5 Oncolytic Viruses and Their Potential as a Therapeutic Opportunity in Osteosarcoma

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Abstract

Osteosarcoma remains an unmet medical need. Oncolytic viruses are gaining traction as novel cancer therapeutics. These viruses are either naturally nonpathogenic or engineered to be safe by specific genetic deletions yet retain the ability to infect and kill human cancer cells and elicit anticancer immunity. Some versions are being specifically designed and tested in patients with osteosarcoma, though due to their generalized mechanism of action most are being tested in patients across a broad range of cancer types. The activity of these viruses is impacted not only by the susceptibility of tumor cells to infection but also by the tumor microenvironment (TME) and by tumor immunogenicity. Here we review the field of oncolytic viruses with a particular emphasis on highlighting any available data in preclinical osteosarcoma models or in patients with osteosarcoma. While in general the viruses have been shown safe to administer to patients by a variety of routes, their therapeutic efficacy to date has been limited. Given the low rate of adverse events and the likely absence of long-term side effects, the utility of oncolytic viruses will most likely be realized when used in combination with other agents.

Keywords

Osteosarcoma · Oncolytic virus · Virotherapy · Viroimmunotherapy

Introduction

Oncolytic viroimmunotherapy represents a novel class of biologic "drugs" wherein modified viruses are utilized for the treatment of many types of cancers. More specifically, oncolytic viruses are live viruses that are used to (i) infect and lyse tumor cells, (ii) induce an antitumor immune response, and (iii) express foreign transgenes, with the ability to self-propagate through tumors. In 1904, George Dock reported complete remission for a patient with leukemia after an influenza infection [27]. This report and other similar clinical observations lead to several clinical trials utilizing virotherapy in the treatment of cancer in the 1950s [48]. Several patients experienced tumor reduction or clinical improvements; however, due to side effects (infections) and the emergence of chemotherapy, oncolytic virother-

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apy did not flourish in that era [44, 48, 94]. It was not until 1991, after successful treatment with genetically attenuated and altered herpes simplex virus (HSV) in a murine glioma model, did oncolytic virotherapy intrigue begin a resurgence [68]. Since that time, with continued advances in genetic manipulation, oncolytic virotherapy has gained significant traction with numerous RNA and DNA virus options being developed and tested via intravenous or intratumoral injection. Talimogene laherparepvec (T-VEC, also known as Imlygic), a genetically modified herpes simplex virus (HSV) type 1 with expression of a human GM-CSF transgene, was FDA approved in 2015 for the treatment of metastatic melanoma after observations of durable primary and abscopal tumor site regressions [4]. With continued adequate safety profiles of administration of genetically altered oncolytic virotherapy in adults and the pediatric population, the future of virotherapy for the treatment of numerous kinds of cancers is promising [4, 76, 92, 95].

Osteosarcoma is the most common bone tumor in the pediatric, adolescent, and young adult populations. Current standard of care includes chemotherapy and surgery, with overall 5-year survival of 70% in localized disease [65]. However, for patients with metastatic osteosarcoma, overall 5-year survival is <20% with no improvements in therapy options in the last 50 years despite numerous attempts. Novel therapeutic approaches are much needed to help improve the outcomes for these patients. In this chapter, we will review the novel therapy of oncolytic viroimmunotherapy for the treatment of osteosarcoma as well the tumor microenvironment and its influences on oncolytic viroimmunotherapy.

Tumor Microenvironment

The immune system's interaction with a tumor is a complex and sophisticated network of connections. It is the communication of stimulation or exhaustion that tips the balance of tumor suppression and growth in the body.

Immunologic Landscape and Immunoediting

The innate immune system consisting of dendritic cells, macrophages, natural killer cells, neutrophils, basophils, and eosinophils is the frontline defense against foreign antigens. The adoptive immune system consisting of B lymphocytes, CD4+ helper T lymphocytes, and CD8+ cytotoxic T lymphocytes is directly activated by the antigen presenting cells of the innate immune system [69]. So-called inflamed tumors are generally composed of high levels of tumor-infiltrating lymphocytes (TIL), including CD8+ cytotoxic T cells, high expression of PD-1 on tumor-infiltrating immune cells, genomic instability generating numerous tumor antigens, and the presence of preexisting antitumor immune response. In contrast, a "noninflamed" tumor exhibits a low mutational burden, low expression of major histocompatibility complex I (MHC-I), and high expression of immunosuppressive cytokines, such as TGFβ [40] (Fig. 5.1).

Cancer immunoediting is the theory that described the interplay of the immune system and the tumor in three different phases [90]. "Elimination" occurs when the immune system recognizes tumor-specific antigens, whether from the products of mutated genes or overexpressed normal genes or genes encoding viral proteins, which activate the innate and adaptive immune systems to eradicate the tumor. Tumor mutational burden (TMB) has been shown to increase tumor-specific antigens leading to a more "inflamed" TME. Numerous adult solid tumors, melanoma, renal cell carcinoma, and non-small-cell lung cancer have shown an association with increased TMB and improved survival leading to its consideration as a predictive biomarker for some immunotherapeutic agents [16, 82, 87]. If all the cancer cells are not destroyed, some may have the ability to lay quiescent, leading to the "equilibrium" phase. During this phase, the tumor is neither growing nor being completely eliminated. Finally, some tumor cells have the ability to evade the immune system and "escape"

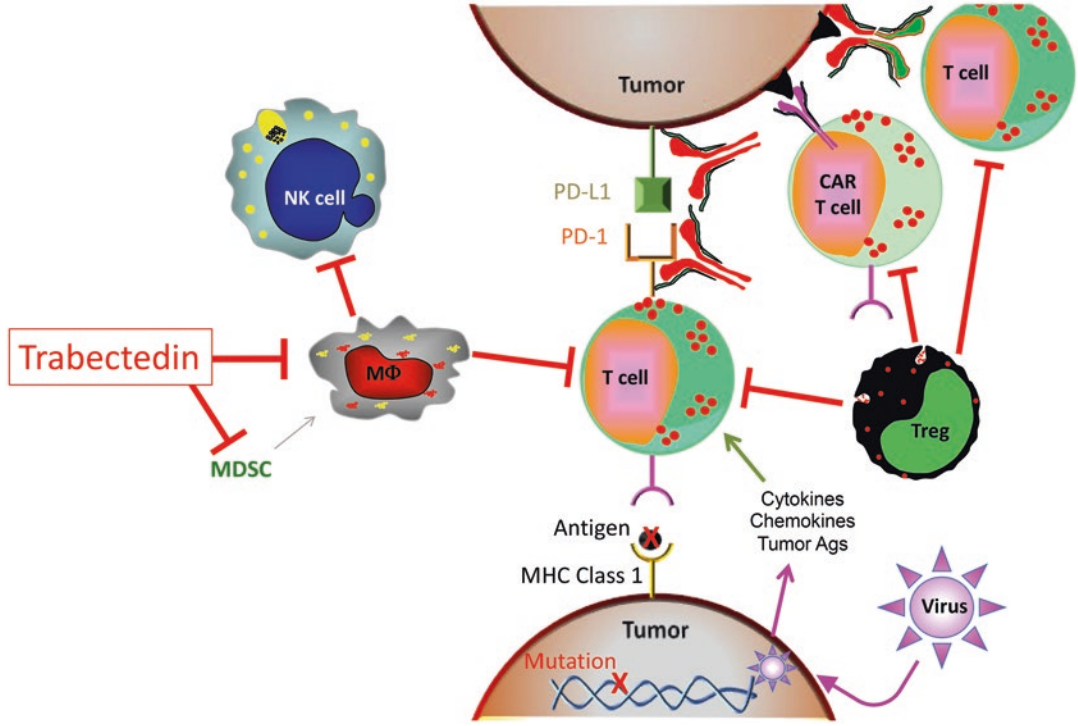

Fig. 5.1 Tumor microenvironment and immunomodulatory therapies. The tumor microenvironment in general, and there is much evidence for osteosarcoma in particular, is replete with immune cells that act to either promote or repress anti-cancer immune responses. The relative importance of each molecule or cell likely varies from patient to patient, and possibly even from region to region within the same tumor. Thus, in the future it may be important to personalize immunotherapy for each patient based on detailed analyses of the microenvironment on biopsy. A variety of small molecule, antibody, cellular and viral therapies have been established or are under investigation to promote innate and adaptive anticancer immunity and/or reverse immune suppression, as described in the text. Green arrows represent immunologic stimulation; red stops represent immunologic suppression; red Y represent antibody therapy. NK cell, natural killer cell; Treg, T-regulatory cell; MHC, major histocompatibility complex; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; CAR-T, chimeric antigen receptor T cell; MDSC, myeloid-derived suppressor cells; MΦ, macrophage

the immune system to proliferate [83, 98]. Numerous mechanisms exist for tumor evasion including recruitment of T-regulatory cells, myeloid-derived stem cells, or macrophages, downregulation of MHC-I, and upregulation of inhibitor ligands on tumor cells including TIM-3, PD-L1, and LAG-3 [42, 69]. Over the recent years, the TME of many different cancers has been found to be an important aspect of prognosis and possible indications for therapeutic options. In numerous adult solid tumors, the higher the immunoscore and immunoediting, or the percentage of tumor-infiltrating lymphocytes, the lower the metastatic rate and the longer the survival [6, 71].

Osteosarcoma TME

Tumor Mutational Burden

Within the pediatric population, it has been found that the majority of the tumors have a low tumor mutational burden [15, 55, 93]. Osteosarcoma is a genetically chaotic tumor with numerous deletions, somatic copy-number alterations, chromothripsis, and a few point mutations leading it to have a relatively high mutational burden among pediatric cancers [42, 89, 91]. However, when comparing to melanoma with a median frequency of somatic mutations per megabase pair of

approximately 13, osteosarcoma still only has approximately 1.2 [16, 19]. Thus, it is unlikely that the mutational burden will play a big role in the predictive value of response to immunotherapies.

Immunoevasion

Despite the fact that osteosarcoma does have a higher mutational burden than other pediatric tumors and thus has some potential for increased recognition by the immune system, osteosarcoma is still utilizing other mechanisms to evade the immune system. A requirement for the adaptive immune system is utilization of the MHC class I. Osteosarcoma has been shown to downregulate MHC class I which would hide it from the cytotoxic T cells and thus promote growth [38]. Osteosarcoma has also been found to have a varying degree of T cell exhaustion. Numerous studies have found an increased PD-L1 expression, while others have not confirmed these findings [36, 52, 64, 70, 75]. A consistent finding, however, is that expression of PD-L1 has an association with lower survival [52, 75]. In regard to the immune cell infiltrates of osteosarcoma, it has been found that a higher CD8+ cytotoxic T cell infiltration is associated with a superior survival, and more specifically, a low CD8/Foxp3 ratio has been identified as a poor prognostic feature that is independent from metastatic status or response to chemotherapy [70, 75] [31]. Finally, osteosarcoma has been found to have dysregulation with the TME innate immune system. Macrophages, specifically M-2 polarized, have been correlated with higher TIM-3 and PD-1 expression [36]. TAMs have been shown to inhibit the T cell response, recruit immunosuppressive cell populations, inhibit dendritic cells, and lead to vascular dysfunction with limited T cell migration [25]. Interestingly, inhibition of M-2-polarized macrophages has been found to enhance proliferation of T cells within the osteosarcoma TME and also prevent metastasis in a murine model [36, 104].

Oncolytic Virotherapy in Osteosarcoma

The main goal of oncolytic viroimmunotherapy is to utilize naturally occurring or genetically modified viruses which replicate selectively within a tumor cell without damage to healthy tissues. The first step includes direct lysis of the cancer cells causing immunogenic cell death which leads to release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). DAMPs and PAMPs lead to the release of pro-inflammatory cytokines and activation of innate immune cells [1]. Lastly, antigen stimulation from the interaction of the innate and adaptive immune cells leads to T cell priming and TIL recruitment.

Oncolytic DNA Viruses

Adenovirus

Adenovirus (AdV) is a non-enveloped, dsDNA virus that results most commonly as a mild upper respiratory tract infection. Over 40 serotypes have been identified in AdV, but the most common strains utilized in oncolytic viroimmunotherapy are serotypes 2 and 5. During infection, adenovirus binds to cellular receptors such as coxsackie-adenovirus receptor (for attachment) and integrins (for entry). The virus will then be endocytosed and brought to the nucleus while disassembling its viral capsid. Viral transcription occurs in three phases. In the early phase, the viral genome early-region 1A (E1A) or early-region 1B (E1B) is transcribed. E1A protein activates the cellular repair or apoptotic pathway mediated by p53, while E1B protein prevents early death by binding to p53 and inducing degradation or binding the antiapoptotic factor BCL2. The first oncolytic adenovirus called dl1520 (also known as ONYX-015 and Cl-1042) contained a deletion of the E1B gene and thus no formation of the E1B-55kD protein leading to growth arrest [20]. Initial preclinical studies showed that dl1520 was effective in decreasing

tumor size and thus was the first AdV to go to clinical trial [12, 84]. In the clinical trial, AdV was combined with systemic chemotherapy in patients with advanced sarcoma. Only one partial response was seen in a patient with malignant peripheral nerve sheath tumor [73]. Concerns for dl1520 arose when its specificity was found to be independent of p53 status [20]. Another site for gene inactivation within osteosarcoma is the Rb mutation. The AdV, Ad5-Δ24, removes the binding site for the Rb protein with a 24-base pair deletion in the E1A region [32]. This deletion results in virus replication that is selective for cells that have a defective Rb mutation. A study utilizing AdΔ24 alone in human osteosarcoma cell line in vitro and in vivo demonstrated antitumor efficacy and persistence of viral particles within the tumors. Unfortunately, no complete cures were observed in vivo [102]. Therefore, in another in vitro and in vivo study, standard chemotherapy agents to treat osteosarcoma were used in combination with virus. AdΔ24 alone again showed some efficacy; however, when cisplatin was added, the antitumor efficacy was enhanced [66]. AdΔ24, renamed DNX-2401, was utilized in a Phase I clinical trial with recurrent malignant glioma with 12% demonstrating a ≥95% reduction in tumor size and 20% of patients survived >3 years after treatment. No current clinical trials are open for osteosarcoma.

Another approach to adenovirotherapy includes placing genes essential for replication under the control of a tumor-specific promotor. Osteocalcin is a protein hormone found in the bone, limited to osteoblasts in healthy humans, but has high activity in osteosarcoma [51]. Ad-OC-E1A is a constructed AdV with the osteocalcin promoter. It has been tested in canine osteosarcoma cells with enhanced killing in vitro and a therapeutic benefit in vivo [41]. Ad-OC-E1A also showed efficacy in human osteosarcoma cells in vivo and in vitro with significant tumor reduction in a pulmonary metastatic model [58]. A Phase I/II trial was planned for AD-OC-E1A in osteosarcoma patients, but the study has yet to be published [9].

Telomerase activity is another target for regulated expression as 44–81% of bone and soft-tissue sarcomas have been detected to have activity [37]. Human telomerase reverse transcriptase (hTERT) gene expression stabilizes telomere lengths and is highly expressed on cancer cells but not normal tissues [50]. OB-301, telomelysin, is an AdV that utilizes the hTERT promoter to restrict replication to cells with high telomerase activity [37]. OBP-301 was tested on osteosarcoma cell lines and demonstrated insensitivity to the antitumor effects unlike other bone and soft-tissue sarcomas [57, 88]. Thus, enhanced OBP-301, with added expression of wild-type p53 (OBP-702), or combination with a p53-expressing replication-deficient adenovirus was utilized in vitro and in vivo osteosarcoma cell lines. OBP-702 was found to induce more profound tumor delays and cell killing. Through a Phase I clinical trial, OBP-301 was well tolerated in patients with advanced solid tumors; however, no patients with osteosarcoma were enrolled [72].

Finally, AdV has been manipulated to alter the TME. IL-24 has exhibited antitumor activity in numerous types of cancer like osteosarcoma (58). In one study, an hTERT promoter AdV was equipped with IL-24 (OA-IL-24), thus resulting in high levels of IL-24 in the TME. Utilizing osteosarcoma cells, OA-IL-24 demonstrated higher killing in vitro and suppressed tumor growth in vivo. More importantly, OA-IL-24 increased sensitivity to doxorubicin [61]. Lastly, an oncolytic AdV named VCN-01 utilized the defective pRB restrictive pathway with an addition of an expression cassette of human PH20 gene, which expressed hyaluronidase leading to degradation of extracellular matrix hyaluronic acid [67]. An association between high hyaluronic acid levels and low survival rates and development of chemoresistance is seen in some tumors [67]. VCN-01 demonstrated potent antitumor effect in vivo and in vitro including in a metastatic model. VCN-01 is currently being utilized in two clinical trials (NCT02045602 and NCT2045589) in adults with refractory solid tumors.

Herpes Simplex Virus

Herpes simplex virus (HSV) is an enveloped, double-stranded linear DNA virus with a 152 kb genome. Due to its large genome and nonessential joint regions, a large portion can be removed without affecting viral potency, resulting in space for the insertion of transgenes [34]. HSV is part of the Herpesviridae family with characteristic oral or genital ulcerations manifesting with infection. HSV attaches to host cell membrane through binding of surface glycoproteins to several different cellular receptors as well as heparan sulfate proteoglycans followed by a conformational change that triggers the fusion of the viral envelope to the host cellular membrane. The virion is then released into the cytosol to be degraded and delivered to the nucleus where replication will occur followed by death of the host cell [22]. Most oncolytic HSV-1 have been engineered to have a mutation of the gene encoding ICP34.5, which is the neurovirulence gene which results in marked attenuation in normal cells but not most tumor cells. Oncolytic HSV continues to have an intact thymidine kinase (tk) to allow for treatment with antiherpetic agents in the event of a viral outbreak [22]. Some oncolytic HSVs have inactivated UL39 that encodes ICP6 protein, which is required for viral DNA replication and highly expressed in rapidly dividing cells [59].

Several oncolytic HSVs (talimogene laherparepvec (T-VEC), HSV1716, NV1020, G207, M032, rRp450, etc.) are being utilized in preclinical and clinical studies and have been efficacious in numerous tumor types including sarcomas, melanomas, and colon, breast, lung, and hepatic tumors [11]. NV1020 and G207 have been utilized preclinically in osteosarcoma with only modest sensitivity [11]. HSV1716 has been utilized in preclinical and clinical studies. Preclinical data suggest the favorable responses observed in various solid tumor types are based on not only a direct lytic effect but also an antitumor immune response [56].

HSV1716 was also utilized in a Phase I clinical trial evaluating intratumoral injection in children and young adults with non-central nervous system tumors including osteosarcoma. Despite no durable responses, a patient with osteosarcoma experienced an immunologic flare-up as a result of viral injection [95]. T-VEC is similar to HSV1716 except with the deletion of ICP47, which normally blocks antigen presentation to MHC classes I and II, and contains the coding sequence for human granulocyte-macrophage colony-stimulating factor in place of ICP34.5 [21]. A Phase I trial of T-VEC in children with non-central nervous system solid tumors is underway (NCT02756845).

Vaccinia Virus

Vaccinia virus is an enveloped, double-stranded linear DNA virus with a genome of 190 kb and is a member of the poxvirus family. The original use of vaccinia virus eradicated smallpox in 1979. With its large genome, as with HSV, the ability to delete nonessential genes makes vaccinia virus attractive as an oncolytic virotherapy option. After utilizing numerous proteins for viral entry into cells, vaccinia virus is unique among the other DNA viruses as viral replication occurs independently in the cytoplasm [24]. The utilization of vaccinia growth factor (VGF) via epidermal growth factor receptor allows for viral spread to uninfected tissues [24].

To engineer vaccinia virus toward oncolytic properties, deletion of VGF restricts viral infection to cells with epidermal growth factor receptors, which is often observed in cancer cells [14]. For further attenuation, deletion of the J2R gene encoding for viral thymidine kinase (tk) leads vaccinia virus to be dependent on cells with overexpression of cellular tk, which is also often observed in cancer cells [78]. With both gene deletions, the resulting oncolytic virus was named double-deleted vaccinia virus and is very specific for cancer cells and thus increasing the safety profile [96].

In addition to a double-deleted vaccinia virus, a single-deleted vaccinia virus with additional gene insertions has also been developed. JX-594, also called Pexa-Vec, includes thymidine kinase gene deletions, granulocyte-macrophage colony-stimulating factor insertion to induce a systemic

antitumor immune response, and lac-Z gene insertion under control of the p7.5 promoter [23]. JX-963 is a double-deleted vaccinia virus with the insertion of granulocyte-macrophage colony-stimulating factor.

Preclinical studies have shown high cytotoxicity of Pexa-Vec in vitro against osteosarcoma [39, 63]. In a metastatic osteosarcoma model, double-deleted vaccinia vaccine demonstrated reduced lung metastatic lesions with significantly prolonged survival [63]. Numerous other solid tumors in the pediatric and adult population have shown antitumor activity with numerous vaccinia virus including rhabdomyosarcoma, fibrosarcoma, and fibrohistiocytoma [39]. Several clinical trials in adult patients have proven the safety of oncolytic vaccinia virus with one Phase II trial demonstrating improvement in survival in patients with advanced liver cancer [23]. A Phase I clinical trial utilizing Pexa-Vec including pediatric patients with unresectable refractory solid tumors demonstrated that Pexa-Vec was safe in this population [23]. As far as we are aware, no clinical trials to date have included osteosarcoma patients.

Protoparvovirus

Protoparvovirus H-1 (H-1PV) is a wild-type virus occurring naturally in rats and is a single-stranded, non-enveloped DNA virus with a genome of 5.1 kb. The genome contains only two main transcription units, nonstructural proteins (essential in the replication and cytotoxicity of the virus) and viral capsid proteins [99]. There have been numerous preclinical data suggesting H-1PV oncolytic properties and preference toward tumor cells; however, no one mechanism has been found likely due to numerous mechanisms combined [5]. Wild-type H-1PV or a mutant and deletion of 114 in-frame nucleotides called Del H-1PV have been studied with osteosarcoma. In the pioneering clinical trial utilizing H-1PV in the 1960s, two adolescent patients with osteosarcoma were injected with wild-type H-1PV with no adverse side effects noted. However, both succumbed to their disease shortly thereafter [97]. We found only one other report of utilizing H-1PV in osteosarcoma cells. A preclinical in vitro study utilizing wild-type H-1PV and Del H-1PV demonstrated more effective cytotoxicity of osteosarcoma cells with wild-type versus Del. This result is in contrast to all other preclinical studies reported that suggest Del H-1PV is more effective [33]. More preclinical in vitro and in vivo studies need to be performed utilizing H-1PV.

Oncolytic RNA Viruses

Reovirus

Reovirus is a member of the Reoviridae family and is a non-enveloped double-stranded RNA virus that has low pathogenicity in adults with no exhibited clinical symptoms [45]. Reovirus shows selectivity toward infection of malignant cells with an activated Ras-signaling pathway, which is typically present in osteosarcoma cells [47]. In a preclinical study of canine solid tumors, osteosarcoma was susceptible to reovirus but not to the degree as other solid canine solid tumors [45]. Reolysin is a formulation of reovirus type 3 Dearing strain developed by Oncolytics Biotech and is the only clinically utilized reovirus. This formulation was utilized in vitro and in vivo with significant results in sarcoma cell lines, including osteosarcoma. Stable disease was seen in the osteosarcoma murine models with partial responses when combined with cisplatin [43]. Numerous clinical trials utilizing Reolysin have been performed for many different solid tumors in the adult population with a very tolerable toxicity profile and minimal side effects. A Phase I trial in pediatric patients of Reolysin alone or in combination with cyclophosphamide included three osteosarcoma patients. Both Reolysin alone and in combination were well tolerated; however, no objective responses were seen [53]. A Phase II trial with Reolysin given intravenously to patients with bone and soft-tissue sarcomas metastatic to the lungs has been completed, but no completed published results have been reported to our knowledge.

Semliki Forest Virus

Semliki Forest virus is an enveloped positive-stranded RNA virus of the Togaviridae family [49]. Wild-type Semliki Forest Virus is pathogenic to small rodents and mice but is nonpathogenic in humans [8]. A mutated version of Semliki Forest Virus, SFVA7, has been mutated at its opal codon and amino acids in the nonstructural genome, and rodents, mice, or humans remain asymptomatic with injection. SFVA7 was further altered to express green fluorescent protein, VA7-EGFP, and compared against adenovirus, Ad5Δ24, in osteosarcoma xenografts. In vitro, VA7-EGFP was superior to Ad5Δ24 with more extensive cell death in a shorter period of time with a lower multiplicity of infection. In vivo, VA7-EGFP demonstrated significantly improved survival compared to Ad5Δ24 [49].

Vesicular Stomatitis Virus

Vesicular stomatitis virus (VSV) is a negative-sense RNA virus of the rhabdovirus family. Infection with vesicular stomatitis virus is typically seen in cattle, horses, and swine, with rare, insignificant disease in humans. Oncolytic properties of VSV are due to the sensitivity to interferon with replication only in cells with defective interferon responses, mostly malignant cells [46]. In vitro study of VSV compared to reovirus demonstrated that osteosarcoma was highly susceptible to VSV infection and had more effective cytotoxicity at similar multiplicity of infection [74]. Another study utilized isolated limb perfusion to reduce the viral amount dispersed to the rest of the body. This technique leads to viral gene expression largely limited to osteosarcoma cells without infection of other tissues local or distant, while the primary tumor site demonstrated significant tumor growth delays [54]. A recombinant VSV with near-infrared fluorescent protein, Katushka (rVSV-K), inserted into the genome has been studied in two in vitro studies. First, rVSV-K was utilized in a metastatic osteosarcoma model where it was determined that metastatic lesions had slower growth and prolonged survival compared to control mice, but also the metastatic cells were easily visualized in whole blood samples [46]. Following this study, the same group demonstrated that rVSV-K was also useful in surgical resections with margins significantly larger in the rVSV-K group than in the nonfluorescent group [86].

Measles Virus

Measles virus is a negative single-stranded RNA virus belonging to the Paramyxoviridae family. Measles virus is highly contagious with high morbidity and mortality without a prior exposure via vaccination. Measles virus enters a cell through interactions of its H protein, CD46, and signaling lymphocyte-activating molecule. Oncolytic selectivity is achieved via overexpression of CD46 on most tumor cells. In one in vitro study, an osteosarcoma cell line was resistant to measles virus due to inhibition of viral replication and entry [10]. However, in another in vitro and in vivo study, there were numerous osteosarcoma cell lines sensitive in vitro and antitumor activity with one highly metastatic model in vivo. They found that all six osteosarcoma cell lines were susceptible to measles virus and it was highly cytotoxic. In one metastatic xenograft model, they found reduction of metastatic lesions, prolonged tumor growth at the primary site, and prolonged survival with utilization of measles virus [28].

Poliovirus

Poliovirus is a non-enveloped, positive-stranded RNA virus belonging to the Picornaviridae family which causes paralytic poliomyelitis in humans. Poliovirus attachment and entry are mediated by a single molecule, CD155, which is upregulated in most solid tumors [13]. After confirmation that several osteosarcoma cell lines expressed CD155, poliovirus was shown to induce apoptosis in vitro [7]. As far as we are aware, no in vivo or clinical trials have been performed utilizing osteosarcoma and poliovirus.

Newcastle Disease Virus

Newcastle disease virus is a negative, single-stranded RNA virus belonging to the Paramyxoviridae family and results in a contagious viral bird disease with humans unaffected. PV701 is an attenuated strain with oncoselectivity based on defective interferon signaling. In vitro, PV701 shows a cytotoxic effect on osteosarcoma cells [80]. Newcastle disease virus has been utilized in clinical trials for patients with solid tumors and well tolerated [62]. No clinical trials to our knowledge have included osteosarcoma patients.

Maraba Virus

Maraba virus is a negative single-stranded RNA virus belonging to the Rhabdoviridae family and is found in sand flies with no detection outside of South America currently [77]. The most likely mechanism for malignancy precedence is the utilization of the ubiquitous low-density lipoprotein receptor, which is expressed on a wide range of malignancies [77]. In a comparative in vitro analysis of Maraba virus compared against HSV-1, adenovirus, reovirus, and VSV, Maraba virus exhibited more cytotoxicity. Currently, as far as we are aware, there are no active clinical trials utilizing Maraba virus for osteosarcoma patients.

Combination Therapy

Oncolytic virotherapy has shown numerous promising results in preclinical and clinical studies; however, it has become apparent that single-agent therapy will unlikely eradicate disease. Combination therapy is attractive as a way to overlap immunogenic cell death and enhance responses to therapy. These combination therapies with oncolytic viruses are currently underway. Investigators have begun to study T-VEC in combination with checkpoint inhibition into the clinical trials with good results in melanoma and greater efficacy and also tolerability [29]. Numerous other oncolytic viruses are being combined with checkpoint inhibition preclinically and clinically [17, 60, 81]. In one preclinical study utilizing osteosarcoma murine models, combination of HSV-1 and anti-programmed death ligand-1 demonstrated prolonged survival in an "inflamed" TME murine model compared to a "noninflamed" TME model (Wedekind and Cripe, unpublished results). To our knowledge, no other checkpoint inhibition with oncolytic virus therapy combinations is currently published for osteosarcoma.

Another intriguing combination is two viruses in combination. Newcastle disease virus in combination with adenovirus demonstrated enhanced cytotoxicity in vitro and superior antitumor efficacy in vivo compared to either virus alone in an osteosarcoma model [18]. Another group utilized combination of fowl pox viruses, an enveloped linear double-stranded DNA virus, and Newcastle disease virus. They determined the combination of these two viruses had increased cytotoxicity to osteosarcoma cell line in vitro with prolonged survival and tumor growth in vivo [103]. Despite a limited number of current combination therapies for osteosarcoma, there are numerous potentials that warranted exploration in the future and will be discussed in the future directions section.

Challenges to Oncolytic Virotherapy

Despite much preclinical and clinical data supporting the utilization of oncolytic virotherapy, there are challenges to overcome. One of the main limitations to oncolytic virotherapy is the mode of delivery. The ideal situation would be for intravenous administration to reach metastatic sites; however, there are concerns about the amount of viral particles that reach tumor sites as the liver can sequester much of the particles [3]. To avoid this limitation, virus may be administered via intratumoral injection. For tumors that are very superficial and accessible, intratumoral injection would not be a limitation; however, numerous patients have inaccessible tumors or tumors that are very large. For osteosarcoma in particular, with pulmonary metastatic lesions, intratumoral injections raise more complications

such as the risk of pneumothorax. More data need to be collected to determine the extent of an abscopal effect, as melanoma treated with T-VEC demonstrated abscopal effects that may render injection of distant sites unnecessary [30].

Another complicating factor is that neutralizing antibodies can dampen the efficacy of oncolytic viruses [35]. It has been demonstrated that 50–80% of humans have antibodies against HSV-1 and 90% against reovirus [101]. In a Phase I clinical trial utilizing oncolytic measles virus, the dose level was very high before any efficacy was seen due to neutralizing antibodies [85]. In another Phase I trial, maximum neutralizing antibodies were reached by one-third of patients in 1 week and by almost two-thirds by 2 weeks. Thus, the recommendation for the systemic treatment of reovirus is to administer numerous high doses in the first week of treatment before the neutralizing antibodies can diminish its effect. This limitation reconfirms the need for combination therapy [35, 100].

Future Directions

The future of oncolytic virotherapy for osteosarcoma has numerous possibilities. As there has been limited data in regard to the use of oncolytic virotherapy for osteosarcoma, there is much that needs be learned. The use of combination therapy needs to be explored preclinically with osteosarcoma to determine the best applications for clinical trials. In a preclinical in vivo and in vitro study, trabectedin was observed to inhibit osteosarcoma tumor growth and metastatic growth [79]. Trabectedin in combination with HSV-1 oncolytic virus has shown complete regression of tumors in a Ewing sarcoma mouse model [26]. There is potential that this combination may show benefit in osteosarcoma. Another future combination therapy that has great potential but will require thoughtfulness with its execution is CAR-T cell therapy and oncolytic virotherapy [2]. There are numerous options and pitfalls for combining these two therapies, but through this combination, there may be the potential to counteract immunosuppression within the TME, reverse tumor immunosuppression, or improve the CAR-T homing and activation [2]. Continuing to gain knowledge of the effects of oncolytic virotherapy within the TME and the utilization of combination therapies to enhance the body's immune response are necessary to propel oncolytic virotherapy as a future treatment option for patients with osteosarcoma.

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