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Immune Environment and Osteosarcoma

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Abstract

Immune niche with its huge cell diversity including more specifically tumour infiltrating lymphocytes (TILs), tumour-associated macrophages (TAMs) regulate osteosarcoma (OS) microenvironment. TAMs exert differential activities in the tumour development according to their polarisation. Indeed, in oncology, M1-polarised macrophages are considered as anti-tumour effectors, and M2-polarised macrophages are defined as pro-tumour modulators by increasing the neoangiogenic process. TAM density is correlated with tumour cell proliferation, invasion, metastasis and poor prognosis in various epithelial and haematological cancers and in bone metastasis. Similarly, tumour infiltrating lymphocytes play a key role in tumour development by inducing a local tolerant environment favourable for the tumour growth. The present chapter will describe the main roles of the immune system in the pathogenesis of osteosarcoma and the most recent therapeutic development based on its regulation.

Keywords: osteoimmunology, osteosarcoma, macrophage, lymphocyte, microenvironment

1. Introduction

In recent years, there has been a dramatic increase in the importance given to the theory that the tissue microenvironment participates in determining the “bone niche” in the progression of bone tumours and in establishing resistance processes to conventional therapies. Originally, the concept of tumour niche has emerged based on the “seed and soil theory” proposed by Stephan Paget at the end of the nineteenth century [1, 2]. This tumour niche is defined as a specific microenvironment promoting the emergence of cancer initiating cells and providing all the factors required for their quiescence, proliferation and migration.

Therefore, the tumour microenvironment is composed of a complex, interconnected network of protagonists, including soluble factors such as cytokines, extracellular matrix components, interacting with fibroblasts, endothelial cells, immune cells and various specific cell types depending on the location of the cancer cells (e.g. osteoblasts, osteoclasts in the bone tissue or pulmonary epithelium in case of lung metastasis). This cellular diversity defines three main “niches” depending on their functional implication: an *immune niche* involved in local immune tolerance, a *vascular niche* associated with tumour cell extravasation/migration and a *metastatic niche* (e.g. bone, lung and liver) hosting the metastatic tumour cells [3, 4].

The concept of “bone niche” was initially described in the context of haematological malignancies, such as leukaemia [5] or multiple myeloma [6], and then extended to bone metastases, such as breast or prostate cancers [7]. As all tumours, the pathogenesis of osteosarcoma (OS) is closely related to the microenvironment in which the tumour grows. Even though the aetiology of OS has not been clearly established, its development has the special feature of being strongly associated with the “soil” described by Paget. In physiological and pathological bone tissue, the various cells communicate together by direct contacts involving adhesion molecules or channels, but also in an autocrine/paracrine/endocrine manner involving cytokines and growth factors [8]. Among these glycoproteins, the triad osteoprotegerin (OPG)/Receptor Activator of NF- κ B (RANK)/RANK Ligand (RANKL) plays a pivotal role in OS development [9]. In case of OS, there is effectively a dysregulation in this balance between OPG/RANK/RANKL, provoking exacerbated local bone remodelling (**Figure 1**). As a result, numerous factors initially trapped in this matrix are released, which in turn stimulate sarcoma cell proliferation, leading to the establishment of a vicious cycle between bone and tumour cells [10]. These events are associated with early and late events in the metastatic process by promoting the neoangiogenesis and extravasation of tumour cells [11, 12]. But until today, the characterisation of the microenvironment of OS has not been fully documented [13, 14]. The immune niche, with its huge cell diversity including more specifically tumour infiltrating lymphocytes (TILs) and tumour-associated macrophages (TAMs), regulates the OS microenvironment [15, 16]. TAMs exert different effects on tumour development because of their polarisation. In oncology, M1-polarised macrophages are considered to be anti-tumour effectors, and M2-polarised macrophages are defined as pro-tumour modulators as they increase the neoangiogenic process [17–19]. The density of TAMs is correlated with tumour cell proliferation, invasion, metastasis and poor prognosis in various epithelial and haematological cancers and in bone metastases [20].

Osteoimmunology is a recent term proposed for describing the complex immune environment controlling the bone remodelling and related diseases [21]. Several reports have underlined the therapeutic interest to use immunotherapies or immunomodulatory-based therapies for OS. In this context, the number of new drugs activating the immune system has exploded in the last 10 years, and numerous phase I and II clinical trials are in progress in OS. The present chapter will describe the main roles of the immune system in the pathogenesis of OS and the most recent therapeutic development based on its regulation.

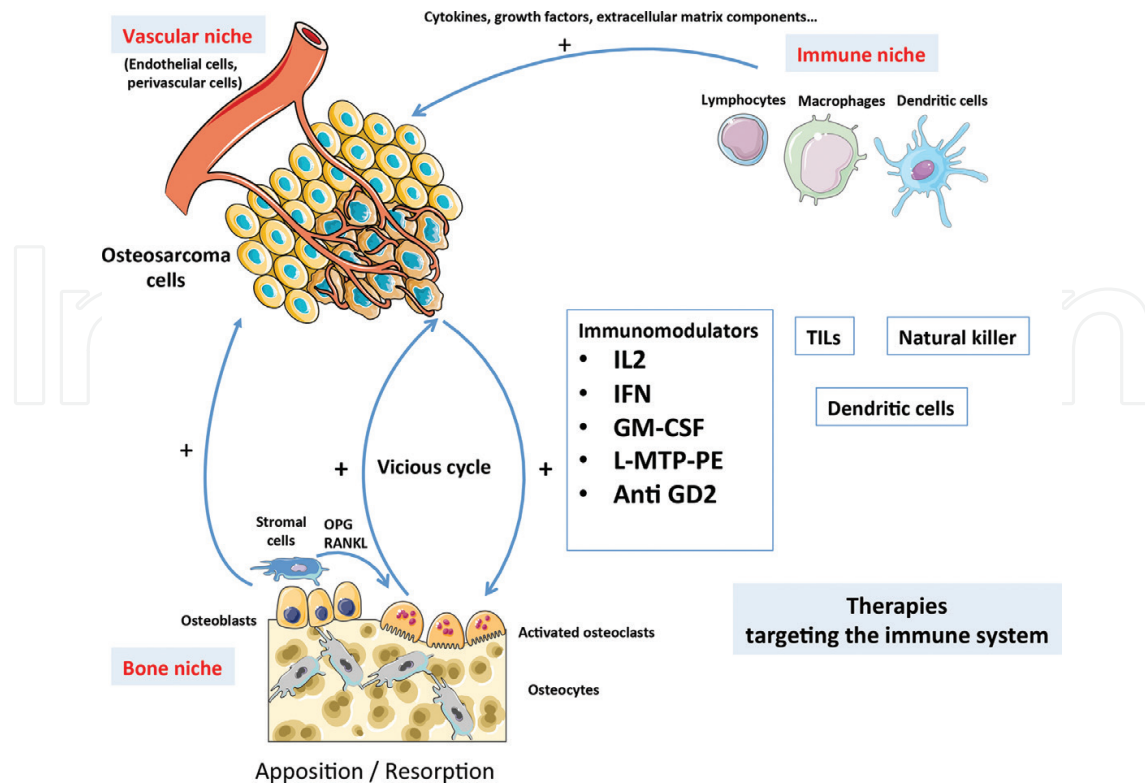


Figure 1. Osteosarcoma and its niches. In osteosarcoma, the microenvironment plays a pivotal role in the pathogenesis of tumour cells. It facilitates the transport of gas and nutrients to cancer cells and extravasation to their metastatic location (vascular niche), induces a tolerant environment (immune niche) and dysregulates bone remodelling (bone niche), in which the molecular OPG/RANKL/RANK triad plays a key role in regulation. OPG and RANKL are produced by osteoblasts and/or stromal cells, whereas RANK is expressed at the surface of osteoclasts and their precursors. The immune niche is one of the source of therapeutic targets against osteosarcoma.

2. The immune niche in osteosarcoma

As said above, the niche of OS is composed of a complex network of diverse cells, which interact together by direct contacts, or in an autocrine/paracrine/endocrine manner involving cytokines and growth factors. This chapter will focus specifically on the main cellular protagonists: lymphocytes, macrophages and the principal associated cytokines.

2.1. Tumour infiltrating lymphocytes (TILs)

Heterogeneity of tumour cells with various osteosarcoma sub-entities further complicates identification of robust biomarkers with broad clinical application [22]. Analysis of the tumour microenvironment in patients with a variety of haematological pathologies and solid tumours such as bone metastases and soft tissue sarcomas has revealed that a major subset of tumours shows evidence of a T cell-infiltrated phenotype [23, 24]. Selected T lymphocytes migrate from secondary lymphoid organs to the tumour sites and invade the tumour tissues. They are composed of various T lymphocyte subpopulations, which exhibit highly specific immunological

reactivity compared to circulating and non-infiltrating lymphocytes [25]. The final immune response resulting from the activation of T lymphocytes is complex and depends on the nature of these T cells (e.g. Treg, CD4⁺) and the presence of the other immune protagonists such as macrophages.

As other immune cells, lymphocytes seem to play an essential role in osteosarcoma growth and prognosis, but publications reporting this population in OS remain rare [16, 22, 26]. The presence of T lymphocytes in human OS tissues was previously studied by Trieb et al. by immunohistochemical techniques [26]. Phenotypic analyses have revealed that the infiltrating lymphocytes into OS were 95% CD3⁺ and 68% CD8⁺. At this time, human CD4⁺ Treg was unknown and not included in the study, which did not detect any correlation of TILs with OS outcome. A few years later, Theoleyre et al. supported the presence of specific T subpopulations in OS: TILs isolated from OS samples exhibited lytic activity *in vitro*, which were apparently no efficient in patients [16]. The study of the microenvironment has a strong impact on targeted patient treatment for which little progress has been achieved since introduction of neo-adjuvant chemotherapy 30 years ago. Prognostic biomarkers for risk stratification at the time of diagnosis are missing and are a major drawback in clinical testing of novel therapeutic agents. Alternatively, analysis of the tumour microenvironment for osteosarcoma outcome-related biomarkers might be less dependent from the osteosarcoma subtype. Recently, Fritzsche et al. have reported for the first time CD8⁺/FOXP3⁺ ratio as strong prognostic factor at time of OS diagnosis, pointing out the functional key role of Treg in OS pathogenesis. Multivariate analysis showed that this novel parameter was independent from tumour metastasis and response to neoadjuvant chemotherapy and could be validated in an independent patient cohort with current state of diagnosis and treatment of OS [22]. It has been suggested in other solid tumours (e.g. colon cancers) that intensity of tumour microenvironment infiltration with T-cells, especially cytotoxic tumour infiltrating CD8⁺T-cells (CD8⁺ TILs) allows more powerful prognostic staging than traditional staging. The characterisation of this simple immune system-based biomarker has been termed the “immunoscore”, and this is currently tested for some tumours in a multicenter study [27, 28].

Lymphocytes are immune cells regulated by diverse cytokines, in particular the triad OPG/RANK/RANKL, which represents a setting up a fertile soil for cancer cells as well [9]. Bone remodelling results from a balance between two opposite cellular activities: (i) osteoblasts in charge of the synthesis of new organic extracellular matrix, which will become progressively mineralized and (ii) osteoclasts specialised in the degradation of the mineralised extracellular matrix, process named bone resorption. Osteoclastogenesis and osteoclast activities are regulated by a master cytokine called NF-κB Ligand (RANKL), member of the tumour necrosis factor (TNF) superfamily (official TNF nomenclature: TNFSF11) [9]. RANKL is a soluble and/or membrane cytokine expressed by osteoblasts and stromal cells, binds to RANK a membrane receptor expressed at the surface of mature osteoclasts and their precursors. RANKL binding to RANK induces specific NF-κB-dependent signal transduction pathways and stimulates osteoclast differentiation, survival and resorption activity. RANKL binds to a soluble receptor named osteoprotegerin (OPG), which is a ubiquitous protein and acts as a decoy blocking RANKL binding to RANK [29]. OPG is then considered as a strong inhibitor of osteoclastogenesis and bone resorption. LGR4 is the last receptor of RANK recently identified. LGR4 expressed at

the osteoclast/osteoclast precursor membrane is a negative regulator of RANKL-RANK activation (see review in Ref. [9]). It is widely accepted that the multiple components of the bone niche (e.g. soluble factors, extracellular matrix) strongly contribute to the bone tumour initiation and the metastatic process [30, 31]. RANKL influences the microenvironment of cancer cells by acting on the local immunity. Indeed, the major role of RANKL in the immune system has been initially identified in RANKL-knockout mice in which the development of secondary lymphoid organs was impaired especially the lymph nodes [32] but also at the “central” level where the maturation of thymic epithelial cells necessary for T cell development was affected [33]. RANKL is also involved in the modulation of the immune response by inducing T cell proliferation [34] and dendritic cells survival [29]. Indeed, activated T cells through RANKL expression stimulate dendritic cells, expressing RANK, to enhance their survival and thereby increase the T cell memory response [34]. More recently, Khan et al. demonstrated that RANKL blockade can rescue melanoma-specific T cells from thymic deletion and increases anti-tumour immune response as shown in melanoma [35]. In 2007, Mori et al. reported for the first time functional RANK expression in human OS cells strengthening the involvement of the RANK/RANKL/OPG axis in OS [36, 37]. Moreover, in animal OS models, OPG gene transfer prevents the formation of osteolytic lesions associated with OS development, in reducing the tumour incidence and the local tumour growth, leading to a fourfold increase in mice survival 28 days post-implantation [38]. This opened a new door of novel therapeutic approaches for OS.

2.2. Tumour-associated macrophages (TAMs)

The mononuclear phagocyte system is composed by heterogeneous populations participating in the body's first line of defence against pathogens and parasites [39]. This system includes cells circulating cells into the body fluids such as monocytes, and integrates resident cells such as macrophages, dendritic cells (DC) and their precursors located in the bone marrow [40]. Their production and activities are controlled by numerous cytokines and growth factors but are more specifically regulated by the two ligands of macrophage-colony stimulating factor (M-CSF, cFMS or CD115): M-CSF (or CSF-1) and interleukin (IL)-34 [41, 42].

TAMs are the predominant leukocytes infiltrating solid tumours and can represent up to 50% of the tumour mass. These cells play a pivotal role in tumour behaviour illustrated by the significant link between TAM number and density and the prognosis [18, 43, 44]. Whereas TAMs can exert anti-tumour activities, the ambiguous role of macrophages in tumour progression is reflected in the finding that TAMs can also actively contribute to each stage of cancer development and progression [45]. Macrophage subtypes are conventionally classified in M1 considered as the classical population and M2 identified as an alternative subpopulation in link to their differentiation/activation state. However, if the parallel between M1 and M2 can be drawn with the Th1 and Th2 T lymphocyte classification, Th1/Th2 cells do not regulate macrophage polarisation. On the contrary, macrophage subtypes are versatile, are non-permanent cell populations, initiate and influence T lymphocyte polarisation resulting in differential immune response (e.g. Th1 response against virus and bacteria, Th2 response against parasites) [46]. Indeed, M1 and M2 macrophages are characterised by specific profiles of cytokine secretion. M1 macrophages are characterised by the secretion of IL-12, IL-1, IL-6, TNF or ROS, considered as pro-inflammatory mediators and directly involved in the control

of infections and anti-tumour activities. On the opposite, M2 macrophages constitute a heterogeneous population (M2a, b and c) inducing a Th2 immune response and secreting IL-4, IL-10 and IL-13. M2 macrophages are pro-angiogenic, pro-fibrotic and pro-tumorigenic.

The exact role of macrophages in OS is still unclear and controversial. Some studies have defined TAMs as anti-tumour effectors. Buddingh et al. thus demonstrated that higher TAM infiltration was associated with better overall survival in high-grade osteosarcoma. However, the authors did not observe any differences between metastatic and non-metastatic osteosarcomas, and TAMs exhibited both M1 and M2 characteristics [47]. On the contrary, the impact of macrophages in tumour development has been also suspected. Lewis and Pollard distinguished the anti-tumour M1-macrophages from M2-macrophages leading to tumour growth and invasion, angiogenesis, metastasis and immune-suppression [18]. OS development may thus be accompanied by a switch in the phenotype of infiltrating TAMs, from anti-metastatic M1-macrophages to pro-metastatic M2-macrophages. This hypothesis is in agreement with the *in vivo* work described by Xiao et al. who showed a switch in macrophage subpopulations in a mouse model of human osteosarcoma from M1-macrophages during the first week of tumour growth, to M2-macrophages after 2–3 weeks [48]. In addition, Pahl et al. demonstrated that human M1-like macrophages can be induced to exert direct anti-tumour activity against osteosarcoma cells, mediated by TNF- α and IL-1 β [49]. In the same manner, Ségaligny et al. demonstrated that osteosarcoma cells expressed IL-34, increasing the recruitment of M2-polarised macrophages into the tumour tissue, which correlates with tumour vascularization and the metastatic process [50]. TAMs accumulate in tumour microenvironment and according to their M2 or M1 phenotype contribute to tumour growth, angiogenesis and metastasis [51]. RANK is present at the cell membrane of monocytes/macrophages and RANKL acts as chemoattractant factor for these cells [52]. M2 subtype is strongly associated with the angiogenic process and interestingly RANK is mainly expressed by M2 macrophage subtype [53]. RANK/RANKL signalling in M2 subtype modulates the production of chemokines promoting the proliferation of Treg lymphocytes in favour of an immunosuppressive environment [54]. In breast carcinoma, RANKL is mainly produced by CD4⁺CD25⁺ T lymphocytes expressing Foxp3 and corresponding to Treg lymphocytes. In this context, a vicious cycle is established between TAMs, Treg and tumour cells resulting in the tumour growth, the spreading of cancer cells and the amplification of the metastatic process [55].

2.3. Recent therapeutic developments based on the regulation of the immune system of osteosarcoma: immunomodulating drugs

The therapeutic protocol currently used for osteosarcoma was established by Rosen et al. at the end of the 1970s [56]. It comprises preoperative (neoadjuvant) chemotherapy associating mainly four drugs (doxorubicin, cisplatin, methotrexate and ifosfamide) [57], and followed by surgical removal of all detectable disease (including metastases), and postoperative (adjuvant) chemotherapy, preferably within the setting of clinical trials [58]. OS is considered resistant to applicable doses of radiation [59, 60]. Supplemental therapeutic approaches such as chemoembolisation or angioembolisation, thermal ablation, radiofrequency ablation and cryotherapy are experimental or palliative [59]. Unfortunately, patients, who are diagnosed with metastatic disease or who relapse post-therapy have an extremely poor prognosis, with little to no

improvements in survival seen over the past 30 years [61]. Several reports have underlined the therapeutic value of using immunotherapies or immunomodulatory-based therapies for osteosarcoma [15, 62–64]. In this context, the number of new drugs activating the immune system has exploded in the last 10 years and numerous phase I and II clinical trials are in progress in osteosarcoma (**Figure 1**). In this chapter, the most recent therapeutic developments targeting the regulation of T lymphocytes and macrophages will be exposed.

2.3.1. T-cell therapies

Detection of specific T lymphocyte populations in the tumour microenvironment and in human tumour tissues defines an immunoscore and leads to patient stratification based on this immunophenotyping. These analyses have identified new predictive biomarkers and new therapeutic targets, which have stimulated the development of immunotherapies [65].

2.3.1.1. Disialoganglioside (GD2)

Monoclonal antibodies targeted against cell surface antigens specific to tumour cells have been proven to be effective in patients with breast cancer, lymphoma and neuroblastoma [66–68]. Usually these bispecific antibodies are engineered antibodies linking a tumour antigen recognition domain to a second domain that activates a receptor on immune effector cells, typically T cells. The expression of the glycosphingolipid GD2 is restricted to the central and peripheral nervous system, skin (melanocyte) and mesenchymal cells located in the stroma [69–71]. In addition to the healthy tissues, GD2 was detected in neuroblastomas, melanomas, sarcomas, lung and central nervous system tumours, in which a variable number of cancer cells express this antigen [72–75]. Based on this relative restricted distribution, GD2-targeting appeared very quickly as an interesting immunotherapy, especially for high-risk neuroblastomas, for which anti-GD2 antibodies improved significantly patient survival [68, 76]. In patients with stage IV neuroblastoma, anti-GD2 antibody ch14.18 has been shown to improve EFS effectively when given in the setting of minimal residual disease. The rationale for using this antibody in patients with OS lies in that 95% of osteosarcoma express GD2 [77]. Consequently, given the success of anti-GD2 mAb therapy in neuroblastoma, studies exploring the use of these mAbs in OS are underway [78]. Current trials include the GD2mAbs humanized3F8 (NCT01419834 and NCT01662804) and hu14.18K322A (NCT00743496) [61].

2.3.1.2. Nivolumab

Nivolumab is an immunomodulator, which acts by blocking the activation of the programmed cell death-1 (PD-1) receptor, induced by one of its two ligands (PD-L1) on activated T cells [79]. Numerous preclinical investigations have demonstrated that inhibition of the interaction between PD-1 and PD-L1 enhances the T-cell response, resulting in increased anti-tumour activity. PD-L1 or PD-1 blockade with monoclonal antibodies results in strong and often rapid anti-tumour effects in several mouse models. A high PD-L1 expression has been identified in OS cell lines [80], and PD-1 expression on CD4 and CD8 T-cells was found higher in OS patients than in healthy controls and in patients with metastasis at diagnosis, high tumour stage or bone fracture [81]. A phase I/II trial will be conclude in 2016 on refractory

solid tumours and sarcomas including osteosarcoma. A total of 242 patients will be enrolled and treated with Nivolumab IV over 60 minutes twice a month. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity.

2.3.2. Immune and dendritic cell vaccine

Dendritic cells have the specific ability to initiate and modulate adaptive immune responses [82]. This specificity, associated with their role in antigen presentation, has led to their use in vaccine approaches in cancer. Matured autologous dendritic cells loaded with tumour lysates derived from tumour tissue were used as the vaccine product. In a pre-clinical model of osteosarcoma, it has been demonstrated that killer dendritic cells were able to induce an adaptive anti-tumour immune response with a decrease in tumour development after cross-presentation of the tumour cell-derived antigen [83]. A phase I clinical trial demonstrated the feasibility and good tolerance of dendritic cells pulsed with MAGE-A1, MAGE-A3 and NYESO-1 full length peptides in combination with decitabine. Anti-tumour activity was observed in some patients [84]. In 2012, 12 osteosarcoma patients were vaccinated with tumour lysate pulsed dendritic cells, but evidence of a clinical benefit was observed in only two of these patients [85]. These authors concluded that osteosarcoma patients may be relatively insensitive to DC-based vaccine treatments. A new clinical trial was initiated, enrolling 56 patients (>1 year) with confirmed sarcoma, either relapsed or without known curative therapies, and treated with autologous dendritic cells pulsed with tumour lysate. NCT02409576 is a pilot trial ("Pilot Study of Expanded, Activated Haploidentical Natural Killer Cell Infusions for Sarcomas (NKEXPSARC)") analysing the effect of donor NK cells on clinical response determined by imaging. About 20 patients (aged 6 months–80 years) will be included between 2015 and 2016. The patients will receive lymphodepleting chemotherapy with cyclophosphamide (1 day) followed by fludarabine (5 days) and each patient will receive IL-2 1 day before infusion of the NK cell (total six doses).

2.3.3. Mifamurtide (liposomal-muramyl tripeptide phosphatidyl-ethanolamine L-MTP-PE)

As it has been discussed above, the density of TAM is linked to a poor diagnosis. In osteosarcoma, Buddingh et al. showed that macrophages exhibit M1 and M2 phenotypes and demonstrated a link between M2 macrophages and angiogenesis. Similarly, in preclinical models of osteosarcoma, the recruitment of the M2 subtype is correlated with tumour angiogenesis and lung metastasis [47, 50].

L-MTP-PE is a synthetic analogue of muramyl dipeptide of the bacterial cell walls, which was identified as a powerful activator of monocyte/macrophage lineage. Indeed, L-MTP-PE induces the expression of pro-inflammatory mediators such TNF- α , IL-1, IL-6, lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM1) leading to an M1 macrophage response. The therapeutic interest of L-MTP-PE was widely studied in osteosarcoma [86, 87]. The largest clinical experience with combination chemotherapy and L-MTP derives from the Intergroup 0133 osteosarcoma study. No difference in survival was found for patients who received ifosfamide in addition to the standard three-drug chemotherapy (doxorubicin, cisplatin and methotrexate). But this study did suggest that L-MTP had a beneficial impact on survival, improving the 5-year overall survival rate from 70 to 78% ($p = 0.03$) [88]. However, no significant difference in survival was observed

between the two groups of treatment concerning the patients with metastatic disease (40% without L-MTP versus 53% with L-MTP, $p = 0.27$). Based on these results, the European Medicines Agency granted L-MTP an indication for the treatment of non-metastatic osteosarcoma in 2009; the American Food and Drug Administration (FDA) did not. L-MTP is also approved for use in Turkey, Mexico and Israel. Recently, Biteau et al. have proved the efficacy of association of zoledronate and L-mifamurtide combination in osteosarcoma. This association induced an additional and in some cases synergistic inhibition of primary tumour progression [89].

2.3.4. *Inhaled granulocyte-macrophage colony stimulating factor (GM-CSF)*

GM-CSF is one of the master regulators of myeloid cell lineage by controlling their differentiation, proliferation and activities. Indeed, this growth factor exerts immunomodulatory and immunostimulatory activities by stimulating the functional activities of neutrophil granulocytes but also of macrophages and DC. More specifically, GM-CSF promotes the recruitment and cytotoxic functions of macrophages, stimulates natural killer and dendritic cells, and consequently, upmodulates the number of CD4 T lymphocytes [90]. Arndt et al. have recently performed a phase I clinical trial using inhaled GM-CSF in patients with first isolated pulmonary recurrence of OS. Unfortunately, even though the clinical studies demonstrated the safety of administered GM-CSF (e.g. aerosolized delivery), no biological benefit (e.g. local immunomodulation in lung metastases) or improved clinical outcome was demonstrated. A future larger prospective randomised trial may demonstrate improved outcomes in OS patients probably [91, 92].

3. Conclusion

The therapies focused on the immune niche partially represent the potential therapeutic targets available for OS nowadays. Blood vessels, bone cells and tumour cells are targeted as well in the current clinical trials. However, in the future, the key to success will lie in better understanding and characterisation of the disease, leading to better patient stratification and, consequently, to personalised medicine.

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References

- [1] Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer* 2003;3:453–8.
- [2] Paget S. The distribution of secondary growths in cancer of the breast. *Cancer Metastasis Rev* 1989;8:98–101.
- [3] Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumour cells? *Cell Stem* 2015;16:225–38.
- [4] Ordóñez-Morán P, Huelsken J. Complex metastatic niches: already a target for therapy? *Curr Opin Cell Biol* 2014;3:29–38.
- [5] Iwasaki H, Suda T. Cancer stem cells and their niche. *Cancer Sci* 2009;100:1166–72.
- [6] Basak GW, Srivastava AS, Malhotra R, Carrier E. Multiple myeloma bone marrow niche. *Curr Pharm Biotechnol* 2009;10:345–6.
- [7] Wan L, Pantel K, Kang Y. Tumour metastasis: moving new biological insights into the clinic. *Nat Med* 2013;19:1450–64.
- [8] Landskron G, De la Fuente M, Thuwajit P, Hermoso MA. Chronic inflammation and cytokines in the tumour microenvironment. *J Immunol Res* 2014;2014:149185.
- [9] Renema N, Navet B, Heymann MF, Lezot F, Heymann D. RANK-RANKL signalling in cancer. *Biosci Rep*. 2016;36:e00366.
- [10] Grimaud E, Soubigou L, Couillaud S, Coipeau P, Moreau A, Passuti N, et al. Receptor activator of nuclear factor kappa B ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. *Am J Pathol* 2003;163:2021–31.
- [11] Ungefroren H, Sebens S, Seidl D, Lehnert H, Hass R. Interaction of tumour cells with the microenvironment. *Cell Commun Signal* 2011;9:18.
- [12] Zhu L, McManus MM, Hughes DPM. Understanding the biology of bone sarcoma from early initiating events through late events in metastasis and disease progression. *Front Oncol* 2013;3:230.
- [13] Alfranca A, Martinez-Cruzado L, Tornin J, Abarrategi A, Amaral T, de Alava E, et al. Bone microenvironment signals in osteosarcoma development. *Cell Mol Life Sci* 2015;72:3097–113.
- [14] Ando K, Heymann MF, Stresing V, Mori K, Rédini F, Heymann D. Current therapeutic strategies and novel approaches in osteosarcoma. *Cancers* 2013;5:591–616.
- [15] Endo-Munoz L, Evdokiou A, Saunders NA. The role of osteoclasts and tumour-associated macrophages in osteosarcoma metastasis. *Biochim Biophys Acta* 2012;1826:434–42.
- [16] Theoleyre S, Mori K, Cherrier B, Passuti N, Gouin F, Rédini F, et al. Phenotypic and functional analysis of lymphocytes infiltrating osteolytic tumours: use as a possible therapeutic approach of osteosarcoma. *BMC Cancer* 2005;5:123.

- [17] Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010;32:593–604.
- [18] Lewis CE, Pollard JW. Distinct role of macrophages in different tumour microenvironments. *Cancer Res* 2006;66:605–12.
- [19] Qian BZ, Pollard JW. Macrophage diversity enhances tumour progression and metastasis. *Cell* 2010;141:39–51.
- [20] Rogers TL, Holen I. Tumour 469 macrophages as potential targets of bisphosphonates. *J Transl Med* 2011;9:177.
- [21] Criscitiello C, Viale G, Gelao L, Esposito A, De Laurentiis M, De Placido S, Santangelo M, Goldhirsch A, Curigliano G. Crosstalk between bone niche and immune system: osteoimmunology signaling as a potential target for cancer treatment. *Cancer Treat Rev* 2015;41(2):61–8.
- [22] Fritzsche B, Fellenberg J, Moskovszky L, Sapi Z, Krenacs T, Machado I, Poeschl J, Lehner B, Szendroi M, Bosch AL, Bernd L, Csoka M, Mechtersheimer G, Ewerbeck V, Kinscherf R, Kunz P. CD8⁺/FOXP3⁺-ratio in osteosarcoma microenvironment separates survivors from non-survivors: a multicenter validated retrospective study. *Oncoimmunology*. 2015;4(3):e990800.
- [23] Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, Martino S, Wang M, Jones VE, Saphner TJ, Wolff AC, Wood WC, Davidson NE, Sledge GW, Sparano JA, Badve SS. Prognostic value of tumour-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol* 2014;32(27):2959–66.
- [24] Sorbye SW, Kilvaer T, Valkov A, Donnem T, Smeland E, Al-Shibli K, Bremnes RM, Busund LT. Prognostic impact of lymphocytes in soft tissue sarcomas. *PLoS One*. 2011;6(1):e14611.
- [25] Rosenberg SA. Progress in human tumour immunology and immunotherapy. *Nature* 2001;411:380–4.
- [26] Trieb K, Lechleitner T, Lang S, Windhager R, Kotz R, Dirnhofner S. Evaluation of HLA-DR expression and T-lymphocyte infiltration in osteosarcoma. *Pathol Res Pract* 1998;194(10):679–84.
- [27] Galon J, Pages F, Marincola FM, Angell HK, Thurin M, Lugli A, Zlobec I, Berger A, Bifulco C, Botti G, et al. Cancer classification using the Immunoscore: a world-wide task force. *J Transl Med* 2012;10:205.
- [28] Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, Bruneval P, Trajanoski Z, Fridman WH, Pages F, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol* 2011;29:610–8.
- [29] Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi Y. TRANCE (tumour necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member

predominantly expressed in T cells, is a dendritic cell specific survival factor. *J Exp Med* 1997;186:2075–80.

- [30] Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998;95:3597–602.
- [31] Ikeda T, Kasai M, Utsuyama M, Hirokawa K. Determination of three isoforms of the receptor activator of nuclear factor-kappa B ligand and their differential expression in bone and thymus. *Endocrinology* 2001;142:1419–26.
- [32] Mueller CG, Hess E. Emerging functions of RANKL in lymphoid tissues. *Front Immunol* 2012;3:261.
- [33] Akiyama T, Shimo Y, Yanai H, Qin J, Ohshima D, Maruyama Y, Asaumi Y, Kitazawa J, Takayanagi H, Penninger JM, Matsumoto M, Nitta T, Takahama Y, Inoue J. The tumour necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* 2008;29:423–37.
- [34] Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF Receptor and its ligand enhance T cell growth and dendritic cell function. *Nature* 1997;390:175–9.
- [35] Khan IS, Mouchess ML, Zhu ML, Conley B, Fasano KJ, Hou Y, Fong L, Su MA. Enhancement of an anti-tumour immune response by transient blockade of central T cell tolerance. *J Exp Med* 2014;211:761–8.
- [36] Mori K, Rédini F, Gouin F, Cherrier B, Heymann D. Osteosarcoma: current status of immunotherapy and future trends. *Oncol Rep* 2006;15(3):693–700.
- [37] Mori K, Le Goff B, Berreur M, Riet A, Moreau A, Blanchard F, Chevalier C, Guisle-Marsollier I, Léger J, Guicheux J, Masson M, Gouin F, Rédini F, Heymann D. Human osteosarcoma cells express functional receptor activator of nuclear factor-kappa B. *J Pathol* 2007;11:555–62.
- [38] Lamoureux F, Richard P, Wittrant Y, Battaglia S, Pilet P, Trichet V, Blanchard F, Gouin F, Pitard B, Heymann D, Redini F. Therapeutic relevance of osteoprotegerin gene therapy in osteosarcoma: blockade of the vicious cycle between tumour cell proliferation and bone resorption. *Cancer Res* 2007;67(15):7308–18.
- [39] van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. *J Exp Med* 1968;128:415–35.
- [40] Taylor PR, Gordon S. Monocytes heterogeneity and innate immunity. *Immunity* 2003;19:2–4.
- [41] Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, et al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* 2008;320:807–11.

- [42] Heymann D. Interleukin-34: an enigmatic cytokine. *IBMS BoneKey* 2010;7:406–13.
- [43] Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2002;196(3):254–65.
- [44] Zhang QW, Liu L, Gong CY, Shi HS, Zeng YH, Wang XZ, Zhao YW, Wei YQ. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One* 2012;7(12):e50946.
- [45] Laoui D, Van Overmeire E, De Baetselier P, Van Ginderachter JA, Raes G. Functional relationship between tumor-associated macrophages and macrophage colony-stimulating factor as contributors to cancer progression. *Front Immunol* 2014;5:489.
- [46] Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* 2000;164:6166–73.
- [47] Buddingh EP, Kuijjer ML, Duim RA, Bürger H, Agelopoulos K, Myklebost O, et al. Tumour-infiltrating macrophages are associated with metastasis suppression in high-grade osteosarcoma: a rationale for treatment with macrophage activating agents. *Clin Cancer Res* 2011;17:2110–9.
- [48] Xiao Q, Zhang X, Wu Y, Yang Y. Inhibition of macrophage polarization prohibits growth of human osteosarcoma. *Tumour Biol* 2014;35:7611–6.
- [49] Pahl JHW, Kwappenberg KM, Varypataki EM, Santos SJ, Kuijjer ML, Mohamed S, et al. Macrophages inhibit human osteosarcoma cell growth after activation with the bacterial cell wall derivative liposomal muramyl tripeptide in combination with interferon- γ . *J Exp Clin Cancer Res* 2014;33:27.
- [50] Ségaligny AI, Mohamadi A, Dizier B, Lokajczyk A, Brion R, Lanel R, et al. Interleukin-34 promotes tumour progression and metastatic process in osteosarcoma through induction of angiogenesis and macrophage recruitment. *Int J Cancer* 2015;137:73–85.
- [51] Cook J, Hagemann T. Tumour-associated macrophages and cancer. *Curr Opin Pharmacol* 2013;13:595–601.
- [52] Breuil V, Schmid-Antomarchi H, Schmid-Alliana A, Rezzonico R, Euller-Ziegler L, Rossi B. The receptor activator of nuclear factor (NF)- κ B ligand (RANKL) is a new chemotactic for human monocytes. *FASEB J* 2003;17:2163–5.
- [53] Kambayashi Y, Fujimura T, Furudate S, Asano M, Kakizaki A, Aiba S. The possible interaction between receptor activator of nuclear factor κ -B ligand expressed by extramammary paget cells and its ligand on dermal macrophages. *J Invest Dermatol* 2015;135:2547–50.
- [54] Fujimura T, Kambayashi Y, Furudate S, Asano M, Kakizaki A, Aiba S. Receptor activator of NF- κ B ligand promotes the production of CCL17 from RANK⁺ M2 macrophages. *J Invest Dermatol* 2015;135:2884–7.
- [55] Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, Karin M. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature* 2011;470:548–53.

- [56] Rosen G, Tan C, Sanmaneechai A, Beattie EJ Jr, Marcove R, Murphy ML. The rationale for multiple drug chemotherapy in the treatment of osteogenic sarcoma. *Cancer* 1975;35:936–45.
- [57] Bacci G, Longhi A, Fagioli F, Briccoli A, Versari M, Picci P. Adjuvant and neoadjuvant chemotherapy for osteosarcoma of the extremities: 27 year experience at Rizzoli Institute, *Eur J Cancer* 2005;41:2836–45.
- [58] Chou AJ, Geller DS, Gorlick R. Therapy for osteosarcoma: where do we go from here? *Paediatr Drugs* 2008;10:315–27.
- [59] Errani C, Longhi A, Rossi G, et al. Palliative therapy for osteosarcoma. *Expert Rev Anticancer Ther* 2011;11:217–27.
- [60] Schwarz R, Bruland O, Cassoni A, Schomberg P, Bielack S. The role of radiotherapy in osteosarcoma. *Cancer Treat Res* 2009;152:147–64.
- [61] Roberts SS, Chou AJ, Cheung NK. Immunotherapy of Childhood Sarcomas. *Front Oncol* 2015;5:181.
- [62] DeRenzo C, Gottschalk S. Genetically modified T-cell therapy for osteosarcoma. *Adv Exp Med Biol* 2014;804:323–40.
- [63] Li Z. Potential of human $\gamma\delta$ T cells for immunotherapy of osteosarcoma. *Mol Biol Rep* 2013;40:427–37.
- [64] Tarek N, Lee DA. Natural killer cells for osteosarcoma. *Adv Exp Med Biol* 2014;804:341–53.
- [65] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor micro-environment. *Nat Immunol* 2013;14(10):1014–22.
- [66] Coiffier B, Lepage E, Brière J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346(4):235–42.
- [67] Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353(16):1659–72.
- [68] Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med* 2010;363(14):1324–34.
- [69] Hersey P, Jamal O, Henderson C, Zardawi I, D'Alessandro G. Expression of the gangliosides GM3, GD3 and GD2 in tissue sections of normal skin, naevi, primary and metastatic melanoma. *Int J Cancer* 1988;41(3):336–43.
- [70] Martinez C, Hofmann TJ, Marino R, Dominici M, Horwitz EM. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. *Blood* 2007;109(10):4245–8.
- [71] Svennerholm L, Boström K, Fredman P, et al. Gangliosides and allied glycosphingolipids in human peripheral nerve and spinal cord. *Biochim Biophys Acta* 1994;1214(2):115–23.

- [72] Cheung N, Kushner B, Yeh S, Larson S. 3F8 monoclonal antibody treatment of patients with stage 4 neuroblastoma: a phase II study. *Int J Oncol* 1998;12(6):1299.
- [73] Cheung N, Lazarus H, Miraldi FD, et al. Ganglioside GD2 specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma. *J Clin Oncol* 1987;5(9):1430–40.
- [74] Heiner JP, Miraldi F, Kallick S, et al. Localization of GD2-specific monoclonal antibody 3F8 in human osteosarcoma. *Cancer Res* 1987;47(20):5377–81.
- [75] Cheung NKV, Canete A, Cheung IY, Ye JN, Liu C. Disialoganglioside GD2 anti-idiotypic monoclonal antibodies. *Int J Cancer* 1993;54(3):499–505.
- [76] Cheung N-KV, Cheung IY, Kushner BH, et al. Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-cis-retinoic acid in highrisk patients with stage 4 neuroblastoma in first remission. *J Clin Oncol* 2012;30(26):3264–70.
- [77] Roth M, Linkowski M, Tarim J, et al. Ganglioside GD2 as a therapeutic target for antibody-mediated therapy in patients with osteosarcoma. *Cancer* 2014;120(4):548–54.
- [78] Gorlick R, Janeway K, Lessnick S, Randall RL, Marina N. COG Bone Tumor Committee. Children's Oncology Group's 2013 blueprint for research: bone tumors. *Pediatr Blood Cancer* 2013;60(6):1009–15.
- [79] Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. *Int J Clin Oncol*. 2016;21(3):462–73.
- [80] Shen JK, Cote GM, Choy E, et al. Targeting programmed cell death ligand 1 in osteosarcoma: an auto-commentary on therapeutic potential. *Oncoimmunology* 2014;3(8):e954467.
- [81] Zheng W, Xiao H, Liu H, Zhou Y. Expression of programmed death 1 is correlated with progression of osteosarcoma. *APMIS* 2015;123(2):102–7.
- [82] Constantino J, Gomes C, Falcão A, Cruz MT, Neves BM. Antitumor dendritic cell-based vaccines: lessons from 20years of clinical trials and future perspectives. *Transl Res* 2016;168:74–95.
- [83] Chauvin C, Philippeau JM, Hémond C, Hubert FX, Wittrant Y, Lamoureux F, Trinité B, Heymann D, Rédini F, Josien R. Killer dendritic cells link innate and adaptive immunity against established osteosarcoma in rats. *Cancer Res* 2008;68:9433–40.
- [84] Krishnadas DK, Shusterman S, Bai F, Diller L, Sullivan JE, Cheerva AC, George RE, Lucas KG. A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. *Cancer Immunol Immunother* 2015;64:1251–60.
- [85] Himoudi N, Wallace R, Parsley KL, Gilmour K, Barrie AU, Howe K, Dong R, Sebire NJ, Michalski A, Thrasher AJ, Anderson J. Lack of T-cell responses following autologous

tumour lysate pulsed dendritic cell vaccination, in patients with relapsed osteosarcoma. *Clin Transl Oncol* 2012;14:271–9.

- [86] Kleinerman ES, Jia SF, Gri NJ, Seibel NL, Benjamin RS, Ja EN. Phase II study of liposomal muramyl tripeptide in osteosarcoma: the cytokine cascade and monocyte activation following administration. *J Clin Oncol* 1992;10(8):1310–6.
- [87] Ando K, Mori K, Corradini N, Redini F, Heymann D. Mifamurtide for the treatment of nonmetastatic osteosarcoma. *Expert Opin Pharmacother* 2011;12(2):285–92.
- [88] Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the children’s oncology group. *J Clin Oncol* 2008;26(4):633–8.
- [89] Biteau K, Guiho R, Chatelais M, Taurelle J, Chesneau J, Corradini N, Heymann D, Redini F. L-MTP-PE and zoledronic acid combination in osteosarcoma: preclinical evidence of positive therapeutic combination for clinical transfer. *Am J Cancer Res* 2016;6(3):677–89.
- [90] Lamplot JD, Denduluri S, Qin J, Li R, Liu X, Zhang H, Chen X, Wang N, Pratt A, Shui W, Luo X, Nan G, Deng ZL, Luo J, Haydon RC, He TC, Luu HH. The current and future therapies for human osteosarcoma. *Curr Cancer Ther Rev* 2013;9(1):55–77.
- [91] Arndt CA, Koshkina NV, Inwards CY, Hawkins DS, Krailo MD, Villaluna D, Anderson PM, Goorin AM, Blakely ML, Bernstein M, Bell SA, Ray K, Grendahl DC, Marina N, Kleinerman ES. Inhaled granulocyte-macrophage colony stimulating factor for first pulmonary recurrence of osteosarcoma: effects on disease-free survival and immunomodulation. A report from the Children’s Oncology Group. *Clin Cancer Res*. 2010;16(15):4024–30.
- [92] Heymann MF, Brown HK, Heymann D. Drugs in early clinical development for the treatment of osteosarcoma. *Expert Opin Investig Drugs* 2016;25(11):1265–80.

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