OPEN ACCESS

GAVIN PUBLISHERS

Journal of Oncology Research and Therapy

Seetharam M, et al. J Oncol Res Ther: JONT-157.

DOI: 10.29011/2574-710X. 000057

Review Article

# Immunotherapy for Sarcomas-The Past, Present and Future

**Mahesh Seetharam[1*], Kantha R. Kolla[1], Sant Chawla[2]**

[1]Department of Oncology and Hematology, Mayo Clinic, Phoenix Arizona, USA

[2]Sarcoma Oncology Center, California, USA

[*]**Corresponding author:** Mahesh Seetharam, Department of Oncology and Hematology, Mayo Clinic, Phoenix Arizona, USA. Email: Seetharam.Mahesh@mayo.edu

**Citation:** Seetharam M, Kolla KR, Chawla S (2018) Immunotherapy for Sarcomas-The Past, Present and Future. J Oncol Res Ther: JONT-157. DOI: 10.29011/2574-710X. 000057

**Received Date:** 25 April, 2018; **Accepted Date:** 11 May, 2018; **Published Date:** 21 May, 2018

## Abstract

Immunologic approach to cancer management has evolved significantly over past century. Beginning from old practice of provoking infection to inhibiting negative regulatory pathways, immunotherapeutic approach has undergone dramatic changes. Starting from Coley's toxin to cytokine-based therapies, adoptive cell therapy and targeting immune checkpoints are some of the actively pursued immunological strategies. Results from studies conducted in metastatic melanoma and renal cell carcinoma, paved way for their approval in those cancers and also generated interest in other cancers including sarcomas. In this review article, we focus on mechanism of immune system and their role in health and disease focusing on cancers. We also review the preclinical and clinical studies pertaining to cytokine based therapies, role of vaccine therapies, adoptive cell therapy and immune checkpoint inhibitors in the management of bone and soft tissue sarcomas.

**Keywords:** Checkpoint Inhibitors; Immunotherapy; Soft Tissue Sarcomas; Osteosarcoma; Vaccine Therapy

## Abbreviations

| | | |
|---|---|---|
| DC | : | Dendritic Cell |
| TCR | : | T Cell Receptor |
| MHC | : | Major Histocompatibility Complex |
| MDSC | : | Myeloid Derived Suppressor Cell |
| Treg | : | Regulatory T Cell |
| TAM | : | Tumor Associated Macrophage |
| TGF-beta | : | Transforming Growth Factor Receptor-Beta |
| CTLA-4 Protein-4 | : | Cytotoxic T- Lymphocyte Associated |

## Introduction

Sarcomas accounts for < 1% of adult solid malignant tumors and about 21% of pediatric solid malignant tumors [1]. Sarcomas can be classified into 2 categories: Soft Tissue Sarcomas (STS) and bone sarcomas. Incidence of sarcomas is 2-4 people/ 100,000 population. Incidence ratio of bone sarcomas to STS is 1:4 [2]. Median age of diagnosis of STS is 59 years with bimodal distribution peaking in the 5$^{th}$ and 8$^{th}$ decades. Extremities are the most commonly involved structures among STSs, accounting for 40% of all STSs, with more predominance in lower limbs (28%) than upper limbs (12%). Size, increasing age, high grade, presence of local recurrence or metastasis at the time of diagnosis, positive surgical margins are the adverse prognostic factors for STSs [3,4]. According to National Cancer Data Base of the American College of Surgeons, the relative 5 years' survival rate is 53.9% for osteosarcoma; 75.2 % for chondrosarcoma; 50.6% for Ewings sarcoma; 66% for patients with bone and STS [5]. The median overall survival is around 15 months in metastatic or recurrent locally advanced sarcoma patients [6].

In 1891, Dr. William Coley reported a case of unresectable small cell sarcoma of the neck. The patient's sarcoma completely regressed after he had severe episode of erysipelas. It was hypothesized that the systemic response against erysipelas might have led to the regression of patient's tumor. Later, it was proposed that erysipelas activated innate immunity through Toll-like receptors, which was followed by activation of acquired immunity specific against sarcoma. Over the last century, Coley's work influenced many scientists to work in the field of cancer immunology [7,8].

In a study conducted on patients with osteosarcoma, it was found that 10-year survival in patients with osteosarcoma with infection was 84.5% Vs 62.3% in patients with osteosarcoma without infection. These findings imply correlation between deep postoperative infection and survival rate in osteosarcoma patients [9,10]. Applying cancer immunotherapies for treating patients with sarcomas had begun with cytokine therapies in 1980s with significant advances in the recent few years with adoptive immunotherapy [11].

A prospective study was conducted on patients with HIV and Kaposi sarcoma who received HAART therapy to evaluate the immune response. After a 52 weeks of follow up on HAART therapy, decrease in both HIV-1 and Kaposi Sarcoma (KS) titres. Also seen was increase in CD4+ T cells [12,13] Table 1.

| | TAA | Comment |
|---|---|---|
| Protein products | SYT-SSX fusion protein | Synovial sarcoma |
| | p53, erbB2, MDM2, c-KIT | Sarcoma associated with Li Fraumeni syndrome |
| Viral antigens | HHV8 | Kaposi sarcoma |
| Chromosomal translocations | t(X:18)(p11;q11) | Synovial sarcoma |
| | t(11;22)(q24;12) | Ewing sarcoma |
| Cancer testis antigens | NY-ESO1 | Synovial sarcoma |

**Table 1:** Sarcoma Tumor Associated Antigens (TAA) [14].

## Immune System

Immune responses include innate and adaptive immune responses. Various types of cancer testis antigens, which act as potential targets that induce cytotoxic immune responses, have been recognized in sarcoma patients [14]. Phagocytic cells (neutrophils and macrophages) and natural killer cells are the main components of innate immune system [15]. They provide initial defense against invading microbes [16]. Cytokines, which are small molecular proteins, dendritic cells and macrophages play critical role in innate immunity [17]. T and B lymphocytes are the key components of adaptive immunity [16]. Adaptive immunity includes humoral and cell-mediated immunity. T lymphocytes recognize short peptides as antigens which are presented by Major Histocompatibility Complexes (MHCs) on the cell surface of dendritic cells [18]. Following antigen recognition, signal from CD40 or Toll Like Receptors (TLRs) activate dendritic cells, and this process then leads to activation of naïve CD8 T cells to effector and memory CD8 T cells [19]. Macrophages, dendritic cells and B cells act as Antigen-Presenting Cells (APCs) [20]. B cells and macrophages present endogenous and internalized exogenous antigens with MHC I and MHC II molecules respectively and thus, B cells and macrophages possess ability to activate CD4 T cells when they internalize extracellular antigens [21,22]. Dendritic cells present both endogenous and exogenous antigens with MHC class I molecules to activate CD8 T cells. This called as cross-presentation, is required to defend against virus infected cells and tumor cells [23] **(**Figure 1**)**.

Cancer Testis Antigens (CTAs), melanocyte differentiation antigens, mutated proteins, overexpressed proteins, and viral antigens are the common tumor antigens recognized by immune system [24].

### Immune Inhibitory Mechanisms (Immune Checkpoints)

CTLA-4, a protein receptor expressed on T cells, has a structure similar to CD28 and thus competes with CD28 to bind to CD80/86 on dendritic cells and leads to down regulation of T cell activation [25,26]. Regulatory T cells (Tregs) define $CD4^{+}CD25^{+}Foxp3^{+}$ T cells and via high expression of CTLA-4 suppress activation of cytotoxic lymphocytes [27]. Programmed cell death protein 1 (PD 1), an immune checkpoint receptor is highly expressed on cytotoxic T lymphocytes [28]. Tumor cells stimulate the expression of PD-Ligand 1(PD-L1). The association of PD1 with PD-L1 downregulates the function of T cells [29].

Tim-3 and LAG3 are the cell surface molecules of activated effector T cells, that downregulate tumor immunity [30]. Inflammation in the tumor microenvironment or genetic alterations leads to activation of STAT3 [31]. STAT3 activation stimulates tumor cells and Tregs to express immune checkpoint molecules such as PD-L1, that results in inhibition of T cell activity [32] Figure 1.

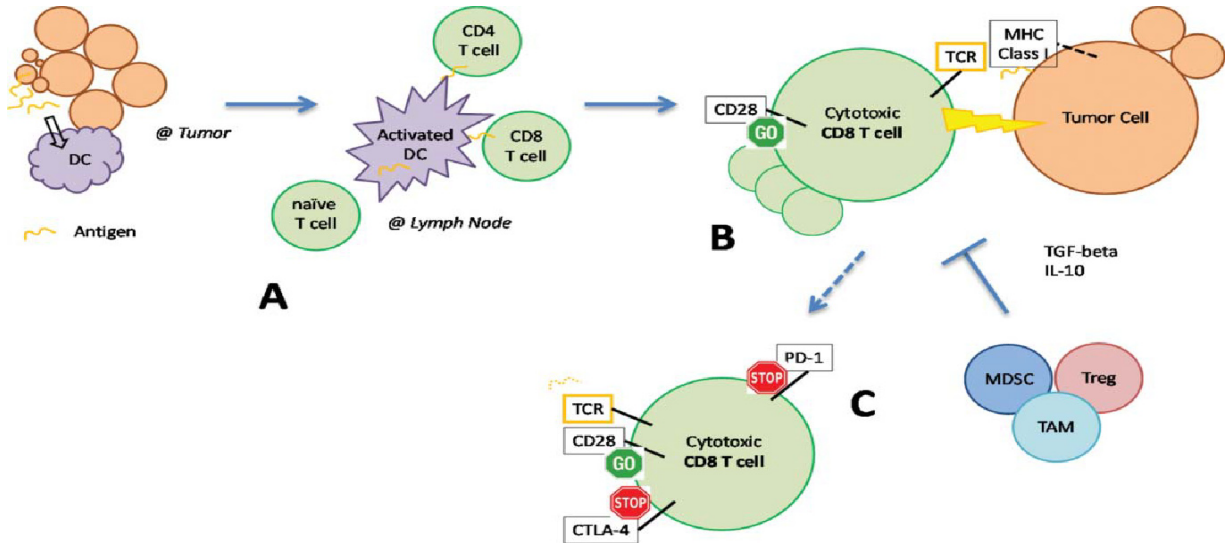


**Figure 1:** Showing adaptive immune response to cancer and mechanisms which inhibit the anti-tumor immune response. Immunotherapeutic strategies enhance various components of the immune response: **(A)** cancer vaccines manipulate DCs to enhance antigen presentation, **(B)** adoptive cell therapy utilizes expanded populations of cytotoxic T cells, **(C)** immune checkpoint blockade removes the brakes (CTLA4, PD-1) on T cells to maintain their activation status and cytotoxic function [33].

## Preclinical Strategies

**I.** Fusion proteins: Fusion proteins are generated by joining two or more genes that originally code for separate proteins. They usually occur as a result of chromosomal translocations. Though there may be tolerance to epitopes of separate proteins, the fusion area acts as a foreign antigen. This can act as a target to natural immune response or to the immune response induced through immunotherapy. Synovial Sarcoma (SS), Clear Cell Sarcoma (CCS), and Desmoplastic Small Cell Tumor (DSRCT) are some of the soft-tissue malignancies that contain fusion proteins which occur as a result of chromosomal translocations that fuse 5' region of one gene with the 3' region of another gene. The fusion breakdown sequences associated with CCS, SS, and DSRCT may act as tumor-specific neoantigens. In-vitro studies were conducted to test this hypothesis, in which peptides related to fusion breakpoints were designed and looked for their ability to bind to class I HLA molecules. Results showed specific binding of two SS breakpoint peptides with HLA-B7 antigen and binding of a SS peptide and a CCS peptide to HLA-B27 molecule. A peptide from DSRCT breakpoint was bound to HLA-A3 molecule. These findings suggest that sequences in the fusion region of sarcomas have ability to bind to class I HLA molecules and can act as neoantigens. Thus, these may serve as a source to develop novel immunotherapies to tumors bearing these translocations and for sarcoma patients with appropriate HLA molecules [34].

**II.** Cytokine induced killer cells: Cytokine Induced Killer (CIK) cells are a class of immune effector cells that possess mixed T and natural killer (NK) cell phenotypes. Due to inability to eliminate chemoresistant Cancer Stem-Like Cells (sCSC), which cause relapses and drug resistance, unresectable metastatic sarcomas become incurable. A preclinical study was conducted to evaluate the efficacy of cytokine induced killer cells against autologous sarcomas, including putative sCSCS. Tumor killing against autologous sarcomas was evaluated both *in vitro* and within an immunodeficient mouse model. Results from *in vitro* studies showed lysis of autologous and allogeneic sarcoma cells by CIK cells. Results from *in vivo* studies showed infiltration of tumors by CIK cells and also inhibition of tumor growth by CIK cells, when compared to control animal group. Thus, these results suggest CIK cells as a novel class of immunotherapy for use in metastatic disease [35].

**III.** Tuftsin: Tuftsin is a tetrapeptide fraction of immunoglobulin G. it binds to neutrophils and macrophages and stimulates their phagocytic activity. A preclinical study was conducted on Swiss albino mice having fibrosarcoma to evaluate the immunomodulator effect of tuftsin on antitumor activity of etoposide. Results showed that tuftsin-bearing liposomized etoposide (Tuft-Lip-ETP) significantly reduced tumor volume, upregulated the expression of p53wt and also delayed tumor growth. These results suggest that drug loaded liposomes when incorporated with tuftsin can be treatment option for

various cancers such as in fibrosarcoma [36].

**IV.** Imatinib in GIST: Imatinib mesylate, a tyrosine kinase inhibitor, targets mutated KIT oncoproteins and elicits 80% clinical response in GIST (gastrointestinal stromal tumors) patients. A preclinical study was conducted in mouse models with GIST. Imatinib reduced tumor cell expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (Ido), which resulted in activation of CD8+ T cells and apoptosis of regulatory T cells (Tregs). Immunosuppressive enzyme Ido (indoleamine 2,3-dioxygenase), causes the development, stabilization, and activation of T-regs and suppresses effector T cells. Ido is usually produced by tumor cells and dendritic cells [37]. Simultaneous immunotherapy enhanced the effectiveness of imatinib in mouse GIST. Thus these results demonstrate that concurrent immunotherapy may enhance outcomes in cancers treated with targeted agents [38].

**V.** Tumor associated macrophages: Macrophages can be classified as either classically (M1; proinflammatory and secrete TNF and IL-12) or alternatively (M2; anti-inflammatory and secrete IL-10) activated. A preclinical study was conducted using spontaneous mouse model having GIST and freshly procured human GISTs. It was found that imatinib (KIT oncoprotein inhibitor) induced a process where TAM interaction with apoptotic tumors cells led to induction of CCAAT/Enhancer Binding Protein (C/EBP) transcription factors. This resulted in polarization of TAM towards M2 like phenotype. In human GISTs which eventually developed resistance to imatinib, it was found that TAMs reverted to an M1-like phenotype and expressed similar gene profile as TAMs from untreated human GISTs. Thus, polarization of TAM depends on activity of tumor cell oncogene and implies important role in immunotherapeutic strategies in human cancers [39,40].

**VI.** Synergy between immune inhibitory molecules PD-1 and LAG-3: Inhibitory receptors such as CTLA-4, PD-1 and LAG-3 play a pivotal role in immune escape mechanisms in cancers. Preclinical studies were done in mice to evaluate the interaction between inhibitory pathways. It was found that dual anti-LAG-3/anti-PD-1 antibody treatment resulted in better outcomes compared to single antibody treatment in most of the established tumors. Lag3 (-/-) Pdcd 1 (-/-) mice demonstrated significant increased survival. These results suggested a strong synergy between PD-1 and LAG-3 inhibitory pathways and suggests dual blockade as a promising combination strategy for cancer treatment [41].

**VII.** Impact of radiotherapy on immune mechanisms: A retrospective analysis was conducted before and after radiotherapy using quantitative reverse transcription PCR analysis and immunohistochemistry on paired formalin-fixed paraffin-embedded tumor samples from 35 sarcoma patients. Results showed that radiotherapy induced upregulation of several immune effectors and cancer-testis antigens and also downregulation of immune suppressors. These findings suggest designing of newer therapeutic regimens by combining standard radiotherapy with immunotherapeutic agents [42].

**VIII.** Cancer-associated antigen(s) in urine of sarcoma patients: In the studies conducted by Francesctti et al, tumor-associated antigens were detected in concentrated and dialyzed urine of sarcoma patients with high tumor burden. It was also found that the antigenic activity reappeared before tumor recurrence. Thus, urine from cancer patients with large tumor burden may act as a source of tumor-associated antigen and may also play a role in detecting subclinical tumor recurrence [14].

**IX.** Synergistic effect of TNF-alpha microspheres and IL-12: preclinical studies using splenocytes from MCA205 bearing mice were treated with IL-12 and TNF-alpha PLAM (poly-lactic acid microspheres). Results showed local and sustained release of IL-12 and TNF-alpha using PLAM synergistically activated NK and cytotoxic T-cell response. As per the authors, intratumoral TNF-α and IL-12 could be a therapeutic option along with benefit to overcome toxicities associated with systemic delivery [43].

**X.** Impact of combination of mixed heat shock protein/peptide vaccine and cyclophosphamide plus interleukin-12 in sarcoma: Heat Shock Proteins (HSP) are produced by cells as a response to exposure to stressful conditions. They can induce innate and adaptive immune responses. A preclinical study was conducted on mice to evaluate the antitumor activity of mixture of HSP/Ps (mHSP/Ps, HSP60, HSP70, Gp96 and HSP 110) enhanced with cyclophosphamide (CY) and interleukin-12 (IL-12). Results from mice vaccinated with enhanced vaccine (mHSP/Ps and CY plus IL-12) showed 80% tumor regression and long-term survival. This suggests that the combination regimen could be a promising therapeutic option for tumor treatment [44].

**XI.** Syngeneic Monoclonal Antibodies (MoAb): Syngeneic Monoclonal Antibodies (MoAb) were generated by fusing spleen cells from MSC regressor mice to myeloma SP2/0. Antibodies were given to animals with established tumors. It was found that antibodies given to animals with established tumors resulted in prolonged life span. Whereas, antibodies given to animals with very large tumor burdens did not result in significant prolongation in life span [45].

**XII.** The impact of hyperthermia and immunotherapy was evaluated in mice having MCA 105 sarcomas. Mice received whole body hyperthermia alone, immunotherapy using tumor sensitized lymphocytes or immunotherapy plus whole body

hyperthermia. Results showed significant reduction in growth of primary extremity sarcomas and pulmonary metastasis as a result of hyperthermia [46].

**XIII.** In another animal model, mice bearing fibrosarcoma (MCA 105 and 106 tumors) were treated with adoptive transfer of spleen cells from immunized mice. This resulted in regression of established tumor [47].

**XIV.** Another study was done to evaluate presence of sarcoma antigens in serum and urine. The study was conducted using micro complement fixation test to demonstrate antigenic activity of urine and serum of sarcoma patients. The urine and serum samples from sarcoma patients showed positive reactivity against autologous and allogeneic sarcoma serum, whereas no activity was noticed in samples from healthy volunteers [48].

## Immunotherapeutic Strategies in Sarcoma:

### (a) Cytokine Based Immunotherapy

High dose IL-2 is an approved treatment for metastatic melanoma and renal cell carcinoma [49]. Interleukin-2 causes activation and expansion of CD4 and CD8 T cells. A prospective study was conducted in pediatric patients with progressive and metastatic solid tumors (2 had Ewing's sarcoma, 4 patients had metastatic osteosarcoma), who received IL-2 therapy. At a median follow up of 28 months, 2 patients with osteosarcomas had complete response. Adverse events included fatigue, anorexia, diarrhea [50].

In an adjuvant trial conducted by Muller et al, patients with high grade osteosarcoma were given semi-purified, leukocyte Interferon-α (IFN-α), as adjuvant therapy. At median follow up of 12 yrs, the 10 year metastases free and sarcoma specific survival rates were 39% and 43% respectively, suggestive of beneficial effect of IFN-α as adjuvant therapy in high grade osteosarcoma [51].

A phase II study was conducted in 20 patients with advanced bone sarcomas, to evaluate the efficacy of human IFN-α therapy. 3/20 patients demonstrated partial response. Fever, anorexia, myalgia was the commonly noted adverse events [52].

### (b) Muramyl Tripeptide

Liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE or Mifamurtide), when given along with chemotherapeutic agents, acts as an immunostimulatory agent in adjuvant and metastatic settings [53]. Mifamurtide, a liposomal muramyl tripeptide phosphatidyl ethanolamine is a synthetic analog of muramyl dipeptide. Recognition of L-MTP-PE by intracellular pattern recognition molecule NOD2 (nucleotide binding oligomerization domain containing protein-2) results in production of IL-1β, IL-6 and TNF-α through the activation of NF-Kβ signaling in monocytes and macrophages [54].

MTP targets tumors that metastasize to tissues having high levels of macrophages. Sarcomas exhibit tendency to metastasize to lungs and thus are considered suitable targets for muramyl tripeptide therapy.

A phase II trial was conducted in patients with relapsed osteosarcoma, to evaluate the efficacy of LMTP-PE. Infusion of LMTP-PE resulted in increased TNFα and IL-6 supportive of the biologic activity and immune stimulation effect in patients with osteosarcoma [55].

In a randomized clinical trial, newly diagnosed patients with osteosarcoma were randomly assigned to receive MAP alone (cisplatin, doxorubicin and methotrexate), MAP+IFO (ifosfamide), MAP+LMTP-PE and MAP+IFO+L-MTP-PE. Results found that addition of L-MTP-PE to chemotherapy resulted in improved 6-year overall survival rate 70% to 78% (p=0.03) (HR: 0.71) (95% CI: 0.52-0.96) [56].

### (d) Vaccine

Cancer vaccines work by stimulating patients' own immune system to act against tumor. Sarcomas express wide diversity of immunogenic proteins and antigens such as cancer testis antigens family (NYESO-1, MAGE-A3, PRAME, LAGE-1), gangliosides (GM2, GD2 and GD3), sarcoma specific fusion proteins (SSX, FOX01, EWSR1 and TLS CHOP), and heat shock proteins [57,58]. This makes sarcomas, an ideal target for vaccine therapies [59,60,61]. Numerous vaccine strategies targeting the above mentioned antigens, tumor lysate, dendritic cells pulsed with antigens and heat shock proteins have been developed [62,63]. Common sarcoma specific fusion proteins are SYT-SSX, FOX01, EWSR1, TLS CHOP, whereas NYESO-1, SSX2/3, MAGE, GAGE and WTI were considered as partially specific fusion proteins [64]. It was found that tumor Ag expression is more frequently seen in high grade late stage tumors. NY-ESO-1 is commonly expressed in synovial sarcoma, osteosarcoma and leiomyosarcoma. LAGE-1 (cancer testes antigen) is commonly seen in leiomyosarcoma, liposarcoma and synovial sarcoma [65].

Gangliosides GM2, GD2 and GD3 are the most abundantly expressed gangliosides in sarcomas, and thus act as attractive targets for vaccine therapy [58]. Synovial sarcomas contain t(x;18), which represents fusion of SYT (at 18q11) with either SSX1 or SSX2 [65,66]. Cancer vaccines are usually combined with co-stimulatory adjuvants like GM-CSF or IL-2 to enhance the immune system [59] Table 2-5.

| | |
|---|---|
| MAGE-A | CT16 |
| mMAGE_B | CT17 |
| BAGE | MMA-I |
| GAGE-A | CAGE |
| SSX-2 | SAGE |
| NY-ESO-1 | CT10 |
| SCP-1 | HOM-TES-85 |

**Table 2:** List of Cancer Testis Antigens [65].

| | |
|---|---|
| NY-ESO | Synovial, myxoid round cell liposarcoma, uterine leiomyosarcoma, osteosarcoma |
| LAGE | Myxoid round cell liposarcoma, non-myxoid liposarcoma, osteosarcoma |
| PRAME | synovial sarcoma, non-myxoid liposarcoma, myxoid liposarcoma |
| MAGE-A3 | uterine leiomyosarcoma, non-uterine leiomyosarcoma |

**Table 3:** Cancer testis antigens expressed in sarcomas [65].

| Sarcoma subtypes | NY-ESO | LAGE | MAGE-A3 | MAGE-A4 | MAGE-A9 | PRAME | SSX-2 |
|---|---|---|---|---|---|---|---|
| osteosarcoma | + | + | + | + | | | |
| Ewing's sarcoma | + | + | + | + | | | |
| Chondrosarcoma | + | + | + | + | | | |
| Synovial sarcoma | + | + | + | + | + | + | + |
| liposarcoma | + | + | + | + | + | | + |
| Leiomyosarcoma | | + | + | + | + | + | |

**Table 4:** Cancer testis antigens commonly expressed in soft tissue sarcomas and bone sarcomas [67].

| | NY-ESO positive |
|---|---|
| Synovial sarcoma | +++++ |
| GIST | + |
| Leiomyosarcoma | - |
| MPNST | + |
| SFT | - |
| Cellular schwannoma | - |
| Dermatofibrosarcoma protuberans | + |
| Angiosarcoma | + |
| Ewing sarcoma | - |

**Table 5:** NY-ESO expression among sarcomas [68].

- A phase I/II trial was conducted in 86 patients with sarcoma to evaluate the efficacy of autologous tumor cell lysate vaccine therapy. Results showed 8 of the 23 evaluable patients demonstrated positive Delayed Type Hypersensitivity (DTH) reaction. Patients with +DTH had median survival time of 16.6 months Vs. 8.2 months in DTH negative group [69].
- In a study conducted using cell lysate vaccine therapy in osteosarcoma patients post amputation, delay in the development of pulmonary metastases was noted [70].
- A Phase I vaccine trial using SYT-SSX junction peptide was conducted in six patients with disseminated synovial sarcoma. 3/6 patients had increase in frequency of peptide specific CTLs (cytotoxic T lymphocytes); 4/6 patients showed induction of peptide specific CTLs. Stable disease was noted in 1 patient. No serious adverse events were noted [71].
- A phase I study was conducted in 15 pediatric patients with relapsed solid malignancies (including bone and STS) after standard treatment to evaluate the efficacy of autologous dendritic cells pulsed with tumor cell lysate. Results showed 70% of patients had +DTH reaction. One patient had complete remission; 5 patients had stable disease during the follow up period of 16-30 months [61].
- Ganglioside vaccine: A phase 2 study was done in 136 patients with metastatic sarcoma following metastasectomy. Patients were randomly assigned to receive immunological adjuvant OPT821 (that augments T cell response) with a KLH conjugated ganglioside vaccine that targets GM2, GD2 and GD3 or placebo (control group). Results showed that median progression free survival and 1-year progression free survival rates were 6.4 months and 35% respectively; while no significant difference was noted between the 2 groups. At a median follow-up of 18 months, the 1-year overall survival rate was >90%. Serologic responses to GM2 and GD2 were noted in 98% and 21% of patients in vaccine vs. control group respectively [72].
- In a phase II trial, patients with bone and soft tissue sarcoma (9 different subtypes and 11 different HLA class 1A phenotype) were vaccinated with 4 of 31 available HLA-matched peptides. Cellular and humoral responses were seen against vaccinated peptides. 30% patients had stability of disease. 1 patient with synovial sarcoma had progression free survival of 33 months. Median overall survival time of all 20 patients was 9.6 months [73].

- In a pilot study, patients with translocation positive recurrent or metastatic Ewing's sarcoma or alveolar rhabdomyosarcoma were assigned into 3 groups. All of them received autologous T cells, influenza vaccinations and dendritic cells pulsed with tumor specific peptides. Group 1 and 2 received moderate and low dose Recombinant Human Interleukin-2 (rhIL-2) respectively [74], while group 3 patients did not receive rhIL-2. Results found that immune response to vaccinating peptide was noted in 39% patients. The 5-year overall survival was 43% in immunotherapy group and 31% control group. Toxicity was minimal [69].
- In a clinical trial, patients with NY-ESO-1 positive metastatic melanoma or metastatic synovial cell sarcoma refractory to standard treatments received TCR (T Cell Receptor) transduced T Cells+IL-2. Results found that objective clinical response were noted in 4/6 patients with synovial cell sarcoma and in 5/11 patients with melanoma. 2/11 melanoma patients demonstrated complete regression that lasted over 1 year. Partial response (that lasted for 18 months) was noted in 1 patient with synovial cell sarcoma. These findings demonstrate efficacy of TCR based gene therapies against NY-ESO-1 [75].
- In a phase 2 trial, patients with sarcoma received autologous tumor cell vaccines using cancer cells from metastatic site along with either GM-CSF or interferon-gamma. Results found that following vaccination, 50% of patients had +ve DTH reaction. Compared to patients without DTH conversion, patients with DTH+ve reaction demonstrated long median survival. (8.3 Vs 16.6 months) [76].

**(e) Adoptive Cell Therapy**

Basic principle: In adoptive cell transfer, immune cells that possess antitumor activity are administered to patients for treatment. The underlying mechanism is expansion and infusion of tumor infiltrating lymphocytes or use of Chimeric Antigen Receptors (CAR) or the use of genetically modified lymphocytes. Lymphocytes can be genetically modified with the help of gamma retroviruses or lentiviruses. These genetically modified lymphocytes with TCRs (T cell receptors) can recognize tumor antigens or encode molecules that can increase their antitumor activity. Later, these lymphocytes acquire antitumor activity [77]. Simultaneous expansion of immunosuppressive T cells (T regulatory cells) may occur during the process of T cell expansion. These regulatory T cells may decrease the efficacy of this adoptive immunotherapy approach.

Thus, to avoid this, a conditioning regimen of chemotherapy is mostly used prior to the initiation of adoptive immunotherapy.

- A patient with locally advanced, chemotherapy refractory epithelioid sarcoma received autologous, *in vitro* expanded peripheral blood natural killer cells. This resulted in regression of disease and improvement in quality of life. This process of Autologous Immune Enhancement Therapy (AIET), included isolation of natural killer and T cells from patients own (autologous) peripheral blood, isolated, activated and expanded. This finding suggests that AIET could be a beneficial treatment for patients with advanced epithelioid sarcoma [78].
- A study was conducted by Dr. Steve Rosenberg and colleagues in patients with synovial cell sarcoma. Patients received genetically engineered T cells combined with IL-2. Results showed that 2/3$^{rd}$ of the patients had objective clinical response; partial response that was durable for 18 months was noted in 1 patient [79]. This study shows that sarcomas may show response to adoptive immunotherapy protocols [77].
- In a phase I, II study 19 patients with HER2+ bone sarcomas received Chimeric Antigen Receptor (CAR) modified T cell therapy. 4/17 evaluable patients showed disease stability for 12 weeks- 14 months. Three of the patients had tumor necrosis $\geq$ 90%. Median OS was 10.3 months for all the patients. No dose limiting toxicities were reported [80].

**(f) Immune Checkpoint Inhibitors**

Immune checkpoints act as regulators of immune system. Studies have found that tumors, by activating suppressive immune checkpoint pathways, diminish the immune response to the tumor. CTLA4, PD-1, and PD-L1 act as the most common immune checkpoint inhibitors.

CTLA4 (cytotoxic T-lymphocyte-associated protein 4), is commonly expressed in regulatory T cells. But, it's expression is upregulated in conventional T cells after activation- which commonly happens in cancers. When bound to CD80 or CD86 on the surface of antigen presenting cells, it acts as an off switch and downregulates immune responses.

Programmed cell death protein 1 (PD-1), acts by suppressing T cell inflammatory activity, promotes apoptosis in antigen specific T-cells, and reduces apoptosis in regulatory T cells.

Programmed Death-Ligand 1 (PD-L1) by binding to PD-1 or B7.1, transmits an inhibitory signal which results in reduction of proliferation of antigen-specific CD8+ T cells and/or CD4+ helper cells.

Histochemical analysis of patients with soft tissue sarcoma was done to evaluate the PD1 and PDL1 tumor expression. Results

showed that 100% of undifferentiated sarcomas demonstrated positive expression; whereas 10% was noted in myxoid liposarcoma; these results suggest that certain subtypes may be more sensitive to immune checkpoint blockade with anti PD1 and anti PDL-1 [81].

- A phase II study was conducted in patients with recurrent synovial sarcomas, to evaluate the efficacy of anti CTLA 4 Ab, ipilimumab. Results showed no serologic evidence of an immune response and progression of disease [82].
- In a phase 1 study conducted on patients with advanced solid tumors, 1 of the enrolled patients had leiomyosarcoma and other patient had malignant peripheral nerve sheath tumor. Patients received pembrolizumab (anti PD-1 mAb). Both patients had stable disease [83] Table 6.

| Histological type | N | PD1 positive | PD-L1 positive |
|---|---|---|---|
| leiomyosarcoma | 20 | 45% | 70% |
| synovial sarcoma | 16 | 63% | 75% |
| undifferentiated sarcoma | 11 | 100% | 82% |
| myxoid liposarcoma | 10 | 10% | 30% |
| well differentiated liposarcoma | 4 | 50% | 25% |
| dedifferentiated liposarcoma | 3 | 67% | 67% |
| Ewing sarcoma | 6 | 67% | 67% |
| malignant peripheral nerve sheath tumor | 6 | 50% | 50% |
| angiosarcoma | 5 | 80% | 80% |
| myxofibrosarcoma | 4 | 25% | 25% |
| epithelioid sarcoma | 4 | 100% | 75% |
| alveolar rhabdomyosarcoma | 4 | 75% | 100% |
| pleomorphic rhabdomyosarcoma | 2 | 100% | 100% |
| clear cell sarcoma | 1 | 100% | 100% |
| Studies with anti PD-1 therapy in sarcomas: | | | |

**Table 6:** Expression PD1 and PD-L1 in soft tissue sarcoma subtypes [81].

### (g) Pembrolizumab in Sarcoma

With the impressive response to immune checkpoint inhibitors in melanoma, head and neck cancer, MSI high solid tumors to name a few, there have been interest in evaluating this strategy in sarcomas [83]. Some of the evaluated predictive markers of response to immunotherapy has included PDL-1 expression, tumor mutation burden and Microsatellite Instability (MSI). The MSI status if high (microsatellite unstable), has gained FDA approval for use of pembrolizumab (anti-PD1). The data regarding Tumor Mutation Burden (TMB) and microsatellite stability has been sparse in sarcoma. The PDL-1 level in bone and soft tissue sarcoma has ranged between 15-60% based on the subtype of sarcoma with the highest levels in undifferentiated pleomorphic sarcoma [84,85].

The single arm study by Dr. Tawbi et al. looked at 80 patients with soft tissue or bone sarcomas, treated with pembrolizumab 200 mg once every 3 weeks. These patient had previously received at least three lines of treatment. After follow up for 17.8 months, seven out of 40 patients (18%) with soft tissue sarcoma had an objective response. This included 4 patients with undifferentiated pleomorphic sarcoma, two with liposarcoma and one with synovial sarcoma. Of the bone sarcomas, only 2/40 had an objective response, including one with osteosarcoma and one with chondrosarcoma.

The progression free survival in the soft tissue group was 55%, higher than expected 40% for an active regimen. Based on this study, pembrolizumab was deemed active in undifferentiated pleomorphic and liposarcomas. There was increased PDL-1 expression in undifferentiated pleomorphic sarcoma which might explain the response to pembrolizumab [83,84].

### (h) Pembrolizumab with Nivolumab

The recent article published by Dr. D'Angelo et.al looked at combination of Nivolumab with Ipilimumab against nivolumab monotherapy in a phase I randomized trial of metastatic bone and soft tissue sarcoma. The single agent arm had 5% objective response rate, and was 16% with the combination. The most common toxicity was anemia, dehydration, increased lipase level. Serious adverse events were seen in 19% in monotherapy arm vs. 26% with the combination. As the combination met the pre-specified endpoint, it was recommended to be studied in a randomized trial [85].

### (i) Role of Radiation Therapy

Local treatment using measures such as radiation therapy can result in release of tumor antigens. Mechanism of induction of immunotherapy through radiation therapy (gamma radiation particularly) could be through (1) tumor cell apoptosis can result in processing and presentation of antigens by dendritic cells; (2) immunologically active cytokines may be released; (3) immune cell tumor infiltration through increase in tumor vascular permeability [42].

### (j) Heat Shock Proteins: (HSP)

Cellular stresses such as hypoxia, heat or pharmacologic agents stimulate synthesis of Heat Shock Proteins (HSPs). HSPs act as intracellular chaperones and bind, assemble and disaggregate proteins [86]. When HSPs are present extracellularly, they act as potent immune stimulators, by enhancing antigen processing and presentation [87]. In a study conducted by Multhoff G et al, it

was found that heat shock inducible tumor specific HSP 72 cell surface expression resulted in increased sensitivity to lysis through NK cells stimulated by IL-2. It was also found that, this was not dependent on tumor cell expression of MHC 1 [88].

**(k) Combination Therapies**

In a study conducted by Finkelstein SE et al, patients with soft tissue sarcoma received combination of intratumoral dendritic cells and fractionated external beam radiation. Results showed that 52.9% of patients had tumor specific immune response lasting 11-42 weeks; 70.6% of patients were progression free survival after 1 year; treatment resulted in significant accumulation of T cells in the tumor. Minimal toxicity profile was noted. These results suggest combination therapy as a promising treatment regimen [89,90]. Retrospective analysis of tumor samples from sarcoma patients before and after radiotherapy showed that radiotherapy caused significant enhancement of effector immune cells and CTAs, and simultaneous downregulation of immune suppressors. Thus, radiotherapy may provide supplemental support for immune defense in sarcomas and implies therapeutic role of combination therapy [42].

## Discussion

Immunotherapy has had a long beginning with use of interleukin and interferon, but only recently there have been significant developments to improve outcomes in a meaningful way. The approval of immune checkpoint inhibitors based on improved overall survival in different tumor types as opened a myriad of opportunities and trials evaluating use of different immune strategies including blockade of PD-1, PDL-1, TIM-3, LAG-3. Although interleukin and interferon had been used for many years in renal cell carcinoma and melanoma, toxicity has limited its more widespread use.

With the approval of the newer immunologic approaches targeting CTLA-4 (Ipilimumab), PD-1 (Nivolumab and Pembrolizumab) and PDL-1 (Avelumab and Atezolizumab) for a wide range of malignancies, there has been a clinically significant impact on outcomes. The prognosis of many of these cancers have improved such as with stage IV melanomas with 5 yr survival close to 35%, which was in the single digits before. There has been intense efforts with a number of clinical trials evaluating these drugs in a number of other cancers and also in combination with other agents including other immune checkpoint inhibitors, chemotherapy, radiation therapy to name a few.

Recent work has focused on combining IDO inhibitors, anti-TIM3 and anti-LAG3 with anti PD-1 therapies to enhance the efficacy of already approved therapies, and there will be even more in the near future with the enhanced understanding of the molecular underpinnings of many of these cancers.

Despite the positive outcomes in many cancer types, the data in sarcoma with use of immunotherapy has not been robust yet to promote its use in sarcomas. The clinical trials with single agent pembrolizumab suggested activity in undifferentiated pleomorphic sarcoma and liposarcoma. It is currently being studied with many other combinations including doxorubicin, gemcitabine, olaratumab axitinib, radiation therapy. Furthermore, the combination of nivolumab and ipilimumab data published recently also showed activity in bone and soft tissue sarcoma [85].

Enhanced understanding of tumor biology and newer targeted therapies are welcome news, and is a major breakthrough in treatment of these diseases. Unfortunately, the benefits of these drugs need to be balanced against the cost of treatment as combination immunotherapy approaches come with a very high cost. As these newer drugs get promoted to treatment of earlier stages of cancer for adjuvant therapy, concerted efforts need to be made to also try keeping the cost down. It is possible that using these immuno-oncologics earlier in the disease course will improve outcomes with better longer term outcomes and potential savings in health care, but that needs longer follow up.

There is a lot of effort ongoing to understand better the appropriate patients for these treatments. PDL-1 expression at least for now could give some idea about the utility of these drugs based on published information, and also tumor mutation burden and microsatellite status have also stood out to be predictive of response to immunotherapy. But, there is a lot more work to be done and knowledge to be gained which allows better patient selection to maximize the benefits to patients with the highest chance of response and have better predictive tools for non-responders.

## Conclusion

Immunotherapy, with approval of anti-PD1 and anti-PDL1 therapies has been a major scientific advancement in treatment of many different cancers and improved outcomes in many cancer types, and will continue to evolve with understanding of tumor biology. There are many more immuno-oncologic targets being evaluated with hope to enhance the benefit of the already approved immunotherapy drugs. The early data of anti PD-1 therapies in certain sarcoma subtypes are very encouraging, but need to understand the molecular heterogeneity of the different subtypes to improve outcomes. Predictive markers of response including PDL-1, tumor mutation burden and microsatellite status have helped select patients who are more likely to respond to immunotherapy, but certainly needs more work and understanding of mechanism of action and resistance. The role of immuno-oncologics in sarcoma is evolving and will likely be in combination with chemotherapy, radiation therapy or other immuno-oncologics, with the hope to have a meaningful benefit in this very heterogeneous cancer.

## References


1. Burningham Z, Hashibe M, Spector L, Schiffman JD (2012) The epidemiology of sarcoma. Clin Sarcoma Res 2: 14.
2. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics. CA Cancer J Clin 68: 7-30.
3. Zagars GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, et al. (2003) Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 1225 patients. Cancer 97: 2530-2543.
4. Salo JC, Lewis JJ, Woodruff JM, Leung DH, Brennan MF (1999) Malignant fibrous histiocytoma of the extremity. Cancer 85: 1765-1772.
5. Damron TA, Ward WG, Stewart A (2007) Osteosarcoma, chondrosarcoma, and Ewing's sarcoma: National Cancer Data Base Report. Clin Orthop Relat Res 459: 40-47.
6. Italiano A, Mathoulin-Pelissier S, Cesne AL, Terrier P, Bonvalot S, et al. (2011) Trends in survival for patients with metastatic soft-tissue sarcoma. Cancer 117: 1049-1054.
7. Kluwe J, Mencin A, Schwabe RF (2009) Toll-like receptors, wound healing, and carcinogenesis. J Mol Med (Berl) 87: 125-138.
8. Modlin RL (2012) Innate immunity: ignored for decades, but not forgotten. J Invest Dermatol 132(3 Pt 2): 882-886.
9. Jeys LM, Grimer RJ, Carter SR, Tillman RM, Abudu A (2007) Post-operative infection and increased survival in osteosarcoma patients: are they associated? Ann Surg Oncol 14: 2887-2895.
10. Sottnik JL, U'Ren LW, Thamm DH, Withrow SJ, Dow SW (2010) Chronic bacterial osteomyelitis suppression of tumor growth requires innate immune responses. Cancer Immunol Immunother 59: 367-378.
11. Rosenberg SA, Spiess P, Lafreniere R (1986) A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 233: 1318-1321.
12. Wilkinson J, Cope A, Gill J, Bourboulia D, Hayes P, et al. (2002) 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 76: 2634-2640.
13. Gill J (2002) 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 31: 384-390.
14. Francescutti V, Skitzki JJ (2012) Sarcomas and the immune system: implications for therapeutic strategies. Surg Oncol Clin N Am 21: 341-355.
15. de Visser KE, Eichten A, Coussens LM (2006) Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6: 24-37.
16. Medzhitov R, Janeway CA Jr (1997) Innate immunity: the virtues of a nonclonal system of recognition. Cell 91: 295-298.
17. Kawai T, Akira S (2007) TLR signaling. Semin Immunol 19: 24-32.
18. Smith-Garvin JE, Koretzky GA, Jordan MS (2009) T cell activation. Annu Rev Immunol 27: 591-619.
19. Murali-Krishna K, Altman JD, Suresh M, Sourdive DJD, Zajac AJ, et al. (1998) Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity 8: 177-187.
20. Steinman RM (1991) The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9: 271-296.
21. Corr M, Slanetz AE, Boyd LF, Jelonek MT, Khilko S, et al. (1994) T cell receptor-MHC class I peptide interactions: affinity, kinetics, and specificity. Science 265: 946-949.
22. Scholl PR, Geha RS (1994) MHC class II signaling in B-cell activation. Immunol Today 15: 418-422.
23. Heath WR, Carbone FR (2001) Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol 19: 47-64.
24. Chiriva-Internati M, Pandey A, Saba R, Kim M, Saadeh C, et al. (2012) Cancer testis antigens: a novel target in lung cancer. Int Rev Immunol 3: 321-343.
25. Sasse AD, Sasse EC, Clark LG, Ulloa L, Clark OA (2007) Chemoimmunotherapy versus chemotherapy for metastatic malignant melanoma. Cochrane Database Syst Rev: Cd005413.
26. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, et al. (1994) Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity 1: 793-801.
27. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, et al. (2008) CTLA-4 control over Foxp3+ regulatory T cell function. Science 322: 271-275.
28. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, et al. (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439: 682-687.
29. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, et al. (2010) Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 207: 2187-2194.
30. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264.
31. Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, et al. (2009) CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science 326: 986-991.
32. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, et al. (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 206: 3015-3029.
33. Tseng WW, Somaiah N, Engleman EG (2014) Potential for immunotherapy in soft tissue sarcoma. Hum Vaccin Immunother 10: 3117-3124.
34. Worley BS, van den Broeke TL, Goletz TJ, Pendleton DC, Daschbach EM, et al. (2001) Antigenicity of fusion proteins from sarcoma-associated chromosomal translocations. Cancer Res 61: 6868-6875.
35. Sangiolo D, Mesiano G, Gammaitoni L, Leuci V, Todorovic M, et al. (2014) Cytokine-induced killer cells eradicate bone and soft-tissue sarcomas. Cancer Res 74: 119-129.
36. Khan MA, Khan A, Owais M (2006) Prophylactic use of liposomized tuftsin enhances the susceptibility of Candida albicans to fluconazole in leukopenic mice. FEMS Immunol Med Microbiol 46: 63-69.

37. Munn DH, Mellor AL (2013) Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol 34: 137-143.

38. Balachandran VP, Cavnar MJ, Zeng S, Bamboat ZM, Ocuin LM, et al. (2011) Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. Nat Med 17: 1094-1100.

39. Cavnar MJ, Zeng S, Kim TS, Sorenson EC, Ocuin LM, et al. (2013) KIT oncogene inhibition drives intratumoral macrophage M2 polarization. J Exp Med 210: 2873-2886.

40. Ruffell D, Mourkioti F, Gambardella A, Kirstetter P, Lopez RG, et al. (2009) A CREB-C/EBPbeta cascade induces M2 macrophage-specific gene expression and promotes muscle injury repair. Proc Natl Acad Sci USA 106: 17475-17480.

41. Burgess M, Gorantla V, Weiss K, Tawbi H (2015) Immunotherapy in Sarcoma: Future Horizons. Curr Oncol Rep 17: 52.

42. Sharma A, Bode B, Studer G, Moch H, Okoniewski M, et al. (2013) Radiotherapy of human sarcoma promotes an intratumoral immune effector signature. Clin Cancer Res 19: 4843-4853.

43. Sabel MS, Arora A, Su G, Mathiowitz E, Reineke JJ, et al. (2007) Synergistic effect of intratumoral IL-12 and TNF-alpha microspheres: systemic anti-tumor immunity is mediated by both CD8+ CTL and NK cells. Surgery 142: 749-760.

44. Guo Q, Yuan M, Peng J, Cui X, Song G, et al. (2011) Antitumor activity of mixed heat shock protein/peptide vaccine and cyclophosphamide plus interleukin-12 in mice sarcoma. J Exp Clin Cancer Res 30: 24.

45. Kennel SJ, Lankford T, Flynn KM (1983) Therapy of a murine sarcoma using syngeneic monoclonal antibody. Cancer Res 43: 2843-2848.

46. Geehan DM, Fabian DF, Lefor AT (1993) Immunotherapy and whole-body hyperthermia as combined modality treatment of a subcutaneous murine sarcoma. J Surg Oncol 53: 180-183.

47. Shu SY, Rosenberg SA (1985) Adoptive immunotherapy of newly induced murine sarcomas. Cancer Res 45: 1657-1662.

48. Gupta RK, Morton DL (1979) Detection of cancer-associated antigen(s) in urine of sarcoma patients. J Surg Oncol 11: 65-74.

49. Atkins MB, Regan M, McDermott D (2004) Update on the role of interleukin 2 and other cytokines in the treatment of patients with stage IV renal carcinoma. Clin Cancer Res 10: 6342s-6346s.

50. Schwinger W, Klass V, Benesch M, Lackner H, Dornbusch HJ, et al. (2005) Feasibility of high-dose interleukin-2 in heavily pretreated pediatric cancer patients. Ann Oncol 16: 1199-1206.

51. Müller CR, Smeland S, Bauer HC, Saeter G, Strander H (2005) Interferon-alpha as the only adjuvant treatment in high-grade osteosarcoma: long term results of the Karolinska Hospital series. Acta Oncol 44(5): 475-480.

52. Edmonson JH, Long HJ, Frytak S, Smithson WA, Itri LM (1987) Phase II study of recombinant alfa-2a interferon in patients with advanced bone sarcomas. Cancer Treat Rep 71: 747-748.

53. Kager L, Pötschger U, Bielack S (2010) Review of mifamurtide in the treatment of patients with osteosarcoma. Ther Clin Risk Manag 6: 279-286.

54. Geddes K, Magalhães JG, Girardin SE (2009) Unleashing the therapeutic potential of NOD-like receptors. Nat Rev Drug Discov 8: 465-479.

55. Kleinerman ES, Jia SF, Griffin J, Seibel NL, Benjamin RS, et al. (1992) Phase II study of liposomal muramyl tripeptide in osteosarcoma: the cytokine cascade and monocyte activation following administration. J Clin Oncol 10: 1310-1316.

56. Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, et al. (2008) Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival--a report from the Children's Oncology Group. J Clin Oncol 26: 633-638.

57. Wilky BA, Goldberg JM (2014) Immunotherapy in sarcoma: a new frontier. Discov Med 17: 201-206.

58. Chang HR, Cordon-Cardo C, Houghton AN, Cheung NK, Brennan MF (1992) Expression of disialogangliosides GD2 and GD3 on human soft tissue sarcomas. Cancer 70: 633-638.

59. Harrison C (2014) Vaccines: Nanorings boost vaccine adjuvant effects.

60. Vergati M, Intrivici C, Huen NY, Schlom J, Tsang KY (2010) Strategies for cancer vaccine development. J Biomed Biotechnol.

61. Dillman R, Barth N, Selvan S, Beutel L, de Leon C, et al. (2004) Phase I/II Trial of Autologous Tumor Cell Line–Derived Vaccines for Recurrent or Metastatic Sarcomas. Cancer Biother Radiopharm 19: 581-588.

62. Burgess M, Tawbi H (2015) Immunotherapeutic approaches to sarcoma. Curr Treat Options Oncol 16: 26.

63. Pollack SM, Jones RL, Farrar EA, Lai IP, Lee SM, et al. (2014) Tetramer guided, cell sorter assisted production of clinical grade autologous NY-ESO-1 specific CD8(+) T cells. J Immunother Cancer 2: 36.

64. Ladanyi M (2001) Fusions of the SYT and SSX genes in synovial sarcoma. Oncogene 20: 5755-5762.

65. Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT (2002) Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev 188: 22-32.

66. D'Angelo SP, Shoushtari AN, Agaram NP, Kuk D, Qin LX, et al. (2015) Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol 46: 357-365.

67. Uehara T, Fujiwara T, Takeda K, Kunisada T, et al. (2015) Immunotherapy for Bone and Soft Tissue Sarcomas. Biomed Res Int: 820813.

68. Lai JP, Robbins PF, Raffeld M, Aung PP, Tsokos M, et al. (2012) NY-ESO-1 expression in synovial sarcoma and other mesenchymal tumors: significance for NY-ESO-1-based targeted therapy and differential diagnosis. Mod Pathol 25: 854-858.

69. Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Yanik GA, D, et al. (2001) Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res 61: 8513-8519.

70. Marcove RC, Mike V, Huvos AG, Southam CM, Levin AG (1973) Vaccine trials for osteogenic sarcoma. A preliminary report. CA Cancer J Clin 23: 74-80.

71. Kawaguchi S, Wada T, Ida K, Sato Y, Nagoya S, et al. (2005) Phase I vaccination trial of SYT-SSX junction peptide in patients with disseminated synovial sarcoma. J Transl Med 3: 1.

72. Carvajal RD, Agulnik M, Ryan CW, Milhem MM, George S, et al. (2014) Trivalent ganglioside vaccine and immunologic adjuvant versus

adjuvant alone in metastatic sarcoma patients rendered disease-free by surgery: A randomized phase 2 trial. Journal of Clinical Oncology 32: 10520-10520.

73. Takahashi R, Ishibashi Y, Hiraoka K, Matsueda S, Kawano K, et al. (2013) Phase II study of personalized peptide vaccination for refractory bone and soft tissue sarcoma patients. Cancer Sci 104: 1285-1294.

74. Mackall CL, Rhee EH, Read EJ, Khuu HM, Leitman SF, et al. (2008) A pilot study of consolidative immunotherapy in patients with high-risk pediatric sarcomas. Clin Cancer Res 14: 4850-4858.

75. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, et al. (2011) Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 29: 917-924.

76. Dillman RO, Wiemann M, Nayak SK, DeLeon C, Hood K, et al. (2003) 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 26: 367-373.

77. Rosenberg SA (2011) Cell transfer immunotherapy for metastatic solid cancer--what clinicians need to know. Nat Rev Clin Oncol 8: 577-585.

78. Ratnavelu K, Subramani B, Pullai CR, Krishnan K, Sugadan SD, et al. (2013) Autologous immune enhancement therapy against an advanced epithelioid sarcoma: A case report. Oncol Lett 5: 1457-1460.

79. Tolcher AW, Sarantopoulos J, Patnaik A, Papadopoulos K, Lin CC, et al. (2009) Phase I, pharmacokinetic, and pharmacodynamic study of AMG 479, a fully human monoclonal antibody to insulin-like growth factor receptor 1. J Clin Oncol 27: 5800-5807.

80. Ahmed, N, et al. (2015) Human Epidermal Growth Factor Receptor 2 (HER2) -Specific Chimeric Antigen Receptor-Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. J Clin Oncol 33: 1688-1696.

81. Kim JR, Moon YJ, Kwon KS, Bae JS, Wagle S, et al. (2013) Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One 8 q: e82870.

82. Maki RG, Jungbluth AA, Gnjatic S, Schwartz GK, D'Adamo DR, et al. (2013) A Pilot Study of Anti-CTLA4 Antibody Ipilimumab in Patients with Synovial Sarcoma. Sarcoma: 168145.

83. Patnaik A, Kang SP2, Rasco D3, Papadopoulos KP3, Elassaiss-Schaap J, et al. (2015) Phase I Study of Pembrolizumab (MK-3475; Anti-PD-1 Monoclonal Antibody) in Patients with Advanced Solid Tumors. Clin Cancer Res 21: 4286-4293.

84. Tawbi HA, Burgess M, Bolejack V, Tine BAV, Schuetze SM, et al (2017) Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol 18: 1493-1501.

85. D'Angelo SP, Mahoney MR, Van Tine BA, Atkins J, Milhem MM, P, et al (2018) Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol 19:416-426.

86. Chirico WJ, Water MG, Blobel G (1988) 70K heat shock related proteins stimulate protein translocation into microsomes. Nature 332: 805-810.

87. Vanbuskirk A (1989) A peptide binding protein having a role in antigen presentation is a member of the HSP70 heat shock family. J Exp Med 170: 1799-1809.

88. Multhoff G, Botzler C, Wiesnet M, Eissner G, Issels R (1995) CD3-large granular lymphocytes recognize a heat-inducible immunogenic determinant associated with the 72-kD heat shock protein on human sarcoma cells. Blood 86: 1374-1382.

89. Finkelstein SE, Iclozan C, Bui MM, Cotter MJ, Ramakrishnan R, et al. (2012) Combination of external beam radiotherapy (EBRT) with intratumoral injection of dendritic cells as neo-adjuvant treatment of high-risk soft tissue sarcoma patients. Int J Radiat Oncol Biol Phys 82: 924-932.

90. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, et al. (2017) Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Mol Cancer Ther 12: 2598-2608.