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The use of immunotherapy in the treatment of soft tissue sarcoma

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Abstract

Soft-tissue sarcomas (STS) are cancers that develop in soft tissues, such as muscle and fat tissue, joints and tendons, nerve endings, and blood vessels. The most common location for a STS tumor is in an individual's limbs. While no single disease-driving factor has been identified, high levels of chemical and radiation exposure and certain genetic susceptibility factors, increase the risk of developing STS. As is the case with many cancers, STS tumor cells use various mechanisms to evade the immune response and decrease immune cell activation. Immunotherapy aims to activate the immune response against cancerous cells. There are many different types of immunotherapy treatment that target different arms of the immune system. Because of the versatility in their mechanisms of action, immunotherapies can be designed to work against many different tumor types. Immunotherapy has been increasingly used to inhibit the growth of sarcomas, including STS. This review will discuss conventional therapies for STS and describe how the introduction of immunotherapy has expanded the treatment options and improved the outcomes for patients. Different immunotherapies that have been used in the treatment of STS will be presented, including T cell receptor therapy, antibody biologics, and delivery using nanoparticle technology. The impact that STS immunotherapies have had on patient survival and their quality of life will be discussed. Immunotherapeutics present significant possibilities for STS patients in terms of increased survival and improved quality of life.

Keywords

Soft tissue sarcoma, Immunotherapy, TCR therapy, Cancer, Antibody biologics, Nanoparticles, CAR-T cells, Tumor associated antigens, Autophagy, Checkpoint inhibition

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I. Introduction

Soft Tissue Sarcoma (STS) is a subset of cancers in which tumors develop within soft tissues (1). STS develops within adipose tissue, muscles, nerve endings, joints, tendons, and blood vessels, arising most often in the limbs and abdomen (1). Over 60 different types of STS have been identified, which need varying treatments (2). Among those with STS, the most common diagnoses are liposarcoma, often found in fatty tissue; synovial sarcoma, typically found in the extremities; and rhabdomyosarcoma, typically found within skeletal myoblast cells (3-6).

The development of sarcoma is very rare, with an incidence of 1-5 per 1 million individuals, and a positive diagnosis can significantly affect a patient's quality of life (7). This heterogenous group of cancers makes up less than 1% of malignant tumor diagnoses (7). Diagnosis of STS typically differs among different groups within race, age, and gender (8). Patients with sarcoma often experience significant change in their daily activities, leading to a reduced quality of life (9). While the effects of STS on the quality of life vary depending on socio-demographics and tumor location, STS patients associate the disease with emotional, mental, and physical struggles, including pain, fatigue, and reduced role functioning (9). Most commonly, the mental and emotional hardships result from a lack of physical movement and cognitive functioning (9). Furthermore, decreased social interactions contribute to a feeling of isolation in some sarcoma patients (9).

As STS progresses, survival rates of patients decrease (10). Survival rate is significantly affected by tumor size and grade; which are typical measures of cellular abnormality (11). Patients with a high-grade STS tumor larger than 8 cm have a three times greater risk of death when compared to patients with a smaller tumor size (11). A high-grade tumor patient with presurgical metastatic disease has a three times greater chance of a sarcoma related death compared to a patient without a pre-surgical metastatic disease (11). This data emphasizes the significantly deleterious effect that STS progression has on patient survival rate.

Previous studies have suggested a correlation between both environmental and genetic factors with the development of soft tissue sarcoma tumors (12). Identified risk factors for STS development include exposure to high levels of certain chemicals or radiation and certain genetic susceptibility factors (12). Some types of STS have a higher likelihood of developing in individuals with a previous family history of cancer or inherited mutations in tumor-suppressing pathways (13). For example, germ line mutations of the p53 tumor suppressor gene is commonly identified as a link to the development of STS (14). Additionally, fusion of the PAX3-FKHR and PAX7-FKHR genes increase the likelihood of the development of sarcoma tumors (15). Non-inherited, spontaneous mutations in these pathways and others can also play a role in the development of STS (16). Additionally, several genes, including PDGFR, p53, NF1, CDK4, and MDM2 have been found to influence the

progression, rather than the development of STS (17).

When cancer-causing mutations occur, a response by the immune system often prevents the growth and survival of the mutated cells through a process called immunosurveillance (18). Such processes frequently block cancer development, but mutations that drive immune escape lead to cancer that is selectively more difficult to target, and often immune resistant (19). As cancerous tumors begin to develop, cytotoxic immune cells, like natural killer cells and CD8+ cells, identify and attack the tumor cells expressing the mutated protein (20). Through the exposure to, and recognition of, cancerous antigens, immunity is further strengthened by antigen-specific responses of B-cells and T cells and the dispersion of cytokines (21). Genetic mutations in these preventative immune-response pathways can increase the risk of cancer development, including STS (22). Sarcoma is conventionally treated with chemotherapy, radiation therapy, and surgical resection of the tumor (23). Immunotherapy is a relatively recent development that enhances the immune response against cancerous cells. Enhancement of the immune response may be induced by adoptive T cell transfer, antibody blockade, and targeted delivery of therapeutic factors *via* nanoparticles (24). This review will provide an overview of conventional sarcoma treatments and how their survival and relapse rates compare with new immunotherapy treatments. The mechanisms of action of the various immunotherapy treatments will be explained, and the patient populations that have benefited from these therapies will be critically analyzed. Future directions of immunotherapy for STS patients will be discussed. Understanding the current state of STS treatment will aid in deciding the most successful and effective treatments for patients and inform future research.

II. Conventional treatment methods

Tumor resection surgery has been the most commonly used treatment for STS, especially for small, low-grade tumors (25). The specific surgical procedure used depends on the cancer stage and the tumor grade (25). A Mohs micrographic surgery is used when the tumor is located in an area in close proximity to normal tissue, and when sparing this normal tissue is important (26). This procedure is done by cutting away thin layers of the tumor leaving as much unaffected tissue as possible (26). On the contrary, a wide local excision of the tumor also removes some of the non-tumor tissue around it (27). In a limb-sparing surgery, the procedure is done to remove a tumor in a limb without amputation (27). In such procedures, radiation therapy or chemotherapy is often used to reduce the size of the tumor prior to the surgery (27). The final surgical option is amputation of the affected limb, and is necessary in rare cases and conditions of STS (27).

Radiation therapy and chemotherapy are other treatments commonly used in STS. In radiation therapy, high energy x-rays and radiation are used to kill cancer cells, reduce tumor size, and/or keep tumors from enlarging (28). External radiation therapy uses a medical linear accelerator (LINAC), that focuses radiation energy at the localized tumor area from outside

the body (28). Internal radiation therapy involves injecting a radioactive substance near the tumor using needles, wires, or catheters (28). Radiation therapy is typically used to treat sarcoma prior to surgery, as it can effectively treat tumors of smaller size and reduce the size of larger tumors, thereby reducing complications in surgery (29). On the other hand, chemotherapy kills the cancer cells or stops them from growing using drugs. Chemotherapeutic drugs can be injected into the localized tumor area or administered orally (30). Chemotherapy as a treatment is typically used prior to surgery, as it delays further progression and metastasis (31). Radiation therapy, chemotherapy, and surgery have been the mainstays of STS treatment since the 1970s. (32).

III. T cell receptor therapy

T cell receptor (TCR) therapy is a treatment involving the adoptive transfer of genetically modified white blood cells (T cells) to target specific tumor antigens (33). TCR therapy modifies the patient's own T cells (34). The development of these genetically modified lymphocytes requires sophisticated processes involving patient screening, leukapheresis, the generation of a transduced TCR product, and the adoptive transfer of the modified T cells into the patient (33). The effectiveness of T cells activation against targeted antigens is determined by the TCR's affinity for the target peptides or receptors (33).

T cells can be modified with sequences from chimeric antigen receptors (the product is designated as CAR T cells) and expanded using the ex vivo culture of T cells obtained directly from the cancerous tumor or from circulating mononuclear cells (35-36). This approach generates T cell responses that are very similar to natural responses. These engineered cells have increasingly been seen as revolutionary in successfully and efficiently eliminating tumor cells (37). Recently, a new approach was developed featuring the use of TCR sequences from T cells with a known anti-tumor specification and affinity (38).

Commonly expressed in sarcoma, preferentially expressed antigen in melanoma (PRAME) is a highly attractive cancer target within solid tumors typically treated with TCR modified cells (39). In a recent study testing whether individuals with STS can benefit from PRAME-TCR-gene therapy, 26 soft tissue synovial sarcoma patients expressed PRAME specific T cells that could be targeted for gene therapy (40). With the addition of interferon (IFN) treatment, an increased number of PRAME-expressing cells were recognized (40). The specific restricted HLA region of soft tissue synovial sarcoma, HLA-A*02:01, was more easily identified when exposed to the interferon treatment in 85% of the sarcoma patients (40). High levels of PRAME specific T cell expression were identified in all of the STS patients; with ~85% demonstrating a homogenous expression pattern (40). However, low levels or heterogenous levels of HLA-I were expressed by the tumor cells, a common mechanism used by cancerous cells to evade the immune response (40). Therefore, IFN treatment may amplify the function of these engineered T cells by increasing tumor HLA-I expression. PRAME-specific T cells have been shown to kill cancerous sarcoma tumors when

there is a high expression of HLA-I (40). By increasing the expression of HLA-I in PRAME specific T cells through IFN treatment, PRAME-specific TCR-gene therapy may be significantly effective against STS (40).

In another study designed to identify key biologic patterns associated with the pathogenesis of sarcoma, T cells with receptors that recognized antigens in sarcoma tumors were engineered (41). To determine patterns within T cell receptors and target cells, RNA from the tumor samples was extracted and analyzed (41). Seven gene regions were defined within the analysis of the tumors, with 367 genes being significantly different between groups (41). Across all tumors and STS types, human leukocyte antigen subtypes (HLA-A), HLA-B, and HLA-C) were essential genes that facilitated the identification of tumor cells (41). CD3d and CD8a, both T cell markers, were associated with T cell mediated inflammation, and the expression of these markers was highest in STS tumors (41). Analysis of the tumor's gene expression correlated rapid growth with a high expression of genes that are associated with antigen presentation and T cell response (41). Generated genetic changes within these T cell receptors significantly slowed the growth of the sarcoma tumors (41). Certain tumors increase immune recognition via a highly expressed immunogenic protein NY-ESO-1 (42). Adoptive transfer of NY-ESO-1-specific T cells is a possible route for T cell receptor therapy by the identification and lysis of tumor cells bearing the NY-ESO-1 antigen (42). Future immunotherapy practices, including TCR therapeutic treatments, could target other STS tumor-specific antigens. Additional immune manipulation to increase the amounts of T cell infiltration into the tumor may be required in the future development of TCR therapy to treat STS (41).

Rhabdomyosarcoma (RMS) is a common type of soft tissue sarcoma occurring within the skeletal muscle that develops from primitive mesenchymal cells (43). In recent years RMS has been associated with high rates of metastatic and relapsed phases in adolescents and children (44). While immunotherapy could be used as a therapeutic treatment for sarcoma, the low level of major histocompatibility complex (MHC) molecules expressed in RMS often reduce the ability of T cells to recognize these cells (45). As a potential approach to use TCR therapy to treat RMS, T cells were engineered to express tumor specific receptors. These receptors were characterized by an antibody-derived single-chain variable fragment (scFv) that was in close proximity with the signaling domain in the cytoplasm of the T cell CD3 chain receptor (35). The proximity of the antibody chain and the cytoplasmic signaling domain allowed for the engineered T cells to better engage the tumor antigens (35). The engineered T cells demonstrated MHC-mediated cytotoxic activity towards the antigen expressing target cells (46).

The pathogenesis of RMS is greatly influenced by the platelet-derived growth factor receptor α (PDGFRA) (47). The PDGFRA receptor is activated to control the growth of muscle cells in the presence of PDGF-AA or PDGF-CC ligands (48). PDGFRA plays a vital role in the control of cancer cell survival, proliferation, and migration (49). In a recent study, chimeric

antigen receptor (CAR) T cells were modified using a PDGFRA specific single-chain variable fragment (scFV) region bound to the 4-1BB and CD3-ζ chain receptor domains (49). The generated T cells displayed a strong ability to abrogate the cellular metabolism of PDGFRA+ RMS cells both *in vitro* and *in vivo* (49). The expression of PDGFRA was identified in 63.3% of the RMS patients at different expression levels (49). PDGFRA CAR-T cells expanded in response to specific tumor antigens when cultured with RMS cells (49). In comparison to the growth of non-transduced cells, PDGFRA CAR-T cells decreased the growth of RMS. Increased amounts of targeted antigen levels in RMS cells were positively correlated with the cytotoxic capability of CAR-T cells (49). The use of generated PDGFRA CAR-T cells significantly improved the tumor regression rate and prolonged patient survival.

The use of costimulatory molecules with CAR-T cells led to a significant increase in cytokine production by enhancing the activation and response of T cells (50). Increased production of cytokines, including IL-2, TNF-α, and IFN-γ, by CAR-T cells increased T cell engagement to tumor specific antigens in an autocrine manner (49). The expression of these various cytokines with the CAR-T cells was strongly correlated with the ability of the T cells to eliminate the tumor cells (49). The engineered PDGFRA CAR-T cells significantly decreased the overexpression of PDGFRA in RMS and caused their lysis (49). Due to the ability of the CAR-T cells to cause the regression of RMS and increase the lifespan of T cells, PDGFRA transfer into CAR-T cells has the potential as a TCR therapy treatment for RMS, and these results may extend to patients with other types of STS.

The possibility of using chimeric antigen receptor (CAR) T-cell therapy as a therapeutic approach for a great variety of cancers is very promising (51). When treating relapsed or refractory human malignancies, CAR T-cells have proven to be increasingly successful (52). These CAR T cells can be thought of as a "living drug" and act by directly attaching to the specific antigens that reside on the tumor cell's surface, initiating a cytolytic response to eliminate these cells (53). The initial processes of CAR T-cell therapy binds the ScFv recognition domain to an intracellular part of the CD- 3ζ chain essentially signaling a trigger to T cell activation. This allows for the identification of, and attachment to antigens (54). The concept including the ScFv recognition domain makes possible for CAR cells to attach to cell-specific tumor antigens (54). As the CAR T cells produce an immune synapse that is required for any cytotoxic activity, Fas and FAS ligand, perforin and granzyme, and cytokines are used to make the stroma of the tumor more sensitive to any antigen or vaccine (55). To generate a successful response, the CAR T cells must penetrate through the tumor cells, detect the specific antigen, perform the cytotoxic function, and develop memory T cells with a long-term immunity, all within the specific stroma sensitive time period (56). The simulation of these anti-tumor T cells requires three different signals: a signal from the interactions between the T cell receptor and the specific tumor antigen, a signal from an

interaction between costimulatory molecules and the ligands on the tumor cell, and a final signal triggered by inflammatory cytokines (57).

Pharmaceutical companies are developing better iterations of CAR-T therapy. Combining different technologies, BioNTech SE, established a “highly tumor-specific CAR-T cell therapy product” designated as a CAR T cell Amplifying RNA Vaccine (CARVac) (58). The approach combines an autologous CAR-T cell therapy targeting the oncofetal antigen Claudin-6 (CLDN6) and a CLDN6-encoding CARVac, to improve persistence and functionality of the adoptively transferred cells. By encoding for antigens that are specific to the tumor being targeted, the vaccine can potentially increase CAR-T cell activity achieving a greater therapeutic effect (58). Their investigational product, BNT211, is a specific T cell therapy directly affecting CAR-T cells against the antigen Claudin-6 (CLDN6) (58). The CLDN6 antigen is present on various solid tumor cancers including sarcoma, gastric cancer, ovarian cancer, and testicular cancer. Six weeks post the infusion of BNT211, 4 out of 5 patients showed a partial response and all 5 patients exhibited tumor shrinkage (58). Antitumor response increased with a greater dose of the CAR-T cell amplifying vaccine (58).

IV. Antibody biologics

Antibodies used in immunotherapies are genetically modified to block cellular pathways and functions of tumors (59). Antibody biologics have been evaluated and validated in multiple clinical studies demonstrating their ability to initiate lysis of tumor cells (60). Rather than interfering with the rapid division of malignant cells - as chemotherapy does - antibody biologics are used to treat cancer by interfering with intricate multi-step intracellular pathways involved in the development and growth of tumors (59). Antibodies can block cell-surface receptors or target proteins produced by tumor cells (61). Since these engineered antibodies bind to the receptors of specific tumor antigens, antibody biologics are typically classified as a “targeted therapy” (59).

The use of monoclonal antibodies to activate the immune system was first used in the treatment of low grade B cell lymphoma and, with approval from the United States Food and Drug Administration, a variety of monoclonal antibodies have since been approved for use in treating a diverse range of tumors and malignancies (62). Antibody biologics have more recently been used for the treatment of sarcoma (62). The specific pathways targeted and modified by monoclonal antibodies often vary depending on the location, types, and proliferation of the sarcoma cancer itself (62).

In many cancers, tumors hijack the programmed cell death 1 (PD-1) pathway in order to suppress the body's immune control of the tumor (63). In several trials of an anti-PD-1 treatment, PD-1 expression within the tumor was significantly reduced along with a concomitant increase in antitumor activity (63). In a one year clinical trial, 57 patients with advanced levels of STS were treated with 50 mg of Metronomic cyclophosphamide (CP) twice in a 21 day cycle: every day for the first

week, and then every other week, with an additional 200 mg of pembrolizumab on day 8 (63). Three of the 50 patients included in the analysis were progression free after receiving the treatment for a six month time period (63). With the completion of the trial, the average progression free rate, in which tumor growth ceased, was 1.4 months, and the overall survival rate increased by 9.2 months (63). There was a significant increase in the ratio of kynurenine to tryptophan between tumor samples that were collected prior to the treatment and those collected post treatment (63). The increase in kynurenine was indicative of a possible expansion and function of regulatory T cells; modulating tumor lysis (63).

The anti-CD20 antibody Mabthera® (rituximab) is being increasingly used to treat lymphoproliferative disorders (64). It has been used to treat Kaposi sarcoma, a subtype of STS that develops in the lymph nodes, blood vessels, and mucocutaneous sites within a patient's organs and extremities (64). Kaposi sarcoma typically grows within the mantle zone of B cells, which express CD20, a non-glycosylated cell surface phosphoprotein (64-65). In a singular case study, a patient was followed for more than three years after being diagnosed, to assess the ability of anti-CD20 monoclonal antibody therapy to achieve a durable remission of Kaposi sarcoma (66). The patient in the trial had previously been treated with chemotherapy, daunorubicin, and cidofovir, but the patient's condition had not significantly improved (66). Administration of rituximab completely ablated B cells (66). The lack of B cells could allow for a complete remission because of reduced signaling needed for the growth of STS (67). The depletion of the CD19+ cells in the patient lasted a total of 14 weeks (67). B cells only reappeared in the patient's bloodstream at levels similar to pre-treatment, six months after rituximab administration (66). These findings, although anecdotal and/or from a small number of patients, demonstrate the possibility of using antibody biologics as a treatment for STS.

Some humanized antibodies have been produced in mice to produce antitumor effects by inhibiting growth signals for tumor cells (61). FZD10 has been identified as a cell-surface receptor within the Wnt pathway, which is implicated in the development of STS (68). It was determined that transcript levels of FZD10 in vital organs and the presence of the protein on the cell surface are both markers for the development of cancer associated with the FDZ10 gene (69). The over expression of FZD10 has been reported in the diagnosis of many primary STS cancers (70). Studies have demonstrated a reduced growth of soft tissue sarcoma in mice after receiving an injection of a polyclonal antibody, TT641, directed against FZD10 (69). The reduced growth of STS was correlated with the initiation of apoptosis in the tumor cells (9). This suggested that TT641 alone had a cytotoxic effect on STS cancerous cells (69). The results demonstrated the possibility of using a humanized antibody against FDZ10 as an STS therapeutic treatment.

V. Nanoparticle Drug Delivery

Nanoparticles are very small drug-containing particles that bind to the tumor cellular membranes, the cytoplasm, nuclear receptor

sites, or are released into the microenvironment, making it possible to deliver high concentrations of specific drugs to cancerous cells (70). The nanoparticles, ranging from 20 to 3000 nm in size, disperse individually through the cancerous cells (71). These nanoparticle carriers are biodegradable, and the drug molecules are absorbed through the cell surface (71). Drugs released from nanoparticles can either reach the tumorous cells through active or passive targeting (72). Active targeting involves a drug carrier system that is directly conjugated to a tissue or cell ligand, while passive targeting utilizes leaky endothelial junctions to reach the target area (72). Several factors play an important role in the effective delivery of tumor suppressing drugs by nanoparticles (72). The size of the particles must be large enough to avoid being absorbed from the blood capillaries, veins, and arteries (72). However, they must be small enough so that they are not cleared by phagocytic macrophages (72). Additionally, the successful delivery of drug-loaded nanoparticles is dependent on pH, temperature, solubility of the drug, drug diffusion in the matrix, desorption of the surface and drug, and the combination of both the erosion and diffusion processes of the drug (72). Nanomedicine, through the variety of nanoparticles that localize the distribution of chemotherapeutics to a tumor site have significant potential for use in sarcoma therapy, diagnosis, and treatment (73).

While the use of nanoparticles to deliver antitumor drugs to a specific tumor area has been successful in degrading smaller tumors, it is typically paired with combinations of conventional treatments, like surgery or chemotherapy, when targeting larger tumor sites (74). In a three year study, 129 patients with STS were administered nanoparticles loaded with olaratumab and doxorubicin, or doxorubicin alone (75). For patients who received the treatment with both olaratumab and doxorubicin, the average progression free time was 6.6 months, while the progression free time for those with the doxorubicin treatment only was 4.4 months (75). The presence of both drugs increased the time of tumor progression (75). The patients who received the olaratumab and doxorubicin treatment had a mean survival time of 26.5 months, significantly greater than those who were given the doxorubicin treatment, whose mean overall survival time was 14.7 months (75). Following years of limited progress in regard to STS treatment, this clinical trial suggested that the combination of both olaratumab and doxorubicin formulated in nanoparticles significantly increased the progression-free and overall survival time without undue drug toxicity (75).

The nanoparticle delivery of drugs has become an effective treatment when paired with lesser doses of radiation therapy for patients with advanced STS (76). In phase 2 and 3 trials, the success and safety of using the hafnium oxide (HfO_2) nanoparticles NBTXR3, activated by small doses of radiotherapy, was found to be beneficial (77). In the clinical trial, 87 patients with local STS tumors received NBTXR3 nanoparticle drug treatment and were compared with 89 patients receiving only radiotherapy treatment (77). The number of patients in which the treatment resulted in a complete

ablation of tumors doubled with the combined therapy (77). Serious adverse events occurred during the treatments, however there were no deaths that resulted from the treatment itself (77). Despite the adverse events, the trial validated the possibility of utilizing nanoparticle drug delivery in conjuntion with low radiation doses to treat STS.

VI. Oncolytic Viruses

Oncolytic viruses represent a relatively new type of therapeutic treatment that encourage anti-tumor responses with 2 requirements: the viral oncolysis of specific cancerous cells, and the presence of an anti-tumor host cell (78). While the cellular mechanisms behind the processes have not been fully studied, they are dependent on the interaction between cancer cells and the anti-tumor elements, the induction of apoptosis and signals of adaptive anti-tumor immunity (78). Oncolytic viruses function by directly targeting and attacking host tumor cells (78). The viral replication of antitumor cells, antiviral response elements, and the regulation of cellular receptor targeting all play a significant role in the success of viral oncolytic activity (78). The potential of oncolytic viruses killing cancerous cells also varies and is affected by the type of virus, the dose administered, and the responsiveness of tumor cells to apoptosis (78). Oncolytic viruses can be modified to decrease the likelihood of further cancer development, while increasing the potential for immune recognition and lytic activity of the anti-tumor cells (78). Adenovirus replicates in the cancerous cells using different mechanisms (78). One mechanism is by deleting both viral genes E1A and E1B, which promote the degradation of p53 (79). This causes a productive viral replication of the virus by inhibiting cell cycle arrest (79). The second mechanism is the replacement of viral promoters with viral replication of genes that are overexpressed in the cancer cells, in order to promote cytolytic activity that directly attacks the tumor (79).

The use of oncolytic viruses in sarcoma has been in studied in clinical trials and investigations with different subtypes of sarcoma, stage of diagnosis, and the clinical development of the virus (80). Talimongene Laherparepvec (T-VEC), an oncolytic viruses, was recently approved as a therapeutic treatment for sarcoma by the FDA. T-VEC includes a genetically modified HSV-1 that consists of “very specific virus-attenuating mutations” along with a coding region for the “granulocyte-macrophage colony-stimulating factor” (GM-CSF) (81). In preclinical studies, the T-VEC displayed a strong lytic effect against the tumor cell lines (82). The results and findings from the trial also suggested further research towards other factors that could have contributed to the clinical response, such as the dose of the virus, the method of administration, and combination with immunotherapy (81). A second type of virus is the H101 modified adenovirus that was recently approved in China. H101 is a type 5-adenovirus with a deleted E1B-55kD gene (83). Deletion of the E1B-55kD gene allowed for the virus to inactivate p53 to allow for efficient cell replication (83). A clinical study evaluating the ability of H101 to produce an anti-tumor response and toxicity in combination with chemotherapy against more developed cancers was has been performed (83). Findings suggest

that the administered injection of the H101 adenovirus displayed an anti-tumor response to malignant tumors when paired with chemotherapy (83). The use of H101 adenovirus to deactivate a binding to, and activation of p53, has been increasingly referred to as a promising treatment approach to treat cancers that are in more developed stages (83).

While oncolytic viruses are an effective treatment option for sarcoma there are still several factors that limit efficacy (78). The process of delivery, the presence of neutralizing antibodies, and the specific subtype of the cancer can all reduce the efficacy of the administered virus (78). Due to possible previous exposure to the virus being administered, the viral strain may be rapidly cleared by the host immune cells thereby decreasing its efficacy (78). Another limitation is the ability of the oncolytic virus to degrade the interstitial matrix and extracellular DNA (81). In many studies, the ability to evaluate and analyze the interactions of sarcoma and the immune system using human transplanted sarcoma cell lines is limited (78). Humanized mouse models allow for a better understanding of the interactions between the immune system and sarcoma. However, a rodent's immune system is still significantly different from a human immune system thereby introducing uncertainty in predicting the oncolytic virus's effect in clinical studies (84). Some other barriers that have led to therapy failure are the high interstitial fluid pressures and microvascular densities that exist in the TME. (85) Results from multiple clinical trials suggest that oncolytic viruses have limited success as a monotherapy to treat sarcoma. Their efficacy may be increased when combined with other treatment types (78).

VII. Mechanisms of Sarcoma Resistance

Despite the increases in the discovery of new therapeutic treatments in treating sarcoma and the understanding of the cancer itself, the tumor's ability to become resistant to certain treatments remains a challenge (86). There have been a variety of mechanisms identified demonstrating how tumor cells are able to avoid the cytotoxic effect of therapeutic agents (86). Alterations in the genetics of sarcoma cancer cells can have a significant effect on oncologic signal pathways and the development of antitumor resistance (86).

In a specific case study a patient with leiomyosarcoma produced a lasting response toward checkpoint inhibition for more than 2 years, however began to experience treatment failures in a single metastatic area (87). Genetic analysis revealed that with a loss of the PTEN and a resistance to an amplification of the MDM2 gene there was a reduced tumor antigen expression (88, 89).

An additional potential for sarcoma resistance to immunotherapeutic treatments lies within the TME (tumor microenvironment) (90, 91). Sarcomas have been found to obtain increasingly high expression levels of two major suppressive cytokines VEGF (vascular endothelial growth factor) and TGFB1 (transforming growth factor beta 1) (90, 91). The presence of VEGF in sarcoma tumor was correlated with a variety of resistance mechanisms; such as increased suppressive

immature phenotypes of TAMs, DCs, and T regulatory cells; the impairment of T cell trafficking and migration, and the alteration of several physiologic process (92). Additionally, the interaction between tyrosine kinase inhibitors and anti-VEGF activity contributed to various checkpoint blockades in bone sarcoma and STS (87). Similarly, TGFB was identified to directly antagonize T cell function by increasing the expression of FOXP3 on T cells, inhibiting the function of natural killer cells, decreasing the expression of MHC, and triggering suppressive phenotypes of TAMs (93). The role that TGFB plays in sarcoma still remains uncertain, however many studies have shown that its expression triggers epithelial-mesenchymal transition -type transcription factors, which in turn encourage the progression and metastasis of osteosarcoma cells (94).

VII. Future directions/ Successes in Immunotherapies

Over the years, sarcoma has been one of the most complex and difficult to treat cancers in the United States, with little to no improvement (95). However, in recent years, there has been a slow, yet quantifiable increase in the success rate of treatments (96, 97). The National Cancer Institute's surveillance, epidemiology, and end results conducted a study reporting the survival and improvement rates of STS over a 20-year period (98). Patients in the later years of the study presented with, on average, more advanced disease than patients in the earlier years, potentially due to the increase in the age of the studied population. In addition, there was an increase in the average tumor size (98). Despite the increase in severity, the overall survival rate of STS progressively improved over the 20-year period (98). From periods 1991 to 1996, 1997 to 1993 and 2004 to 2010 the overall rate improved to 28%, 40% and 62% respectively (98). The study did not separate findings based on treatment type, but overall, the results demonstrate that there has been significant improvement in sarcoma treatments and survival rates, giving hope to patients and families in light of the continued development of STS therapeutics.

Recent studies have found that TAA (tumor-associated antigens) are released in greater quantities when tumor cells undergo necrosis or autophagy rather than apoptosis (99). Necrosis is a type of Immunogenic cell death (ICD) where death receptors recognize undesirable signals from both intra and extra-cellular environment to begin the process of necroptosis (99). When signaling from CASP8 in apoptosis is malfunctioning, necroptosis serves as a backup in assembling TNFR1 simulation as a response to a viral infection (99). Several studies showed that many highly active cancer cells displayed lower levels of RIPK3 (an effector of necroptosis) suggesting necroptosis to be a tumor suppressor that directly affects the function of immune cells (100). Therefore, the activation of a genetically modified RIPK3-mediated phosphatase phosphoglycerate mutase 5 (PGAM5) could help activate T cell anti-tumor responses through the independent process of nuclear translocation of the nuclear factor within activated T cells and the dephosphorylation of dynamin-related protein 1 (Drp1) within the necroptotic pathway (101). Several studies have revealed that RIPK3, a

key effector of necroptosis, can modulate the expression of cytokines and the process of tissue repair (102). The processes of necroptosis can be initiated through simulated and modified apoptosis-inducible ligands suggesting a possibility for using different CAR-T products to engineer a necroptopic lytic response from cancer cells (103). In addition, recombinant adenovirus was shown to produce necroptotic effects when combined with doxorubicin, promoting cell death (103).

During the process of autophagy, the immune system reacts to cancer cells by releasing large amount of TAA (tumor associated antigens) and cytokines (104). Autophagy is a process in which the lysosome degrades various cellular components and debris in stressful conditions (104). Autophagy consists of three different mechanisms: macro autophagy, micro autophagy, and chaperone-mediated autophagy (CMA)\ (105). During both the micro and macro autophagy processes, smaller proteins, individual component of organelles, and organelles themselves are degraded by lysosomes to maintain a state of homeostasis (106, 107). The autophagic degradation is mediated through receptors like p62 and NBR1 (108, 109). The process of autophagy could be enhanced by immunotherapeutic treatments to treat sarcomas, along with other cancers, by degrading different immune checkpoint proteins, promoting the release of cytokines, and the release of anti-tumor factors (104). More specifically, the degradation of PD-L1 through autophagy initiates the production of T cells attacking cancerous cells, essentially improving the effectiveness of immunotherapy (110). High levels of PD-L1 expression in tumor have been correlated to the inactivation of T cells which, in turn, increases resistance of the cancer cell to immune responses (110). Using the autophagic process to block the PD-L1 checkpoint can therefore improve the efficacy of immunotherapy (110). The induction of PD-L1 degradation increased the potential of vaccines and viruses in immunotherapeutic treatments for sarcoma (104).

With a goal of targeting cancerous tumors through immunotherapy, genetic changes to activate the immune system can lead to the development of autoimmune disease. Autoimmunity is a resulting effect from a constantly and progressive breakdown of checkpoint mechanisms that protect healthy cell from immune attack (111). The use of genetically modified cytokines to treat tumors produces moderate response and adverse events with IFNs (interferons) (112). Altered levels of IFNs have been correlated with different autoimmune diseases (ADs) including arthritis and lupus (113). A lack of IFN production resulted in a deficit of regulatory T cells that help maintain an immune self-tolerance by replicating and activating autoreactive immune cells (114). Foxp3, a nuclear transcription factor, was identified as a highly selective marker for regulatory T cells and plays a significant role in its development (115). A decrease in the specific T regulatory cells, CD4+, CD25+ and CD4+FOXP3+, is correlated with greater susceptibility to autoimmune diseases (115, 116). Additionally, the deregulation of apoptosis and the immune system's inability to self-repair itself can be attributed to different mutations in the p53

gene, leading to the development of arthritis, bowel disease, and generalized inflammation (116).

VIII. Conclusion

Through the continued improvement of conventional STS treatments and the development of new immunotherapy treatments, there has been an increase in the number of surviving and treated sarcoma patients (96-98). Every new development in immunotherapy gives patients another therapeutic option, increasing their chances of a successful treatment. Due to a variety in tumor location and grade, conventional treatment methods are limited in therapeutic potential. The use of immunotherapies to treat different cancers, including STS, has expanded treatment options to include a variety of new and more effective therapeutic practices. The identification of biomarkers in STS and the development of targeted therapeutic options could lead to a more personalized approach to STS treatment. However, such biomarkers have yet to be identified. The use of immunotherapy as a treatment for STS has already benefited patients and increased survival. Further development of these therapies has a significant potential for those patients diagnosed with STS.

Abbreviations

PAX3-FKHR: Paired box gene 3 - Forkhead Homologue in Rhabdomyosarcoma
PDGFR: Platelet-derived growth factor receptor
P53: Protein tumor gene 53
NF1: Neurofibromatosis 1
CDK4: cyclin-dependent kinase 4
MDM2: murine double minute 2
HLA: Human leukocyte antigens
CD3d and CD8a: Cluster of differentiation 3 delta and cluster of differentiation 3 alpha
CD8+: cytotoxic T lymphocyte
NY-ESO-1: New York Esophageal Squamous Cell Carcinoma-1
4-1Bb: Tumor necrosis factor ligand superfamily member 9
CD3-ζ: Cluster of differentiation 3 zeta chain
IL-2: Interleukin 2
TNF-α: Tumor Necrosis Factor Alpha
IFN-γ: Interferon Gamma
ScFv: Single-chain Variable Fragment
FAS: FS-7 Associated Surface Antigen
CD20: Cluster of Differentiation 2
FZD-10: Frizzled 10 protein
Wnt: Wingless-related Integration Site
PTEN: Phosphatase and Tensin Homolog
FOXP3: Forkhead Box P3
CASP8: Caspase 8

TNFR1: Tumor Necrosis factor receptor 1
RIPK3: Receptor-Interaction Protein Kinase 3
ICD: International Classification of Diseases
P62: Ubiquitin-Binding Protein
NBR1: Neighbor of BRCA1 gene 1 protien
E1A/E1B: Adenovirus Early Región 1A and Adenovirus Early Region 1B

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