Review article

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

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Received:
31.08.2023
Accepted:
10.09.2023

DOI: 10.24292/01.OR.132100923

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].

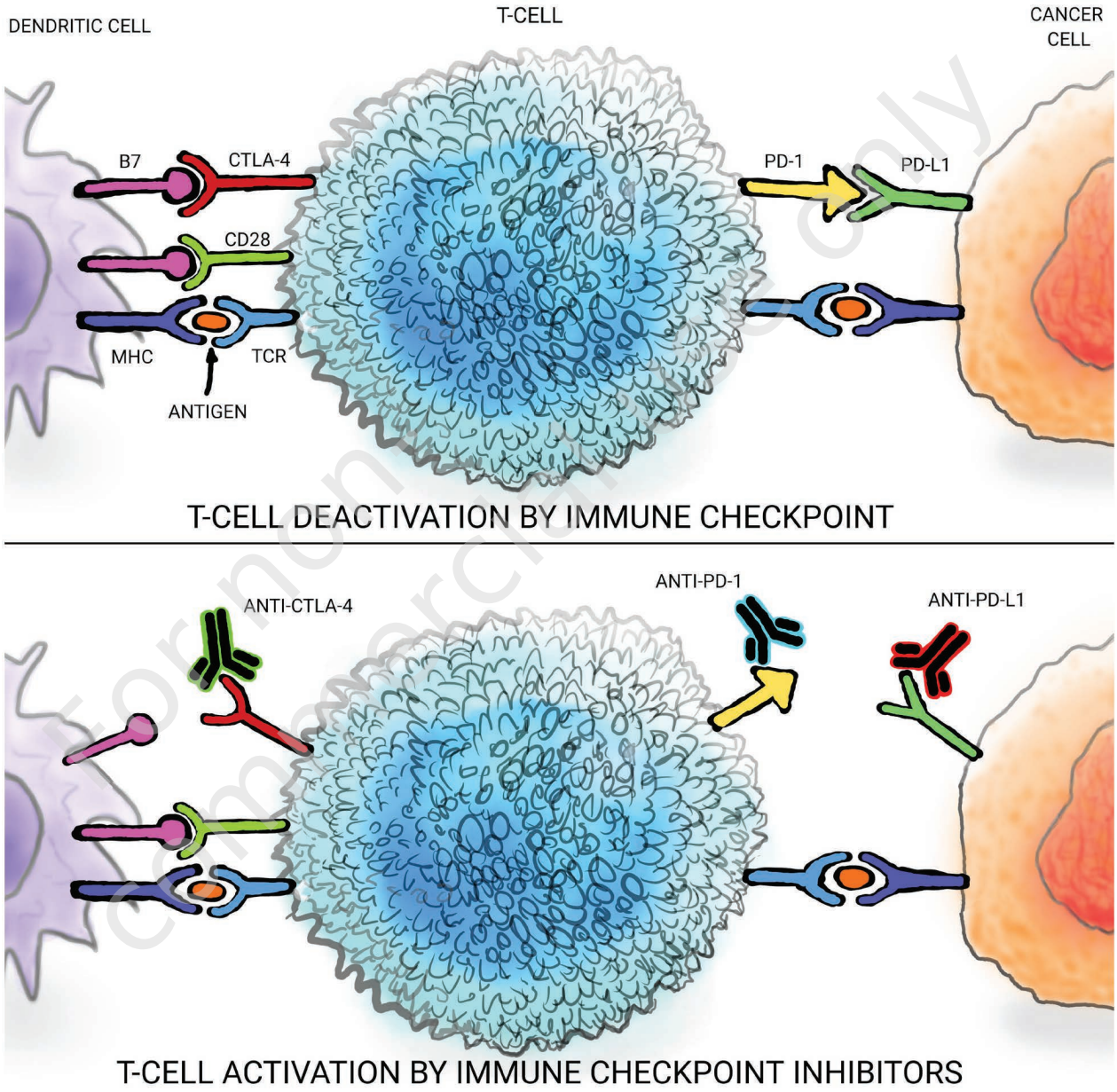
To utilize the mechanisms of autophagy and immune checkpoints in anticancer therapy, Ge et al. [89] designed a pH-sensitive nanocarrier that released the natural derivative of curcumin and the BMS1166 in the acidic environment of the osteosarcoma. The curcumin derivative activated the autophagic cell 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-1β 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.

caspase-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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Authors' contributions:
Kamil Poboży: 20%; Julia Domańska: 20%; Paweł Domański: 20%; Wojciech Konarski: 20%; Tomasz Poboży: 20%.

Conflict of interests:
The authors declare that there is no conflict of interest regarding the publication of this article.

Financial support:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics:
The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed with the content of the manuscript as written. The paper complies with the Helsinki Declaration, EU Directives and harmonized requirements for biomedical journals.