Chapter 6
Immune Therapy for Sarcomas

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Abstract Absolute lymphocyte count (ALC) recovery rapidly occurring at 14 days after start of chemotherapy for osteosarcoma and Ewing sarcoma is a good prognostic factor. Conversely, lymphopenia is associated with significantly decreased sarcoma survival. Clearly, the immune system can contribute towards better survival from sarcoma. This chapter will describe treatment and host factors that influence immune function and how effective local control and systemic interventions of sarcoma therapy can cause inflammation and/or immune suppression but are currently the standard of care. Preclinical and clinical efforts to enhance immune function against sarcoma will be reviewed. Interventions to enhance immune function against sarcoma have included regional therapy (surgery, cryoablation, radiofrequency ablation, electroporation, and radiotherapy), cytokines, macrophage activators (mifamurtide), vaccines, natural killer (NK) cells, T cell receptor (TCR) and chimeric antigen receptor (CAR) T cells, and efforts to decrease inflammation. The latter is particularly important because of new knowledge about factors influencing expression of checkpoint inhibitory molecules, PD1 and CTLA-4, in the tumor microenvironment. Since these molecules can now be blocked using anti-PD1 and anti-CTLA-4 antibodies, how to translate this knowledge into more effective immune therapies in the future as well as how to augment effectiveness of current interventions (e.g., radiotherapy) is a challenge. Barriers to implementing this knowledge include cost of agents that release immune checkpoint blockade and coordination of cost-effective outpatient sarcoma treatment. Information on how to research clinical trial eligibility criteria and how to access current immune therapy trials against sarcoma are shared, too.

Keywords Sarcoma • Absolute lymphocyte count • Lymphopenia • Mifamurtide • CAR T cells • PD1 • CTLA-4 • Immune checkpoint blockade • (Mal)adaptive immune response • Glutamine disaccharide (Healios)

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Table 6.1 Immune function (lymphocytes) and sarcoma survival

Parameter	Observation	Reference
ALC[a]	ALC >500, then better EWS[b] survival	[2, 3]
On d14 after		
Initial cycle of chemotherapy	ALC >800, then better osteosarcoma survival	[4, 5]
Lymphopenia at diagnosis	Significantly decreased survival	[1]
PMN/Lymph ratio	High PMN/Lymph ratio has worse survival in STS[c]	[6]

ALC absolute lymphocyte count, *EWS* Ewing sarcoma, *STS* soft tissue sarcoma

6.1 Background

Lymphopenia is frequent in advanced cancers including advanced soft tissue sarcomas and has been associated with poor survival (5 vs 10 months; $p < 0.01$; Ref. [1]). Better lymphocyte recovery or resilience after starting chemotherapy for Ewing sarcoma or osteosarcoma is also predictive of better survival [2–5]. The higher pretreatment neutrophil to lymphocyte ratio predicts a worse prognosis; conversely, more lymphocytes (i.e., a lower neutrophil:lymphocyte ratio) were associated with significantly better survival ($p < 0.05$) for patients with soft tissue sarcomas [6].

So if immune function contributes to better survival, how can this be realized? The promise and prospect of having increased immune response for not only destruction of existing macroscopic >3 mm deposits seen on imaging, but also for therapy of micrometastases and surveillance to prevent recurrences has been recently reviewed for childhood sarcomas [7]. This chapter applies to patients with sarcomas of all ages (Table 6.1).

6.2 Factors Influencing Immune Function

Medical and physical (local control) treatments for sarcoma can contribute to immune dysfunction. A recent randomized trial of epidural versus general versus combined epidural + general anesthesia for osteosarcoma limb salvage surgery showed the combination as associated with more prompt recovery of t-lymphocyte subsets and restoration of immune function [8]. Chemotherapy and radiation commonly are associated with lymphopenia. The severity of lymphopenia associated with therapy has significantly inferior outcomes for a variety of cancers including pancreatic adenocarcinoma ($p = 0.001$; Ref. [9]). In patients with newly diagnosed solid tumors, >40% developed severe and persistent treatment related lymphopenia (TRL) within 2 months; TRL was associated with poor survival (HR 2.1; $p < 0.0001$; Ref. [10]). Commonly used cytotoxic agents used against sarcomas which are associated with immune suppression and lymphopenia include alkylators (cyclophosphamide, ifosfamide, cisplatin), anthracyclines (doxorubicin), taxanes (docetaxel), and vincristine. Dexamethasone is also often used as a short 1–3 day "pulse" to

counteract acute side effects of chemotherapy including nausea and anaphylactoid reactions to docetaxel.

Because of location and difficulty in achieving complete resection with adequate margins, radiation is a commonly used modality in the treatment of high-grade soft tissue sarcomas (6308/10,290)—and is associated with significantly improved survival compared to the no radiotherapy group ($p < 0.001$; Ref. [11]). However, radiotherapy (RT) is associated with lymphopenia and galectin-1 secretion by tumors [12, 13]. Sometimes lymphopenia related to radiation is long-lasting. Galectin-1, a potential mediator of radiation-induced lymphopenia, can be detected in blood. Research detailing effect of location, dose, and schedule of radiation associated with galectin-1 may be instructive.

6.3 Cytokines and Inflammation

Cytokine action is most effective at short distances and regionally. However, if "supra-physiologic doses" of a cytokine such as IL-2, G-CSF, GM-CSF, or erythropoietin are given repeatedly and/or using long-acting formulations, inflammatory effects associated with white blood cell proliferation and activation may possibly become counterproductive. This is because of recent evidence showing that inflammation contributes to an "adaptive immune response," the production of PD1 and CTLA-4 [14–19]. Programmed cell death ligand (PD-L1) and PD-1 interaction is the immune system's checkpoint to decrease potential autoimmune "off-target" effects. In sarcomas, there is evidence of variable expression of both tumor infiltrating lymphocytes with PD-L1 expression and PD1 in the tumor microenvironment [20, 21]. One could hypothesize that if inflammation occurs in a tissue harboring sarcoma micrometastases such as lung, this could potentially be counterproductive. Interestingly, it appears that metastatic, but primary osteosarcomas express PD-L1 [22]. Figure 6.1 illustrates how inflammation including iatrogenic inflammation (surgery, radiation, chemotherapy) may contribute towards less immune function for the control of sarcomas during current therapy as well as new agents to block immune checkpoint inhibitory molecules.

6.4 Nutrition and Immune Function: Glutamine Appears to be a Key Player

Nutrition can contribute toward better or worse immune function. The major fuel for both lymphocytes and enterocytes is glutamine. Catabolic situations (poor appetite, nausea, NPO for medical procedures) lead towards a "glutamine shuttle" in which muscle must produce glutamine to maintain enteral health and immune function. Glutamine-enriched diets support muscle glutamine metabolism without

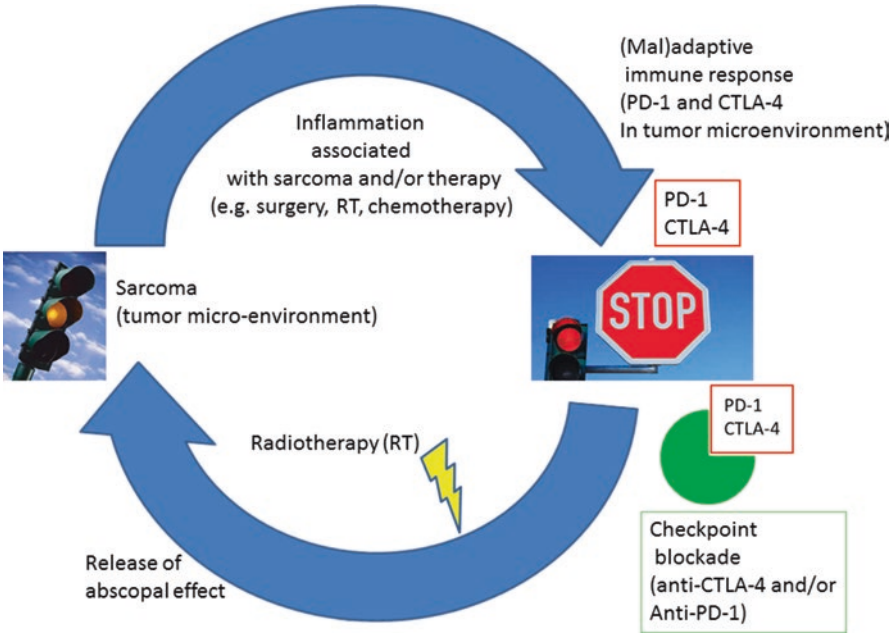

Fig. 6.1 Paradigm of inhibition of immune via inflammation from interventions and tumor growth check immune function versus release of checkpoint inhibition by anti-PD-1 and/or anti-CTLA-4 (checkpoint blockade) to facilitate abscopal (out-of–field) responses with radiation. Thus RT may possibly act like a "tumor vaccine"

stimulating tumor growth [23, 24]. Glutamine can accelerate healing of small intestine and improve outcome after radiation including whole abdominal radiation [25–28]. Elegant studies by Klimberg's group [29, 30] have shown that not only does glutamine improve tolerance of chemotherapy but may also improve methotrexate efficacy. Glutamine is particularly effective in reduction of stomatitis and oral, pharyngeal, and esophageal mucositis if it is in a suspension with a disaccharide that facilitates mucosal absorption [31–33]. A powder containing glutamine and trehalose is now commercially available (Healios). Concerns about glutamine "feeding the tumor" were not born out using a genetically engineered mouse model in which mice routinely developed cancer, glutamine supplementation did not "feed the tumor"; supplementation was associated with upregulation of p53 signaling, inhibition of Akt, lower levels of IGF-1R, and higher levels of PTEN and mdm-2 proteins [34]. Lim et al. showed glutamine supplementation prevented DMBA-induced squamous cell cancers [35]. Thus better nutrition which could include glutamine supplementation may not only reduce chemotherapy-associated toxicity, but also may result in a favorable therapeutic index against cancer [36–39]. Finally, oral glutamine could reduce radiation morbidity in breast conservation [40]. Whether a similar result could be obtained after pre-op radiation for sarcomas remains to be determined.

Iatrogenic factors that contribute to inflammation are many. Chemotherapy alone is an ineffective approach to control osteosarcoma [41] and other sarcomas except GIST. Although chemotherapy may become the main therapeutic intervention for months before or after surgery, chemotherapy cycles can be associated with repeated bouts of poor appetite, catabolic states, and inflammation (e.g., mucositis, enteritis, skin toxicity). C-reactive protein (CRP), a biomarker of inflammation, is associated with the diagnosis, prognosis, and causes of cancer [42]. Surgery also invariably elicits an inflammatory response. Elevated CRP before sarcoma surgery has been associated with decreased survival in patients with soft tissue sarcoma and bone sarcomas including chondrosarcoma, osteosarcoma, and Ewing sarcoma [43–47].

CRP level has been recently correlated with failure-free survival after prostate cancer radiotherapy [48]. Inflammation from radiation is also "part of the package deal" of an adequate local control plan for sarcoma. It appears that radiotherapy is a mixed blessing for sarcoma control. Sharma et al. found that radiotherapy of human sarcoma promotes an intratumoral immune effector signature [49]. Although radiotherapy (RT) is recommended for large, deep, high-grade soft tissue sarcomas, only 6308 of 10,290 soft tissue sarcoma patients received RT. Lack of RT was associated with lower long-term survival ($p < 0.001$; Ref. [11]). Similarly, in metastatic Ewing sarcoma, patients that received adequate local control, especially those with both RT and surgery had better outcomes [50].

With chemotherapy and radiation, there may be tumor evolution to become resistant to apoptosis that is chemotherapy-related, but also to evade immune surveillance (e.g., loss of HLA expression, loss of antigen expression, and/or selection for more stem cell-like phenotype such as aldehyde dehydrogenase expression [51–55]).

6.5 Current Sarcoma Treatment Paradigm

In order to successfully eliminate sarcoma stem cells, local control measures remain the cornerstone for elimination of primary and metastatic disease. Local control measures can be thought of as "physical" and include surgery, RT, heat (radiofrequency ablation, RFA), freezing (cryoablation), and electric current (electroporation).

Control of sarcoma micrometastases has relied on antiproliferative agents in chemotherapy sensitive bone and soft tissue sarcomas [56–62] and now targeted tyrosine kinase inhibitors and agents including pazopanib [63–69].

If adjuvant therapy is actually eliminating all micrometastases or assisting the immune system by control of rapidly proliferating cells and buying time for immune system to finally effectively "mop-up" remaining non-proliferating cancer stem cells is a matter of conjecture. The following will summarize and detail only some of the immune approaches against sarcoma.

Regional therapies may not only kill tumor stem cells but also leave antigen in place to facilitate local and systemic immune responses [11, 70–87]. Cytokines and

macrophage activators act on different immune cells to facilitate more sustained and possibly effective immune responses [88–102]. Augmentation of immune response against sarcoma using antibodies has been tried against osteosarcoma and chondrosarcoma [103–118]. There have been "FANG" now known as VIGIL vaccine trials in Ewing sarcoma [119, 120] and NY-ESO vaccine has been used in sarcoma [121]. The above efforts are summarized in Table 6.2.

Perhaps the most complex, yet promising approach with potential for systemic immune surveillance against cancer involves transfer of immune cells with anti-sarcoma specificity. Table 6.3 summarizes some current investigational efforts (from clinicalTrials.gov and Ref. [103, 122–126]).

The final section of this chapter will describe the potential for RT to augment anti-sarcoma immune function. An abscopal response refers to an out-of-field effect of radiotherapy that is systemic, not just local [127]. Preclinical models and clinical observations using radiotherapy including stereotactic ablative radiotherapy have shown that PD-1 and/or CTLA-4 restrains radiotherapy-induced abscopal effects [13, 49, 128–132]. Perhaps the most elegant demonstration of the synergy of dual checkpoint blockade with anti-PD-1 + anti-CTLA-4 with RT was by Minn's group [133]. In this study both apparently durable complete responses including abscopal responses after RT in three different models systems were significantly better using dual checkpoint blockade with both anti-PD-1 and anti-CTLA-4 [133]. Thus it would appear that combining radiation and checkpoint inhibition may possibly become a new systemic therapy for solid tumors [127, 131, 132, 134–137]. Use of these agents in sarcomas is just beginning (Table 6.4). In 2015, there are no clinical trials of dual checkpoint inhibition and RT in sarcoma open yet. Thus, enhancing immune function within the current paradigm of RT may become an important part of a multidisciplinary approach towards sarcoma (Fig. 6.1).

6.6 Summary and Conclusion

Better immune function can improve sarcoma survival. Sarcoma experts and caregivers will need to become forward observers call in the most effective means to treat this group patients with rare cancers in a variety of locations. The future is to reconcile, translate, and integrate our knowledge that immune function is very important to survival from sarcoma with known benefit from surgery, chemotherapy, and radiotherapy (RT). This will result in new treatments and improved paradigms when developing sarcoma multidisciplinary plans.

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Table 6.2 Agents and therapies which affect immune function against sarcomas: physical means, activators, antibodies, and vaccines

Agent or therapy	Effects on immune function	References
Physical means		
Modality	Comment and reference(s)	
Surgery	Part of multidisciplinary approach [70]	
RFA	Feasible, may improve disease free survival but results in denatured tumor antigens [71–74]	
Electroporation	Seems effective in Kaposi sarcoma [75], nonthermal [76–78]	
Cryoablation	Tumor cell death and antigen preservation [79, 80]	
Ultrasound	Specialized equipment needed [81, 82]	
Radiotherapy (RT)	Preservation of tumor antigens and a common pre-op modality [11, 70, 85]; increase in size during RT does not affect prognosis [86]. Stereotactic body radiotherapy is a reasonable option for metastases [83, 84]. Immune response to RB1-regulated senescence limits radiation-induced osteosarcoma [87]	
Activators of immune function against sarcomas		
Activator	Mechanism and reference(s)	
GM-CSF + Furin	Macrophages increase and antigen presentation; furin decreases TGF-beta in vaccine microenvironment	
Aerosol GM-CSF	Aerosol decreases toxicity but was ineffective against osteosarcoma [88]	
G-CSF	Granulocyte increases; Ewing sarcoma expresses G-CSF and the receptor for G-CSF [89]	
IL-2	NK and T cell activation and proliferation against sarcoma [90, 91]	
	Works with NK cells as aerosol [92, 93]	
Mifamurtide	Macrophage activator requires prolonged schedule of administration for best effects [94–96]. L-MTP-PE phosphatidyl serine lipid is an address signal for "apoptosis" [102]; Improved osteosarcoma survival [97–102]	
Antibodies and fusion proteins against sarcomas		
Antibody	Disease, reference(s)	
Anti-GD2 antibody	Osteosarcoma [103–105]	
Anti-TP-3-PAP	Preclinical antibody x immunotoxin conjugate [106, 107]	
Apo2L/TRAIL	Possible activity in chondrosarcoma [108, 109]	
Denosumab	Giant cell tumor [110–112]	
Anti-IGF-1R	Ewing sarcoma, osteosarcoma, sarcoma [94, 113–118] NCT02306161	
Olaratumab	FDA approved for relapsed sarcomas. See also NCT02677116 and NCT02659020	
Vaccines		
Sarcoma	Antigen/adjuvant and reference(s)	
Ewing Sarcoma	bi-shRNAfurin and GM-CSF [119, 120]	
Sarcoma	NY-ESO+ dendritic cell [121]	

Table 6.3 Cellular therapy against sarcomas

Sarcoma type	Cells recognizing antigen(s)	Reference
Osteosarcoma	CAR T cells against GD2	NCT-02107963 (NCI)
Osteosarcoma	iC9-GD2-CAR-VZV-CTL T Cells	NCT-01953900 (Baylor/TCH)
Osteosarcoma	CAR T cells against Her-2	[103, 122–124]
Osteosarcoma	Activated T cells armed with GD2 x CD3 bispecific antibody	NCT 02173093
Synovial Sarcoma	TCR T cells against NY-ESO	[125, 126] NCT01343043 and TATCTASOM (NCT02239861)
Sarcoma	SCT and NK cells	NCT02100891
		NCT01847468
		NCT01287104
Sarcoma	Expanded, Activated NK cells	NCT02409576

Table 6.4 Clinical trials of checkpoint blockade in sarcomas: anti-PD1 and/or anti-CTLA-4 (November 2015)

Intervention(s)	Disease	ClinicalTrials.gov info
Nivo + ipi	Kaposi sarcoma	NCT02408861
		NCT02408861
Nivo +/− ipi	Metastatic or unresectable sarcoma (adults)	NCT02500797
Nivo +/− ipi	Recurrent or refractory sarcomas (younger patients)	NCT02304458
Nivolumab	Uterine leiomyosarcoma	NCT02428192
Ipi + dasatinib	GIST, stage IV soft tissue sarcoma	NCT01643278
Pem	Advanced sarcomas	NCT02301039
Pem + cyclophos	Advanced sarcomas	NCT02406781
Pem + Chemo	Advanced sarcomas	NCT02331251
Pem + p53 vaccine	Sarcoma	NCT02439263

Nivo Nivolumab (anti-PD-1), *Pem* Pembrolizumab (anti-PD-1), *Ipi*: Ipilimumab, (anti-CTLA-4)

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