Immunotherapy for osteosarcoma: Where do we go from here?

Mary F. Wedekind1,2 | Lars M. Wagner3 | Timothy P. Cripe1,2

1Division of Hematology, Oncology, and Blood and Marrow Transplant, Department of Pediatrics, Nationwide Children's Hospital, Columbus, Ohio
2Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio
3Division of Hematology-Oncology, Department of Pediatrics, Kentucky Children's Hospital, Lexington, Kentucky

Correspondence
Mary F. Wedekind, Division of Hematology, Oncology, and Blood and Marrow Transplant, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205.
Email: mary.wedekind@nationwidechildrens.org

Abstract
Osteosarcoma is the most common bone tumor in children and young adults, with few advances in survival and treatment, especially for metastatic disease, in the last 30 years. Recently, immunotherapy has begun to show promise in various adult cancers, but the utility of this approach for osteosarcoma remains relatively unexplored. In this review, we outline the mechanisms and status of immunotherapies currently in clinical trials as well as future therapies on the horizon, and discuss their potential application for osteosarcoma.

KEYWORDS
immunotherapy, osteosarcoma, sarcoma

1 | INTRODUCTION

Osteosarcoma is the most common cancer originating in the bone, and typically affects adolescents and young adults. Although the primary tumor is often surgically resected, patients remain at high risk for eventually developing pulmonary metastases unless adjuvant chemotherapy is administered. Even then, only two-thirds of patients with initially localized disease are expected to be cured, with long-term survival occurring in <30% of patients with metastatic or recurrent tumors.1 The inability to effectively optimize existing treatments or identify new active agents has prevented any improvement in outcome for over three decades. Given these limitations, novel treatment approaches are needed.

Several lines of evidence suggest that osteosarcoma may be susceptible to immune-based therapies. Osteosarcoma tumors have a higher percentage of CD8+ infiltrating lymphocytes than other sarcoma subtypes,2 and the degree of infiltration correlates positively with survival.3 Osteosarcomas have a high level of genomic instability, with some tumors expressing the programmed cell death protein-1 ligand (PD-L1),4 suggesting potential sensitivity to inhibitors of the programmed cell death protein-1 (PD-1)/PD-L1 axis.5–7 In addition, there are multiple cell surface proteins that are potentially targetable with antibodies. But perhaps the most compelling data come from past experience with mifamurtide, which is an analog of bacterial cell walls that can trigger the activation of monocytes and macrophages and improve tumor control. Mifamurtide is currently approved in Europe for the treatment of osteosarcoma, based in part on a randomized phase III study showing improved overall survival in patients with osteosarcoma receiving mifamurtide plus conventional chemotherapy.8

In this review, we outline the mechanisms and current status of immunotherapies that are now in clinical trials, as well as those on the horizon for the treatment of osteosarcoma (Figure 1).

1.1 | Cancer immunotherapy

The immune system is a highly sophisticated organization of cells that work together to provide protection against foreign threats (e.g. infection, tumor) while maintaining tolerance against self. The interplay between the patient's immune system and cancer is complex and includes immune surveillance, immune cell infiltration, and tumor cytolyis by the host, which are counteracted by tumor defenses that dampen the immune response through the release of inhibitory cytokines and downregulation of surface markers.9 A popular model that captures this interplay is termed cancer “immunoediting,” which consists of the following three different phases: elimination,
experience in osteosarcoma and future directions

Mechanism

Antibody targeting of cell surface proteins

Tumor vaccines and dendritic cells

Experience in osteosarcoma and future directions

Tumor vaccines and dendritic cells

Experience in osteosarcoma and future directions

1.2 | Antibody targeting of cell surface proteins

1.2.1 | Mechanism

The use of antibodies to target cancer cell surface proteins is attractive given the multiple antigens that are potentially targetable in osteosarcoma, the safety and ready availability of these "off-the-shelf" treatments, and the past success seen in pediatric cancers such as neuroblastoma and acute lymphoblastic leukemia. Monoclonal antibodies (mAb) attach to specific tumor surface antigens and activate natural killer (NK) cells and macrophages to release cytotoxic granules to kill tumor cells in a process known as antibody-dependent cellular cytotoxicity. In contrast, bispecific T-cell engagers (BiTE) are antibodies which contain two single-chain variable fragments connected by a flexible linker that brings in close proximity the CD3 receptor of the T cell with the tumor antigen, resulting in T-cell activation and subsequent cancer cell cytolysis. A third approach involves the coupling of an antibody with a cytotoxic agent such as vedotin to selectively deliver chemotherapy to cancer cells.

1.2.2 | Experience in osteosarcoma and future directions

Several mAb have already been tested in clinical trials for patients with osteosarcoma, including the use of trastuzumab to target HER2, cixutumumab to target insulin-like growth factor 1, and glembatumumab vedotin to target the glycoprotein nonmetastatic B (NCT02487979). Although solid rationale existed for each trial, these strategies have not showed sufficient antitumor activity to warrant further testing. Specific reasons for these disappointing findings are unknown, but may include the incomplete or low expression of tumor antigens, or compromised cellular toxicity due to inhibitory stimuli within the tumor microenvironment.

Despite these early disappointments, further studies are ongoing with antibodies against other cell surface proteins such as disialoganglioside (GD2), which is widely expressed in both primary and recurrent osteosarcoma tumors. As shown in Table 1, a variety of antibody-based studies are now ongoing that incorporate anti-GD2 mAb with other immunoadjuvants such as sargramostim or interleukin-2, or utilize BiTE antibodies against GD2. In addition, targeting of the RANK ligand with denosumab is being explored in an ongoing clinical trial for patients with recurrent osteosarcoma (NCT02470091), based on the role of the RANK ligand in regulating bone turnover, activating downstream signaling, and modulating gene expression.

1.3 | Tumor vaccines and dendritic cells

1.3.1 | Mechanism

Tumor vaccines were one of the original modalities tested as cancer immunotherapy, and are designed to induce an antitumor response through the exposure of tumor antigens. Vaccines have included whole cells, lysates, proteins, DNA, RNA, and peptides. Dendritic cells (DC) are antigen presenting cells (APCs) that have the ability to activate T cells and cause the proliferation of cytotoxic T lymphocytes (CTLs). Matured autologous DC can be loaded with the particle(s) of choice, treated with immunoadjuvants ex vivo, and then re-injected into the patient.

1.3.2 | Experience in osteosarcoma and future directions

DC vaccines have produced delays in disease progression and even the regression of established osteosarcomas in animal models. However, only limited activity was seen in the two clinical trials using DC vaccines pulsed with autologous tumor cell lysate in patients with recurrent osteosarcoma. These studies did, however, show that this strategy is safe and can activate the immune system to some extent. It is unknown whether vaccines would be more effective for osteosarcoma in a setting of minimal residual disease, or whether combination with other immunotherapies would improve tumor control. In an effort
to increase efficacy, investigators are adding decitabine to upregulate cancer antigen expression (NCT01241162), or gemcitabine to increase the tumor cell cytotoxicity and decrease myeloid-derived suppressor cells (NCT01803152).

### 1.4 Oncolytic viruses

#### 1.4.1 Mechanism

Oncolytic viruses are attenuated viruses genetically engineered to only replicate in malignant cells. This strategy is appealing for solid tumor therapy because these viruses are not dependent on the expression of specific tumor cell antigens. In addition to direct cytotoxicity, the administration of oncolytic virus creates a pro-inflammatory tumor microenvironment leading to antigen presentation and APC maturation with subsequent epitope spreading.28

#### 1.4.2 Experience in osteosarcoma and future directions

Preclinical studies using various different oncolytic virotherapies have demonstrated activity against some adult cancers. In the clinic, talimogene laherparepvec (T-VEC) was found to produce responses in patients with melanoma even in noninjected metastatic tumors, and the success of this strategy has led to T-VEC being the first FDA-approved oncolytic virus.29 A trial combining T-VEC with the anti-PD-1 antibody pembrolizumab is now being tested in patients with osteosarcoma due to lack of efficacy.30 Other oncolytic virotherapies that are in preclinical development.30–32

### 1.5 Adoptive cell therapy

#### 1.5.1 Mechanism

Adoptive cell therapy provides a patient with cytolytic cells to cause an antitumor response.23 These therapies are designed to counteract the various ways tumor cells evade the host immune system. For example, malignant cells may downregulate their expression of HLA and tumor antigens, thus making them unable to be recognized by T cells. To overcome this issue, T cells can be engineered to respond with high affinity to specific antigens without the need for peptide recognition in the context of HLA presentation. These engineered T cells are termed chimeric antigen receptor T cells (CAR-Ts), and are composed of an extracellular domain derived from a monoclonal antibody specific for a tumor surface antigen, a spacer domain, a transmembrane domain, and an intracellular signal-transducing chain of the T cell receptor.33,34 In sarcoma trials, the process includes harvesting autologous peripheral blood mononuclear cells using apheresis, activation with CD3 and CD28 antibodies together with recombinant interleukin-2, and then transduction with retroviral particles encoding the target antigen, such as HER2.35 T-cell lines are then further expanded with interleukin-2, tested to confirm immunospecificity, and then reinfused to the patient.15 The use of CAR-Ts has recently been approved by the US Food and Drug Administration for the treatment of relapsed pediatric acute lymphoblastic leukemia, and applications for sarcomas are now being explored.

Other adoptive cell therapy options include NK cells and tumor-infiltrating lymphocytes (TILs). NK cells are lymphocytes in the innate immune system with both cytotoxic and regulatory functions. Unlike T- and B cells, NK cells recognize targets without prior exposure. TILs are another form of adoptive cell therapy in which highly specific T cells migrate into tumors, and upon exposure to tumor antigens, directly kill tumor cells and release cytokines which further mediate the antitumor response.36,37

#### 1.5.2 Experience in osteosarcoma and future directions

CAR-Ts generated against IGF-1R and tyrosine kinase-like orphan receptor 1 prolong survival in murine models of osteosarcoma.38 A phase I/II trial of HER-2 CAR-T therapy included 16 patients with relapsed osteosarcoma and demonstrated no dose-limiting toxicities, with the persistence of HER2-CAR T-cells for at least 6 weeks in the majority of evaluable patients, some of whom experienced prolonged

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**TABLE 1** Clinical trials for antibody therapy targeting cell surface proteins

| Trial identifier | Eligible disease(s) | Treatments | Estimated enrollment | Clinical phase | Result or primary outcome | Status |
|-----------------|---------------------|------------|----------------------|---------------|--------------------------|--------|
| NCT01419834     | High-risk neuroblastoma GD2 positive tumors | Humanized 3F8 anti-GD2 monoclonal antibody | 74 | I | Ongoing | Ongoing, recruiting |
| NCT02484443     | Recurrent osteosarcoma | Dinutuximab + GMCSF | 44 | II | Ongoing | Ongoing, recruiting |
| NCT02502786     | Recurrent osteosarcoma | Humanized 3F8 anti-GD2 antibody + GMCSF | 39 | II | Ongoing | Ongoing, recruiting |
| NCT00831844     | Relapsed or refractory solid tumors | Anti-IGF-1R monoclonal antibody | 116 | II | Ongoing | Completed |
| NCT02487979     | Recurrent or refractory osteosarcoma | Glembatumumab vedotin | 38 | II | Ongoing | Ongoing, not recruiting |
| NCT02173093     | Neuroblastoma, osteosarcoma | Anti-GD2 BiTE therapy | 40 | I/II | Ongoing | Ongoing, recruiting |
| NCT01662804     | Neuroblastoma GD-2 positive solid tumors | Humanized 3F8 mAb + IL-2 | 14 | I | Ongoing | Ongoing, not recruiting |

GMCSF, granulocyte-macrophage colony-stimulating factor.
stable disease.35 There are currently two ongoing trials with GD2-targeted CAR-T cells (NCT 01953900 and NCT02107963).

There are also encouraging preclinical data showing the benefit of NK cells in animal models of osteosarcoma,44–46 and several NK trials are ongoing (Table 2). Although the adoptive transfer of TILs can result in the reduction of bulky metastatic melanoma,47 the extraction and expansion of these cells has been problematic. However, improvements in methodology53 may make this treatment more feasible in the future for patients with osteosarcoma.

Other future adoptive cell therapies include the use of unmodified CD8 lymphocytes, T cells with engineered high-affinity receptors, and gδ T cells. As CTLs are the leading cells in immune surveillance, it is ideal to use these CD8 lymphocytes for treatment. Targeting the cancer antigens NY-ESO-1 and MAGE-A3 with specific lymphocytes has shown initial success against soft tissue sarcoma and non-small cell lung cancer (NSCLC).39–41 Culturing unmodified CD8 lymphocytes with decitabine to induce MAGE-A and NY-ESO-1 led to substantial tumor regressions in mouse models of osteosarcoma.42 Another potential adoptive therapy option is the use of T cells with genetically modified receptors for targeting cancer antigens such as MART-1, gp-100, and NY-ESO-1, with favorable outcomes in melanoma and synovial sarcoma.43,44 Although these strategies are exciting, they have not yet been tested in clinical trials for patients with osteosarcoma.

Finally, investigators have explored the use of gδ T cells, which may bridge the innate and adaptive responses, and which have an affinity to recognize and lyse osteosarcoma cells.45 Preclinical studies demonstrated that gδ T-cell treatment of mouse models of osteosarcoma had dramatic tumor regression.46 Further in vitro and in vivo studies demonstrated significant enhancement of tumor killing when zoledronic acid was combined with the gδ T cells against osteosarcoma tumors, leading to a potential combination therapy for patients with osteosarcoma,45,47 although not yet clinically validated.

### 1.6 | Checkpoint inhibitors

#### 1.6.1 | Mechanism

Without ex vivo expansion, endogenous TILs often fail to control tumors because malignant cells escape immune surveillance by dampening the immune response via checkpoint ligands.48 Checkpoint inhibitors reverse this process by reinvigorating the T-cell-mediated antitumor responses against tumor antigens through the major histocompatibility complex,16 with the greatest response directed at neoantigens that are distinct from those on host tissues.49 The complex genome and chromosomal instability seen in osteosarcoma tumors has not been proven to lead a high mutational burden; however, high levels of genetic instability have the potential to generate neo-epitopes that are the substrate for immune-mediated killing, thus making this tumor attractive for therapy with checkpoint inhibitors, including those targeting CTLA-4, PD-1, and PD-L1.5,7,50

CTLA-4 is a transmembrane glycoprotein receptor expressed on Tregs and memory T cells, and after binding to CD80/86 on DC results in functional inhibition.51 CLTA-4 expression is increased in patients with osteosarcoma compared to healthy subjects, leading to the proposed use of CTLA-4 inhibitors in patients with osteosarcoma.52,53 PD-1 is another transmembrane immunoglobulin family member expressed on T cells, with the highest expression seen in chronically activated T cells.54 PD-1 serves as a "brake" of the immune system by suppressing CTLs and activating Treg cells,55 and its ligand PD-L1 is expressed on a subset of osteosarcoma tumor cells as well as immune cells contained within osteosarcoma tumor samples.56 The success of checkpoint inhibitors for several different adult cancers has driven

### TABLE 2  Clinical trials for adoptive cell therapy, dendritic cell therapy, and vaccines

| Trial identifier | Eligible disease(s) | Treatments | Estimated enrollment | Clinical phase | Result or primary outcome | Status |
|------------------|---------------------|------------|----------------------|----------------|--------------------------|--------|
| NCT02107963      | GD2 positive solid tumors | Anti-GD2 CAR-T-cell therapy | 15 | I | Awaiting results | Completed |
| NCT01953900      | Refractory or metastatic GD2 positive sarcoma | Anti-GD2 CAR-T cells in VZV | 26 | I | Ongoing study | Ongoing, not recruiting |
| NCT02409576      | Metastatic EWS, metastatic OS intermediate, and high-risk RMS | Haploidentical NK cell infusions | 20 | I | Ongoing study | Ongoing, recruiting |
| NCT01803152      | Bone sarcoma, soft tissue sarcoma | DC vaccination, DC vaccination + gemcitabine pretreatment | 56 | I | Ongoing study | Suspended pending amendment |
| NCT01241162      | Neuroblastoma, EWS, OS, RMS, SS | Decitabine + cancer antigen vaccine | 19 | I | Awaiting results | Completed |
| NCT02819843      | Melanoma, Merkel cell carcinoma, other solid tumors | T-VEC, T-VEC + radiotherapy | 34 | II | Ongoing study | Ongoing, recruiting |
| NCT00931931      | Non-CNS solid tumors | Oncolytic herpes virus (HSV1716) | 18 | I | Awaiting results | Completed |

CNS, central nervous system; OS, osteosarcoma; RMS, rhabdomyosarcoma; SS, synovial sarcoma; VZV, varicella zoster virus.
considerable interest in their potential application for osteosarcoma treatment.

1.6.2 Experience in osteosarcoma and future directions

Merchant et al. reported a pediatric phase I study of the CTLA-4 inhibitor ipilimumab in children with relapsed solid tumors, including eight with osteosarcoma. They showed similar toxicity and pharmacokinetics as adults, with an increase in activated and cycling CTLs without an increase in Tregs. Unfortunately, no objective antitumor responses were observed. The Sarcoma Alliance for Research through Collaboration consortium recently tested pembrolizumab for the treatment of sarcomas, and reported partial response in one (4%) of the 22 patients with recurrent osteosarcoma. Numerous studies are now testing various combinations of CTLA-4, PD-1, and PD-L1 inhibition, as discussed below and listed in Table 3.

1.7 Combination therapies

The complexity of the immune system coupled with the disappointing activity seen with the single-therapy approaches studied to date suggest that combination strategies will be necessary to optimize immunotherapy for osteosarcoma. A wide variety of pathways in the tumor microenvironment may contribute to resistance to checkpoint inhibitors, such as T-cell cytotoxicity interruptions, downregulation of MHC, altered DC migration, upregulation of CD80 and CD86, downregulation of PD-L1, and expression of proteins such as indoleamine 2,3-dioxygenase (IDO), LAG3, and TIM3. It will likely be crucial to determine these pathways in individual patients to optimize these treatments.

Rational combination therapies can be designed by capitalizing on specific mechanisms of particular immunotherapeutics. For example, CTLA-4 and PD-1 affect different components of the immune response. CTLA-4 has a primary role in activation of the CD4 effector compartment, specifically inducing expansion of the ICOS+ Th1-like CD4 effector subset, while PD-1 predominately modulates CTL proliferation. Studies combining PD-1 and CTLA-4 blockade for metastatic melanoma have shown clinical benefit (30% of patients exhibiting >80% tumor reduction), leading to the FDA approval of nivolumab and ipilimumab for metastatic melanoma without BRAF mutation. In osteosarcoma, preclinical murine metastatic models have shown clinical benefit (30% of patients exhibiting >80% tumor reduction), leading to the FDA approval of nivolumab and ipilimumab for metastatic melanoma without BRAF mutation. In osteosarcoma, preclinical murine metastatic models have shown clinical benefit (30% of patients exhibiting >80% tumor reduction), leading to the FDA approval of nivolumab and ipilimumab for metastatic melanoma without BRAF mutation. In osteosarcoma, preclinical murine metastatic models have shown clinical benefit (30% of patients exhibiting >80% tumor reduction), leading to the FDA approval of nivolumab and ipilimumab for metastatic melanoma without BRAF mutation. In osteosarcoma, preclinical murine metastatic models have shown clinical benefit (30% of patients exhibiting >80% tumor reduction), leading to the FDA approval of nivolumab and ipilimumab for metastatic melanoma without BRAF mutation. In osteosarcoma, preclinical murine metastatic models have shown clinical benefit (30% of patients exhibiting >80% tumor reduction), leading to the FDA approval of nivolumab and ipilimumab for metastatic melanoma without BRAF mutation.

Checkpoint inhibitors may also increase the activity of CAR-T cells and BiTE antibodies, which may be limited by increased Tregs, immunosuppressive cytokines, genomic instability, loss of target antigen expression, anti-antibody formations leading to T-cell exhaustion, and upregulation of PD-1. Finally, checkpoint inhibitors are also being combined with gene modified T-cell therapy and cancer vaccines in phase I trials (NCT02070406 and NCT02775292), based on encouraging preclinical data.

2 BIOMARKERS

A major challenge for immunotherapy is the identification of biomarkers that predict response, so that treatments can be tailored for the greatest benefit. For checkpoint inhibitors, various proposed predictors of response include the tumor immune phenotype (expression of PD-1 and PD-L1, as well as the presence of TILs), the somatic genomic features such as mutational burden and microsatellite instability, the gut microbiome, and the HLA class I genotype.

Unfortunately, PD-L1 expression has not been consistently predictive of response in either melanoma or NSCLC. Adding further complexity, PD-L1 expression is heterogeneous in both primary and metastatic lesions. Whether higher levels of TILs are predictive of response to PD-1-targeted therapy is being assessed but is as yet unknown. Tumor mutational burden is another potential biomarker for PD-1 therapies, given the greater number of neoantigens. Specifically, tumors with mismatch repair deficiency (MMRD) have a striking response rate to pembrolizumab. However, this biomarker is subject to other influences such as chemotherapy and radiation, and outside of MMRD does not always suffice as a stand-alone predictor. In the end, it is most likely to be a composite of biomarkers that will be utilized together to be predictive.

For treatments based on a specific antigenic target, that target must be widely expressed on tumor cells but not on host cells for optimum benefit. However, even widespread target expression does not guarantee activity. Reliable predictive factors for other immunotherapies remain elusive at present.

2.1 Targeting immunosuppression in the microenvironment

There are numerous factors causing immunosuppression within the tumor microenvironment, and some of these can potentially be targeted for therapy. Transforming growth factor-beta (TGF-β) is a growth factor present in the tumor microenvironment of osteosarcoma that stimulates tumor growth. The expression of TGF-β is higher in patients with osteosarcoma than that in healthy individuals, higher in metastatic disease versus localized disease, and correlates with a
Table 3: Clinical Trials of Checkpoint Inhibitor Therapy

| Trial identifier | Eligible disease(s)                                                                 | Treatments                                                                 | Estimated enrollment | Clinical phase | Result or primary outcome                                                                 | Status                  |
|------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------|---------------------|----------------|------------------------------------------------------------------------------------------|-------------------------|
| NCT02301039      | Recurrent, unresectable, and/or metastatic sarcoma (SARCO28)                       | Anti-PD-1 antibody                                                        | 146                 | II             | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02263508      | Melanoma                                                                           | T-VEC + anti-PD-1 antibody                                                | 660                 | III            | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02304458      | Recurrent or refractory solid tumors or sarcomas                                    | Nivolumab, nivolumab + ipilimumab                                         | 352                 | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02332668      | Melanoma, PD-L1 positive solid tumors, relapsed, refractory Hodgkin lymphoma       | Anti-PD-L1 antibody                                                        | 310                 | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02813135      | Recurrent solid tumors, any recurrent/ refractory malignancy                        | Anti-PD-1 antibody, other therapeutics based on molecular profiling       | 285                 | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02541604      | Recurrent solid tumors                                                             | Anti-PD-L1 antibody                                                        | 100                 | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT03006848      | Recurrent osteosarcoma                                                             | Anti-PD-L1 antibody                                                        | 40                  | II             | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT01445379      | Melanoma, PD-L1 positive solid tumors, relapsed, refractory Hodgkin lymphoma       | Anti-PD-L1 antibody                                                        | 310                 | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT01968109      | Solid tumors                                                                       | Anti-LAG-3 antibody                                                        | 1,000               | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT01738139      | Advanced cancers                                                                   | Anti-CTLA-4, anti-CTLA-4 + tyrosine kinase inhibitor                       | 96                  | I              | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02070406      | Advanced local or metastatic solid tumor                                           | Anti-CTLA-4 antibody + gene modified T cell + dendritic cell vaccine      | 12                  | I              | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02992964      | bMMRD positive tumors                                                               | Anti-PD-1 antibody                                                         | 20                  | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02901145      | Recurrent solid tumors                                                             | Anti-PD-1 antibody + cyclophosphamide                                       | 30                  | I/II           | Not yet open                                                                              | Not yet open            |
| NCT02775292      | Stage IV or locally advanced tumors expressing NY-ESO-1                             | Anti-PD-1 antibody + gene modified T cells + NY-ESO-1 vaccine             | 12                  | I              | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02301039      | Advanced bone sarcoma and soft tissue sarcoma                                      | Anti-PD-L1                                                                | 146                 | II             | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT027723955     | Advanced solid tumors                                                              | Anti-PD-L1 antibody + GSK3359609                                           | 304                 | I/II           | Ongoing                                                                                  | Ongoing, recruiting     |
| NCT02793466      | Relapsed or refractory solid tumors                                               | IgG1 monoclonal antibody (blocks interaction of PD-L1 with PD-1)         | 36                  | I              | Ongoing                                                                                  | Ongoing, recruiting     |

bMMRD, bialleic mismatch repair deficiency.

lack of response to chemotherapy. Preclinical osteosarcoma murine models showed antitumor effects when TGF-β blockade was combined with DC, due to immune response reconstitution. A second approach uses the tyrosine kinase inhibitor axitinib, a vascular endothelial growth factor inhibitor and platelet-derived growth factor receptor inhibitor, to facilitate T-cell trafficking into the tumor microenvironment, and a trial of this agent combined with pembrolizumab is currently underway (NCT02636725). Other strategies explored in adults include combining PD-1 agents with specific inhibitors of IDO, given the known immunosuppressive properties of IDO including the arrest of T-cell proliferation and induction of Tregs. This combination strategy may be attractive for osteosarcoma, given the ubiquitous expression of IDO reported in primary osteosarcoma tumors.
3 | CONCLUSION

Progress toward improving outcomes in patients with osteosarcoma has been limited in the last three decades by the failure to identify either new active agents or ways to optimize the use of existing drugs, not unlike many adult solid tumors before the advent of immunotherapies. Several biological features of osteosarcoma suggest that modulation of the immune response could lead to benefits, and the wide variety of therapeutic approaches now available make this an exciting time for immunotherapy. However, the sheer complexity of the immune system and the nuances of the tumor-specific microenvironment underscore how daunting this task is. As seen with conventional chemotherapy drugs, tumors utilize multiple pathways to resist immunotherapy, suggesting that combination approaches will be needed to achieve meaningful and durable responses. Success will likely require further elucidation of the mechanisms of resistance to existing immunotherapies, the development and testing of rational combination treatments to overcome this resistance, and the identification of predictive biomarkers to help guide appropriate use of these treatments. Although much work remains to be done, the hope is that immunotherapy can lead to breakthroughs that will revolutionize osteosarcoma therapy in the same way that adult cancer therapy has been transformed.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Mary F. Wedekind http://orcid.org/0000-0001-9707-7175
Lars M. Wagner http://orcid.org/0000-0003-4717-9960
Timothy P. Cripe http://orcid.org/0000-0002-8595-3577

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