ligand interacts with the cell surface Frizzled receptor, the β-catenin is not degraded by proteasome, but it enters into the nucleus and acts as a nuclear transcription factor, leading to activation of genes involved in Wnt pathway. In the bone the Wnt-β-catenin signalling promotes the maturation of osteoblasts and the survival of osteocytes and indirectly inhibits osteoclastogenesis by inducing the expression of OPG by osteocytes.

In osteoclast precursors, osteoclastogenesis is a process linked to the activation of the nuclear receptor NF-kB (RANK, receptor activator of nuclear factor-kB) induced by interaction with its ligand, the RANK-L protein (ligand of receptor activator of nuclear factor-kB), produced by osteocytes [12]. However, the osteocytes also produce the osteoprotegerin (OPG) protein (Fig. 1.1) that by binding to RANK-L inhibits the interaction with RANK and consequently osteoclast differentiation. In bone tissue microenvironment, the RANK-L and OPG ratio is a key factor in the differentiation of osteoclasts and so in bone resorption [13, 14].

All these actions are summarized in Fig. 1.2.

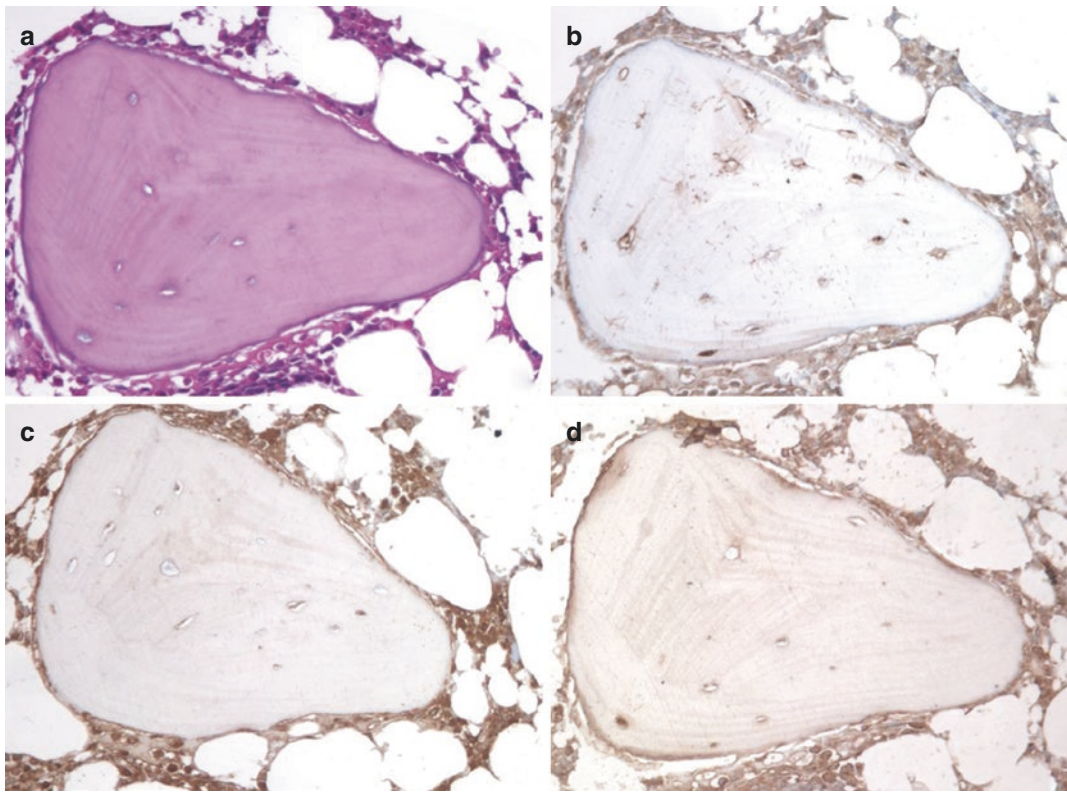

Fig. 1.1 (**a** HE 20X; **b** SOST 20X; **c** OPG 20X; **d** RANKL 20X) Sclerostin (**b**) and OPG (**c**) are clearly expressed in mature osteocytes, while RANKL (**d**) is only occasionally seen in the same cells

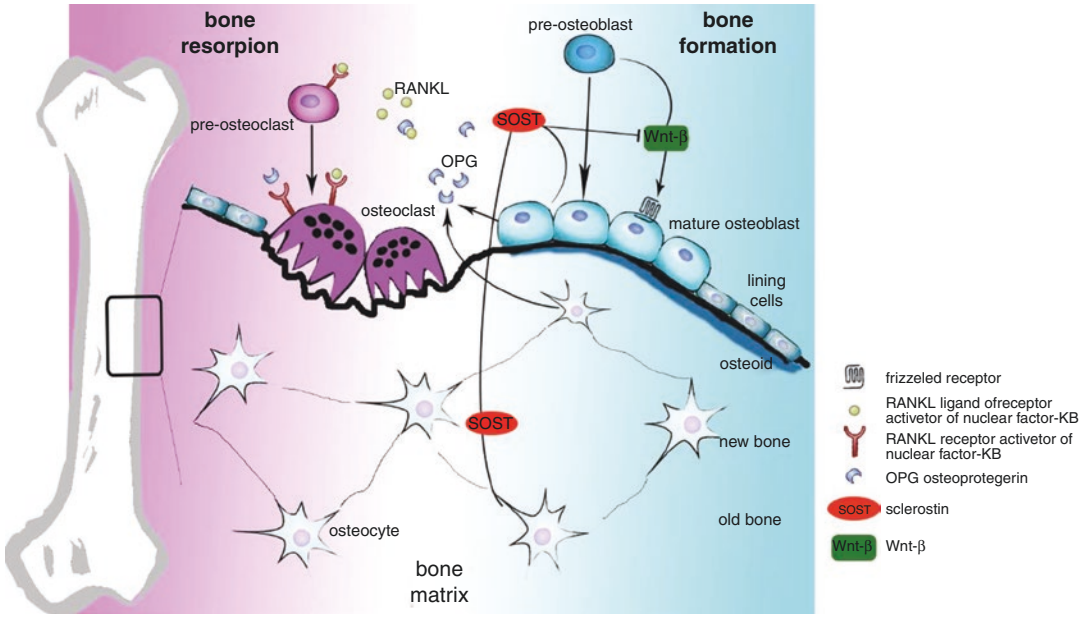

Fig. 1.2

During the development of metastasis, the malignant cells undergo genetic and epigenetic alterations that allow it to move away from the primary tumour site in order to enter into the bloodstream and eventually develop a secondary tumour at the other site. The molecular mechanisms related to the development of metastases and to the spread of circulating tumour cells (CTC) are not yet completely understood.

Through the vessels, the CTC arrive in highly vascularized bone marrow, and through the interaction with the haematopoietic cells and stromal microenvironment, they contribute to their survival. Metastatic tumour cells stimulate the osteolysis and/or proliferation of osteoblasts with bone formation. Such bone remodelling stimulus acts on the osteocytes leading to the release of several growth factors that promote the proliferation of stromal, haematopoietic and neoplastic cells.

Metastatic cancer cells, moreover, produce metalloproteases (MMPs) that degrade the matrix (type I collagen), and this stimulates the osteocytes to produce other factors, such as the sclerostin, that activate bone remodelling in a vicious circle (Fig. 1.3).

Initially, it was thought that only osteoblasts and osteoclasts were directly activated by metastatic cancer cells, but to date it has been shown that osteocytes also are involved in bone turnover induced by cancer cell through the Wnt pathway and sclerostin secretion [15, 16]. Moreover several studies suggested that osteocytes not only play a key role in the regulation of bone marrow microenvironment [17–20] but also are involved in the proliferation of metastatic tumour cells [21, 22] through the production of cytokines and growth factors (Fig. 1.4).

1.2.1 Diagnosis of Bone Metastasis

Metastases are the most common type of secondary bone malignant tumour. Any malignant tumour can give rise to bone metastases [23–27]. In 25–30% of cases, the bone lesions may be the first manifestation of malignancy [27–29]. In the latest years, new technologies allowed an early detection of metastasis and helped to identify primary tumour site through imaging techniques and tumour marker identification [23, 27, 28, 30, 31]. Usually, histological examinations are not performed on bone metastasis of known origin. However, this was used to define prognostic and/or predictive markers for target cancer therapy (Fig. 1.5).

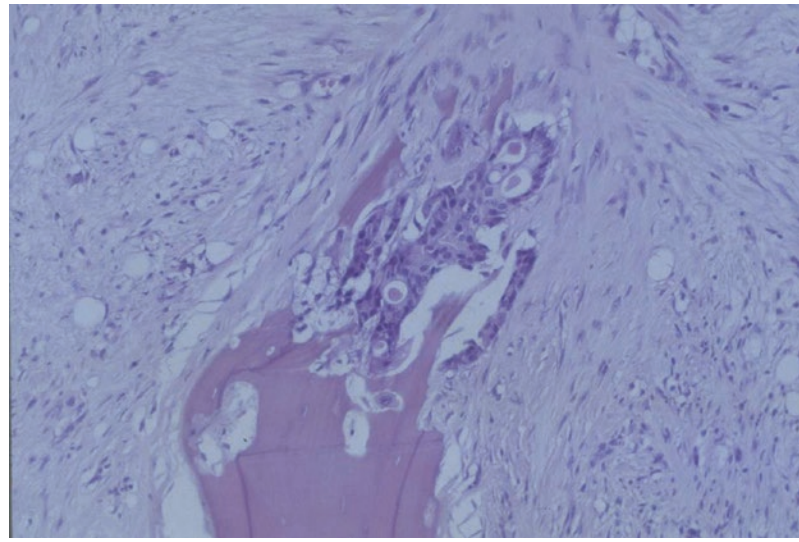

Fig. 1.3 (HE 20X) The intimate relationship between this bony trabecula resorption and metastatic cancer cells strongly suggests a crosstalk between bone cells and tumour cells involved in remodelling

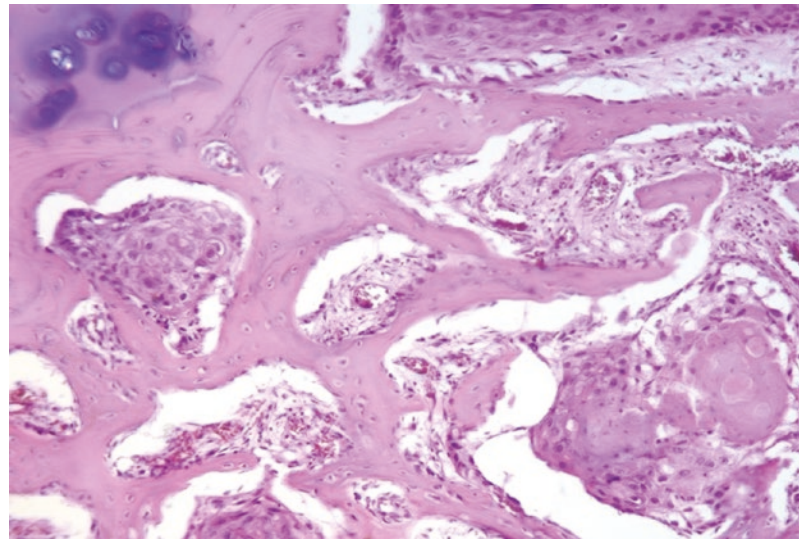

Fig. 1.4 (HE 10X) This metastatic squamous carcinoma grows up in marrow spaces permeating the bony trabeculae. Cytokines and growth factors are certainly involved in such extensive invasion without important bone destruction

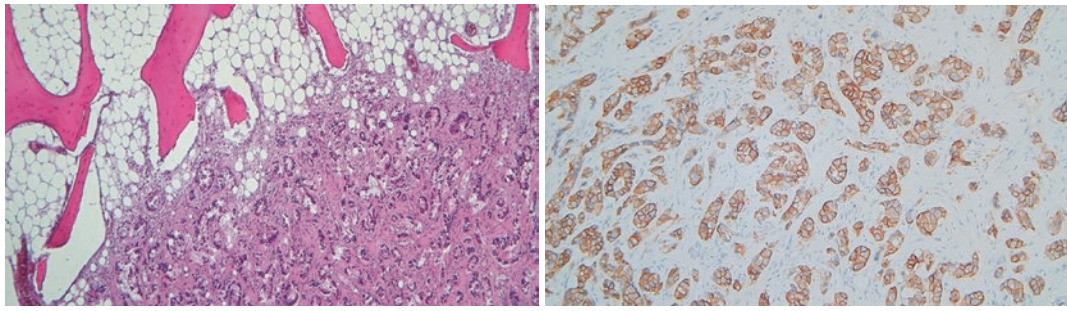

Fig. 1.5 (Left HE 20X, right Her2 40) This bone metastatic breast carcinoma is tested for Her2 expression as a predictive and prognostic indicator

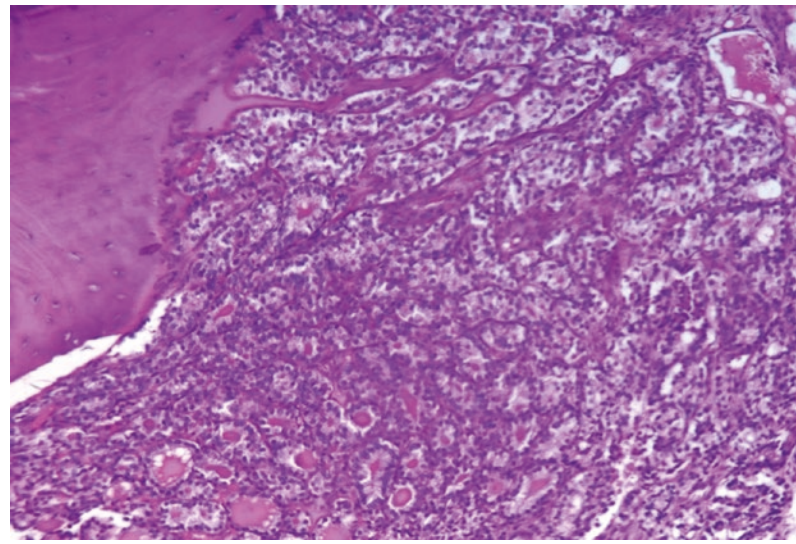

Fig. 1.6 (HE 20X) Peculiar morphology in this case of metastatic follicular thyroid carcinoma allows an easy diagnosis of the primary location to be performed

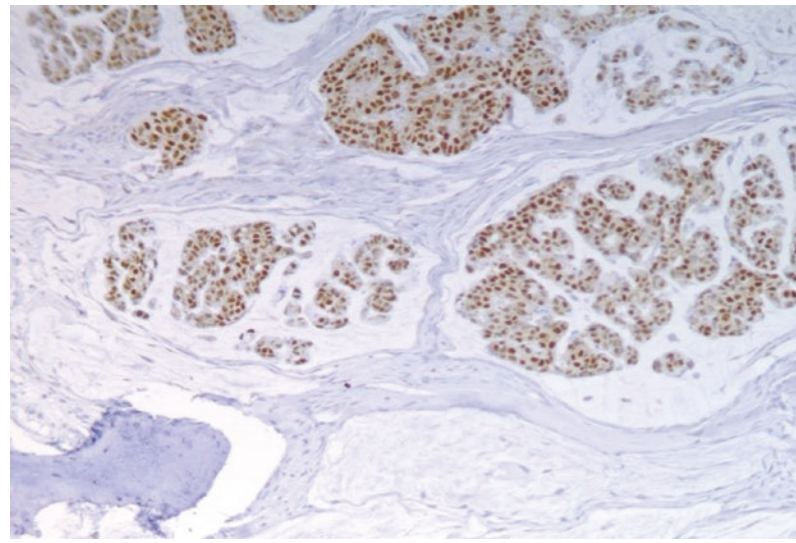

Fig. 1.7 (Oestrogen 20X) Immunohistochemical detection of the presence of oestrogen receptors in this bone metastatic breast carcinoma allows a diagnosis of the primary location to be supposed

At the time of diagnosis, in 10–30% of patients with bone metastases, the primary tumour is not identified [24, 31–35]; despite clinical history, physical examinations and routine laboratory or imaging exams, the site of the primary tumour is not detected [35]. Therefore, in these cases the histologic findings derived from bone biopsy could be diagnostic (Fig. 1.6).

Sometimes, a metastatic bone lesion could have such a histological appearance of undifferentiated tumours not to allow a precise pathological classification using haematoxylin-eosin stain. Therefore, using the immunohistochemistry method (IHC) with labelled antibodies, it is possible to identify the immunophenotype of metastatic cells and to determine the origin of primary tumour [34, 36].

For example, oestrogen receptor, progesterone receptor and gross cystic disease fluid protein (GCDFP) are positive in breast carcinoma (Fig. 1.7), thyroid transcription factor-1 (TTF-1) in lung carcinoma, prostate-specific antigen (PSA) in prostatic carcinoma, renal cell carcinoma marker (RCCMA) and CD10 in renal carcinoma and thyroglobulin in thyroid carcinoma [37]. A simplified correspondence among immunohistochemical markers and possible primary tumour in bone metastasis of unknown origin is reported in Table 1.1 [38].

Table 1.1 Immunohistochemical panel in bone metastasis of unknown origin

Primary tumour	Ck	LCA	Vim.	Ck7	Ck20	TTF1	GCDFP	ER	CDX2	PSA	S100	CD56	Synap.	P63	MiTF	PSA	RCCma	Thyr	Urop	WT1
Lung carcinoma	+	–	–	+	–	+	–	–	–	–	–	–	–	+/–	–	–	–	–	–	–
Breast carcinoma	+	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Gastrointestinal carcinoma	+	–	–	+/–	+	–	–		+	–	–	–	–	–	–	–	–	–	–	–
Prostate carcinoma	+	–	–	–	–	–	–			+	–	–	–	–	–	+	–	–	–	–
Melanoma	–	–	+	–	–	–	–	–	–		+		–	–	+	–	–	–	–	–
Ovarian serous carcinoma	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+
Renal clear cell carcinoma	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	
Thyroid carcinoma	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Squamous cell carcinoma	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Transitional cell carcinoma	+	–	–	+/–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–
Neuroendocrine carcinoma	+	–	–	–	+/–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
Sarcoma	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lymphoma	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

CK pan-cytokeratin, *LCA* leukocyte common antigen, *Vim* vimentin, *TTF1* thyroid transcription factor 1, *GCD* gross cystic disease fluid protein 15, *ER* oestrogen receptor, *CDX2* caudal type homeobox 2, *MiTF* microphthalmia-associated transcription factor, *PSA* prostate-specific antigen, *Synap* Synaptophysin, *RCCma* renal cell carcinoma marker, *Thyr* thyroglobulin, *Urop* uroplakin and *WT1* Wilms tumour 1

Acknowledgements The authors are grateful to Dr. Martina Leopizzi for helping in building iconography and to Dr. Carmen Mazzitelli and Dr. Caterina Chiappetta for the critical review of the manuscript.

References

1. Stopeck AT, Lipton A, Body JJ, Steger GG, Tonkin K, de Boer RH, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol. 2010;28:5132–9.
2. Fizazi K, Lipton A, Mariette X, Body JJ, Rahim Y, Gralow JR, et al. Randomized phase II trial of denosumab in patients with bone metastases from prostate cancer, breast cancer, or other neoplasms after intravenous bisphosphonates. J Clin Oncol. 2009;27:1564–71.
3. Ulrich U, Rhiem K, Schmolling J, Flaskamp C, Paffenholz I, Salzer H, et al. Cross-linked type I collagen C- and N-telopeptides in women with bone metastases from breast cancer. Arch Gynecol Obstet. 2001;264:186–90.
4. Brown JE, Cook RJ, Major P, Lipton A, Saad F, Smith M, et al. Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer, and other solid tumors. J Natl Cancer Inst. 2005;97:59–69.
5. Coleman R, Body JJ, Aapro M, Hadji P, Herrstedt J. Bone health in cancer patients: ESMO clinical practice guidelines. Ann Oncol. 2014;25(Suppl 3):iii124–37.
6. Van Poznak CH, Temin S, Yee GC, Janjan NA, Barlow WE, Biermann JS, et al. American Society of Clinical Oncology executive summary of the clinical practice guideline update on the role of bone-modifying agents in metastatic breast cancer. J Clin Oncol. 2011;29:1221–7.
7. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fossa SD, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med. 2013;369:213–23.
8. Martin RB. Does osteocyte formation cause the nonlinear refilling of osteons? Bone. 2000;26:71–8.
9. Robling AG, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem. 2008;283:5866–75.
10. Tu X, et al. Sost downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. Bone. 2012;50:209–17.
11. Leupin O, et al. Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. J Biol Chem. 2011;286:19489–500.
12. Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. In: Burr D, Allen M, editors. Basic and applied bone biology. Atlanta: Elsevier; 2014. p. 27–45.
13. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000;289:1504–8.
14. Khosla S. Minireview: the OPG/RANKL/RANK system. Endocrinology. 2001;142:5050–5.
15. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell and more. Endocr Rev. 2013;34:658–90.
16. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011;26:229–38.
17. Xiong J, et al. Matrix-embedded cells control osteoclast formation. Nat Med. 2011;17:1235–41.
18. Nakashima T, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med. 2011;17:1231–4.
19. Suva LJ. Sclerostin and the unloading of bone. J Bone Miner Res. 2009;24:1649–50.
20. Wijenayaka AR, et al. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. PLoS One. 2011;6:e25900.
21. Compton JT, Lee FY. A review of osteocyte function and the emerging importance of sclerostin. J Bone Joint Surg Am. 2014;96:1659–68.
22. Zhou JZ, et al. Differential impact of adenosine nucleotides released by osteocytes on breast cancer growth and bone metastasis. Oncogene. 2015;34:1831–42.
23. Vandecandelaere M, Flipo RM, Cortet B, Catanzariti L, Duquesnoy B, Delcambre B. Bone metastases revealing primary tumors. Comparison of two series separated by 30 years. Joint Bone Spine. 2004;71(3):224–9.
24. Nottebaert M, Exner GU, von Hochstetter AR, Schreiber A. Metastatic bone disease from occult carcinoma: a profile. Int Orthop. 1989;13(2):119–23.
25. Cooke KS, Kirpekar M, Abiri MM, Shreefter C. US case of the day. Skeletal metastasis from poorly differentiated carcinoma of unknown origin. Radiographics. 1997;17(2):542–4.
26. Piccioli A. Breast cancer bone metastases: an orthopedic emergency. J Orthop Traumatol. 2014;15(2):143–4.
27. Katagiri H, Takahashi M, Inagaki J, Sugiura H, Ito S, Iwata H. Determining the site of the primary cancer in patients with skeletal metastasis of unknown origin: a retrospective study. Cancer. 1999;86(3):533–7.
28. Piccioli A, Capanna R. Il trattamento delle metastasi ossee. Linee Guida S.I.O.T; 2008.
29. Wedin R, Bauer HC, Skoog L, Söderlund V, Tani E. Cytological diagnosis of skeletal lesions. Fine-needle aspiration biopsy in 110 tumours. J Bone Joint Surg Br. 2000;82(5):673–8.
30. Xu DL, Zhang XT, Wang GH, Li FB, Hu JY. Clinical features of pathologically confirmed metastatic bone tumors—a report of 390 cases. Ai Zheng. 2005;24(11):1404.
31. Conroy T, Platini C, Troufleau P, Dartois D, Lupors IE, Malissard L, et al. Presentation clinique et facteurspronostics au diagnostic de metastases osseuses. A propos de d'uneserie de 578 observations. Bull Cancer. 1993;80:S16e22.
32. Papagelopoulos P, Savvidou O, Galanis E, et al. Advances and challenges in diagnosis and management of skeletal metastases. Orthopedics. 2009;29(7):609–20.

33. Shih LY, Chen TH, Lo WH. Skeletal metastasis from occult carcinoma. J Surg Oncol. 1992;51(2):109–13.
34. Airoldi G. Cancer of unknown primary origin: utility and futility in clinical practice. Ital J Med. 2012;6:315–26.
35. Hemminki K, Riihimäki M, Sundquist K, Hemminki A. Site-specific survival rates for cancer of unknown primary according to location of metastases. Int J Cancer. 2013;133(1):182–9.
36. Bitran JD, Ultmann JE. Malignancies of undetermined primary origin. Dis Mon. 1992;38(4):213–60.
37. Oien KA, Dennis JL. Diagnostic work-up of carcinoma of unknown primary: from immunohistochemistry to molecular profiling. Ann Oncol. 2012;23(Suppl 10):271–7.
38. Simon MA, Karluk MB. Skeletal metastases of unknown origin. Diagnostic strategy for orthopedic surgeons. Clin Orthop Relat Res. 1982;166:96–103.