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REVIEW

Osteosarcoma Overview

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ABSTRACT

Osteosarcoma (OS) is the most common primary malignancy of bone and patients with metastatic disease or recurrences continue to have very poor outcomes. Unfortunately, little prognostic improvement has been generated from the last 20 years of research and a new perspective is warranted. OS is extremely heterogeneous in both its origins and manifestations. Although multiple associations have been made between the development of osteosarcoma and race, gender, age, various genomic alterations, and exposure situations among others, the etiology remains unclear and controversial. Noninvasive diagnostic methods include serum markers like alkaline phosphatase and a growing variety of imaging techniques including X-ray, computed tomography, magnetic resonance imaging, and positron emission as well as combinations thereof. Still, biopsy and microscopic examination are required to confirm the diagnosis and carry additional prognostic implications such as subtype classification and histological response to neoadjuvant chemotherapy. The current standard of care combines surgical and chemotherapeutic techniques, with a multitude of experimental biologics and small molecules currently in development and some in clinical trial phases. In this review, in addition to summarizing the current understanding of OS etiology, diagnostic methods, and the current standard of care, our group describes various experimental therapeutics and provides evidence to encourage a potential paradigm shift toward the introduction of immunomodulation, which may offer a more comprehensive approach to battling cancer pleomorphism.

Keywords: Bone cancer; Bone sarcoma; Metastatic osteosarcoma; Osteosarcoma; Treatment for sarcoma

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INTRODUCTION

Osteosarcoma (OS) is an osteoid-producing malignancy of mesenchymal origins. This high-grade tumor is the most common primary malignancy of bone and is often fatal in both children and adults. While primary bone cancers represent less than 0.2% of all cancers [1], according to the National Cancer Institute SEER (Surveillance, Epidemiology, and End Results) program, their frequency has been increasing by 0.3% per year over the last decade [2]. While OS occurs most frequently in patients between 5 years of age and early adulthood, incidence peaks again in the older (>65) populations and has been associated with pre-existing Paget's disease and prior radiation therapy [3–7]. Collectively, the metaphysis of the lower long bones, specifically the distal femur and proximal tibia, are the most commonly involved primary sites, with patients over 25 displaying a greater variety of bony locations [3].

Metastatic disease is classified by location as either pulmonary or extrapulmonary and is the major cause of OS-related death [8–10]. While bony metastases are associated with poorer prognoses (with reports of 13% survival at 5 years [11]), the lung is involved in approximately 80% of cases [12] and subsequent respiratory compromise is responsible for most of the death toll [13]. Compared with a potential cure rate of over 60% in patients presenting with nonmetastatic disease [12, 14], those with detectable metastases at the time of diagnosis (approximately 15–20% [15, 16]) have the poorest overall prognoses [17–22], with reports of 5-year survival rates as low as 19% [17, 23]. Moreover, even in the subset of patients free of primary metastases, 40% will go on to eventually develop a secondary metastasis [12]; in one study, survival rates of patients with nonmetastatic high-grade OS with subsequent metastases were 23% at 5 years and 0% at 4 years for pulmonary and bony metastases, respectively [24].

In contrast to distant pulmonary and extrapulmonary metastases, skip lesions (also known as skip metastases or synchronous regional bone metastases) are local and yet potentially more serious metastatic complications. These small, anatomically isolated cancerous foci are distinct from the primary tumor and located either within the same bone or transarticular to it [25, 26]. Classically, they are associated with extensive metastatic dissemination, robust therapeutic resistance, and particularly poor prognoses [27–29].

Similar to those with metastatic OS, patients with recurrent disease have comparably dismal 5-year post-relapse-overall-survival (PROS) rates [18]. In addition, features such as axial tumor site [30, 31], male sex [30], and advancing age [31] have all shown correlation with inferior patient outcomes. Notably, the pre-operative histologic response to neoadjuvant chemotherapy, as judged by the extent of tumor necrosis, offers some of the most important predictive value regarding overall patient survival [30, 32–34].

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

ETIOLOGY AND GENOMICS

OS etiology is complex and not well understood. The molecular pathogeneses and genetics of OS are vast and extremely

heterogeneous [35], with discrepancies in the literature regarding its demographic and environmental influences further complicating the story. Most cases are sporadic; however, increased risk has been associated with multiple germline mutation disorders including hereditary retinoblastoma [36, 37], Rothmund–Thomson syndrome [38, 39], Li Fraumeni syndrome [40], and Bloom syndrome [41], among others.

Classically, alterations in the retinoblastoma (Rb) gene have been strongly associated with a predisposition for developing OS [42–44] and loss of heterozygosity has been reported to occur in up in 40–70% of cases [45–49]. Additionally, altered p53 loci, which have been reported to occur in approximately 10–39% of cases [49–59], display synergistic tumorigenic properties [50, 60–62]. Whole genome sequencing studies have attempted to elucidate recurrent chromosomal structural patterns, most recently with loci at 6p21.3 and 2p25.2 displaying potentially genome-wide significance [63, 64]. However, despite these and countless other genetic similarities discovered across cell lines, OS continues rapidly modifying its genotype, thus making potential targeted molecular therapeutics increasingly impractical. To date, no single genetic target has proven therapeutically successful, and this wealth of information has yet to lead to a significant decrease in mortality [35].

As per the central dogma, this genotypic heterogeneity has translated into a wide variety of macromolecular biomarker expression profiles with potentially useful clinical implications. Indeed, phenotypic trends have been found and characterized across various OS cell lines. Multiple studies have identified characteristic protein and mRNA expression profiles showcasing anomalous levels of ErbB-2 [65, 66], cathepsin D [67], FBXW7 [68], miR-421 [69], and HMGB1 [70], among others. It has also been suggested that matrix-Gla protein expression may play a role in facilitating tumor spread to the lungs [71]. However, as of yet, the true diagnostic, etiologic, and clinical significance of these biomarkers is ongoing and controversial.

Apart from genetic mutations and expression profiles, studies have also discovered correlations between poor prognosis (or increased incidence) and various demographics such as male gender [72], old age [73, 74], height [75], and African American descent [76]. Others have suggested an association between bone growth and incidence rates [75, 77–80], but the relative strength and importance of this relationship have been challenged [81]. Environmental factors that have been associated with increased risk of future OS development include exposure to radiation [6, 7], teriparatide usage [82], and consumption of fluorinated drinking water during childhood [83, 84]; however, more recent studies have disputed the latter two relationships [85–88].

DIAGNOSIS AND PATHOLOGY

Due to the complex nature of primary bone malignancies, diagnosis of OS is best accomplished via a comprehensive multidisciplinary approach [89]. Often, the first sign of potential bone malignancy is intermittent pain around the affected area with or without a palpable mass [90]. When involving areas around the knee joint, pain exacerbated by weight bearing may manifest as a limp; occasionally, patients will present with a recent bout of bone trauma [91]. As previously mentioned, any older adult with a history of Paget's disease has an increased risk of

developing OS, most notably of the pelvis [92], and this transformation is associated with a poor prognosis [93].

Various serum markers have been investigated for their utility in diagnosing and tracking progression and recurrence. Alkaline phosphatase (ALP) and lactose dehydrogenase (LDH) are useful serum biomarkers, with ALP carrying the most diagnostic value in OS [94, 95]. ALP has also been shown to correlate positively with tumor volume, which carries additional useful prognostic implications [94–96]. LDH has also been shown to correlate with tumor volume; however, the correlation is weaker than for ALP and is mainly a result of nonspecific metabolic demand [95]. If disease is suspected, the first step is to gather plain radiographs of the involved bone and adjacent joint. Any abnormal films then warrant further radiological investigation of soft tissue involvement and possible primary lung metastasis via magnetic resonance imaging (MRI) and computed tomography (CT), respectively [97]. Bone scintigraphy (BS) is often used in conjunction with CT to identify metastases and the presence or absence of metastatic disease remains one of the most important predictors of patient outcome [97, 98]. For a more detailed, flow chart-style approach to patient work-up, please refer to the guidelines put forth by the National Comprehensive Cancer Network (NCCN) for bone cancer, Version 2.2017 [99].

Although they have not yet taken the place of BS in OS diagnosis, various experimental test procedures such as positron emission tomography (PET) scans are currently being investigated for their potentially superior ability to diagnose and track the progression of bone cancers. In 2009, Costelloe et al. demonstrated that combined PET/CT could be used to reliably predict the progression-free survival (PFS), overall survival, and extent of tumor necrosis in OS [100]. Studies have also suggested enhanced sensitivity with the use of fludeoxyglucose-(^{18}F-FDG)-PET-CT over BS for detecting metastases [101]. Hyung-Jun Im et al. [102] used a similar technique to show that initial baseline metabolic tumor volume and total lesion glycolysis have independent prognostic value in determining survival in pediatric osteosarcoma populations. Importantly, combined PET/CT scanning has been shown to accurately determine the extent of histological osteonecrosis and may offer a less-invasive alternative to the typical biopsy-requiring Huvos grading system [103], as previously described [104]. Please refer to Table 1 for a list of test properties, including sensitivity, specificity, and a brief note on diagnostic application (as indicated by the study from which those numbers were derived). Note, however, that despite the promising aspects of these non-invasive techniques, a biopsy is always required to confirm the diagnosis [105]. As such, proper disease management mandates tissue collection with the correct identification of the specific histologic subtype [106]. While many subtypes exist and correlate specifically to corresponding chemotherapeutic responses [107], the unifying histological feature is the presence of osteoid-producing malignant cells [105].

Incisional or core needle biopsy is the final step in the diagnostic process [97] and the tumor is staged using the Musculoskeletal Tumor Society staging scheme [108] or the American Joint Commission on Cancer (AJCC) system. In the AJCC system, the tumor is described based on four important factors represented by letters [109]: T (primary tumor size), N (spread to regional lymph nodes), M (metastasis), and G (grade). Each letter is then assigned a particular number (e.g., T1) that

Table 1 OS serological and radiological test properties with diagnostic applications

Test	Sensitivity	Specificity	Application
Serum ALP [95]	0.78	0.94	Correlation ($r = 0.5$) with tumor volume
			Most descriptive for osteoblastic subtype
Serum LDH [95]	0.82	0.97	Correlation ($r = 0.4$) with tumor volume
			Describes tumor metabolic demand
Spiral CT [195]	0.75	1.00	Pulmonary metastases
FDG-PET [195]	0.5	0.98	Pulmonary metastases; confirmation of CT abnormality
PET/CT [196]	0.95	0.98	Bony metastases (examination-based analysis)
BS [196]	0.76	0.97	Bony metastases (examination-based analysis)
PET/CT + BS [196]	1.00	0.96	Bony metastases (examination-based analysis)
FDG-PET/CT [197]	0.947	Not reported	Initial staging or assessment of recurrent disease

describes the tumor's pathologic extent (T, N, and M) and histological appearance (G). Once assigned its corresponding TxNxMxGx code, the tumor can then be grouped into its corresponding stage, with Stage 1A being the most localized and Stage IVB the most invasive. This information can be used to determine prognosis, assess response to therapy, and monitor disease progression [110].

Osteosarcoma subtypes include osteoblastic, chondroblastic, fibroblastic, small cell, telangiectatic, high-grade surface, extra-skeletal, and other lower-grade forms including periosteal and parosteal [111]. Based upon their histological appearances, OS subtypes can be grouped into three categories: high-grade, intermediate-grade, and low-grade. Parosteal OS is a low-grade subtype that is fibroblastic in appearance and limited to the bone surface; however, with time, it may progress to involve deeper structures. For this and other low-grade subtypes, treatment involves surgery alone and carries a favorable prognosis. Periosteal OS is chondroblastic on histology and is the only subtype that falls into the intermediate-grade category. Depending on extent of invasion, treatment for intermediate-grade OS often includes systemic chemotherapy. High-grade OS, which includes the classic osteoblastic subtype, is the fastest-growing and most aggressive group. The majority of OS subtypes fall under this category and include osteoblastic, chondroblastic, fibroblastic, small cell, telangiectatic, high-grade surface, and extra-skeletal. Telangiectatic OS is notable for its profuse vascularity and scant osteoid production, which often complicates tissue biopsy and radiographic identification, respectively [112]. Telangiectatic OS also carries with it an anatomical predilection to the epiphyseal region of the bone. All high-grade OS should be considered micrometastatic at diagnosis and treated with surgery and systemic chemotherapy.

TREATMENT

The current standard for osteosarcoma treatment employs neoadjuvant chemotherapy, surgery, and then post-operative adjuvant chemotherapy.

Multiple different chemotherapy regimens containing anywhere from two to seven drugs have been used [97, 113, 114]. The four drugs that have shown consistent activity are cisplatin, doxorubicin, high-dose methotrexate with leucovorin rescue, and isofosfamide with or without etoposide. A recent meta-analysis showed that patients who were treated with three drugs had a superior outcome to those that received two drugs. However, there was no benefit in using four drugs compared to three drugs [115]. Indeed, adding standard or high-dose ifosfamide significantly increased toxicity while having negligible effects on outcome [116, 117], which was recently confirmed by an international trial that showed that there was no benefit in adding high-dose ifosfamide plus etoposide to the cisplatin, doxorubicin, methotrexate combination (MAP) [118]. Therefore, MAP multi-agent chemotherapy is the first-line treatment and the standard of care at the present time. For a more thorough investigation of OS chemotherapeutics, please refer to the meta-analysis conducted in 2011 by Anninga et al. [115].

Neoadjuvant (pre-operative) chemotherapy greatly increases relapse-free survival (RFS) of patients with non-metastatic disease [119, 120]. Furthermore, it allows for tumor categorization into histological response subgroups, which has significant prognostic and clinical value and provides the opportunity to alter the post-operative treatment strategies [121]. The goal for a positive treatment with neoadjuvant chemotherapy is to achieve at least 90% necrosis on the surgically resected tumor [121]. If the percent necrosis is below 90% at resection after neoadjuvant treatment, then the post-resection adjuvant chemotherapy regimen can be altered. However, changing the chemotherapy regimen post-operatively based on response has not been shown to have a positive impact on patient outcome [118]; indeed, another finding from the EURAMOS-1 randomized control trial series has reported increased toxicity and secondary malignancies from intensifying chemotherapy regimens (adding both ifosfamide and etoposide to MAP therapy, i.e., MAPIE) for poor responders [122]. For full treatment guidelines with detailed workup flow charts, please refer to the NCCN guidelines for bone cancer, Version 2.2017 [99].

SURGERY AND RADIATION THERAPY

Surgical excision usually involves tumor resection with negative margins, as multiple studies have linked positive margins with an increased risk of local recurrence (LR) and inferior survival [123]. Classically, Enneking et al. described four different types of surgical margins: intralesional, marginal, wide, and radical [108]. An intralesional margin, as the name suggests, is obtained when the specimen is taken from within the tumor itself. Although these margins have little therapeutic utility, they are often used for biopsies and have diagnostic purposes. Marginal and wide margins remove the lesion *en bloc*, with wide leaving a substantial border of normal tissue. This border of normal tissue surrounding the tumor is absolutely paramount in the treatment of OS. Despite these definitions, however, the proper margin to obtain on a case-by-case basis has been continually met with controversy; to date, no general consensus has been born out of the literature. In 2012, a retrospective cohort of 47 patients found no significant difference in LR between groups with close (tumor <5 mm from resection margin) and wide (tumor >5 mm from resection margin) margins [124]. The following year, Jeon et al. suggested that negative tumor

margins correlate with significantly lower risks of LR in bone and perineurovascular resection planes while having little influence on LR in soft tissue [125]. Furthermore, there is also evidence to support that the risk of LR is higher in patients treated with closer margins [112]. In either case, when adequate margins cannot be achieved, amputation should be considered [126]. Naturally, limb-salvage is greatly preferred over amputation; over 85% of patients are candidates for this type of procedure [127–130].

In patients with disseminated disease, the complete resection of pulmonary metastases is vital when possible [131, 132], as lung metastectomy has been shown to significantly prolong survival in this population [133, 134]. Patients with recurrent unresectable metastases usually have poorer prognoses, even when treated aggressively with pre-operative chemotherapy [135]. For recurrent or refractory disease, some studies have suggested that incorporating etoposide into the chemotherapy treatment regimen may be beneficial [136]; however, these data are controversial and associated with severe toxicities [137]. Whenever possible, surgical resection of recurrent disease is first-line over systemic therapy, which is less effective and reserved for unresectable cases [20]. Radiation therapy may also be used to help clear microscopic or minimal residual disease when substantial surgical resection is not possible [138]; however, for a majority of cases, radiation is not used.

NEW THERAPEUTIC APPROACHES

New effective OS therapies have plateaued over the last several decades; this lack of new treatment strategies is reflected by unchanging survival rates [3, 139]. As OS cells tend to exhibit extreme genetic pleomorphism, therapeutic attempts to target specific cell receptors and intracellular signaling molecules have not significantly increased survival. In addition, and likely as a result of its pleomorphism, OS cells exhibit strong chemotherapy resistance, most notably in the 15–20% of patients initially presenting with detectable metastases at the time of diagnosis [15], and who consequently have the poorest prognoses [17–22]. Therefore, MAP adjuvant treatment strategies have had minimal beneficial effects on this subset of patients [140]; as such, a number of alternative therapeutic modalities have been investigated.

Various biologics and small molecules have been used to target cell-surface receptors and downstream signaling pathways involved in OS pathogenesis. For example, as HER2 is often expressed in a subset of OS cell lines and has been associated with poorer prognoses [141], a phase II trial was conducted using trastuzumab to target HER2-positive OS; however, despite minimal drug reactions and additional toxicity, no significant difference in groups was observed [142]. Pappo et al. conducted a similar phase II trial targeting another receptor commonly expressed by malignant cells, IGF-1R [143]; again, clinical responses were underwhelming [144]. The PI3K/mTOR pathway [145, 146] and mitogen-activated protein kinases [146, 147] have also recently been recognized as potential targets and their therapeutic significance is currently under investigation.

Over the past few decades, increasing evidence has suggested that platelets and other mesenchymal cells, notably the PDGFR-alpha-R for OS [148], can assist tumor cell pathogenesis [149]. Indeed, Labelle et al. [150] showed that platelet-tumor cell interactions, mediated by activation of transforming growth factor (TGF)-beta/SMAD (small mothers against

decapentaplegic) and nuclear factor (NF)-kappa-B, could promote metastasis by inducing an epithelial–mesenchymal-like transition. As such, Takagi et al. [151] was able to significantly inhibit platelet-induced OS cell proliferation by blocking Akt-mediated downstream signaling using sunitinib. Likewise, sunitinib has since been shown to reduce tumor burden and lung metastasis in mice [152]; however, the clinical significance of these initial data is yet to be determined.

Elucidating the mechanisms of OS's robust chemoresistance has yielded other potentially promising therapeutic targets. Recently, it has been shown that HMGB1-induced autophagy contributes to OS chemotherapy resistance [153]; hence, this and other chemoresistance-promoting pathways provide the means for new therapeutic approaches and their inhibitors deserve further investigation. Other potential therapeutics currently under investigation include zoledronic acid [154] and even the natural phenolic compound, curcumin [155].

Due to the vast heterogeneity of OS molecular profiles [35–40, 63, 64], the future of OS treatment may be moving away from targeted anti-oncogenic paradigms and toward more generalized immunomodulatory/immunoeditory approaches [156]. Cancer immunotherapy, although still in its infancy, attempts to enhance tumor immunogenicity and stimulate tumorocidal activity, thereby reallocating the burden of disease clearance back to the patient's own body. Nonspecific immunogens, cytokines, adoptive T-cells, vaccines, oncolytic virotherapies, and checkpoint blockades have all shown potential therapeutic promise [157]. If deemed clinically advantageous, these new immunotherapeutics will likely be administered as adjuvants and integrated into the current standard of care.

Muramyl tripeptide (MTP), which has been shown to activate NF-κB [158] and increase circulating levels of TNF alpha and interleukin-6 (IL-6) in patients with OS [159], can be packaged within liposomes [160] and injected [161, 162]. This liposomal product (Mifamurtide) allows for particle ingestion by monocytes and macrophages and the subsequent activation of their cytotoxic function against tumor cells [163]. Tumor-associated macrophages, although mostly thought of as being pro-tumorigenic [164], have been found to play a potentially significant role in preventing metastasis in high-grade OS [165]. In patients with no clinically detectable metastases and in those with resectable disease, the addition of Mifamurtide to multi-agent chemotherapy has been shown to significantly increase event-free survival (EFS) and overall survival with a 29% reduction in the mortality rate at 8 years [166]. The addition of Mifamurtide also improved the outcomes of patients who presented with metastases at the time of diagnosis [167]. A therapeutic synergism of MTP with zoledronic acid on primary tumor progression has also been suggested [168].

There have also been attempts to target immunotherapy directly to the lungs via the use of aerosols, which have the potential benefit of lowering systemic toxicity by being delivered directly to the site of action [169]. OS relapse most commonly occurs as pulmonary metastasis [105], with patient survival often being below 30% [170, 171] and as low as 14% [20] in these cases. Granulocyte–macrophage colony stimulating factor (GM-CSF), a molecule with multiple roles in immune regulation and phagocyte maturation [172], was recently used in a phase II trial of post-relapse OS patients to investigate its effect on disease-free survival (DFS). However, despite the ability to reach

adequately high doses with minimal side effects, no significant improvement in survival was seen [173]. While these results are disappointing, they may indicate that the immunomodulatory effects of GM-CSF alone were not enough to influence a tumorocidal environment. Unfortunately, there was no investigation of whether GM-CSF treatment resulted in any biologic effect on the lung tumor nodules. Therefore, it is hard to assess whether this therapy resulted in the desired effect in terms of activating an immune response in the lung. Thus, the possibility remains that the lack of therapeutic effect was secondary to the inability of GM-CSF to stimulate an immune response in the lung. Indeed, there is growing evidence to support that the chemical profile of tumor microenvironments comprises an astonishingly complex constellation of signaling molecules in various distributions [174, 175] and it is highly possible that further manipulation may be necessary to achieve an effective tumorocidal environment [176]. As such, Zeidner et al. and Wang et al. have shown that combinations of GM-CSF with interferon (IFN) and IL-12 therapy improved outcomes for chronic myeloid leukemia patients [177] and increased antitumor effects against murine hepatocellular carcinoma [178], respectively. Another more recent immunotherapeutic attempt at combating OS pulmonary metastasis with combination immunotherapy included aerosol IL-2 with adjuvant natural killer (NK) cell infusions, which has shown enhanced efficacy compared to IL-2 or NK cell infusions alone [179]. Other cytokines, including IL-15 and IL-12, have been shown to increase natural killer cell-mediated lysis of chemotherapy-resistant OS cells [180] and suppress pulmonary metastasis formation [181], respectively.

IFN immunotherapy has also shown promise in the treatment of OS. IFN-α, while initially recognized for its ability to inhibit viral replication, is now used in the treatment of a variety of different solid and hematological cancers [182]. In OS, it has been shown to suppress tumor invasion as well as enhance the cytotoxic effects of cisplatin [183]. In 2015, Bielack et al. conducted an international randomized, controlled trial comparing the efficacy of MAP therapy alone versus MAP plus pegylated interferon alpha-2b in 2260 registered patients; however, the results were complicated by insufficient patient adherence and no statistical difference in outcome was found [184]. In 2015, Gao et al. revealed that IFN-lambda1, a relatively new member of the interferon family [185], inhibits the invasive properties of MG-63 human osteosarcoma cell lines in vitro [186]. Another in vitro study showed that INF-gamma can enhance the ability of γδ T cells to target and kill HOS and U2OS OS cell lines [187].

As it is now widely accepted that tumors often promote suppression of the immune system in order to facilitate their pathogenesis [188], many immunotherapies have shown promise by targeting immunoregulatory cell-surface markers. Programmed death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are surface receptors involved in down-regulating the cytotoxic T cell response [189–191] and their blockade has been implicated in the treatment of a variety of cancers [192]. Interestingly, combined blockade of PD-L1 and CTLA-4 has been shown not only to completely eliminate metastatic osteosarcoma in murine models but also to induce immunity to further inoculation [193, 194].

CONCLUSIONS

Over the past 25 years, altering or intensifying the chemotherapy regimens for newly diagnosed osteosarcoma patients has failed to improve the 65–70% long-term survival. The only success in improving patient outcomes was the addition of Mifamurtide to the three-drug or four-drug regimen. Combining Mifamurtide with chemotherapy increased long-term survival from 70 to 78% at 8 years [166] and improved the outcome of patients who presented with metastases at diagnosis [167]. This improvement shows that immunotherapy is effective against this cancer. As OS therapies move forward over the next 5 years, it is likely that both immunostimulation and suppression blockade immunotherapies will play emerging roles. The genetic heterogeneity and morphological adaptability of OS necessitates a more comprehensive treatment approach, as the disease's molecular repertoire is too vast to be treated successfully by targeted therapies alone. Successful treatment will almost certainly require a combination of these different techniques to best achieve an effective tumorocidal environment; the key will lie in recognizing what specific role each immune cell plays and how best to assist its function.

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