4. Immunotherapy in Osteosarcoma Treatment

4.1. The Tumor Immune Microenvironment (TIME)

Since the last decade, treatment modalities like chemotherapy and radiation used for cancer patients have moved towards antibody-based immunotherapies. Understanding TIME is essential for gauging the success of therapies, exploring the biomarkers, selecting the appropriate patient population, and finding new novel targets for therapies. The tumor microenvironment is a composite of various cellular and extracellular matrices. It refers to the immune cells and their associated secretions within the tumor microenvironment, while microenvironment refers to the intercellular material and its humoral components. The tumor-infiltrating immune cells (TIICs) are one of the critical constituents of tumor microenvironment. Basic and clinical research on the tumor microenvironment, which consists of cancerous, stromal, and immune cells, demonstrates the critical role of antitumor immunity in cancer development and progression.

4.1.1. Immune Cell Infiltration

Based on the immune cell infiltration, the TIME is generally described in two classes: infiltrated-excluded (I-E) and infiltrated-inflamed (I-I). In the former class, TIME is usually populated with immune cells. However, the tumor core could be relatively devoid of immune cells like cytotoxic lymphocytes (CTLs). This lack of CTL infiltration in some tumors is associated with the presence of tumor-associated macrophages (TAMs), which surround the tumor margins. The later infiltrated-inflamed (I-I) class has high infiltration of CTLs expressing PD-1 and leukocytes and tumor cells expressing the immune-dampening PD-1 ligand PD-L1. The knack of the organism to identify and eliminate abnormal cells, including cancerous cells, depends on its immunological system. Cancer cells have the immunogenicity that triggers an immune response. This characteristic of cancer cells is a key factor in the success of developing immunotherapeutic approaches. The two most common immune cells infiltrating OS are macrophages and T lymphocytes.

4.1.2. Prognostic Significance

I-E TIME is hypothesized as “cold” as these are poorly immunogenic. These cold tumors have CTLs with low expression of the activation markers GZMB (GRZB) and IFNG and poor infiltration of CTLs into the tumor core, making adaptive immunity less effective. Infiltrated-inflamed (I-I) TIMEs are considered to be immunologically ‘hot’ tumors. A subclass of I-I TIMEs called TLS-TIMEs, due to their cellular similarity with tertiary lymphoid structures (TLSs) like lymph nodes, having diverse lymphocytes, is created with a positive prognosis. The TIME evolves with tumor progression, altering immune cell composition and function, which impacts therapy effectiveness. Poor CD8+ T cell infiltration is a negative prognostic marker associated with metastatic progression and worse outcomes.

4.2. Immune Evasion Mechanisms

A better understanding of immune evasion mechanisms, like avoiding recognition and killing by immune cells used by tumors, is crucial for combating cancers. Tumor-derived Extracellular Vesicles (EVs) are also implicated in the immune evasion mechanism. A

number of immune evasion strategies affect how precursor lesions progress into the invasive phase and aid in cancer's progression from an early to a metastatic stage. By creating an immunosuppressive microenvironment and losing their immunogenicity or antigenicity, cancerous cells might evade the immune system's destruction. An essential stage in the development of cancer is immunoediting. There are three stages in this immunoediting process: "elimination", "equilibrium", and "escape". White blood cells and tumor cells seem to have co-evolved. Cancer can occur under TCR-T cell therapy, possibly due to the loss of part of the HLA-A*02:01 allele in tumor cells after the loss of heterozygosity in tumor cells' MHC. Furthermore, cells can reduce MHC-1 expression through autophagy involving NBR1. MHC-1 can be degraded selectively in the cell by lysosome. At this point, although MHC-1 expressions are downregulated, mutations are rarely detected at the gene level. Numerous studies have since demonstrated that most cancers that returned after CAR-T treatment either stopped expressing the CD19 gene or developed without it. Indoleamine 2,3-dioxygenase (IDO) has been steadily implicated in developing checkpoint inhibitor resistance in malignancies. T-cell failure due to overexpression of IDO provides an environment that is conducive to tumor cell growth and negates the effects of immune checkpoint inhibitors. A mechanism called "cancer immunoediting" is one of the tumors' immune evasion tactics. Antitumor immune responses triggered by antigen recognition in the tumor environment seem to result in cancer immunoediting. When the immune system interacts with cancer cells that have certain genetic alterations in the first place, the clones that have lost these mutations may proliferate selectively and biasedly, which could allow the tumor to evade the immune system. It is possible that cancer cells do not evoke a strong exclusionary immune response for certain tumors because the immune system may not distinguish cancer cells that have lost particular antigens from healthy host cells. When costimulatory molecules are involved, the way mature APCs present antigens to T cells differs from how cancer cell antigens are presented to T cells. The simultaneous existence of a second signal from costimulatory molecules, like CD28, is necessary for the APCs to deliver antigens and trigger T-cell activation during antigen recognition. This second signal regulates the subsequent T-cell response. Antigen stimulation itself may be disregarded when the antigen is given to T cells without the second signal; this is known as unresponsive energy, and it causes the antigen-specific T cell responses to be lost. Even if T-cells are exposed to potent cancer-specific antigens produced from genetic alterations, cancer cells may not elicit T-cell responses because they lack crucial second signals.

4.3. Expression of Immune Checkpoint Molecules

Low expression of MHC-I and upregulation of immune checkpoints is another mechanism of immune evasion used by tumors. Impaired production of MHC class I/Human Leukocyte Antigen (HLA) class I antigens is one way cancers evade T cell-mediated immunosurveillance. For instance, in the EwS TME, HLA-G, and HLA-E, the non-classical MHC-I molecules implicated in the protective maternal–fetal barrier in the placenta are highly upregulated on tumor and myeloid cells. Human autoimmune cells produce a class of receptor proteins called immune checkpoints, which can control the immune system's activity level and help maintain immunological tolerance. Following immunoediting, tumors have also developed a mechanism that suppresses the function of immune cells by releasing signals through immunological checkpoints. ICI is responsible for preemptively inhibiting immunological checkpoints to enable immune cells to continue destroying the tumor. Currently, the most widely utilized inhibitors are PD-1, PD-L1, and CTLA-4. Immune

checkpoint molecules such as programmed death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) were identified in 1990. To have anticancer effects, both compounds inhibit T cell activation. They serve as an organism's defensive mechanism to stop excessive T cell activation and stop autoimmune reactions, and their expression rises in direct proportion to T cell activation. Ligands that attach PD-1 and CTLA-4 inhibit T-cell function. For instance, during antigen presentation, the costimulatory molecule CD80/CD86 produced on APCs binds to CD28 on T cells concurrently to increase T cell activation. On the other hand, active T cells produce CTLA-4, which competes with CD28 to block the costimulatory signal mediated by CD80/CD86.

4.4. Immunotherapeutic Strategies

Understanding and modulating the TIME is essential for enhancing immunotherapy outcomes, such as immune checkpoint blockade (ICB). Upregulating MHC-I expression in pediatric sarcomas could be a viable tactic to trigger antitumor responses mediated by CD8+ T cells. This can be accomplished by activating proinflammatory pathways, such as type I IFNsIFNAR1/2-STAT1/2/3, type II IFN-IFNGR-STAT1, or TNF-TNF receptor-NF-κB. Targeting TAMs may be a promising therapeutic approach in several pre-clinical and clinical trials. These macrophages are tumorigenic as they help in angiogenesis, migration, extravasation, and chemotherapy resistance. Augmentation of the body's immune response is one of the promising strategies in cancer treatment. Modalities like cytokine therapy, adoptive T-cell transfer therapy, and antibodies that elicit both innate and adaptive immune responses are used in cancer immunotherapeutics. Strategies to improve the effectiveness of immunotherapy will need to consider the immune escape mechanisms that cancer use. Chimeric antigen receptor (CAR) T-cell therapy, immune checkpoint inhibitors, and vaccinations are examples of immunotherapeutic approaches. Alongside chemotherapy for treating osteosarcoma, immunotherapy has emerged as a promising avenue in which the body's immune system recognizes and combats cancer cells. Recent immunotherapeutic strategies include immune checkpoint inhibitors, adoptive T cell therapy, and chimeric antigen receptor (CAR)-T cell therapy. Cancer immunotherapies have been proposed as the fourth cancer treatment. In particular, the clinical application of immune checkpoint inhibitors, such as anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, in various cancer types represents a breakthrough in cancer therapy. The concept of immunotherapy has been acknowledged already for centuries. The development of innovative methods to use the immune system for cancer treatment was made possible by the quick rise in molecular biotechnology techniques. Due to their ability to produce long-lasting and successful therapeutic responses in cancer patients, a variety of strategies, such as adoptive cell treatments, monoclonal antibodies, checkpoint inhibitors, and oncolytic viruses, currently represent the most notable developments in cancer treatment. CRISPR/Cas9 technology offers powerful tools for genetic engineering, targeting specific tumor characteristics in osteosarcoma. Its application in silencing genes such as CD44 has been demonstrated to reduce the metastatic potential of osteosarcoma cells, indicative of its potential to interfere with tumor progression. CRISPR/Cas9 can induce precise genetic modifications that enable the development of more effective CAR T-cells by enhancing their specificity towards tumor cells while reducing off-target effects. Moreover, CRISPR/Cas9 has been employed to identify synthetic lethal interactions in osteosarcoma, thus providing insights into vulnerabilities within this heterogeneous tumor type. For example, studies suggest that targeting multiple pathways through gene editing might further synergize with CAR T-cell therapies, leading to a more

effective treatment strategy. The ability to modify the tumor microenvironment through specific gene knockouts or alterations can create a more conducive environment for CAR T-cell infiltration and activity. The immunosuppressive TME in osteosarcoma is characterized by factors that inhibit T-cell function, including various immune cells, stromal components, and extracellular matrix proteins. Several studies have shown that engineering CAR T-cells to express specific receptors or co-stimulatory molecules can enhance their ability to navigate such barriers. For example, employing a switchable CAR strategy could allow for temporal control over T-cell activity, thus enabling a more adaptable response to changing TME dynamics. Additionally, the combination of CRISPR/Cas9 technology to modulate the expression of immunosuppressive factors within the TME presents a novel approach to facilitate CAR T-cell function. Targeting molecules that dampen T-cell responses, such as PD-1 ligands or other inhibitory receptors, may restore T-cell functionality and improve therapeutic outcomes.

4.5. Immune Checkpoint Inhibitors

Immune checkpoint blockers were the first antibody-based immunotherapy agents to be used. These act by blocking the receptor and ligand interaction of various molecules involved in cellular immunity. Checkpoint blockade inhibitors, such as anti-programmed cell death protein 1 and anti-cytotoxic T-lymphocyte-associated antigen-4, chimeric antigen receptor T-cell therapy, and monoclonal antibodies, are effective strategies in treating some types of cancers. These drugs stimulate the immune system to fight cancer cells and have revolutionized cancer treatment by increasing survival in a range of cancer types. Since 2011, many ICIs have been available in the market for treating cancers. T-cells of the adaptive immune system are the focus of immune checkpoint therapy because of their capacity to identify particular antigens. Immune checkpoint inhibitors demonstrate that these are not always effective but are instead only effective in limited cancer populations.

4.5.1. Immune Checkpoint Inhibitors and Osteosarcoma

The landscape of immunotherapy for osteosarcoma is evolving, with multiple strategies being explored to enhance the effectiveness of immune-based treatments. Several studies highlight a multifaceted immunotherapy approach for osteosarcoma, emphasizing the potential of checkpoint inhibition, targeted CAR-T modifications, and combinational strategies to improve patient outcomes.

4.5.2. CAR T-Cell Therapy Targeting Osteosarcoma Antigens

Inducing CXCL10 expression in the EwS TME in order to enhance T cell infiltration may, thus, be a promising therapeutic strategy. Chimeric antigen receptor (CAR)-T cell therapy is a novel form of immunotherapy derived from adoptive T cell transfer therapy. The potential targets for CAR-T cell therapy in osteosarcoma are tumor-associated antigens. These are expressed in both normal and tumor tissues but are more highly expressed in tumor tissues. Many potential targets for CAR-T cell therapy in osteosarcoma include receptor tyrosine kinases (HER2, IGF1R, ROR1, and EphA2), cell surface glycoproteins (CSPG4, FRα, FRβ, and EC17), B7-H3 (CD276), disialoganglioside (GD2), natural killer group 2D (NKG2D), activated leukocyte cell adhesion molecule (ALCAM/CD166), interleukin-11 receptor alpha (IL-11Rα), and fibroblast activation protein (FAP). In therapy with TCR/CAR-T cells, enough high-quality antigen-responsive T cells extracted from tumor tissues in a given patient may

determine the availability and efficacy of TIL treatment. Peripheral blood mononuclear cell (PBMC)-derived lymphocytes, which synthetically express a desired TCR or chimeric antigen receptor (CAR), have been developed and used in clinical settings as a new T cell treatment to overcome these restrictions. T cells that have been transduced with antigen-specific TCRs are used in TCR-T therapy. The process of delivering T cells with a CAR gene, which comprises gene fragments from intracellular TCR domains, a fragment from a cancer antigen-recognizing antibody gene, and other T cell costimulatory molecules, is known as CAR-T therapy.

One promising approach involves a clinical trial using multi-antigen stimulated cell therapy (MASCT-I), which was evaluated in combination with camrelizumab (a PD-1 inhibitor) and apatinib (a VEGFR2-targeting TKI) in patients with advanced sarcomas. This pilot study demonstrated that the combination therapy was safe and showed promising efficacy, particularly in osteosarcoma patients, where the objective response rate (ORR) reached 33.3% and the median progression-free survival (PFS) was 5.7 months.

Meanwhile, CAR-T cell therapy targeting GD2, a surface antigen expressed in osteosarcoma, was investigated in a phase I trial, which revealed that while GD2 CAR-T cells were safe and feasible, their clinical efficacy was limited, with most patients experiencing only stable disease before eventual progression. The study identified key immune determinants influencing CAR-T expansion, including the presence of naïve and memory T cells in pre-treatment samples and a correlation between CXCR3+ monocytes and enhanced CAR-T expansion.

A more advanced switchable CAR-T strategy was also explored to improve CAR-T effectiveness against osteosarcoma. This approach utilized anti-FITC CAR-T cells activated by an anti-B7-H3-FITC monoclonal antibody, enabling tumor-specific targeting and greater control over CAR-T activation. In preclinical models, this system effectively redirected CAR-T cytotoxicity against osteosarcoma tumors, showing enhanced infiltration and antitumor activity in the presence of the switch molecule. It is important to remember that only a small percentage of individuals receiving immunotherapy have desirable therapeutic outcomes. Specifically, solid tumors typically have a tumor microenvironment that suppresses T-cell activation and promotes tumor growth. Cytokine storm and autoimmune events are noteworthy adverse effects of such treatment. Therapeutic targeting of PD-L1 and PD-1 immune checkpoints, which are expressed in ~20% of pediatric sarcoma patients in EwS and OS, has not shown clinical efficacy. However, OS tumors that score in the top quartile of immune infiltration may benefit from ICB. The histological classification of sarcomas affects the clinical response to ICB due to dynamic and variable expression of immune checkpoints.

One of the most commonly reported immune-related toxicities following CAR T-cell therapy is cytokine release syndrome (CRS). This condition is characterized by the rapid release of cytokines into the bloodstream following T-cell activation, which can lead to symptoms ranging from mild flu-like manifestations to severe, life-threatening complications such as hypotension and acute respiratory distress syndrome. CRS is particularly prominent in therapies targeting high antigen loads, which can elicit a robust T-cell response, thereby amplifying cytokine secretion. Additionally, immune effector cell-associated neurotoxicity syndrome (ICANS) is another significant complication, manifesting as neurological symptoms ranging from confusion to seizures and, in severe cases, cerebral edema.

Beyond CRS and ICANS, CAR T-cell therapy can induce “on-target, off-tumor” toxicity. This occurs when CAR T cells, directed against specific antigens, also inadvertently affect normal tissues expressing the same antigens, leading to adverse effects on organs where the targeted antigen is normally present, which can result in additional complications such as organ dysfunction or inflammatory responses in healthy tissues. For instance, targeting the CD19 antigen has been linked to damage to normal B cells and subsequent hypogammaglobulinemia, predisposing patients to infections. Specific concerns in osteosarcoma therapy include the risk of damage to surrounding healthy tissues due to the high expression of similar antigens in those tissues. Research suggests that careful selection of target antigens is vital, given the limited tumor-specific targets available for osteosarcoma.

Strategies are being explored to mitigate these toxicities, exemplified by the development of safety switches and suicide genes in CAR constructs to enhance the safety profiles of these therapies. By addressing these immune-related toxicities, researchers aim to expand the therapeutic window of CAR T-cell therapy for osteosarcoma and other solid tumors, ultimately improving patient outcomes and reducing adverse effects.

Despite being very promising, the low response rates of checkpoint inhibitors in osteosarcoma can be attributed to several interrelated factors, including the tumor microenvironment, immune evasion mechanisms, and the intrinsic properties of osteosarcoma itself. Firstly, osteosarcoma exhibits a relatively low immunogenicity compared to other malignancies, which contributes to its diminished response to immune checkpoint inhibitors (ICIs). Specifically, the tumor microenvironment in osteosarcoma is characterized by a deficiency in immune cell infiltration, particularly tumor-infiltrating lymphocytes (TILs), and the presence of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs). This immunosuppressive environment limits the activation and efficacy of T cells, which are necessary for ICI function, as TILs often exhibit high expression of immune checkpoint molecules like PD-1, further impairing their antitumor activity. Research has shown that the expression levels of various immune checkpoints in osteosarcoma, including PD-L1, are crucial in determining the potential effectiveness of checkpoint blockade therapies.

Moreover, the heterogeneity of osteosarcoma and its complex biology complicate therapeutic responses. Although there is evidence of cytotoxic T-cell infiltration and promising immune responses in some cases, the overall response rates remain disappointingly low, often falling below 10%. The existence of “cold” tumors, which lack sufficient immune cell presence, adversely affects the likelihood of achieving effective antitumor responses with single-agent therapies. In fact, studies indicate that the optimization of the immune landscape, possibly through combination therapies or alternative immunotherapeutic strategies, is necessary to improve the clinical efficacy of ICIs in this cancer type. A critical factor contributing to the unresponsiveness of osteosarcoma to ICIs is the persistence of signaling pathways that facilitate immune evasion. Osteosarcoma cells can exploit various mechanisms to dampen immune responses, including the overexpression of checkpoint molecules and secretion of immunosuppressive cytokines.

4.6. Adoptive Cell Therapy

Immune checkpoint therapy targets T-cells of the adaptive immune system due to their ability to recognize specific antigens. To improve the targeted lethality, adoptive cell therapy involves obtaining the patient's immune cells, growing and amplifying them in vitro, and reintroducing them into the patient. The benefit is that the patient can prevent immunological rejection of foreign cells or antibodies, and the target cells can be eliminated as precisely as with prior immunotherapy. Depending on the cells collected and cultivated, therapies can also be categorized as TCR-T cell treatment, CAR-T cell therapy, lymphokine-activated killer cell therapy, natural killer (NK) cell therapy, etc. The T cells isolated from the patient's body undergo gene editing for the expression of the new generation of T cells to detect the TCR of cancer cells and more precisely and effectively identify the tumor. T cells are then cultivated and given back to the patient.

Natural Killer (NK) Cell Therapy

Natural killer (NK) cells are lymphocytes that may identify cancerous cells by balancing the detection of cell-surface stress and danger markers. NK cells release cytokines and use a variety of methods to cause target cell lysis after being triggered by such recognition. As key mediators of immunotherapeutic modalities such cytokines, antibodies, immunomodulating medications, and stem cell transplantation, NK cells are becoming more widely acknowledged for their function in regulating tumor growth and metastasis. In the absence of major histocompatibility complex (MHC)-restricted receptor–ligand interactions, NK cells can identify a range of stressed cells, including cancer cells. NK cells are crucial mediators of various therapeutic interventions and play a part in regulating the growth and spread of tumors. The fundamental idea behind adoptive natural killer (NK) cell therapy is that adoptive transfer of NK cells can restore a natural deficit in innate immunity, which is caused by a combination of immunosuppressive mechanisms that result in reduced function and cancer-induced decreases in NK cell numbers. The fundamental idea behind adoptive natural killer (NK) cell immunotherapy is that adoptive transfer of NK cells can restore a natural defect in innate immunity, which is caused by a combination of immunosuppressive mechanisms that result in suppressed function and cancer-induced decreases in NK cell numbers.

4.7. Cancer Vaccines

The creation of the HPV vaccination has opened the door for the creation of additional cancer vaccines. The goal of therapeutic cancer vaccines is to induce tumor regression, eradicate minimal residual disease, establish lasting antitumor memory, and avoid non-specific or adverse reactions. The clinical research indicated that patients with particular infectious diseases had a low cancer incidence. This served as the foundation for developing this cancer vaccine. This might be explained by how inflammation and infection expose antigens that cancer cells aberrantly produce. It could also be a secondary effect, in which the cancer cells are impacted by immunological memory accumulated from previous inflammation or infection. Similarly, the incidence of ovarian cancer is reduced by antibodies against aberrant cell surface-associated mucin (MUC1) generated after a mump infection. Additionally, Bacillus Calmette–Guerin has long been utilized as a vaccine against tuberculosis and is currently being used extensively as a therapeutic vaccination against bladder cancer.

Development of Peptide-Based and Dendritic Cell Vaccines

The ability of dendritic cells to initiate immunity and manage and modulate the sort of immune response has made them particularly appealing as vehicles for mRNA delivery in immune treatment techniques. Research has focused on creating an ex vivo population of antigen-loaded dendritic cells capable of inducing strong and enduring CD8+ and CD4+ T-cell responses in cancer patients. Nevertheless, the generation of immunopotent dendritic cells ex vivo for efficient antitumor immune responses cannot be completely replicated. There are various issues related to the development of the Cancer Vaccines. Test designs may have been defective when several traditional cancer vaccines were being developed for clinical use. For instance, patients with a terminal diagnosis whose immune systems were already significantly weakened by exposure to multiple medical therapies, including surgery, chemotherapy, and radiation therapy, or by the advancement of the illness, were frequently used to assess the clinical effects of cancer vaccines. To use vaccines and achieve the desired outcomes in clinical settings, it is also crucial to create auxiliary technologies such as adjuvants, production processes, and delivery systems. Current medication laws may make it more difficult to introduce such cutting-edge technology.

4.8. Oncolytic Viruses

Oncolytic viruses serve as a model to demonstrate their versatile nature and show how they can complement other cancer therapies to gain optimal patient benefits. The use of oncolytic viruses in treating cancer can bring cancer immunotherapy to the next higher level. Viruses due to their selective replication and induction of cytopathic effects are considered suitable for cancer immunotherapy.

Mechanism and Therapeutic Potential

Oncolytic viruses can make tumors immunogenic that the immune system does not appear to recognize. The use of oncolytic viruses in altering the tumor microenvironment and enabling T-cell treatments to function in solid tumors may be an additional strategy worth investigating. The cancer cells are infected and lysed by an oncolytic virus. Oncolytic viruses are either naturally occurring or can be created in a lab by altering naturally occurring viruses. These viral changes ushered in a new age of less harmful virus-based treatments for cancer. The virus fiber knob attaches itself to receptors on the surface of tumor cells during the early stages of viral infection. Depending on the virus's serotype, distinct receptors mediate this interaction. Serotype 3 adenoviruses bind desmoglein-2, CD46, or CD80/86, but serotype 5 adenoviruses primarily bind to the coxsackie and adenovirus receptor (CXADR). While some receptors, like CXADR, are regrettably downregulated in many advanced cancers, others are commonly present in cancer cells. Later, the virus internalizes due to contact between its penton proteins and the integrins of tumor cells. Ultimately, the virus DNA enters the nucleus by a series of steps, where transcription of the early viral proteins (E1–E4) begins. Thousands of new viral progeny arise following late protein expression, break the cell membrane a few days later, and infect other cells until the immune system finally halts the process. Multiple tumor-associated antigens are revealed by immunogenic cell death and presented to the immune system by activated mature dendritic cells. These viruses stimulate T-cell responses, leading to antitumor immunity. Hence, these are considered immunogenic in nature and can be used to start an antitumor immune

response. Tumor cells are relatively more susceptible to viral infections than normal cells. Therefore, the viruses target and destroy tumors. Oncolytic viruses stimulate the effector cells by infecting tumor cells, as well as changing the TME from cold to hot. Direct tumor lysis occurs due to infection, which leads to the release of DAMPS and PAMPS. These are recognized by immune subsets like DCs, NK cells, and so on due to their PRR expression. The inflammatory cytokines are released, which attract other immune cells. The viral replication in cancer cells leads to the expression of TAA. The DC captures TAA and presents these to T cells, which help recruit T cells in tumors causing their ICD. Positive therapeutic results are expected for patients in any CAR-T therapy, checkpoint inhibitors, or cancer vaccines combined with oncolytic viruses. This is due to their role in the production of TAA and TIL.

5. Novel Therapeutic Approaches and Clinical Trials

Osteosarcoma (OS) treatment has evolved significantly, with a focus on innovative therapeutic strategies that aim to improve clinical outcomes and address the challenges of chemotherapy resistance. These strategies include targeting molecular mechanisms of treatment efficacy and resistance, exploring epigenetic therapies, and utilizing gene editing technologies. Personalized medicine approaches, such as genomic profiling, are being developed to tailor treatments based on individual patient characteristics. These innovative strategies aim to transform OS treatment and improve patient survival rates.

5.1. Epigenetic Therapy

Epigenetic therapies, including DNA methyltransferase inhibitors (DNMTi) and histone deacetylase (HDAC) inhibitors, have shown promise in treating osteosarcoma (OS) by modifying gene expression without altering DNA sequences. DNMTi can restore the expression of tumor suppressor genes (TSG) silenced by hypermethylation, while HDACi enhance gene expression through histone modification, potentially improving sensitivity to conventional chemotherapeutics. For instance, DNMTi like MC3343 have shown promising findings in OS treatment. MC3343 blocks tumor proliferation and induces osteoblastic differentiation, differing from the conventional FDA-approved nucleoside inhibitor 5-azacytidine (5azadC). Furthermore, MC3343 exhibits synergistic effects when combined with doxorubicin and cisplatin.

Studies have demonstrated that HDACi, such as AR-42 and panobinostat, are potent inhibitors of OS cell viability and can synergize with standard chemotherapeutic agents like doxorubicin. AR-42 exhibited greater potency than SAHA in inducing apoptosis in osteosarcoma cells. Furthermore, the combination of AR-42 and doxorubicin resulted in a synergistic effect on inhibiting cell viability. Both AR-42 and SAHA triggered apoptosis via the mitochondrial pathway, but AR-42 was more effective at inhibiting pro-survival Akt signaling. While HDACi monotherapy has shown limited success in clinical trials, combination regimens appear more promising.

5.2. Gene Therapy

Gene therapy approaches, particularly CRISPR/Cas9 and Antisense Oligonucleotides (ASOs), offer exciting new pathways for targeting oncogenes, overcoming chemoresistance, and improving patient outcomes. CRISPR/Cas9 technology has emerged as a potential

strategy for correcting osteosarcoma (OS) mutations. Research indicates that CRISPR/Cas9 can effectively silence oncogenes, such as CD44, which is implicated in the metastatic behavior of osteosarcoma cells. In vitro studies have demonstrated that targeting CD44 with CRISPR/Cas9 significantly reduces its expression, leading to decreased cell proliferation and migration in osteosarcoma cell lines. Furthermore, a CRISPR-Cas9-mediated CDK11 knockout has demonstrated reduced cell proliferation, viability, migration, and invasion in OS cell lines. Moreover, knocking out the ABCB1 gene using CRISPR-Cas9 technology restored sensitivity to doxorubicin in the treated OS cells, highlighting the potential of CRISPR/Cas9 as a therapeutic strategy to overcome drug resistance. Recent advances in CRISPR/Cas9 technology have shown promise for correcting mutations in cancer-related genes like TP53 and RB1, which are frequently altered in osteosarcoma.

Antisense oligonucleotides (ASOs) represent another innovative strategy for targeting oncogenes and resistance genes in osteosarcoma. ASOs are short synthetic RNA or DNA molecules that bind to complementary nucleic acid sequences, modulating protein expression through various mechanisms. In osteosarcoma, ASOs have shown potential in targeting drug resistance-related genes, such as FOXC2-AS1, which promotes doxorubicin resistance. Furthermore, ASO targeting the insulin-like growth factor (IGF) signaling pathway has shown potential in preclinical models, suggesting that ASOs could enhance the efficacy of existing chemotherapeutic agents. However, challenges remain in ASO delivery, stability, and cellular uptake.

Despite these advancements, several challenges remain in implementing gene therapy and novel agents in clinical practice. The heterogeneity of osteosarcoma tumors, coupled with the complexity of their genetic profiles, complicates the identification of suitable targets for therapy. Moreover, the delivery mechanisms for gene therapies require optimization to ensure effective uptake by tumor cells while minimizing off-target effects. To address these challenges, researchers have developed tumor-targeted delivery systems and synthetic switches to self-regulate Cas9 expression. Additionally, DNA nanotechnology-based approaches leveraging collaborative effects show the potential to reduce hybridization-dependent off-target effects.

5.3. Personalized Medicine Approaches

Personalized medicine approaches are emerging as promising strategies for treating osteosarcoma (OS) and overcoming chemoresistance. These approaches leverage genomic profiling, patient-specific targeting, and pharmacogenomics to develop customized therapeutic regimens. Genomic profiling of OS has revealed significant heterogeneity across patients, highlighting the need for personalized treatment strategies. Whole-genome sequencing (WGS) of patient tumors can identify somatic copy number alterations (SCNAs) and structural rearrangements that may drive tumor growth. These findings can be used to identify patient-specific therapeutic targets.

This personalized approach enhances therapeutic success and minimizes unnecessary exposure to ineffective treatments. For instance, the identification of specific biomarkers can guide the use of novel agents, such as immune checkpoint inhibitors, by targeting proteins like PD-1, PD-L1, and CTLA-4, which have shown promise in preclinical models of

osteosarcoma. Drug resistance remains a significant challenge, necessitating the identification of biomarkers for patient stratification and targeted treatments.

Pharmacogenomics is crucial in understanding how genetic variations affect drug metabolism and response in osteosarcoma (OS) patients. Studies have identified several genetic variants associated with treatment efficacy and toxicity of chemotherapeutic agents like doxorubicin and cisplatin. For instance, polymorphisms in DNA repair and drug metabolism genes have been associated with treatment response and toxicity in osteosarcoma patients receiving MAP (methotrexate, doxorubicin, cisplatin) chemotherapy. Studies have linked ERCC2/XPD rs1799793 and ABCC2 rs2273697 variants to poor pathological response, while the ERCC1 rs11615 CC genotype was associated with better chemotherapy response and improved overall survival.

Furthermore, ABCC2 rs3740066 and ABCB1 rs1128503 variants were linked to reduced myelotoxicity and increased methotrexate levels, respectively. Variants in ABCC1, ABCC3, and SLC22A1 genes have been associated with drug resistance and metastasis. Identifying such pharmacogenetic markers could help tailor treatment to improve efficacy and reduce toxicity in osteosarcoma patients. Personalized dosing strategies considering these genetic factors can optimize therapeutic outcomes and reduce adverse effects. Research shows that adjusting chemotherapy dosages based on pharmacogenomic data enhances treatment efficacy while minimizing toxicity.

5.4. New Therapies, Challenges, and Limitations

Bringing new therapies, such as immunotherapy and other innovative treatments, to osteosarcoma patients involves a myriad of real-world challenges, including high costs, regulatory approvals, and accessibility issues. Osteosarcoma, a highly aggressive malignancy that predominantly affects young individuals, currently presents significant therapeutic challenges, necessitating the exploration of novel biological agents and therapeutic strategies. Immunotherapy, while promising, is often associated with high treatment costs, which can constitute a substantial barrier for patients, families, and healthcare systems. The financial burden of advanced treatment protocols, such as immune checkpoint inhibitors and monoclonal antibodies, can lead to significant disparities in access, especially in pediatric populations, where the financial implications may impact family stability.

Furthermore, high-cost therapies may not be adequately covered by insurance, significantly limiting patient options. The development and implementation of novel treatments also necessitate extensive research funding, further driving up costs prior to market entry. The pathway to regulatory approval for new cancer therapies is intricate and often lengthy. Novel treatments, particularly in the context of immunotherapy, require rigorous preclinical and clinical testing to demonstrate safety and efficacy. Regulatory bodies, such as the FDA and EMA, necessitate comprehensive data sets, which can prolong the timeline for patient access.

For instance, mifamurtide is an approved therapy in Europe for osteosarcoma, based on a study showing improved overall survival when used with conventional chemotherapy. This intensive regulatory scrutiny can delay the introduction of potentially life-saving therapies, leaving many patients without viable treatment options during crucial phases of their care.

Even when new therapies receive regulatory approval, accessibility remains a pressing concern. Availability may be geographically limited, particularly for patients in rural or underserved areas, further exacerbating health disparities. The integration of novel immunotherapies often requires specialized facilities equipped with the necessary expertise and resources, which may not be universally available.

For patients with metastatic osteosarcoma, whose prognosis can change rapidly, delays in accessing cutting-edge treatments can significantly affect their survival outcomes. The transition from research to clinical application of new therapies for osteosarcoma is fraught with significant barriers. The challenges of high costs, rigorous regulatory pathways, and accessibility issues must be navigated to realize the benefits of these therapies for patients in need. Addressing these barriers requires a collaborative effort among researchers, healthcare providers, policymakers, and pharmaceutical companies to promote equitable access to innovative treatments in osteosarcoma therapy.

6. Summary and Future Directions

6.1. Summary of Therapeutic Advancements

Osteosarcoma remains a formidable challenge, particularly for children and adolescents who often endure physically and emotionally taxing treatments. Over the years, researchers and clinicians have worked tirelessly to break through the barriers posed by chemotherapy resistance. Their efforts have led to promising strategies that target critical resistance mechanisms such as drug efflux transporters, altered drug metabolism, and boosted DNA repair pathways to restore tumor sensitivity and improve patient outcomes. Alongside refinements in conventional chemotherapy, the field has made great strides in exploring targeted therapies that directly focus on key molecular alterations, like aberrant receptor tyrosine kinases and dysregulated PI3K/Akt/mTOR signaling. At the same time, immunotherapy has brought hope by harnessing our innate defenses, exemplified by checkpoint inhibitors, CAR T-cell innovations, and oncolytic viruses.

These advancements offer patients and families newfound optimism for more effective, less toxic treatments. The integration of targeted therapy, immunotherapy, and nanotherapy presents a potential paradigm shift in the treatment of osteosarcoma, particularly in addressing the challenges posed by tumor heterogeneity and metastatic disease. Osteosarcoma, a highly aggressive bone tumor, has a well-documented resistance to conventional chemotherapy, necessitating innovative approaches to enhance treatment efficacy and patient outcomes. Nanotechnology presents a novel approach for enhancing drug delivery and overcoming the challenges associated with systemic treatments. Nanoparticles can be engineered for targeted delivery, thus enhancing the concentration of therapeutic agents directly at tumor sites while minimizing systemic toxicity.

Recent studies have proposed the use of hyaluronic acid-based nanoparticles to deliver chemotherapeutics alongside immunomodulators, leveraging their ability to modify the immunosuppressive tumor milieu. This synergistic approach is critical in enhancing the penetration of chemotherapeutic agents, ensuring effective tumor cell destruction while fostering an environment favorable for immune system activation. Furthermore, the incorporation of targeted nanotherapy could be particularly beneficial when combined with genetic profiling, allowing for personalized treatment regimens based on individual tumor

characteristics. This direction aligns with ongoing advances in genome-informed therapies, which aim to tailor therapeutic interventions to the unique molecular landscape of each patient's tumor, potentially leading to improved response rates and survival outcomes.

6.2. Future Perspectives

Advancing osteosarcoma therapy requires sustained research efforts and robust clinical trials to refine novel agents and integrate them effectively with existing treatments. Ongoing work to identify reliable biomarkers will help match patients with personalized interventions, such as specific molecular inhibitors or immunotherapeutic regimens tailored to tumor genetics. Just as essential is a multidisciplinary collaboration among researchers, clinicians, and the pharmaceutical industry, ensuring a constant exchange of scientific insights and resources necessary to translate preclinical discoveries into clinically actionable therapies. This synergy will be vital for validating combination approaches such as targeted drugs plus immunotherapy and fine-tuning drug delivery systems like nanoparticles to optimize tumor-specific drug accumulation.

6.3. Call to Action

Despite progress, osteosarcoma therapy still faces persistent challenges, underscoring the urgency for innovative strategies. Researchers must continue exploring underappreciated resistance mechanisms, including epigenetic alterations and cancer stem cell populations, to open new therapeutic windows. Clinicians, meanwhile, should adopt a multidisciplinary mindset integrating surgical, pharmacological, and immunological expertise to offer patients comprehensive, individualized treatment plans. Finally, partnerships with industry are essential for expediting drug development and ensuring that cutting-edge therapies reach patients safely and efficiently. We can only continue to improve survival outcomes and quality of life for individuals battling osteosarcoma through collective, concerted efforts.