Curr Oncol Rep (2015) 17: 52
DOI 10.1007/s11912-015-0476-7

SARCOMAS (SR PATEL, SECTION EDITOR)

Immunotherapy in Sarcoma: Future Horizons

Melissa Burgess[1,4] · **Vikram Gorantla**[2] · **Kurt Weiss**[3,4] · **Hussein Tawbi**[1,4]

Published online: 30 September 2015

Abstract Immunologic approaches to cancer are over a century old. Over the years, the strategy has been fine-tuned from inciting infections in subjects to inhibiting negative regulatory signals from the innate immune system. Sarcomas are among the first tumors to be considered for immune interventions. From Coley's toxin to cytokine-based therapies to adoptive cell therapy, there have been numerous immunotherapeutic investigations in this patient population. A promising strategy includes adoptive T cell therapy which has been studied in small cohorts of synovial sarcoma, a subtype that is known to widely express the cancer testis antigen, NY-ESO-1. Additionally, recent data in metastatic melanoma and renal cell carcinoma demonstrate the utility and tremendous efficacy of immune checkpoint blockade with increased rates of durable responses compared to standard therapies. Responses in traditionally "non-immunogenic" tumors, such as lung and bladder cancers, provide ample rationale for the study of immune checkpoint inhibitors in sarcoma. While immunotherapy has induced some responses in sarcomas, further research will help clarify optimal patient selection for future clinical trials and new combinatorial immunotherapeutic strategies.

This article is part of the Topical collection on *Sarcomas*

✉ Melissa Burgess
burgessma@upmc.edu

[1] Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

[2] Medical Oncology, American Oncology Institute, Serilingampally, Hyderabad, TS, India

[3] Division of Musculoskeletal Oncology, Department of Orthopedic Surgery, University of Pittsburgh, Pittsburgh, PA, USA

[4] Sarcoma Program, University of Pittsburgh Cancer Institute, 5150 Centre Avenue, Fifth Floor, Pittsburgh, PA 15232, USA

Keywords Soft tissue sarcoma · Immunosurveillance · Immunotherapy · Programmed death-1 (PD-1) · Tumor-infiltrating lymphocyte (TIL) · Immune checkpoint blockade

Introduction

In 2015, there will be an estimated 15,040 new cases of soft tissue and bone sarcomas resulting in 8230 deaths in the USA. Sarcomas account for nearly 21 % of all pediatric solid cancers but less than 1 % of all adult solid cancers [1]. Not surprisingly, bone and soft tissue sarcomas are respectively the third and fourth leading causes of cancer-related deaths in patients under the age of 20 [2]. Trend analysis from 2001 to 2010 indicates that soft tissue sarcoma-associated death rates are increasing in men [3].

Although sarcomas can be broadly categorized into soft tissue sarcoma (STS) and bone sarcomas, they represent a heterogeneous group of malignancies with more than 50 distinct histologic subtypes with varying age and location of presentation. Studies to better understand sarcomas and treatment modalities to improve outcomes are limited by their rarity and diversity which is reflected in the slow progress made in the systemic treatment of sarcoma. Treatment outcomes generally reflect the extent of disease at presentation, with early localized disease treated curatively with margin-negative surgery and potentially adjuvant systemic therapy, while advanced disease is treated palliatively with metastasectomy, tumor debulking, radiation, and/or chemotherapy.

In spite of recent advances in our understanding of the biology of cancer, the prognosis of metastatic sarcoma remains poor. In patients with metastatic disease or recurrent locally advanced disease, the overall median survival is

around 15 months and about 10 % of cases are alive at 5 years [4]. Response to conventional chemotherapy and radiation therapy varies with some subtypes being sensitive to conventional approaches while other subtypes are fairly resistant. Further complicating treatment is the first-order kinetics demonstrated by cytotoxic agents, which cannot be reliably expected to eliminate last remnant tumor cells and therefore not capable of achieving primary cures except in tumors with high growth fractions such as Ewing sarcoma and some rhabdomyosarcomas [5–7]. This limitation is reflected by relapses despite aggressive adjuvant therapy casting a shadow of uncertainty over the role of adjuvant chemotherapy even in high-risk disease.

The last decade has seen novel agents being explored in a collaborative fashion in the treatment of sarcoma with large randomized controlled phase III clinical trials being conducted rather efficiently and generating solid data. These agents include chemotherapeutic agents, such as trabectedin, palifosfamide, eribulin, and most recently TH-302 and aldoxorubicin. Also, novel targeted therapeutics has made inroads beyond gastrointestinal stromal tumors (GIST) with pazopanib achieving FDA approval as a third-line agent in metastatic sarcoma. Although these agents have demonstrated benefit, it remains limited at best, where the expectations are a few weeks to months of improvement in progression-free survival and largely absent impact on overall survival. The bar may have been set too low and, the goal of achieving a cure in the metastatic setting has remained elusive, perhaps in the absence of potent therapeutic approaches with long-term impact. The promise of individualized therapy is most relevant for a disease like sarcoma where there is inherent heterogeneity and uniqueness of each individual's disease; however, such approaches are useless in the absence of effective therapeutic choices. Immunotherapy is perceived as the epitome of individualized medicine and has recently achieved tremendous success in melanoma, lung cancer, and renal cell cancer to name a few. Increased understanding of cancer immunology, in general, and also as it relates to sarcoma has raised hope that immunotherapy will have utility in treatment of sarcoma in the neoadjuvant, adjuvant, and metastatic settings. This review reflects on the progress made in the past decade and familiarizes the reader with the current state-of-the art of immunotherapy in sarcoma.

Foundations

The concept of the immune system as a surveillance and anti-tumor effector system is over a century old. The anti-tumor effect of the immune system was originally observed by William B. Coley in a patient with metastatic sarcoma [8]. The patient was noted to have cleared his disease following an *Erysipelas* infection. This observation and further experiments involving direct injection of live streptococcus into tumors lead to the development of Coley's toxin—a combination of heat-inactivated streptococcal organisms along with *Serratia marcescens*. This combination was used to treat patients with inoperable sarcomas. Coley's toxin was observed and analyzed over the next few decades including in a controlled study that reported response rates of up to 21 % in primary tumors, as well as metastatic lesions [9, 10]. The sarcoma surgical literature has reported correlations between deep surgical infection and improved overall survival. In a study of 47 dogs with sarcoma, Lascelles et al. found a correlation between survival and the clinical event of a deep surgical infection [11]. Additionally, a large study from the Royal Orthopaedic Hospital in Birmingham, England, showed a statistical (p=0.017) advantage to deep surgical infection in terms of long-term survival [12]. Another study in a mouse model of osteomyelitis reported that tumor growth was inhibited in infected animals [13]. This notion that concurrent infection somehow enhances immune surveillance is controversial; however, in a series of 396 soft tissue sarcoma patients, a protective survival advantage with infection was not found [14]. Certainly, the relationship between surgical infection and enhanced immune surveillance must be evaluated more thoroughly.

At the same time as Coley was testing his hypothesis, Paul Ehrlich in 1909 proposed the concept of the immune system as an anti-tumor mechanism (vaccine). Burnet coined the term tumor surveillance, which implied surveillance of the host for malignant cells presumed to be recognized and destroyed as they emerged [15]. Dunn et al. further expanded on this concept of "immunoediting" [16]. Immunoediting can be thought of as the relationship between the immune system and tumors with respect to elimination, equilibrium, and escape. Data supports that a functional cancer immunosurveillance process exists and serves as extrinsic tumor suppressor—"elimination"; however, the immune system can facilitate tumor progression by sculpting an adaptive phenotype under selective pressure, "equilibrium" that eventually allows the tumor to "escape." Such observations and hypotheses form the bedrock of the application of immunotherapy in oncology today.

Immunogenicity of Sarcoma

The immunogenic nature of sarcoma was evaluated in early experiments, clinical observations, and animal models. Tumor responses to Coley's toxin were attributed to the host response to bacterial endotoxins. The effectors of this response are thought to be TNF-α and interleukin (IL)-12 which are key and early factors in immune response activation. Studies evaluating TNF-α showed that it did not produce the same effect as Coley's toxin and also only seemed to work in patients with immunogenic tumors who had preserved immunity and built

some preexisting immunity to their malignancy—a vaccinated quiescent immune system needing a jump start [17]. Given the significant toxicities associated with systemic delivery of TNF-α and IL-12, clinical studies have evaluated targeted delivery of these agents to the tumor microenvironment to generate an endogenous immune response to sarcoma. Intratumoral delivery via sustained release mechanisms demonstrated tumor regression in mouse models [18]. This response is thought to be dependent on cytotoxic T cells and natural killer (NK) cells activity. IL-12's ability to amplify immune responses has been shown in murine models of sarcoma vaccines [19].

This observation has been supported by evidence showing that tumor infiltration by lymphocytes is associated with improved patient outcomes [20]. Chemokines expressed by tumors causing recruitment of CD8+ T cells into the tumor microenvironment have been investigated in patients with Ewing sarcoma where tumor infiltration was correlated with improved survival compared to stroma infiltration only [21]. Tumor-infiltrating lymphocytes (TILs) were also seen in cases of partial regression of osteosarcoma [22]. In stark contrast, high concentrations of regulatory T cells (Tregs) were seen in patients that presented with metastatic Ewing sarcoma [23]. This could be attributed to inhibition of cytotoxic CD8+ T lymphocytes by Tregs which likely promoted tumor escape [20, 21]. Anti-tumor immune responses, as seen in rare cases of spontaneous tumor regression, represent evidence of the role of the immune system in the elimination of sarcomas [24]. Regression was also seen in cases of STS where unplanned resection was carried out with residual tumor remaining. Only 35 % of patients had residual tumor on re-excision [25]. Similarly, re-excision seemed to confer an immune-mediated benefit in terms of disease-free survival (DFS) in patients with STS of the extremities based on a retrospective review of 1092 patients at Memorial Sloan Kettering Cancer Center (MSKCC). On multivariate analysis, re-excision was a significant predictor of improved DFS even after adjusting for stage [26]. These observations support the theory of incomplete resection leading to priming of the immune system to eliminate sarcoma.

Besides utilizing Tregs to their advantage, tumors also adapt their cell surface markers to escape immune detection and elimination. Major histocompatibility complex I (MHC I) plays a key role in expression of tumor antigens to the cellular arm of the immune system which can lead to the elimination of tumor cells by CD8+ T cells. Interestingly, studies evaluating MHC I expression in sarcoma have shown a significant number of specimens of varying subtypes that lost or down-regulated the MHC class I [27, 28]. Conversely, HLA I expression was associated with improved overall and event-free survival compared to HLA I-negative osteosarcoma which underscores the importance of the immune systems response to sarcoma [27].

Kaposi sarcoma (KS) has provided valuable insight to the role of the immune system in tumor elimination and equilibrium. Its causative agent is the oncogenic human herpes virus-8 (HHV-8) [29]. KS is noted to develop in untreated HIV patients and is considered an AIDS-defining illness. KS is the most common malignancy seen in untreated HIV-positive patients and in the Western world is pathognomonic for untreated HIV [29]. The introduction of highly active antiretroviral therapy (HAART) as an effective treatment has improved outcomes in AIDS patients. A clinical marker for response to HAART is resolution of the KS lesions in patients with recovering immune systems [30]. The advent of HAART therapy has also led to a decrease in the incidence of KS. Given the viral oncogenic etiology and immunosuppressed status of patients, the resolution of KS lesions and also decrease in overall incidence of KS can be attributed to HAART therapy and its effect on bolstering the immune system. This hypothesis was evaluated in prospective studies that showed HAART therapy led to increasing CD4 count, decreasing HHV-8 titers (undetectable in majority cases), and decreasing HIV titers and also response in terms of complete response (CR) and partial response (PR) of KS [31–34]. These investigations confirm that sarcoma responses could be directly related to robustness of the patient's immune system.

Development of sarcoma cell lines and mouse models in the late 1970s and early 1980s led to the generation of preclinical data supporting immunotherapy in humans [35]. Development of methylcholanthrene-induced fibrosarcoma (meth A) cell lines allowed for the evaluation of sarcomas in mice [35]. Vaccination with *Corynebacterium parvum* was noted to be associated with increased tumor rejection and inhibition compared to control [36]. The tumor rejection was found to be a T cell-dependent process. This acquired immunity could be transferred to other mice with syngeneic tumor leading to tumor regression [37]. Similar studies also showed the feasibility of transfer of immunity [38]. Interestingly, it was noted that the suppression of host immune system was required and anti-tumor effect was lost after a certain time of tumor transplantation. It was hypothesized that it was secondary to generation of Tregs [38]. These results were duplicated in mouse models at the National Cancer Institute (NCI) [39]. Adoptive transfer of spleen cells from immunized mice consistently led to the regression of established fibrosarcoma tumors (MCA 105 and 106 tumors) in host mice [39]. The observed immune response was specific for the tumor type and was mediated by sensitized T lymphocytes. It was noted that irradiation of the transferred cells nullified this effect and also successful therapy required prior immune suppression of the host. Interleukin-2 (IL-2) was used to expand and sensitize T cells in vitro. These were then infused into mice with pulmonary metastases, which resulted in resolution of pulmonary disease [40–42]. These innovative experiments form the basis of adoptive cell transfer therapy (ACT).

Collectively, these studies strongly support the clinical application of cellular immunotherapy for sarcoma and are a basis for clinical trials.

Prior Immunotherapeutic Strategies in Sarcoma

Cytokine-Based Immunotherapy

One of the oldest immunotherapeutic approaches to antitumor response is via cytokines which induce activation and proliferation of T cells. Among these strategies are high-dose interleukin-2 (HD IL-2) approved in 1995 for metastatic melanoma or renal cell carcinoma. At the National Cancer Institute (NCI), HD IL-2 alone or in conjunction with lymphokine-activated killer cells was used in 652 patients with metastatic solid tumors, including sarcomas. In this trial, none of the sarcoma patients (n=6) showed a response [43]. In a subsequent small trial of pediatric patients with Ewing sarcoma or osteosarcoma, and in contrast to Dr. Rosenberg's study at the NCI, two of six patients had durable complete responses and both patients had osteosarcoma [44]. These studies were small; therefore, no firm conclusions of the efficacy of HD IL-2 can be drawn.

Interferon (IFN) has been employed in several sarcoma subtypes with intriguing results. A large case series showed activity in patients with high-grade osteosarcoma treated in the adjuvant setting. With a median follow-up of 12 years, the observed 10-year progression-free and sarcoma-specific survivals were 39 and 43 %, respectively, for 178 patients. Additionally, the European and American Osteosarcoma Study Group conducted a randomized trial (EURAMOS-1) of post-operative systemic therapy consisting of methotrexate, doxorubicin, and cisplatin with or without pegylated IFN α-2b (PEG-IFN) in patients who had good histologic response to neoadjuvant chemotherapy for osteosarcoma (≥90 % necrosis). Two thousand two hundred sixty patients agreed to take part in EURAMOS-1 between 2005 and 2011, making it the largest ever clinical trial in osteosarcoma. The primary end point was event free survival (EFS) at 3 years. Additional end points of overall survival and toxicity were secondary. Among 1041 patients that achieved a good response to neoadjuvant chemotherapy, 716 consented to random assignment. Three hundred fifty-seven were randomized to the PEG-IFN arm, but only 271 patients started the experimental treatment. Among those 271, 105 stopped early with refusal and toxicity as the main reasons for never starting the PEG-IFN and for stopping prematurely, respectively. The results did not show benefit of addition of PEG-IFN maintenance to adjuvant chemotherapy (HR 0.83, 95 % CI, 0.61 to 1.12; p=.214); however, the dosing and use of PEG-IFN have generated criticism given its unclear role in sarcoma. Also, the results need to be interpreted with caution given the high attrition rate. Long-term follow-up for overall survival continues [45]. Although no statistically significant results have been reported, and unlike the small HD IL-2 trials, this intergroup study showed that large international clinical trials of rare cancers could be performed and has provided framework for future clinical trials.

Muramyl Tripeptide Phosphatidyl Ethanolamine

Another agent has generated debate as to its efficacy in treatment of osteosarcoma is muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE). L-MTP-PE is a synthetic analogue of bacterial cell wall which causes a monocyte/macrophage activation leading to nonspecific immune modulation similar to bacillus Calmette-Guérin (BCG). L-MTP-PE showed promise in phase I and II trials [46, 47]. Based on these studies, the Children's Oncology Group conducted a large randomized phase III intergroup trial 0133 enrolling 677 osteosarcoma patients. Half the patients received adjuvant chemotherapy with L-MTP-PE as an immune modulator [48, 49]. It was associated with reduction in risk of recurrence and death by 25 and 30 %, respectively. These results were called into question given the 2×2 factorial study design which exposed the study to a potential interaction between L-MTP-PE and ifosfamide. In follow-up analysis, DFS and non-statistically significant overall survival benefit was noted [50]. Although the analysis was not powered to demonstrate benefit in patients with metastatic disease, improvements in outcomes may also be seen in terms of event free and overall survival [51]. This agent is presently available in the European Union countries: the UK, Israel, and Mexico. It is not approved for usage in North America. Hopefully, additional data from the ongoing use of this agent will confirm or deny its efficacy for patients with osteosarcoma.

Targeted Therapy

Humoral immunity or the antibody-mediated immune system is another strategy in the fight against sarcoma. As discussed before, sarcoma presents a target-rich environment with specific epitopes that can be exploited. A pilot study evaluated a human monoclonal antibody that mimics the complement regulatory protein, CD55, which is over-expressed by osteosarcoma. Patients were vaccinated with this antibody in an attempt to generate an immune response against tumor expressing CD55. Although the majority of patients showed T helper responses with minimal side effects, it did not translate into clear benefit [52]. I^{131}-radiolabelled monoclonal antibody 8H9 directed against epitopes specifically expressed in sarcomas is being evaluated in a phase I clinical trial at MSKCC (NCT01099644). Antibodies directed against the insulin-like growth factor-1 receptor (IGF1-R) have been evaluated in patients with Ewing sarcoma with promising results [53–55].

Vaccines

Vaccine-based therapy for the treatment of sarcoma in the metastatic and adjuvant setting is a very attractive modality given its decreased potential for toxicity and the individualized nature of therapy in respect to the patient's immune system and their sarcoma. Given the heterogeneity of sarcomas, they make ideal vaccine targets. Almost 25 % of sarcomas are characterized by specific genomic alterations. They tend to express specific epitopes based on specific translocations or histology that make for attractive targets for an activated immune system. Sarcoma-specific fusion proteins include SSX, FOXO1, EWSR1, and TLS CHOP. Partially specific sarcoma proteins include NY-ESO-1, SSX2/3, MAGE, GAGE, WT1, and GD2/3. Also, given the large masses that sarcoma presents with, tissue is generally readily available to help manufacture patient/disease-specific vaccines.

Multiple vaccine strategies have been used including targeting well-defined antigens, tumor lysate, dendritic cells (DDCs) pulsed with antigen, and most recently heat shock proteins (HSP) combined with sarcoma-specific antigens. DDCs in vivo serve the role of antigen presenting cells and can sensitize the immune system to tumor-specific antigens and elicit a cytotoxic T cell response. A phase 1 trial in 2000 looked at pediatric patients with relapsed solid tumors, most of them sarcomas. DDCs from patients were primed with tumor lysate ex vivo and injected intradermally. It was well tolerated and deemed a feasible approach. The one patient who demonstrated a vigorous response was also characterized by a robust delayed-type hypersensitivity reaction. DDC pulsed with tumor-specific translocation peptide and E7, a binder of HLA-A2, was evaluated in patients with metastatic or recurrent Ewing sarcoma family of tumors and alveolar rhabdomyosarcoma with t (2:13) or t (11:22) translocations [56]. This was given as a consolidation therapy to 30 out of 52 patients. It was well tolerated with improved survival outcomes at 5 years (43 vs. 15 %) (p=0.0004) in patients who underwent immunotherapy versus those that did not. The mediocre response to this strategy could potentially be due to the medium in which DDCs are developed ex vivo resulting in decreased ability of these DDCs to trigger an effective response in vivo [57]. SYT-SSX-derived peptide vaccines were evaluated in patients (n=21) with advanced synovial sarcoma. Interferon-α was given as an adjunct. Although a significant number (n=9) showed increase in cytotoxic T cells, only one patient had decrease in tumor size [58].

Gangliosides are abundantly expressed in sarcomas making them good targets for vaccine therapy. Among them, GM2, GD2, and GD3 are the most prevalent. GM2 is also expressed on a variety of malignancies, whereas GD2 and GD3 expression is restricted to sarcomas and tumors of neuroectodermal origin [59]. One randomized double-blinded, multi-center phase II trial targeted these antigens by utilizing a KLH conjugated trivalent ganglioside vaccine containing GM2, GD2 lactone, and GD3 lactone with the immunological adjuvant OPT-821 versus OPT-821 plus placebo in metastatic sarcoma patients who are rendered disease free. The primary endpoint of PFS was not statistically significantly different; however, serologic responses were seen in the vaccine arm versus placebo (98 vs. 21 %) and minimal toxicities were seen [60].

Lack of efficacy seen in vaccine trials is discouraging given the existence of clear targets and scientifically sound technique to target them. The most likely reason is the downregulation of the T cell response by sarcoma. This setting would be ideal for adjuvant therapies to enhance the activity of the immune system and make the primary therapy, e.g., vaccines, chemotherapy, etc., more potent. Attempts to use adjuvants such as GM-CSF, IL-2, or Freund's adjuvant to increase the immune system response has not translated to increased efficacy [61–63]. Sarcoma deployment of escape mechanisms by co-opting Tregs, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages likely leads to a muted immune response. The future of vaccines is likely in combination with agents that prevent suppression of the immune system by sarcoma.

Adoptive Immunotherapy

Compared to chemotherapy, which is nonspecific, this approach offers a selective method to eliminate sarcoma by involving transfer of high-affinity tumor antigen-specific cytotoxic T cells or NK cells which have been expanded ex vivo. Based on the work done at the NCI, Robbins et al. evaluated adoptive immunotherapy in patients with sarcoma and melanoma expressing the NY-ESO-1 epitope [64]. Patients were conditioned with cyclophosphamide and fludarabine and then infused with autologous T cells designed to recognize NY-ESO-1. Four out of six synovial sarcoma patients achieved a partial response. One patient had a durable response lasting 18 months. Recently, chimeric antigen receptor (CAR) modified T cell therapy has shown promise in hematologic malignancies and may also be a promising therapeutic approach to sarcoma. Nineteen patients with HER2 expressing bone sarcomas including 16 patients with osteosarcoma were enrolled to receive HER2-CAR engineered T cell therapy. All patients tolerated the infusions well with no dose-limiting toxicities. Of 17 evaluable patients, four patients had stable disease on tumor assessment. A subset of patients had biopsies, and among them, three tumors had >90 % necrosis exhibited. Median overall survival was shown at 10.3 months [65]. The data are relatively new, but stimulating and thought provoking. Additional work with adoptive immunotherapy in sarcoma is being executed and may be promising as processes are fine-tuned.

Immune Checkpoint Blockade

Another immunotherapeutic approach that has gained recent popularity is blockade of immunologic checkpoints, or "brakes" employed by tumors allowing them to "escape" the immune system as discussed previously. Melanoma and non-small cell lung cancers, as well as other solid tumors, and hematologic malignancies are known to evolve and co-opt the naturally occurring feedback loop that dampens the immune system response leading to tumor tolerance. Mechanisms that block tumor tolerance are key to not only augmenting the effect of current therapies but to also complete a cure. The FDA approved ipilimumab, an anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) agent, in 2011 for metastatic melanoma. Given its mechanism of action, ipilimumab is now being evaluated in other malignancies including sarcoma. A phase II pilot study trial using ipilimumab in synovial sarcomas expressing the NY-ESO-1 antigen was conducted. In this study, six patients were treated with one course of ipilimumab given at 3 mg/kg every 3 weeks for three doses only. Although there were no Response Evaluation Criteria in Solid Tumors (RECIST) bresponses observed and only one patient demonstrated an immune response, patients tolerated the treatment well with manageable side effects. This study was terminated early for slow accrual, lack of activity, and lack of immune responses [66].

The role of the programmed death-1 (PD-1)/PD-L1 axis in STS is presently being elucidated. Tumor PD-L1 expression was noted in up to 65% of sarcomas of different subtypes [67••, 68••]. In one retrospective study of tumor specimens from 105 cases, it was determined that the degree of PD-1 positivity in tumor-infiltrating lymphocytes and PD-L1 expression in tumor specimens correlated with a poorer prognosis and more aggressive disease [69••]. These studies suggest that PD-1 and PD-L1 staining may be viable biomarkers for prognosis, and possibly treatment, using anti-PD-1 treatment strategies. Additionally, in osteosarcoma, PD-L1 expression has been investigated via quantitative real-time RT-PCR. Eighteen optimized osteosarcoma cell lines were examined and validated in select cell lines. Among 38 osteosarcoma tissue samples, PD-L1 mRNA expression was high ranging over 4 log (>5000-fold difference). Also, high PD-L1 expression was associated with the presence of TILs ($p=0.01$) [70]. In a preclinical study, the activity of anti-PD-1 monotherapy was established in an implanted fibrosarcoma mouse model, and interestingly, this activity was independent of PD-L1 staining [71]. Other studies in fibrosarcoma rodent xenografts have shown modest activity using anti-PD-1 therapy alone, but significantly enhanced activity when combined with a dual checkpoint antibody directed at LAG-3, another integral immune checkpoint of adaptive immunity [72]. These data provide rationale for both monotherapeutic and combinatorial immune checkpoint blockade strategies in future clinical trials. Given these results and the preclinical data showing encouraging activity in STS, an open-label single-arm phase II study (SARC028) utilizing the anti-PD-1 antibody, pembrolizumab, in patients with advanced soft tissue and bone sarcomas is currently enrolling at the University of Pittsburgh and other national sites (NCT02301039). Pembrolizumab is given intravenously at 200 mg every 3 weeks. Endpoints are objective response rate (ORR) by RECIST 1.1 (primary) and progression-free survival, overall survival, safety, and response rates by immune-related response criteria (irRC). A unique and important aspect of the trial is mandated tumor biopsies pre- and then 8 weeks post-treatment with pembrolizumab which will determine PD-L1 tumor expression as well as monitoring of the immune system in both the tumor microenvironment and in the circulation [73].

Determinants of Anti-tumor Responses

Ongoing biomarker studies will help to solidify the most appropriate patient selection for future immunotherapy trials. While PD-L1 expression as a predictor of response to PD-1 blockade is not well understood [74], there is recent generated data correlating the presence of tumor-infiltrating lymphocytes with better response to anti-PD-1 therapy [75••]. Furthermore, it is clear that not only is it important that CD8+ TILs are present but that they are distinctly located at the invasive tumor margin. These findings resulted from collected tissue samples pre- and during anti-PD-1 therapy in 46 metastatic melanoma patients. The data are thought provoking and are prototypical examples for future studies in biomarker development among other tumor types. Additionally, hypothesis-generating data from the Chan lab at MSKCC has shown increased intratumoral mutational burden to correlate with response to ipilimumab therapy in melanoma patients [68••]. The theory is that increased mutational burden and new peptides, i.e., neoantigens, are formed that help to stimulate the immune system and jumpstart an immune response.

Combinatorial Strategies

Anti-tumor response with immune checkpoint blockade will be enhanced with combinatorial strategies. The combination of ipilimumab and nivolumab in advanced melanoma has shown significant clinical benefit, and other immune checkpoint inhibitors targeting TIM3, BTLA, and LAG-3, among others, are already in clinical development. Chemotherapy, radiation therapy, targeted agents, and other immune checkpoint inhibitors, to name a few, partnered with anti-PD-1 or anti-CTLA-4 antibodies may yield meaningful clinical benefit (Fig. 1). Specifically, chemotherapy has been shown to have immunosuppressive properties and the combination

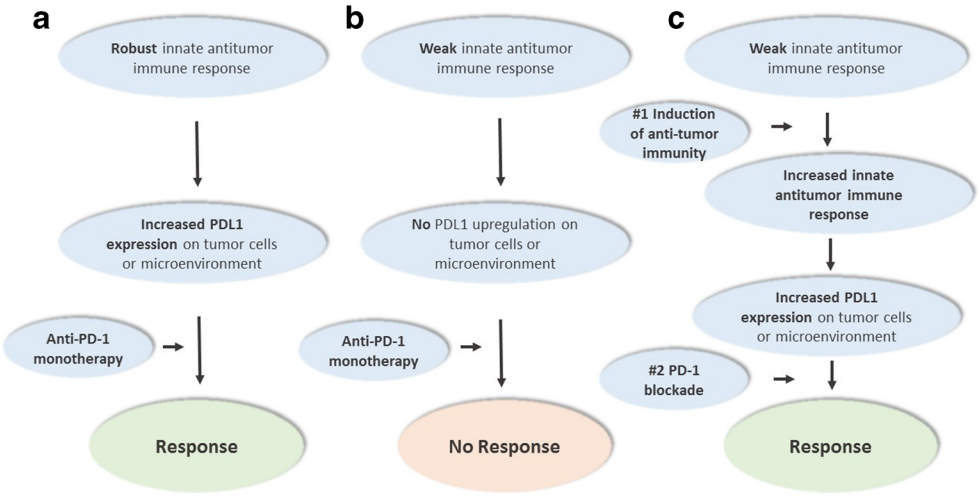

Fig. 1 Potential immunotherapeutic treatment strategies to combat adaptive immune resistance. In tumors, such as melanoma, that elicit a strong endogenous anti-tumor immune response (**a**), single-agent blockade of the PD-1 checkpoint results in anti-tumor response. Conversely, in adaptive immune resistance, weak endogenous immune responses are seen, in other tumors, leading to lack of anti-tumor response despite PD-1 blockade (**b**). Using combinations of anti-PD-1 monotherapy and another immunotherapy approach or other strategy (**c**) may transform a weak endogenous anti-tumor immune response into a more robust one with increased PD-L1 expression and anti-tumor response overcoming adaptive immune resistance

with immune checkpoint inhibitors may be beneficial [76]. It will however be important to minimize potential toxicity. Radiation is often a palliative companion for non-responding patients, and several preclinical studies have shown enhanced effects of immune checkpoint blockade with radiation making this technique an ideal partner for immunotherapy. The "abscopal effect" was discovered as the multiple beneficial immunologic effects on radiation making this also another reasonable combinatorial approach [77]. Specifically, radiation was found to both promote up-regulation of immune effector transcripts and cancer testis antigens, as well as promote increased MHC class I expression among other findings [78]. The role of vascular endothelial growth factor-A (VEGF-A) has been shown to modulate expression of inhibitory checkpoints on CD8+ T cells in tumors such as PD-1. These findings and others serve as justification for future clinical trials combining anti-angiogenic agents with immune checkpoint blockade especially in those sarcomas that are highly vascular [79]. Currently, there are several open or pending clinical trials in renal cell carcinoma (NCT02348008; pembrolizumab plus bevacizumab) and other solid tumors looking at immune checkpoint inhibitors in combination with targeted agents. A lesson learned from melanoma is the application of BRAF inhibition, a common driver mutation, plus immune checkpoint blockade, which creates potential synergy in the treatment of patients with advanced melanoma to hopefully combine the high response rates from BRAF inhibitors and the durable responses from anti-PD-1 and anti-CTLA-4 antibodies. Also, there is evidence that PD-L1 is upregulated on tumor cells when combined with this targeted agent [80, 81]. Phase III data was recently demonstrated showing impressive activity and overall good tolerability of dual anti-PD-1 and anti-CTLA-4 blockade in melanoma patients as well as making this another promising approach in sarcoma [82•, 83•]. Rationale for combinatorial strategies is constructed on diverse scientific research that could translate to potential meaningful clinical benefit; however, much of the data has not yet been reported.

Conclusion

Advanced sarcoma remains a grim disease and newer therapies are clearly needed. Immunologic treatment strategies in sarcoma hold substantial potential and provide a novel approach. Adoptive cellular therapy and immune checkpoint blockade clinical trials are ongoing and future research will help to identify the best delivery of these agents in addition to ongoing biomarker studies that will help to select the optimal patient populations for these studies. Additional work looking at multiple combinations, including immunotherapy plus chemotherapy, radiation, targeted therapy, and more, is the future of immunotherapy research in sarcoma.

Compliance with Ethics Guidelines

Conflict of Interest Melissa Burgess, Vikram Gorantla, Kurt Weiss, and Hussein Tawbi declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Burningham Z, Hashibe M, Spector L, et al. The epidemiology of sarcoma. Clin Sarcoma Res. 2012;2:14.
2. Siegel R, Ma J, Zou Z, et al. Cancer statistics, 2014. CA Cancer J Clin. 2014;64:9–29.
3. Howlader N, Noone A, Krapcho M, et al: SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data submission, posted to the SEER web site, April 2013 2013
4. Italiano A, Mathoulin-Pelissier S, Cesne AL, et al. Trends in survival for patients with metastatic soft-tissue sarcoma. Cancer. 2011;117:1049–54.
5. Grier HE, Krailo MD, Tarbell NJ, et al. Addition of ifosfamide and etoposide to standard chemotherapy for Ewing's sarcoma and primitive neuroectodermal tumor of bone. N Engl J Med. 2003;348:694–701.
6. Nesbit Jr ME, Gehan EA, Burgert Jr EO, et al. Multimodal therapy for the management of primary, nonmetastatic Ewing's sarcoma of bone: a long-term follow-up of the First Intergroup study. J Clin Oncol. 1990;8:1664–74.
7. Gerber NK, Wexler LH, Singer S, et al. Adult rhabdomyosarcoma survival improved with treatment on multimodality protocols. Int J Radiat Oncol Biol Phys. 2013;86:58–63.
8. Coley WB. II. Contribution to the knowledge of sarcoma. Ann Surg. 1891;14:199–220.
9. Nauts HC, McLaren JR. Coley toxins—the first century. Adv Exp Med Biol. 1990;267:483–500.
10. Johnston BJ, Novales ET. Clinical effect of Coley's toxin. II. A seven-year study. Cancer Chemother Rep. 1962;21:43–68.
11. Lascelles BD, Dernell WS, Correa MT, et al. Improved survival associated with postoperative wound infection in dogs treated with limb-salvage surgery for osteosarcoma. Ann Surg Oncol. 2005;12:1073–83.
12. Jeys LM, Grimer RJ, Carter SR, et al. Post operative infection and increased survival in osteosarcoma patients: are they associated? Ann Surg Oncol. 2007;14:2887–95.
13. Sottnik JL, U'Ren LW, Thamm DH, et al. Chronic bacterial osteomyelitis suppression of tumor growth requires innate immune responses. Cancer Immunol Immunother. 2010;59:367–78.
14. Behnke NK, Alamanda VK, Song Y, et al. Does postoperative infection after soft tissue sarcoma resection affect oncologic outcomes? J Surg Oncol. 2014;109:415–20.
15. Burnet M. Immunological factors in the process of carcinogenesis. Br Med Bull. 1964;20:154–8.
16. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22:329–60.
17. Carswell EA, Old LJ, Kassel RL, et al. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 1975;72:3666–70.
18. Sabel MS, Arora A, Su G, et al. Synergistic effect of intratumoral IL-12 and TNF-alpha microspheres: systemic anti-tumor immunity is mediated by both CD8+ CTL and NK cells. Surgery. 2007;142:749–60.
19. Guo QY, Yuan M, Peng J, et al. Antitumor activity of mixed heat shock protein/peptide vaccine and cyclophosphamide plus interleukin-12 in mice sarcoma. J Exp Clin Cancer Res. 2011;30:24.
20. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348:203–13.
21. Berghuis D, Santos SJ, Baelde HJ, et al. Pro-inflammatory chemokine-chemokine receptor interactions within the Ewing sarcoma microenvironment determine CD8(+) T-lymphocyte infiltration and affect tumour progression. J Pathol. 2011;223:347–57.
22. Matsuo T, Shimose S, Kubo T, et al. Extraskeletal osteosarcoma with partial spontaneous regression. Anticancer Res. 2009;29:5197–201.
23. Brinkrolf P, Landmeier S, Altvater B, et al. A high proportion of bone marrow T cells with regulatory phenotype (CD4+ CD25hiFoxP3+) in Ewing sarcoma patients is associated with metastatic disease. Int J Cancer. 2009;125:879–86.
24. Nakayama T, Tsuboyama T, Toguchida J, et al. Natural course of desmoid-type fibromatosis. J Orthop Sci. 2008;13:51–5.
25. Noria S, Davis A, Kandel R, et al. Residual disease following unplanned excision of soft-tissue sarcoma of an extremity. J Bone Joint Surg Am. 1996;78:650–5.
26. Lewis JJ, Leung D, Espat J, et al. Effect of reresection in extremity soft tissue sarcoma. Ann Surg. 2000;231:655–63.
27. Tsukahara T, Kawaguchi S, Torigoe T, et al. Prognostic significance of HLA class I expression in osteosarcoma defined by anti-pan HLA class I monoclonal antibody, EMR8-5. Cancer Sci. 2006;97:1374–80.
28. Mechtersheimer G, Staudter M, Majdic O, et al. Expression of HLA-A, B, C, beta 2-microglobulin (beta 2m), HLA-DR, -DP, -DQ and of HLA-D-associated invariant chain (Ii) in soft-tissue tumors. Int J Cancer. 1990;46:813–23.
29. Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. Nat Rev Cancer. 2010;10:707–19.
30. Ledergerber B, Telenti A, Egger M. Risk of HIV related Kaposi's sarcoma and non-Hodgkin's lymphoma with potent antiretroviral therapy: prospective cohort study (Swiss HIV Cohort Study). BMJ. 1999;319:23–4.
31. Wilkinson J, Cope A, Gill J, et al. Identification of Kaposi's sarcoma-associated herpesvirus (KSHV)-specific cytotoxic T-lymphocyte epitopes and evaluation of reconstitution of KSHV-specific responses in human immunodeficiency virus type 1-infected patients receiving highly active antiretroviral therapy. J Virol. 2002;76:2634–40.
32. Gill J, Bourboulia D, Wilkinson J, et al. Prospective study of the effects of antiretroviral therapy on Kaposi sarcoma-associated herpesvirus infection in patients with and without Kaposi sarcoma. J Acquir Immune Defic Syndr. 2002;31:384–90.
33. Bihl F, Mosam A, Henry LN, et al. Kaposi's sarcoma-associated herpesvirus-specific immune reconstitution and antiviral effect of combined HAART/chemotherapy in HIV clade C-infected individuals with Kaposi's sarcoma. Aids. 2007;21:1245–52.
34. Mosam A, Shaik F, Uldrick TS, et al. A randomized controlled trial of highly active antiretroviral therapy versus highly active antiretroviral therapy and chemotherapy in therapy-naive patients with HIV-associated Kaposi sarcoma in South Africa. J Acquir Immune Defic Syndr. 2012;60:150–7.
35. DeLeo AB, Jay G, Appella E, et al. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc Natl Acad Sci U S A. 1979;76:2420–4.
36. Borberg H, Sauerland D, Jaeckel I. The effect of pretreatment with Corynebacterium parvum on the growth of a chemical induced transplanted murine tumor. Dev Biol Stand. 1977;38:323–7.
37. Mukai K, Horimi T, Orita K. Eradication of syngeneic tumor (Meth A fibrosarcoma) from mice by adoptive immunotherapy of immunized spleen cells induced by Corynebacterium parvum-pyridine extract residue. Acta Med Okayama. 1988;42:301–10.

38. North RJ, Bursuker I. Generation and decay of the immune response to a progressive fibrosarcoma. I. Ly-1+2- suppressor T cells down-regulate the generation of Ly-1-2+ effector T cells. J Exp Med. 1984;159:1295–311.
39. Shu SY, Rosenberg SA. Adoptive immunotherapy of newly induced murine sarcomas. Cancer Res. 1985;45:1657–62.
40. Chou T, Bertera S, Chang AE, et al. Adoptive immunotherapy of microscopic and advanced visceral metastases with in vitro sensitized lymphoid cells from mice bearing progressive tumors. J Immunol. 1988;141:1775–81.
41. Shiloni E, Lafreniere R, Mule JJ, et al. Effect of immunotherapy with allogeneic lymphokine-activated killer cells and recombinant interleukin 2 on established pulmonary and hepatic metastases in mice. Cancer Res. 1986;46:5633–40.
42. Basse P, Herberman RB, Nannmark U, et al. Accumulation of adoptively transferred adherent, lymphokine-activated killer cells in murine metastases. J Exp Med. 1991;174:479–88.
43. Rosenberg SA, Lotze MT, Yang JC, et al. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Ann Surg. 1989;210:474–84. discussion 484-5.
44. Schwinger W, Klass V, Benesch M, et al. Feasibility of high-dose interleukin-2 in heavily pretreated pediatric cancer patients. Ann Oncol. 2005;16:1199–206.
45. Whelan JS, Bielack SS, Marina N, et al. EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. Ann Oncol. 2015;26:407–14.
46. Murray JL, Kleinerman ES, Cunningham JE, et al. Phase I trial of liposomal muramyl tripeptide phosphatidylethanolamine in cancer patients. J Clin Oncol. 1989;7:1915–25.
47. Kleinerman ES, Jia SF, Griffin J, et al. Phase II study of liposomal muramyl tripeptide in osteosarcoma: the cytokine cascade and monocyte activation following administration. J Clin Oncol. 1992;10:1310–6.
48. Meyers PA, Schwartz CL, Krailo M, et al. Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. J Clin Oncol. 2005;23:2004–11.
49. Nardin A, Lefebvre ML, Labroquere K, et al. Liposomal muramyl tripeptide phosphatidylethanolamine: targeting and activating macrophages for adjuvant treatment of osteosarcoma. Curr Cancer Drug Targets. 2006;6:123–33.
50. Meyers PA, Schwartz CL, Krailo MD, et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group. J Clin Oncol. 2008;26:633–8.
51. Chou AJ, Kleinerman ES, Krailo MD, et al. Addition of muramyl tripeptide to chemotherapy for patients with newly diagnosed metastatic osteosarcoma: a report from the Children's Oncology Group. Cancer. 2009;115:5339–48.
52. Pritchard-Jones K, Spendlove I, Wilton C, et al. Immune responses to the 105AD7 human anti-idiotypic vaccine after intensive chemotherapy, for osteosarcoma. Br J Cancer. 2005;92:1358–65.
53. Olmos D, Martins AS, Jones RL, et al. Targeting the insulin-like growth factor 1 receptor in Ewing's sarcoma: reality and expectations. Sarcoma. 2011;2011:402508.
54. Tolcher AW, Sarantopoulos J, Patnaik A, et al. Phase I, pharmacokinetic, and pharmacodynamic study of AMG 479, a fully human monoclonal antibody to insulin-like growth factor receptor 1. J Clin Oncol. 2009;27:5800–7.
55. Kurzrock R, Patnaik A, Aisner J, et al. A phase I study of weekly R1507, a human monoclonal antibody insulin-like growth factor-I receptor antagonist, in patients with advanced solid tumors. Clin Cancer Res. 2010;16:2458–65.
56. Mackall CL, Rhee EH, Read EJ, et al. A pilot study of consolidative immunotherapy in patients with high-risk pediatric sarcomas. Clin Cancer Res. 2008;14:4850–8.
57. Nair S, McLaughlin C, Weizer A, et al. Injection of immature dendritic cells into adjuvant-treated skin obviates the need for ex vivo maturation. J Immunol. 2003;171:6275–82.
58. Kawaguchi S, Tsukahara T, Ida K, et al. SYT-SSX breakpoint peptide vaccines in patients with synovial sarcoma: a study from the Japanese musculoskeletal oncology group. Cancer Sci. 2012;103:1625–30.
59. Zhang S, Cordon-Cardo C, Zhang HS, et al. Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides. Int J Cancer. 1997;73:42–9.
60. Carvajal R, Agulnik M, Ryan CW, et al. Trivalent ganglioside vaccine and immunologic adjuvant versus adjuvant alone in metastatic sarcoma patients rendered disease-free by surgery: a randomized phase 2 trial. J Clin Oncol. 2014;32:5s.
61. Dillman RO, Wiemann M, Nayak SK, et al. Interferon-gamma or granulocyte-macrophage colony-stimulating factor administered as adjuvants with a vaccine of irradiated autologous tumor cells from short-term cell line cultures: a randomized phase 2 trial of the cancer biotherapy research group. J Immunother. 2003;26:367–73.
62. Mahvi DM, Shi FS, Yang NS, et al. Immunization by particle-mediated transfer of the granulocyte-macrophage colony-stimulating factor gene into autologous tumor cells in melanoma or sarcoma patients: report of a phase I/IB study. Hum Gene Ther. 2002;13:1711–21.
63. Dagher R, Long LM, Read EJ, et al. Pilot trial of tumor-specific peptide vaccination and continuous infusion interleukin-2 in patients with recurrent Ewing sarcoma and alveolar rhabdomyosarcoma: an inter-institute NIH study. Med Pediatr Oncol. 2002;38:158–64.
64. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29:917–24.
65. Ahmed N, Brawley VS, Hegde M, et al. Human epidermal growth factor receptor 2 (HER2) -specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol. 2015;33:1688–96.
66. Maki RG, Jungbluth AA, Gnjatic S, et al. A pilot study of anti-CTLA4 antibody ipilimumab in patients with synovial sarcoma. Sarcoma. 2013;2013:168145.
67.•• D'Angelo SP, Shoushtari AN, Agaram NP, et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol. 2014;100:199–204. **This study is one of the studies examining the presence of tumor-infiltrating lymphocytes as well as PD-L1 expression in sarcoma tumors.**
68.•• Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371D23:2189–99. **This important translational study highlights the differences between frequency of mutations and neoepitope signatures in melanoma patients that responded to ipilimumab versus those patients who did not respond. This study revolutionizes how we will write future immune checkpoint inhibitor trials with improved patient selection**.
69.•• Kim JR, Moon YJ, Kwon KS, et al. Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One. 2013;8:e82870. **This article highlights both frequency of PD-1 positive tumor infiltrating lymphocytes TILs and PD-L1 tumor expression in a cohort of mixed sarcoma subtypes. PD-1 positive TILs and PD-L1 tumor expression correlated with poorer overall and event free survival, as well as, more aggressive tumor features.**
70. Shen JK, Cote GM, Choy E, et al. Programmed cell death ligand 1 expression in osteosarcoma. Cancer Immunol Res. 2014;2:690–8.
71. Wei S, Shreiner AB, Takeshita N, et al. Tumor-induced immune suppression of in vivo effector T-cell priming is mediated by the

B7-H1/PD-1 axis and transforming growth factor beta. Cancer Res. 2008;68:5432–8.
72. Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res. 2012;72:917–27.
73. Burgess MA, Crowley, J Tawbi, H. SARC 028: A phase II study of the anti-PD1 antibody pembrolizumab (P) in patients (Pts) with advanced sarcomas. J Clin Oncol 33, 2015
74. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23–34.
75.•• Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515:568–71. **This article demonstrates a predictive biomarker model utilitizing CD8+ T cell infiltrating lymphocytes with anti-PD-1 therapy in patients with melanoma.**
76. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol. 2011;8:151–60.
77. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366:925–31.
78. Sharma A, Bode B, Studer G, et al. Radiotherapy of human sarcoma promotes an intratumoral immune effector signature. Clin Cancer Res. 2013;19:4843–53.
79. Voron T, Colussi O, Marcheteau E, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. J Exp Med. 2015;212:139–48.
80. Kim T, Amaria RN, Spencer C, et al. Combining targeted therapy and immune checkpoint inhibitors in the treatment of metastatic melanoma. Cancer Biol Med. 2014;11:237–46.
81. Hu-Lieskovan S, Robert L, Homet Moreno B, et al. Combining targeted therapy with immunotherapy in BRAF-mutant melanoma: promise and challenges. J Clin Oncol. 2014;32:2248–54.
82.• Wolchok JD, et al.: Efficacy and safety results from a phase III trial of nivolumab (NIVO) alone or combined with ipilimumab (IPI) versus IPI alone in treatment-naive patients (pts) with advanced melanoma (MEL) (CheckMate 067). J Clin Oncol 33, 2015. **This study was the first to show that combined immune checkpoint blockade with anti-PD-1 and anti-CTLA-4 in melanoma patients shows good tolerability and impressive activity.**
83.• Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369D2: 122–33. **This phase I trial examined the combination of the anti-CTLA-4 antibody, ipilimumab, and the anti-PD-1 antibody, nivolumab in patients with advanced melanoma. With a manageable side effect profile, the combination produced durable responses with over half of the patients having objective responses of 53%**.