

Review article

Advances in immunotherapy for osteosarcoma: a review of emerging treatment strategies

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ABSTRACT

Advances in immunotherapy for osteosarcoma have shown promising results, with the use of monoclonal antibodies and immune checkpoint inhibitors. These strategies are aimed at targeting specific molecules and pathways involved in tumour immune evasion and promoting anti-tumour immune responses. Other emerging immunotherapeutic approaches include autophagy and pyroptosis induction, chimeric antigen receptor T-cell therapy, gadolinium-bisphosphonate nanoparticles and dendritic cell-based vaccines. Continued research into these emerging treatment strategies is essential for developing effective therapies for patients with high-grade osteosarcoma.

Key words: osteosarcoma, immunotherapy, immune checkpoint inhibitors, cancer vaccines, autophagy, pyroptosis, chimeric antigen receptor T cells

INTRODUCTION

Osteosarcoma, a malignant mesenchymal cell-derived tumour that produces osteoid [1–3], is a rare yet catastrophic disease and the most frequent primary bone tumour, ranking third among children and adolescents cancers, after lymphomas and brain tumours [1, 4, 5].

Despite the implementation of adjuvant chemotherapy in the 1970s that yielded higher overall 10-year survival rates, survival rates have not improved since the 1990s. The contemporary treatment approach for extremity localized, non-metastatic osteosarcoma involves a combination of surgery and high-dose chemotherapy, resulting in a 5-year event-free survival rate of 60–70%. However, a significant challenge remains in the form of low survival rates for patients with metastases or relapse, as well as those with axial disease [2]. Consequently, there is an urgent need for a better comprehension of this ailment, including improved diagnostic techniques and treatment modalities [1].

This review article aims to provide an in-depth overview of osteosarcoma, covering its etiology, clinical presentation, diagnostic tools, and management options. Subsequently, we investigate the potential of novel treatment approaches to improve the prognosis of high-grade osteosarcoma.

OSTEOSARCOMA

Osteosarcoma, is a type of bone cancer that arises from primitive transformed cells of mesenchymal origin, exhibiting osteoblastic differentiation, and producing malignant osteoid or immature bone [2, 6]. It is the most common histological form of primary bone sarcoma and is most prevalent in children and young adults [7]. The second peak of osteosarcoma occurrence is in individuals over 65 years of age [8]. Several factors increase the risk of osteosarcoma, including familial cases (Li Fraumeni syndrome, retinoblastoma syndrome, Werner syndrome, Bloom syndrome or Diamond-Blackfan anemia) [9–11], bone dysplasias, radiation exposure [12], large doses of strontium-90 [13], and exposure to environmental chemicals such as radium, beryllium, and chromium [14–16]. There is no clear association between water fluoridation and osteosarcoma [17].

The majority of osteosarcoma cases occur in the femur (42%), followed by the tibia (19%), humerus (10%), skull or jaw (8%), and pelvis (8%). In children, osteosarcoma frequently occurs in the metaphysis of long bones [5]. Upon presentation, around 10–20% of patients exhibit observable macrometastatic disease, with the lungs being the most common site of metastasis [18].

Patients with osteosarcoma often first complain of pain that may be worse at night, intermittent, and of varying intensity, or may present as overt localized swelling and a large soft tissue mass. The lymphadenopathy may also be present. A relatively common initial manifestation of osteosarcoma is a pathologic fracture.

Osteosarcoma is typically diagnosed using plain radiograph as the initial imaging modality. Some features of osteosarcoma visible on plain radiograph include the radial “sunburst” appearance and “Codman triangle”, which is a result of the tumour elevating the periosteum [19]. If there are subtle abnormalities or if the plain radiographs are inconclusive, magnetic resonance imaging (MRI) should be performed in patients with a high suspicion of disease. The MRI protocol should include a coronal T1-weighted sequence. Both computed tomography (CT) and MRI are accurate in the local staging of osteosarcoma [20], but MRI is superior in defining the extent of soft tissue involvement [21, 22]. A definite diagnosis of osteosarcoma requires a biopsy of the tumour tissue and subsequent pathological examination [23, 24].

Treatment

Curative therapy for osteosarcoma always involves surgery and the location and size of the primary tumour determine the type of surgical procedure needed [25, 26]. The goal is to achieve a negative margin of resection with a wide local excision that removes the primary tumour along with its reactive zone and a cuff of normal tissue in all planes. Limb-sparing procedures are preferred for extremity lesions to improve functional outcomes as long as complete tumour resection is anatomically possible and adjuvant chemotherapy is used. However, patient selection is critical, and amputation is indicated if there is any doubt that a wide local excision can be accomplished to avoid local recurrence [27–30]. Computer-assisted tumour surgery is beginning to be used for complex surgical resections, particularly for pelvic or sacral tumours [31].

Adjuvant therapy, including chemotherapy and radiation therapy, is a crucial component in managing osteosarcoma. Often, there is subclinical metastatic disease present at diagnosis, which can be eliminated by starting chemotherapy early. Neoadjuvant chemotherapy is used to increase the number of patients who can undergo limb-salvage surgery by reducing tumour burden. The response to neoadjuvant chemotherapy is a critical factor in predicting the outcome of treatment [32].

Radiation therapy is usually not effective against osteosarcoma, and primary radiation therapy alone is often not enough to control local disease, especially for large tumours. Adjuvant radiation

therapy may be considered only for patients with unresectable or incompletely resected primary tumours or for those specific variants of osteosarcoma, which may be more responsive to radiation [33–37].

IMMUNE CHECKPOINTS

One of the key mechanisms of malignant tumours is their ability to evade the immune response [38–40]. This is achieved through the activation of a series of mechanisms that aim to disrupt the activation of T lymphocytes, which are a crucial component of the immune response against tumours. The activation of T lymphocytes mainly depends on the interaction between the T cell receptor (TCR) and antigens presented by the major histocompatibility complex (MHC) and the binding of the co-stimulatory transmembrane receptor CD28 (Cluster of differentiation 28) expressed on T cells to its ligand CD80/86 (Cluster of differentiation 80/86) [41, 42].

These activation mechanisms can be disrupted by immune checkpoints such as programmed cell death protein-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). PD-1 achieves this effect by binding to programmed cell death protein ligand-1 (PD-L1), while CTLA-4 inhibits immune system activation by binding to CD80/86 [41, 43–47]. PD-1/PD-L1 and CTLA-4/CD80/86 checkpoints are utilized by tumours to evade the immune response [38–40, 48, 49].

Other immune checkpoints, such as T-cell immunoglobulin mucin domain-containing protein-3 (Tim-3) [50], indoleamine 2,3-dioxygenase-1 (IDO1) [51], and lymphocyte activation gene-3 (Lag-3) [52], can also hinder the immune response [47].

Immune checkpoint inhibitors

Immune checkpoint inhibitors (ICI) targeting PD-1, PD-L1 and CTLA4 are used in treatment of several cancers such as melanoma, lung cancer, renal cell carcinoma, Hodgkin lymphoma, cutaneous squamous cell carcinoma, and urothelial carcinoma [53]. The discovery of ICIs, such as ipilimumab and nivolumab, has had a particularly significant impact on the treatment of melanoma. Immune checkpoint inhibitors are now frequently used in the treatment of advanced melanoma, and their efficacy is continuously being investigated in clinical trials [54–59]. Figure 1 illustrates the binding sites and mechanism of action of checkpoint inhibitors.

The use of ICIs as mono- or dual therapy in osteosarcoma has not resulted in significant anti-tumour efficacy [47, 60–66]. Potential

reasons for this could be attributed to various barriers such as low expression of PD-L1 [67–69], insufficient tumour-specific antigen presentation [70, 71], limited immune cell infiltration [72–74], and specific extracellular matrix [75–87].

Nevertheless, several preclinical studies and a few clinical trials have demonstrated the anti-tumour potential of combining immune checkpoint inhibitors with several novel strategies. Additionally, recent research has proposed predictive biomarkers of ICIs for osteosarcoma that could aid in the selection of patients who are more likely to benefit from this treatment [47].

Combining immune checkpoint inhibitors with autophagy induction

Autophagy is a process of self-degradation that plays a crucial role in maintaining energy balance during crucial developmental stages and in response to nutrient deficiencies. Additionally, it serves a housekeeping function by eliminating misfolded or aggregated proteins, clearing out damaged organelles like mitochondria, endoplasmic reticulum, and peroxisomes, and getting rid of intracellular pathogens [88]. Recently, augmenting anti-tumour immunotherapy through the use of autophagy has emerged as a promising approach [89–93]. Autophagy, in response to both intracellular and extracellular stressors, can improve antigen presentation and increase the sensitivity of cytotoxic T lymphocytes [94–98].

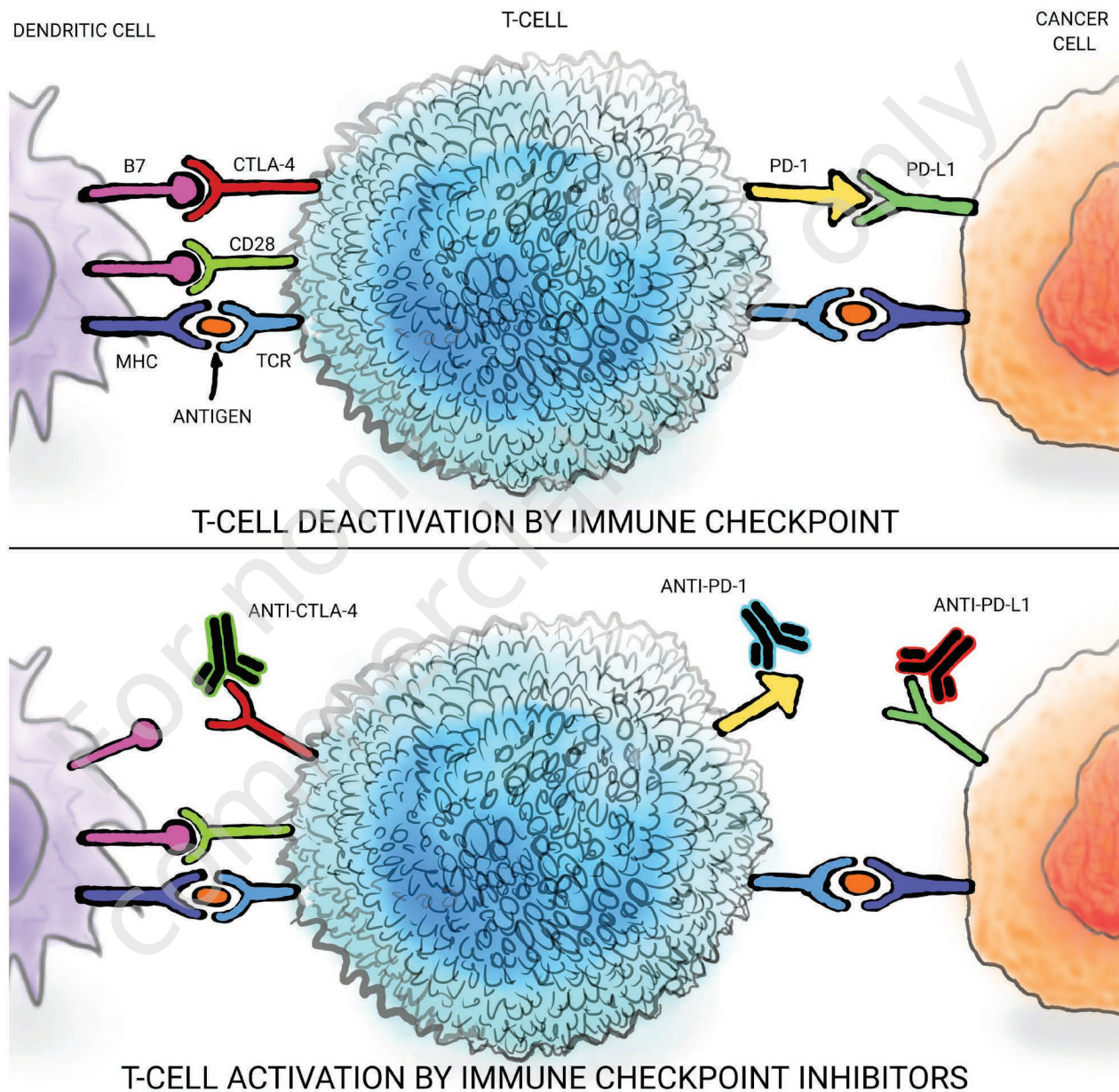
To utilize the mechanisms of autophagy and immune checkpoints in anticancer therapy, Ge et al. [89] designed a pH-sensitive nanocarrier that released the natural derivative of curcumin and the BMS1166 in the acidic environment of the osteosarcoma. The curcumin derivative activated the autophagic cell death and enhanced the immunotherapeutic response of PD-1/PD-L1 blockade. The BMS1166 simultaneously inhibited the PD-1/PD-L1 interaction, increasing tumour immunogenicity and sensitivity to T-cell anti-tumour response.

Administering the nanocarrier to mice with orthotopic osteosarcoma (OS) demonstrated potent anti-tumour effects, resulting in long-term immunity against tumour recurrence. This was accompanied by increased dendritic cell maturation and infiltration of CD8+ T lymphocytes into the tumour [89].

Combining immune checkpoint inhibitors with pyroptosis induction

Pyroptosis is a type of cell death that is initiated by specific inflammasomes, which trigger the cleavage of gasdermin D (GSDMD) and activation of inactive cytokines such as IL-18 and IL-1 β by

Figure 1. Schematic representation of the mechanism of action of checkpoint inhibitors. The interaction between T cells and dendritic cells occurs in lymph nodes, whereas the interaction between T cells and cancer cells occurs in the tumour tissue.



CD28 – cluster of differentiation 28; B7 – B7 protein; CTLA-4 – cytotoxic T-lymphocyte antigen 4; MHC – major histocompatibility complex; PD-1 – programmed cell death protein 1; PD-L1 – programmed death-ligand 1; TCR – T-cell receptor; ANTI-CTLA-4 – antibody directed against CTLA4; ANTI-PD-1 – antibody directed against PD1; ANTI-PD-L1 – antibody directed against PD-L1.

caspace-1. This process leads to cellular swelling, lysis of the plasma membrane, fragmentation of chromatin, and release of proinflammatory substances [99]. More recently, research has shown that pyroptosis may play a role in regulating the proliferation, invasion, and metastasis of tumours, and that this process can be controlled by non-coding RNAs and other molecules [99–103].

Jin et al. [104] proposed a method in 2022 to induce pyroptosis in osteosarcoma cells by selectively modulating the mitochondria,

which could increase the efficacy of anti-tumour treatment when combined with immunotherapy. They developed a polymer micelle made up of poly[2-(N-oxide-N,N-diethylamino)ethyl methacrylate] (OPDEA) and conjugated dichloroacetate (DCA). OPDEA was used to target the mitochondria, and DCA was used to block pyruvate dehydrogenase kinase 1 (PDHK1). This conjugate was found to trigger pyroptosis by inducing oxidative stress in the mitochondria of osteosarcoma cell lines. The researchers also observed that the micelle could stimulate the release of PD-L1.

Therefore, when combined with an anti-PD-L1 monoclonal antibody, the micelle was able to effectively inhibit the proliferation of osteosarcoma cells and sustain T cell activation. This study suggests that targeted modulation of mitochondria to induce pyroptosis could be an effective strategy to improve the anti-tumour efficacy of immunotherapy.

CHIMERIC ANTIGEN RECEPTOR T CELLS (CAR-T)

Chimeric antigen receptor T cell (CAR-T) refers to a T cell with a modified receptor designed to improve its ability to target cancer cells. This gene therapy involves modifying a patient's T cells in a laboratory to equip them with a chimeric antigen receptor (CAR) that allows them to recognize and target cancer cells, after which the cells are reintroduced into the patient's body. CAR-T is used to treat certain types of leukaemia and lymphoma, as well as being studied for solid tumour therapy [105–107].

In 2019 Wang et al. [108] discovered that the expression of CD166 was selectively detected on human osteosarcoma cell lines, indicating its potential as a target for CAR-T cell therapy. The CD166.BBζ CAR-T cells displayed cytotoxicity against osteosarcoma cells in vitro and in vivo, and their injection into mice resulted in the regression of tumours without any obvious toxicity. The findings suggest that CD166.BBζ CAR-T cells may serve as a promising therapeutic strategy for the treatment of osteosarcoma in future clinical practice.

CANCER VACCINES

Cancer vaccines belong to the category of immunotherapy, which harnesses the body's own immune system to identify and eliminate malignant cells. These vaccines function by introducing cancer-associated antigens to the body through various methods such as injection or viral/bacterial vectors. The immune system then recognizes these antigens as foreign and triggers a response to destroy them. The ultimate goal of cancer vaccines is to induce a robust and specific immune response that can prevent the growth of new tumours or eradicate existing ones. The available types of cancer vaccines include peptide vaccines, DNA vaccines, RNA vaccines, and whole-cell vaccines [109]. In cancer vaccines, a frequently utilized approach is to employ dendritic cells that are sourced from the patient [110]. Antigens linked to the tumour are showcased to these cells, which are then infused into the patient. The prepared dendritic cells present the antigens to cytotoxic T cells, which gain the capability to target and eliminate cancer cells in a selective manner [111–113].

GADOLINIUM-BISPHOSPHONATE NANOPARTICLES

Gadolinium is an element commonly used as a contrast agent in MRI scans. It has also been shown to reduce the survival of osteosarcoma cells in vitro in a concentration-dependent manner [114]. Zhang et al. conducted a study in 2022 [115] where they created and synthesized nanoparticles using gadolinium and bisphosphonate. The study demonstrated that internalizing these nanoparticles into osteosarcoma cells increased the tumour's sensitivity to radiotherapy. Furthermore, the nanoparticles stimulated the activation of both the innate and adaptive immune response in the tumour microenvironment, maturation of dendritic cells, and M1 polarization of macrophages. These findings indicated that the nanoparticles have the potential to improve the effectiveness of radiotherapy and immunotherapy in treating osteosarcoma [115].

SYNERGISTIC EFFECTS OF CD47 AND GD2 ANTIBODIES

Anti-tumour immunity is mediated, in part, by macrophages that eliminate tumour cells through phagocytosis. However, CD47 (*cluster of differentiation 47*) is a checkpoint molecule that can inhibit macrophage activity by binding to its receptor SIRPα [116]. The inhibition of CD47 has shown encouraging clinical effectiveness in initial human trials [117–119].

Disialoganglioside GD2 is overexpressed in neuroblastoma and osteosarcoma, and its expression varies in other tumours [120–125]. Unfortunately, anti-GD2 antibodies have not demonstrated significant anti-tumour activity in osteosarcoma or other GD2-positive tumours [126, 127]. On the other hand, the combination of CD47 and GD2 antibodies has been found to have a significant synergistic effect, leading to the recruitment of macrophages associated with the tumour that effectively target the tumour cells. These findings suggest that the combination of CD47 and GD2 antibodies may have potential as a treatment for osteosarcoma [117, 128].

CONCLUSIONS

In conclusion, osteosarcoma remains a devastating disease that poses a significant challenge for patients, their families, and clinicians. Despite significant advances in diagnosis and management, survival rates have remained relatively stagnant since the 1990s.

The emergence of immunotherapy as a potential treatment option is a promising development in the field of osteosarcoma

management. The use of immune checkpoint inhibitors, adoptive T-cell therapy, and vaccines holds great potential for improving outcomes for patients with osteosarcoma. Future research is needed to identify biomarkers to predict response to immunotherapy and to develop combinatorial strategies that can optimize the use of immunotherapeutic agents. Overall, these emerging therapies represent an opportunity to significantly improve the lives of patients with osteosarcoma.

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References

1. Simpson E, Brown HL. Understanding osteosarcomas. *JAAPA*. 2018; 31(8): 15-9. <http://doi.org/10.1097/01.JAA.0000541477.24116.8d>.
2. Luetke A, Meyers PA, Lewis I et al. Osteosarcoma treatment - where do we stand? A state of the art review. *Cancer Treat Rev*. 2014; 40(4): 523-32. <http://doi.org/10.1016/j.ctrv.2013.11.006>.
3. Picci P. Osteosarcoma (osteogenic sarcoma). *Orphanet J Rare Dis*. 2007; 2: 6. <http://doi.org/10.1186/1750-1172-2-6>.
4. Cole S, Gianferante DM, Zhu B et al. Osteosarcoma: A Surveillance, Epidemiology, and End Results program-based analysis from 1975 to 2017. *Cancer*. 2022; 128(11): 2107-18. <http://doi.org/10.1002/cncr.34163>.
5. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treat Res*. 2009; 152: 3-13. http://doi.org/10.1007/978-1-4419-0284-9_1.
6. Sissons HA. The WHO classification of bone tumors. *Recent Results Cancer Res*. 1976; (54): 104-8. http://doi.org/10.1007/978-3-642-80997-2_8.
7. Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015; 136(5): E359-86. <http://doi.org/10.1002/ijc.29210>.
8. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer*. 2009; 115(7): 1531-43. <http://doi.org/10.1002/cncr.24121>.
9. Czarnicka AM, Synoradzki K, Firlej W et al. Molecular Biology of Osteosarcoma. *Cancers (Basel)*. 2020; 12(8): 2130. <http://doi.org/10.3390/cancers12082130>.
10. Fiedorowicz M, Bartnik E, Sobczuk P et al. Molecular biology of sarcoma. *Oncol Clin Pr*. 2018; 14: 307-30. <http://doi.org/10.5603/OCP.2018.0045>.
11. Rickel K, Fang F, Tao J. Molecular genetics of osteosarcoma. *Bone*. 2017; 102: 69-79. <http://doi.org/10.1016/j.bone.2016.10.017>.
12. Shao Z, He Y, Wang L et al. Computed tomography findings in radiation-induced osteosarcoma of the jaws. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010; 109(3): e88-94. <http://doi.org/10.1016/j.tripleo.2009.10.049>.
13. Shvedov VL. Skhematicheskaia model' osteosarkomogeneza, indutsirovannogo 90Sr [Schematic model of genesis of osteosarcoma induced by Sr-90]. *Radiats Biol Radioecol*. 1996; 36(1): 109-18.
14. Rani AS, Kumar S. Transformation of non-tumorigenic osteoblast-like human osteosarcoma cells by hexavalent chromates: alteration of morphology, induction of anchorage-independence and proteolytic function. *Carcinogenesis*. 1992; 13(11): 2021-7. <http://doi.org/10.1093/carcin/13.11.2021>.
15. Dutra FR, Largent EJ. Osteosarcoma induced by beryllium oxide. *Am J Pathol*. 1950; 26(2): 197-209.
16. Mazabraud A. Production expérimentale de sarcomes osseux chez le lapin par injection unique locale de Béryllium [Experimental production of bone sarcomas in the rabbit by a single local injection of beryllium]. *Bull Cancer*. 1975; 62(1): 49-58.
17. Comber H, Deady S, Montgomery E et al. Drinking water fluoridation and osteosarcoma incidence on the island of Ireland. *Cancer Causes Control*. 2011; 22(6): 919-24. <http://doi.org/10.1007/s10552-011-9765-0>.
18. Miller BJ, Cram P, Lynch CF et al. Risk factors for metastatic disease at presentation with osteosarcoma: an analysis of the SEER database. *J Bone Joint Surg Am*. 2013; 95(13): e89. <http://doi.org/10.2106/JBJS.L.01189>.
19. McDonald J, DenOtter TD. Codman Triangle. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; July 30, 2022.
20. Panicek DM, Gatsonis C, Rosenthal DI et al. CT and MR imaging in the local staging of primary malignant musculoskeletal neoplasms: Report of the Radiology Diagnostic Oncology Group. *Radiology*. 1997; 202(1): 237-46. <http://doi.org/10.1148/radiology.202.1.8988217>.
21. Kundu ZS. Classification, imaging, biopsy and staging of osteosarcoma. *Indian J Orthop*. 2014; 48(3): 238-46. <http://doi.org/10.4103/0019-5413.132491>.
22. Sajadi KR, Heck RK, Neel MD et al. The incidence and prognosis of osteosarcoma skip metastases. *Clin Orthop Relat Res*. 2004; 426: 92-6. <http://doi.org/10.1097/01.blo.0000141493.52166.69>.
23. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO Classification of Soft Tissue Tumours: news and perspectives. *Pathologica*. 2021; 113(2): 70-84. <http://doi.org/10.32074/1591-951X-213>.
24. Ritter J, Bielack SS. Osteosarcoma. *Ann Oncol*. 2010; 21(Suppl 7): vii320-5. <http://doi.org/10.1093/annonc/mdq276>.
25. Jaffe N, Carrasco H, Raymond K et al. Can cure in patients with osteosarcoma be achieved exclusively with chemotherapy and abrogation of surgery? *Cancer*. 2002; 95(10): 2202-10. <http://doi.org/10.1002/cncr.10944>.
26. Peabody TD, Gibbs CP Jr, Simon MA. Evaluation and staging of musculoskeletal neoplasms. *J Bone Joint Surg Am*. 1998; 80(8): 1204-18. <http://doi.org/10.2106/00004623-199808000-00016>.
27. Bacci G, Forni C, Longhi A et al. Local recurrence and local control of non-metastatic osteosarcoma of the extremities: a 27-year experience in a single institution. *J Surg Oncol*. 2007; 96(2): 118-23. <http://doi.org/10.1002/jso.20628>.

28. Wittig JC, Bickels J, Kellar-Graney KL et al. Osteosarcoma of the proximal humerus: long-term results with limb-sparing surgery. *Clin Orthop Relat Res*. 2002; 397: 156-76. <http://doi.org/10.1097/00003086-200204000-00021>.
29. Renard AJ, Veth RP, Schreuder HW et al. Function and complications after ablative and limb-salvage therapy in lower extremity sarcoma of bone. *J Surg Oncol*. 2000; 73(4): 198-205. [http://doi.org/10.1002/\(sici\)1096-9098\(200004\)73:4<198::aid-jso3>3.0.co;2-x](http://doi.org/10.1002/(sici)1096-9098(200004)73:4<198::aid-jso3>3.0.co;2-x).
30. Nagarajan R, Neglia JP, Clohisey DR et al. Education, employment, insurance, and marital status among 694 survivors of pediatric lower extremity bone tumors: a report from the childhood cancer survivor study. *Cancer*. 2003; 97(10): 2554-64. <http://doi.org/10.1002/cncr.11363>.
31. Wong KC, Niu X, Xu H et al. Computer Navigation in Orthopaedic Tumour Surgery. *Adv Exp Med Biol*. 2018; 1093: 315-26. http://doi.org/10.1007/978-981-13-1396-7_24.
32. Rosen G, Marcove RC, Huvos AG et al. Primary osteogenic sarcoma: eight-year experience with adjuvant chemotherapy. *J Cancer Res Clin Oncol*. 1983; 106 Suppl: 55-67. <http://doi.org/10.1007/BF00625054>.
33. Ferguson WS, Goorin AM. Current treatment of osteosarcoma. *Cancer Invest*. 2001; 19(3): 292-315. <http://doi.org/10.1081/cnv-100102557>.
34. Stea B, Cavazzana A, Kinsella TJ. Small-cell osteosarcoma: correlation of in vitro and clinical radiation response. *Int J Radiat Oncol Biol Phys*. 1988; 15(5): 1233-8. [http://doi.org/10.1016/0360-3016\(88\)90209-x](http://doi.org/10.1016/0360-3016(88)90209-x).
35. Age and dose of chemotherapy as major prognostic factors in a trial of adjuvant therapy of osteosarcoma combining two alternating drug combinations and early prophylactic lung irradiation. French Bone Tumor Study Group. *Cancer*. 1988; 61(7): 1304-11. [http://doi.org/10.1002/1097-0142\(19880401\)61:7<1304::aid-cncr2820610705>3.0.co;2-i](http://doi.org/10.1002/1097-0142(19880401)61:7<1304::aid-cncr2820610705>3.0.co;2-i).
36. Zaharia M, Caceres E, Valdivia S et al. Postoperative whole lung irradiation with or without adriamycin in osteogenic sarcoma. *Int J Radiat Oncol Biol Phys*. 1986; 12(6): 907-10. [http://doi.org/10.1016/0360-3016\(86\)90384-6](http://doi.org/10.1016/0360-3016(86)90384-6).
37. Whelan JS, Burcombe RJ, Janinis J et al. A systematic review of the role of pulmonary irradiation in the management of primary bone tumours. *Ann Oncol*. 2002; 13(1): 23-30. <http://doi.org/10.1093/annonc/mdf047>.
38. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144(5): 646-74. <http://doi.org/10.1016/j.cell.2011.02.013>.
39. Lei Q, Wang D, Sun K et al. Resistance Mechanisms of Anti-PD1/PDL1 Therapy in Solid Tumors. *Front Cell Dev Biol*. 2020; 8: 672. <http://doi.org/10.3389/fcell.2020.00672>.
40. Vinay DS, Ryan EP, Pawelec G et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Semin Cancer Biol*. 2015; 35 Suppl: S185-98. <http://doi.org/10.1016/j.semcancer.2015.03.004>.
41. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer*. 2019; 19(3): 133-50. <http://doi.org/10.1038/s41568-019-0116-x>.
42. Li B, Chan HL, Chen P. Immune Checkpoint Inhibitors: Basics and Challenges. *Curr Med Chem*. 2019; 26(17): 3009-25. <http://doi.org/10.2174/0929867324666170804143706>.
43. Francisco LM, Salinas VH, Brown KE et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med*. 2009; 206(13): 3015-29. <http://doi.org/10.1084/jem.20090847>.
44. Arasanz H, Gato-Cañas M, Zuazo M et al. PD1 signal transduction pathways in T cells. *Oncotarget*. 2017; 8(31): 51936-45. <http://doi.org/10.18632/oncotarget.17232>.
45. Amarnath S, Mangus CW, Wang JC et al. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. *Sci Transl Med*. 2011; 3(111): 111ra120. <http://doi.org/10.1126/scitranslmed.3003130>.
46. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. *Nat Rev Immunol*. 2018; 18(3): 153-67. <http://doi.org/10.1038/nri.2017.108>.
47. Wen Y, Tang F, Tu C et al. Immune checkpoints in osteosarcoma: Recent advances and therapeutic potential. *Cancer Lett*. 2022; 547: 215887. <http://doi.org/10.1016/j.canlet.2022.215887>.
48. Bhatia A, Kumar Y. Cellular and molecular mechanisms in cancer immune escape: a comprehensive review. *Expert Rev Clin Immunol*. 2014; 10(1): 41-62. <http://doi.org/10.1586/1744666X.2014.865519>.
49. Goel G, Sun W. Cancer immunotherapy in clinical practice – the past, present, and future. *Chin J Cancer*. 2014; 33(9): 445-57. <http://doi.org/10.5732/cjc.014.10123>.
50. Kandel S, Adhikary P, Li G et al. The TIM3/Gal9 signaling pathway: An emerging target for cancer immunotherapy. *Cancer Lett*. 2021; 510: 67-78. <http://doi.org/10.1016/j.canlet.2021.04.011>.
51. Cheong JE, Sun L. Targeting the IDO1/TDO2-KYN-AhR Pathway for Cancer Immunotherapy – Challenges and Opportunities. *Trends Pharmacol Sci*. 2018; 39(3): 307-25. <http://doi.org/10.1016/j.tips.2017.11.007>.
52. Shi AP, Tang XY, Xiong YL et al. Immune Checkpoint LAG3 and Its Ligand FGL1 in Cancer. *Front Immunol*. 2022; 12: 785091. <http://doi.org/10.3389/fimmu.2021.785091>.
53. Twomey JD, Zhang B. Cancer Immunotherapy Update: FDA-Approved Checkpoint Inhibitors and Companion Diagnostics. *AAPS J*. 2021; 23(2): 39. <http://doi.org/10.1208/s12248-021-00574-0>.
54. Hughes T, Klairmont M, Sharfman WH et al. Interleukin-2, Ipilimumab, and Anti-PD-1: clinical management and the evolving role of immunotherapy for the treatment of patients with metastatic melanoma. *Cancer Biol Ther*. 2021; 22(10-12): 513-26. <http://doi.org/10.1080/15384047.2015.1095401>.
55. Carlino MS, Menzies AM, Atkinson V et al. Long-term Follow-up of Standard-Dose Pembrolizumab Plus Reduced-Dose Ipilimumab in Patients with Advanced Melanoma: KEYNOTE-029 Part 1B. *Clin Cancer Res*. 2020; 26(19): 5086-91. <http://doi.org/10.1158/1078-0432.CCR-20-0177>.
56. Lebbé C, Meyer N, Mortier L et al. Evaluation of Two Dosing Regimens for Nivolumab in Combination With Ipilimumab in Patients With Advanced Melanoma: Results From the Phase IIIb/IV CheckMate 511 Trial. *J Clin Oncol*. 2019; 37(11): 867-75. <http://doi.org/10.1200/JCO.18.01998>.
57. Tawbi HA, Forsyth PA, Algazi A et al. Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain. *N Engl J Med*. 2018; 379(8): 722-30. <http://doi.org/10.1056/NEJMoa1805453>.
58. Robert C, Thomas L, Bondarenko I et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011; 364(26): 2517-2526. <http://doi.org/10.1056/NEJMoa1104621>.
59. Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363(8): 711-23. <http://doi.org/10.1056/NEJMoa1003466>. [correction in: *N Engl J Med*. 2010; 363(13): 1290].

60. Paoluzzi L, Cacavio A, Ghesani M et al. Response to anti-PD1 therapy with nivolumab in metastatic sarcomas. *Clin Sarcoma Res.* 2016; 6: 24. <http://doi.org/10.1186/s13569-016-0064-0>.
61. Davis KL, Fox E, Merchant MS et al. Nivolumab in children and young adults with relapsed or refractory solid tumours or lymphoma (ADVL1412): a multicentre, open-label, single-arm, phase 1-2 trial. *Lancet Oncol.* 2020; 21(4): 541-50. [http://doi.org/10.1016/S1470-2045\(20\)30023-1](http://doi.org/10.1016/S1470-2045(20)30023-1).
62. Tawbi HA, Burgess M, Bolejack V et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. *Lancet Oncol.* 2017; 18(11): 1493-501. [http://doi.org/10.1016/S1470-2045\(17\)30624-1](http://doi.org/10.1016/S1470-2045(17)30624-1). [correction in: *Lancet Oncol.* 2017; 18(12): e711, *Lancet Oncol.* 2018; 19(1): e8].
63. Boye K, Longhi A, Guren T et al. Pembrolizumab in advanced osteosarcoma: results of a single-arm, open-label, phase 2 trial. *Cancer Immunol Immunother.* 2021; 70(9): 2617-24. <http://doi.org/10.1007/s00262-021-02876-w>.
64. Georger B, Zwaan CM, Marshall LV et al. Atezolizumab for children and young adults with previously treated solid tumours, non-Hodgkin lymphoma, and Hodgkin lymphoma (iMATRIX): a multicentre phase 1-2 study. *Lancet Oncol.* 2020; 21(1): 134-44. [http://doi.org/10.1016/S1470-2045\(19\)30693-X](http://doi.org/10.1016/S1470-2045(19)30693-X).
65. Merchant MS, Wright M, Baird K et al. Phase I Clinical Trial of Ipilimumab in Pediatric Patients with Advanced Solid Tumors. *Clin Cancer Res.* 2016; 22(6): 1364-70. <http://doi.org/10.1158/1078-0432.CCR-15-0491>.
66. Nuytemans L, Sys G, Creyten D et al. NGS-analysis to the rescue: dual checkpoint inhibition in metastatic osteosarcoma – a case report and review of the literature. *Acta Clin Belg.* 2021; 76(2): 162-7. <http://doi.org/10.1080/17843286.2019.1683129>.
67. Daud AI, Wolchok JD, Robert C et al. Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma. *J Clin Oncol.* 2016; 34(34): 4102-9. <http://doi.org/10.1200/JCO.2016.67.2477>.
68. Ott PA, Bang YJ, Piha-Paul SA et al. T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers: KEYNOTE-028. *J Clin Oncol.* 2019; 37(4): 318-27. <http://doi.org/10.1200/JCO.2018.78.2276>.
69. Garon EB, Rizvi NA, Hui R et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med.* 2015; 372(21): 2018-28. <http://doi.org/10.1056/NEJMoa1501824>.
70. Wu CC, Livingston JA. Genomics and the Immune Landscape of Osteosarcoma. *Adv Exp Med Biol.* 2020; 1258: 21-36. http://doi.org/10.1007/978-3-030-43085-6_2.
71. Young K, Hughes DJ, Cunningham D et al. Immunotherapy and pancreatic cancer: unique challenges and potential opportunities. *Ther Adv Med Oncol.* 2018; 10: 1758835918816281. <http://doi.org/10.1177/1758835918816281>.
72. Zhou Y, Yang D, Yang Q et al. Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced osteosarcoma. *Nat Commun.* 2020; 11(1): 6322. <http://doi.org/10.1038/s41467-020-20059-6>. (correction in: *Nat Commun.* 2021; 12(1): 2567).
73. Chen Z, Li L, Li Z et al. Identification of key serum biomarkers for the diagnosis and metastatic prediction of osteosarcoma by analysis of immune cell infiltration. *Cancer Cell Int.* 2022; 22(1): 78. <http://doi.org/10.1186/s12935-022-02500-6>.
74. Ligon JA, Choi W, Cojocaru G et al. Pathways of immune exclusion in metastatic osteosarcoma are associated with inferior patient outcomes. *J Immunother Cancer.* 2021; 9(5): e001772. <http://doi.org/10.1136/jitc-2020-001772>.
75. Bagchi S, Yuan R, Engleman EG. Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. *Annu Rev Pathol.* 2021; 16: 223-49. <http://doi.org/10.1146/annurev-pathol-042020-042741>.
76. Wu CC, Beird HC, Livingston AJ et al. Immuno-genomic landscape of osteosarcoma. *Nat Commun.* 2020; 11(1): 1008. <http://doi.org/10.1038/s41467-020-14646-w>.
77. Cui J, Dean D, Hornicek FJ et al. The role of extracellular matrix in osteosarcoma progression and metastasis. *J Exp Clin Cancer Res.* 2020; 39(1): 178. <http://doi.org/10.1186/s13046-020-01685-w>.
78. Fernández-Tabanera E, Melero-Fernández de Mera RM, Alonso J. CD44 in Sarcomas: A Comprehensive Review and Future Perspectives. *Front Oncol.* 2022; 12: 909450. <http://doi.org/10.3389/fonc.2022.909450>.
79. Simon T, Li L, Wagner C et al. Differential Regulation of T-cell Immunity and Tolerance by Stromal Laminin Expressed in the Lymph Node. *Transplantation.* 2019; 103(10): 2075-89. <http://doi.org/10.1097/TP.0000000000002774>.
80. Li L, Wei JR, Dong J et al. Laminin γ 2-mediated T cell exclusion attenuates response to anti-PD-1 therapy. *Sci Adv.* 2021; 7(6): eabc8346. <http://doi.org/10.1126/sciadv.abc8346>.
81. Paavola KJ, Roda JM, Lin VY et al. The Fibronectin-ILT3 Interaction Functions as a Stromal Checkpoint that Suppresses Myeloid Cells. *Cancer Immunol Res.* 2021; 9(11): 1283-97. <http://doi.org/10.1158/2326-6066.CIR-21-0240>.
82. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science.* 2015; 348(6230): 74-80. <http://doi.org/10.1126/science.aaa6204>.
83. Xu S, Xu H, Wang W et al. The role of collagen in cancer: from bench to bedside. *J Transl Med.* 2019; 17(1): 309. <http://doi.org/10.1186/s12967-019-2058-1>.
84. Nissen NI, Karsdal M, Willumsen N. Collagens and Cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology. *J Exp Clin Cancer Res.* 2019; 38(1): 115. <http://doi.org/10.1186/s13046-019-1110-6>.
85. Kuczek DE, Larsen AMH, Thorseth ML et al. Collagen density regulates the activity of tumor-infiltrating T cells. *J Immunother Cancer.* 2019; 7(1): 68. <http://doi.org/10.1186/s40425-019-0556-6>.
86. Peng DH, Rodriguez BL, Diao L et al. Collagen promotes anti-PD-1/PD-L1 resistance in cancer through LAIR1-dependent CD8+ T cell exhaustion. *Nat Commun.* 2020; 11(1): 4520. <http://doi.org/10.1038/s41467-020-18298-8>.
87. Chakravarthy A, Khan L, Bensler NP et al. TGF- β -associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. *Nat Commun.* 2018; 9(1): 4692. <http://doi.org/10.1038/s41467-018-06654-8>.
88. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. *J Pathol.* 2010; 221(1): 3-12. <http://doi.org/10.1002/path.2697>.
89. Ge YX, Zhang TW, Zhou L et al. Enhancement of anti-PD-1/PD-L1 immunotherapy for osteosarcoma using an intelligent autophagy-controlling metal organic framework. *Biomaterials.* 2022; 282: 121407. <http://doi.org/10.1016/j.biomaterials.2022.121407>.

90. Limpert AS, Lambert LJ, Bakas NA et al. Autophagy in Cancer: Regulation by Small Molecules. *Trends Pharmacol Sci.* 2018; 39(12): 1021-32. <http://doi.org/10.1016/j.tips.2018.10.004>.
91. Jiang GM, Tan Y, Wang H et al. The relationship between autophagy and the immune system and its applications for tumor immunotherapy. *Mol Cancer.* 2019; 18(1): 17. <http://doi.org/10.1186/s12943-019-0944-z>.
92. Michaud M, Martins I, Sukkurwala AQ et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science.* 2011; 334(6062): 1573-7. <http://doi.org/10.1126/science.1208347>.
93. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature.* 2011; 469(7330): 323-5. <http://doi.org/10.1038/nature09782>.
94. Deretic V, Levine B. Autophagy balances inflammation in innate immunity. *Autophagy.* 2018; 14(2): 243-51. <http://doi.org/10.1080/15548627.2017.1402992>.
95. White E, Mehnert JM, Chan CS. Autophagy, Metabolism, and Cancer. *Clin Cancer Res.* 2015; 21(22): 5037-46. <http://doi.org/10.1158/1078-0432.CCR-15-0490>.
96. Ge Y, Zhou S, Li Y et al. Estrogen prevents articular cartilage destruction in a mouse model of AMPK deficiency via ERK-mTOR pathway. *Ann Transl Med.* 2019; 7(14): 336. <http://doi.org/10.21037/atm.2019.06.77>.
97. Hahn T, Akporiaye ET. α -TEA as a stimulator of tumor autophagy and enhancer of antigen cross-presentation. *Autophagy.* 2013; 9(3): 429-31. <http://doi.org/10.4161/auto.22969>.
98. Li Y, Hahn T, Garrison K et al. The vitamin E analogue α -TEA stimulates tumor autophagy and enhances antigen cross-presentation. *Cancer Res.* 2012; 72(14): 3535-45. <http://doi.org/10.1158/0008-5472.CAN-11-3103>.
99. Fang Y, Tian S, Pan Y et al. Pyroptosis: A new frontier in cancer. *Biomed Pharmacother.* 2020; 121: 109595. <http://doi.org/10.1016/j.biopha.2019.109595>.
100. Wu M, Wang Y, Yang D et al. A PLK1 kinase inhibitor enhances the chemosensitivity of cisplatin by inducing pyroptosis in oesophageal squamous cell carcinoma. *EBioMedicine.* 2019; 41: 244-55. <http://doi.org/10.1016/j.ebiom.2019.02.012>. Erratum in: *EBioMedicine.* 2019; 43: 650. Erratum in: *EBioMedicine.* 2021; 63: 103041.
101. Wang WJ, Chen D, Jiang MZ et al. Downregulation of gasdermin D promotes gastric cancer proliferation by regulating cell cycle-related proteins. *J Dig Dis.* 2018; 19(2): 74-83. <http://doi.org/10.1111/1751-2980.12576>.
102. Wang Y, Gao W, Shi X et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature.* 2017; 547(7661): 99-103. <http://doi.org/10.1038/nature22393>.
103. Wang Y, Yin B, Li D et al. GSDME mediates caspase-3-dependent pyroptosis in gastric cancer. *Biochem Biophys Res Commun.* 2018; 495(1): 1418-25. <http://doi.org/10.1016/j.bbrc.2017.11.156>.
104. Jin J, Yuan P, Yu W et al. Mitochondria-Targeting Polymer Micelle of Dichloroacetate Induced Pyroptosis to Enhance Osteosarcoma Immunotherapy. *ACS Nano.* 2022; 16(7): 10327-40. <http://doi.org/10.1021/acsnano.2c00192>.
105. June CH, O'Connor RS, Kawalekar OU et al. CAR T cell immunotherapy for human cancer. *Science.* 2018; 359(6382): 1361-5. <http://doi.org/10.1126/science.aar6711>.
106. Bonini C, Mondino A. Adoptive T-cell therapy for cancer: The era of engineered T cells. *Eur J Immunol.* 2015; 45(9): 2457-69. <http://doi.org/10.1002/eji.201545552>.
107. Ma S, Li X, Wang X et al. Current Progress in CAR-T Cell Therapy for Solid Tumors. *Int J Biol Sci.* 2019; 15(12): 2548-60. <http://doi.org/10.7150/ijbs.34213>.
108. Wang Y, Yu W, Zhu J et al. Anti-CD166/4-1BB chimeric antigen receptor T cell therapy for the treatment of osteosarcoma. *J Exp Clin Cancer Res.* 2019; 38(1): 168. <http://doi.org/10.1186/s13046-019-1147-6>.
109. Jafari F, Javdansirat S, Sanaie S et al. Osteosarcoma: A comprehensive review of management and treatment strategies. *Ann Diagn Pathol.* 2020; 49: 151654. <http://doi.org/10.1016/j.anndiagpath.2020.151654>.
110. Dillman RO, Cornforth AN, McClay EF et al. Patient-specific dendritic cell vaccines with autologous tumor antigens in 72 patients with metastatic melanoma. *Melanoma Manag.* 2019; 6(2): MMT20. <http://doi.org/10.2217/mmt-2018-0010>.
111. Supra R, Agrawal DK. Immunotherapeutic Strategies in the Management of Osteosarcoma. *J Orthop Sports Med.* 2023; 5(1): 32-40. <http://doi.org/10.26502/josm.511500076>.
112. Harari A, Graciotti M, Bassani-Sternberg M et al. Antitumor dendritic cell vaccination in a priming and boosting approach. *Nat Rev Drug Discov.* 2020; 19(9): 635-52. <http://doi.org/10.1038/s41573-020-0074-8>.
113. Yu Z, Ma B, Zhou Y et al. Allogeneic tumor vaccine produced by electrofusion between osteosarcoma cell line and dendritic cells in the induction of antitumor immunity. *Cancer Invest.* 2007; 25(7): 535-41. <http://doi.org/10.1080/07357900701508918>.
114. Tsai YF, Huang CW, Chiang JH et al. Gadolinium chloride elicits apoptosis in human osteosarcoma U-2 OS cells through extrinsic signaling, intrinsic pathway and endoplasmic reticulum stress. *Oncol Rep.* 2016; 36(6): 3421-6. <http://doi.org/10.3892/or.2016.5174>.
115. Zhang S, Wu Y, Yu J et al. Gadolinium-Bisphosphonate Nanoparticle-Based Low-Dose Radioimmunotherapy for Osteosarcoma. *ACS Biomater Sci Eng.* 2022; 8(12): 5329-37. <http://doi.org/10.1021/acsbomaterials.2c00880>.
116. Jaiswal S, Jamieson CH, Pang WW et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell.* 2009; 138(2): 271-85. <http://doi.org/10.1016/j.cell.2009.05.046>.
117. Theruvath J, Menard M, Smith BAH et al. Anti-GD2 synergizes with CD47 blockade to mediate tumor eradication. *Nat Med.* 2022; 28(2): 333-44. <http://doi.org/10.1038/s41591-021-01625-x>.
118. Sikic BI, Lakhani N, Patnaik A et al. First-in-Human, First-in-Class Phase I Trial of the Anti-CD47 Antibody Hu5F9-G4 in Patients With Advanced Cancers. *J Clin Oncol.* 2019; 37(12): 946-53. <http://doi.org/10.1200/JCO.18.02018>.
119. Advani R, Flinn I, Popplewell L et al. CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. *N Engl J Med.* 2018; 379(18): 1711-21. <http://doi.org/10.1056/NEJMoa1807315>.
120. Long AH, Highfill SL, Cui Y et al. Reduction of MDSCs with All-trans Retinoic Acid Improves CAR Therapy Efficacy for Sarcomas. *Cancer Immunol Res.* 2016; 4(10): 869-80. <http://doi.org/10.1158/2326-6066.CIR-15-0230>.

121. Dobrenkov K, Ostrovnyaya I, Gu J et al. Oncotargets GD2 and GD3 are highly expressed in sarcomas of children, adolescents, and young adults. *Pediatr Blood Cancer*. 2016; 63(10): 1780-5. <http://doi.org/10.1002/pbc.26097>.
122. Schulz G, Cheres DA, Varki NM et al. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. *Cancer Res*. 1984; 44(12 Pt 1): 5914-20.
123. Mount CW, Majzner RG, Sundaresh S et al. Potent antitumor efficacy of anti-GD2 CART cells in H3-K27M+ diffuse midline gliomas. *Nat Med*. 2018; 24(5): 572-9. <http://doi.org/10.1038/s41591-018-0006-x>.
124. Battula VL, Shi Y, Evans KW et al. Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. *J Clin Invest*. 2012; 122(6): 2066-78. <http://doi.org/10.1172/JCI59735>.
125. Cheres DA, Rosenberg J, Mujoo K et al. Biosynthesis and expression of the disialoganglioside GD2, a relevant target antigen on small cell lung carcinoma for monoclonal antibody-mediated cytotoxicity. *Cancer Res*. 1986; 46(10): 5112-8.
126. Hingorani P, Krailo M, Buxton A et al. Phase 2 study of anti-disialoganglioside antibody, dinutuximab, in combination with GM-CSF in patients with recurrent osteosarcoma: A report from the Children's Oncology Group. *Eur J Cancer*. 2022; 172: 264-75. <http://doi.org/10.1016/j.ejca.2022.05.035>.
127. Grant SC, Kostakoglu L, Kris MG et al. Targeting of small-cell lung cancer using the anti-GD2 ganglioside monoclonal antibody 3F8: a pilot trial. *Eur J Nucl Med*. 1996; 23(2): 145-9. <http://doi.org/10.1007/BF01731837>.
128. Anti-GD2 and Anti-CD47 Are Synergistic and Promote Tumor Eradication. *Cancer Discov*. 2022; 12(3): OF8. <http://doi.org/10.1158/2159-8290.CD-2022-011>.

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