Molecular Therapy
Oncology
Review

Translation of oncolytic viruses in sarcoma

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Sarcomas are a rare and highly diverse group of malignancies of mesenchymal origin. While sarcomas are generally considered resistant to immunotherapy, recent studies indicate subtype-specific differences in clinical response to checkpoint inhibitors (CPIs) that are associated with distinct immune phenotypes present in sarcoma subtypes. Oncolytic viruses (OVs) are designed to selectively infect and kill tumor cells and induce intratumoral immune infiltration, enhancing immunogenicity and thereby sensitizing tumors to immunotherapy. Herein we review the accumulated clinical data evaluating OVs in sarcoma. Small numbers of patients with sarcoma were enrolled in early-stage OV trials as part of larger solid tumor cohorts demonstrating safety but providing limited insight into the biological effects due to the low patient numbers and lack of histologic grouping. Several recent studies have investigated talimogene laherparepvec (T-VEC), an approved oncolytic herpes simplex virus (HSV-1), in combination therapy regimens in sarcoma patient cohorts. These studies have shown promising responses in heavily pre-treated and immunotherapy-resistant patients associated with increased intratumoral immune infiltration. As new and more potent OVs enter the clinical arena, prospective evaluation in subtype-specific cohorts with correlative studies to define biomarkers of response will be critical to advancing this promising approach for sarcoma therapy.

INTRODUCTION

Sarcomas are composed of a heterogeneous group of bone and soft tissue malignancies of mesenchymal origin.[1] Sarcomas are rare, accounting for less than 1% of all adult cancer cases in the United States, but disproportionally impact children, accounting for approximately 15% of pediatric solid tumors.[2,3] Patients diagnosed with localized sarcoma are treated aggressively in the frontline setting with treatments that may include surgery, chemotherapy, and radiation therapy (RT). Despite the approval of newer agents (such as pazopanib, eribulin, trabectedin, and tazemetostat), the overall survival of patients with metastatic sarcomas ranges between 12 and 18 months.[4–6] Thus, there is an urgent need for the development of more effective therapeutic options for this orphan group of diseases.

Immunotherapies have been studied, including checkpoint inhibitors (CPIs) and chimeric antigen receptor (CAR) T-cell therapy, with overall modest clinical efficacy observed in sarcomas.[7,8] The SARC-028 study was one of the first clinical trials to evaluate CPI therapy (pembrolizumab) for metastatic sarcoma showing overall response rates of 18% and 5% in soft tissue and bone sarcomas, respectively, with higher response rates observed in specific sarcoma subtypes. Sarcomas are generally considered to be immunologically "cold" tumors, which contributes to the poor responses observed to immunotherapies.[9,10] Low mutational burden,[11] lack of PD-L1 expression on tumor cells,[12] exclusion of immune cells from the tumor niche,[13] and increased presence of immunosuppressive cell types including myeloid-derived suppressor cells (MDSCs) and M2 macrophages,[14] have been identified as factors that drive immune suppression in the sarcoma tumor microenvironment (TME) (Figure 1). Correlative studies reveal immunologic features, e.g., increased tumor PD-L1 expression[15] or the presence of tertiary lymphoid structures in the TME,[13] that are more prevalent in sarcoma subtypes with higher response rates to CPI therapy, e.g., undifferentiated pleomorphic sarcoma (UPS) and alveolar soft part sarcoma (ASPS). ASPS, notably, was found to have high tumor immune score and tumor PD-L1 expression,[16] that likely plays a role in the successful use and approval of CPI therapy (atezolizumab) for this indication.[17]

Oncolytic viruses (OVs) are a novel class of antineoplastic agents. These replication-competent viruses selectively propagate in tumors. They exert their anticancer directly, through the infection and killing of tumor cells, and indirectly by recruiting a milieu of immune cells to the TME to promote antitumor immune response[18] (Figure 1). Reverse engineering allows for manipulation of the viral genome to enhance their tumor tropism, immunostimulatory activity, and improve their safety.[19] Talimogene laherparepvec (T-VEC), an oncolytic herpes simplex virus 1 (HSV-1), became the first oncolytic virus to obtain regulatory body approval in the by the US Food and Drug Administration and the European Medicines Agency. In the pivotal phase III OPTiM trial, patients with unresectable stage IIIB/C-IVM1a melanoma who were randomized to receive intratumoral injections of T-VEC had a significantly superior durable response rate compared with patients who received subcutaneous granulocyte-macrophage colony-stimulating factor (GM-CSF) alone, 16.3% vs. 2.1% respectively.[20] This benefit was maintained on longer follow-up, 19% vs. 1.4% favoring the T-VEC arm.[21] OV therapy is a promising approach to promote release of tumor-associated antigens and inflammation in the TME in sarcomas to enhance responsiveness to immunotherapy approaches.[22] The lack of efficacious systemic therapies, relative resistance to CPI therapy, and often large accessible tumor distribution make sarcomas an ideal target for intratumoral treatment with OVs. Several OVs, both naturally occurring and

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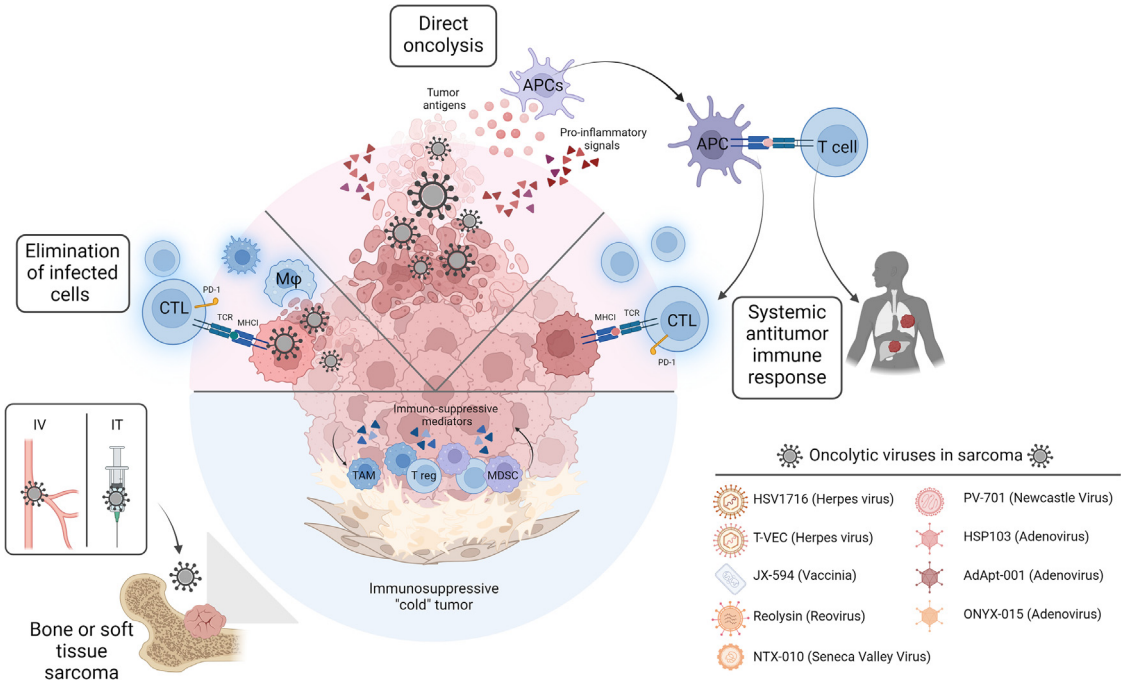

Figure 1. Oncolytic viruses as immunotherapy for sarcoma

Sarcomas show poor tumor immunogenicity, which varies across different specific subtypes. Oncolytic viruses currently investigated in the clinic have been engineered to increase their tumor selectivity and deliver immunogenic payloads, either by intravenous or intratumoral delivery. Upon infection, direct tumor elimination is achieved by direct lysis of tumor cells (oncolysis) and by recruiting both adaptive and innate immune cells to kill infected cells. Viral infection and immunogenic cell death induce pro-inflammatory signals that revert immunosuppression in the tumor microenvironment. Tumor antigens are released, processed by antigen-presenting cells (APCs) and presented in a pro-inflammatory environment, eliciting a systemic tumor-specific response able to eliminate tumors at distant sites. MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; Treg, T regulatory.

engineered, have proven efficacious in preclinical sarcoma models,[23] but only a small number have been tested clinically to treat patients with sarcoma. Table 1 lists the oncolytic viral vectors that have been tested clinically to treat patients with sarcoma, often in combination with other treatment modalities. This review seeks to examine the clinical experience with OV therapy in the treatment of patients with sarcoma and explore approaches that may overcome the barriers to successful implementation of this promising modality.

CLINICAL TRIALS

Following a PubMed database review, we identified 19 clinical trials published between 2002 and 2023 evaluating OV therapy that included at least one patient with sarcoma (keywords: oncolytic virus, virotherapy, solid tumors, phase I, pediatric cancer, soft tissue sarcoma, viro-immunotherapy). There were a total of 194 patients with bone or soft tissue sarcoma who received at least one dose of OV therapy by either intratumoral or intravenous administration, alone or in combination with chemotherapy, radiation, or immunotherapy (Table 1). We excluded two studies that were only available in Chinese,[24,25] interim data reported in conference abstracts,[26] or results published after 2023.[27] There are also several OV trials currently enrolling patients with sarcomas that are ongoing, which may not have reported final results (Table 2).

Thirteen out of 19 trials were phase I studies, three trials were phase I/II, and three were phase II studies. Phase I trials were generally single-agent dose-escalation studies that included small numbers of patients with confirmed sarcoma as part of larger solid tumor cohorts. While the majority of trials reviewed were phase I studies, collectively they accounted for 37% of OV-treated sarcoma patients. Phase II studies generally evaluated fixed dose regimens in larger sarcoma cohorts, accounting for 44% of OV-treated sarcoma patients (Figure 2A). The reviewed clinical trials tested 10 OVs derived from six viral platforms (Table 1), with approximately 80% of sarcoma patients having received OV therapy by the intratumoral route of administration (Figure 2B). HSV-1-based vectors were the most frequently studied oncolytic vectors accounting for 64% of OV-treated patients (>50% received T-VEC) and more than half of patients received OV therapy in a combination therapy regimen. Notably, all phase I/II and phase II trials evaluated OV therapy in the context of combination therapy regimens (Figure 2A). Given the disproportionate impact of sarcoma on children and the poor outcomes in the pediatric setting, we also distinguished trials that enrolled adults vs. cohorts that included children (Table 1). Five clinical trials (with five different OVs) were carried out in patient cohorts that included children and young adults, accounting for ~24% of all OV-treated sarcoma patients.[28–33]

Table 1. Oncolytic viral vectors tested clinically in patients with sarcoma

Viral backbone	Agent name	Clinicaltrials.gov identifier	Phase	References	Age for eligibility	Route of administration	Total number of sarcoma patients
Group C adenovirus	H103[20]		I	Li et al.[80]	18–70	IT	1
	ONYX-015[21]		I/II	Galanis et al.[43]	≥18	IT	6
	Telomelysin[22]		I	Nemunaitis et al.[44]	≥18	IT	2
	AdApt-001	NCT04673942	I	Conley et al.[53]	≥18	IT	13
Herpes simplex virus type 1	HSV1716[32,33]	NCT00931931	I	Streby et al.[33]	≥7 to ≤30	IT	8
				Streby et al.[32]		IV	6
	T-VEC[13,28]	NCT03069378	II	Kelly et al.[51]	≥18	IT	20
		NCT03886311	II	Chawla et al.[36]	≥18	IT	50
		NCT02756845	I	Moreno et al.[31]	≥2 to ≤21	IT	10
		NCT02453191	II	Monga et al.[35]	≥18	IT	30
Vaccinia virus	JX-594[29,55,56]	NCT00625456	I	Breitbach et al.[81]	≥18	IV	1
		NCT01169584	I	Cripe et al.[29]	≥2 to ≤21	IT	1
		NCT02630368	I/II	Toulmonde et al.[52]	≥18	IV	15
Seneca Valley virus	NTX-010[57]	NCT01048892	I	Burke et al.[28]	≥3 to ≤21	IV	3
Newcastle disease virus	PV-701[14,58,59]		I	Pecora et al.[34]	≥18	IV	4
			I	Laurie et al.[82]	≥18	IV	2
			I	Hotte et al.[83]	≥18	IV	1
Reovirus type 3 Dearing	Reolysin[34,60,61]		I	Vidal et al.[84]	≥18	IV	2
		NCT01166542	I/II	Karapanagiotou et al.[85]	≥18	IV	1
		NCT01240538	I	Kolb et al.[30]	≥3 to ≤21	IV	18

Viral vectors and the number of sarcoma patients in the respective clinical trials, patient age range, and route of administration. IT, intratumoral; IV, intravenous.

Figure 3 shows the distribution of the sarcoma subtypes and the distribution of patient cohorts that included only adults (≥18 years old) or those that enrolled children and young adults. In concordance with the expected frequency of sarcoma subtypes, patients with UPS/MFH, liposarcoma, and leiomyosarcoma collectively accounted for 39% of the collective across all 19 trials. Rhabdomyosarcoma, osteosarcoma, and Ewing sarcoma accounted for 20% of the sarcoma subtypes, likely due to the five clinical trials that allowed pediatric-aged patients. Granular detail of the sarcoma subtypes was not always given, particularly in early phase I trials.[34] For example, the subtype of rhabdomyosarcoma was listed in 13 of the 16 rhabdomyosarcoma cases. Similarly, only two of the phase II studies detailed the subtypes of liposarcoma treated on their clinical trials evaluating T-VEC combination therapy regimens.[35,36]

VIRAL CONSTRUCTS

Oncolytic virotherapy utilizes the replication-competent viruses as drug therapy. Tumor selectivity is key and can be achieved by using naturally tumor selective viruses or viral engineering. The description of engineered OVs tested clinically in sarcoma is shown in Table 3. Reolysin, PV701 (Newcastle disease virus), and the Seneca Valley virus NTX-010 are unmodified viruses that have been evaluated clinically, all administered intravenously. PV-701 is a mesogenic, highly purified isolate, of the nonrecombinant MK107 vaccine strain of Newcastle disease virus.[34] Prior studies in related Newcastle disease virus strains demonstrated the tumor selectivity, ability to induce immune-mediated antitumor activity and efficacy in fibrosarcoma xenografts.[37–39] NTX-010 is a naturally occurring picornavirus that selectively infects and propagates in tumors with neuroendocrine features.[40] In a preclinical screen prior to clinical translation, consistent *in vitro* and *in vivo* cytotoxicity was observed in neuroblastoma and rhabdomyosarcoma.[41] All other oncolytic viruses were engineered to enhance their tumor selectivity and/or antitumor efficacy. H103, Telomelysin, ONYX-015, and AdAPT-001 are replication competent (or conditionally replicating) oncolytic adenoviruses.[42–45] H103, ONYX-105, and Telomelysin contain modifications to attenuate or control E1B 55-kDa expression designed to enhance tumor selectivity by restricting virus replication to tumor cells lacking p53 function. H103 additionally encodes the HSP70 gene leveraging the ability of heat shock proteins to chaperone and present tumor antigens to dendritic presenting cells and was shown to mediate its antitumor immunity through CD8+ cytotoxic T-lymphocyte responses.[46] AdAPT-001 is described as a "minimally modified" oncolytic adenovirus with a 50 base pair (bp) deletion in the E1A promoter region, enhancing tumor selectivity without impairing virus fitness, and encoding an immunomodulatory transforming growth factor beta (TGF-β) trap that sequesters

Table 2. Currently active (recruiting or not yet recruiting) clinical trials of oncolytic virotherapy trials, alone or in combination, for which patients with sarcoma are eligible for enrollment

Clinicaltrials.gov identifier	Viral agent (virus type)	Partner modality (if applicable)	Target population	Trial phase
NCT02630368	JX-594 (Vaccinia)	Metronomic cyclophosphamide +/− Avelumab	Soft tissue sarcoma and breast cancer	II
NCT02700230	MV-NIS (Measles virus)		Advanced/metastatic malignant peripheral nerve sheath tumors	I
NCT02923778	T-VEC (HSV-1)	Radiation	Resectable liposarcoma, leiomyosarcoma, undifferentiated pleomorphic sarcoma (UPS)	II
NCT03069378	T-VEC (HSV-1)	Pembrolizumab	Advanced/metastatic cutaneous angiosarcoma, epithelioid sarcoma, UPS, myxofibrosarcoma (MFS)	II
NCT03886311	T-VEC (HSV-1)	Nivolumab and trabectedin	Advanced/metastatic sarcoma including desmoid tumors and chordoma	II
NCT04599062	T-VEC (HSV-1)	Radiation	Resectable stages II-IV soft tissue sarcoma	I/II
NCT03767348	RP1 (HSV-1)	Nivolumab	Locally advanced or metastatic non-melanoma skin cancer (NMSC)	I/II
NCT04714983	DNX-2440 (Adenovirus)		Resectable liver metastases	I
NCT04725331	BT-001 (Vaccinia)	Pembrolizumab	Advanced solid tumors	I/II
NCT05061537	PF-07263689 (Vaccinia)	Sasanlimab	Advanced solid tumors	I
NCT05361954	STI-1386 (HSV-1)		Advanced solid tumors	Ib

and prevents immunosuppression by TGF-β.[45] Two HSV-1-based constructs have undergone clinical translation in patients with sarcoma. HSV1716 and T-VEC share the deletion of ICP34.5, which enhances their tumor selective replication. HSV1716 maintains expression of thymidine kinase, rendering it susceptible to acyclovir as a safety mechanism.[47] T-VEC notably contains two additional modifications: deletion of ICP47 to promote antigen presentation, and arming with the human GM-CSF transgene to promote dendritic cell recruitment and activation.[48] Since the approval of T-VEC for melanoma, its investigational use has been expanded to other malignancies including sarcoma.[48] JX-594 is a vaccinia virus engineered to have increased tumor selectivity through deletion of thymidine kinase, armed with the human GM-CSF transgene. In rabbit and rat models, JX-594 was well tolerated, had minimal to no detectable plaque-forming units in the normal tissues, significantly increased GM-CSF levels in tumor-bearing rabbits, and significantly increased tumor-infiltrating CD4+ and CD8+ cells compared with their controls.[49] Oncolytic reovirus (Reolysin), vaccinia (JX-594), Seneca Valley virus (NTX-010), and HSV-1 vectors (HSV1716 and T-VEC) have been evaluated in cohorts that included children and young adults with sarcoma.

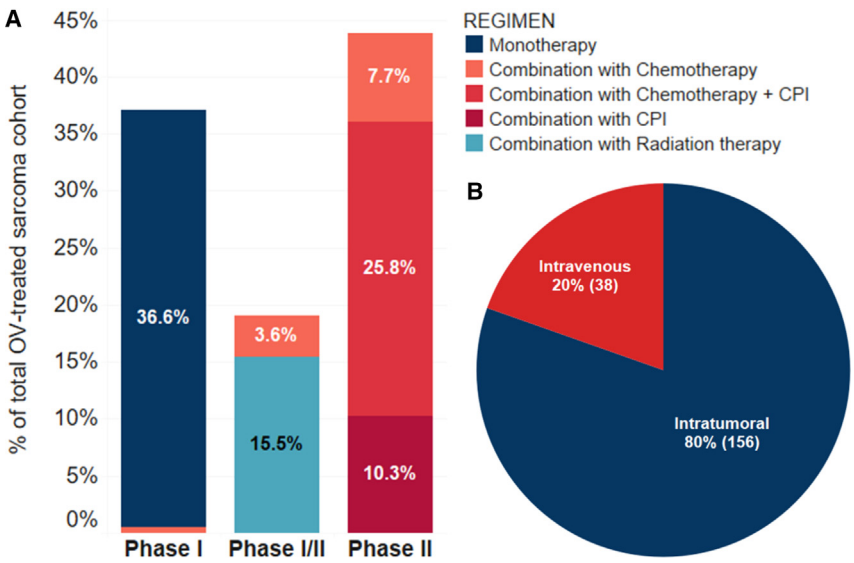

Figure 2. Distribution of oncolytic virus clinical trials that included patients with sarcoma by phase, regimen and route of administration

Clinical trial distribution by phase and regimen (A), and route of administration (B). The distribution of OV-treated sarcoma patients based on their enrollment in phase I, I/II, or II trials. CPI, checkpoint inhibitor; OV, oncolytic virus.

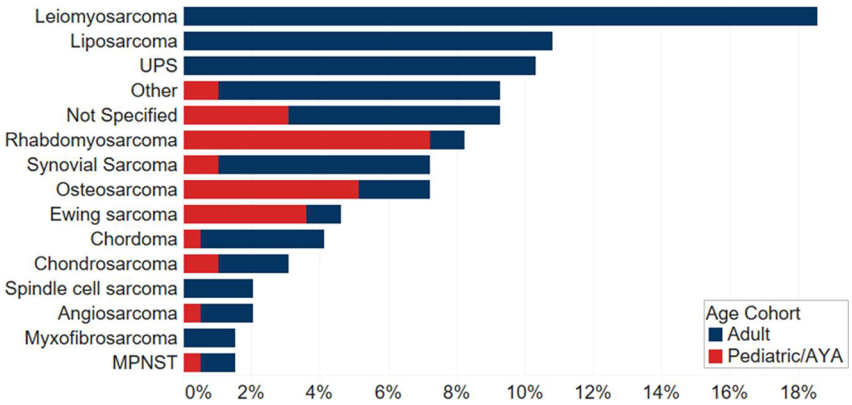

Figure 3. Distribution of the cohort age and sarcoma subtypes oncolytic virus clinical trials that included patients with sarcoma

AYA, adolescent and young adult; UPS/MFH, undifferentiated pleomorphic sarcoma; MPNST, malignant peripheral nerve sheath tumor; Other subtypes include clear cell sarcoma, desmoplastic small round cell tumor, extraskeletal myxoid chondrosarcoma, spindle cell sarcoma, carcinosarcoma, alveolar soft part sarcoma, solitary fibrous tumor, myoepithelioma, endometrial stromal sarcoma, gastrointestinal stromal tumor.

CLINICAL SAFETY

In a recent comprehensive review of 97 OV clinical trials conducted in over 3,000 patients across 20 years, most adverse events attributed to the viruses were low-grade constitutional symptoms or local injection site reactions.[50] In this review, it was noted that high-grade myelosuppression was often associated with cytotoxic chemotherapy. Similarly, while rare, the immune-related adverse events were associated with CPI treatment.[50] Several recent clinical trials were not covered in that comprehensive review, including four phase II trials utilizing T-VEC in combination therapy regimens in larger sarcoma cohorts and a phase I evaluation of T-VEC in children that included pediatric sarcoma patients. Kelly and colleagues evaluated T-VEC in combination with the anti-programmed death protein 1 (PD-1) monoclonal antibody pembrolizumab in patients with metastatic and/or locally advanced sarcoma (NCT03069378). The incidence of grade 3 treatment-related adverse events of 20% was consistent with prior studies of immune CPIs alone.[51] In another study, Monga et al. evaluated weekly administration of T-VEC with concurrent radiation in the neoadjuvant setting in patients with trunk and extremity sarcomas prior to surgical resection (NCT02453191). Toxicities were in keeping with prior studies of intratumoral administration. Notably, there was no increase in the risk of wound-related events above the historic expected rate, with only 27% of patients developing wound-healing complications.[35] Chawla et al. describes the use T-VEC in combination with nivolumab (PD-1 inhibitor) and the chemotherapy trabectedin (TNT regimen) in a phase II study in patients with advanced, previously treated sarcomas (NCT03886311).[36] Adverse events were mainly related to trabectedin and comparable to the safety profile of trabectedin therapy alone.[4] Toulmonde and colleagues described phase II studies testing JX-594 in combination with metronomic cyclophosphamide vs. metronomic cyclophosphamide alone. Treatment was generally well tolerated, with most patients experiencing low-grade fever or fatigue. Only two of 15 patients randomized to the experimental arm experienced grade 3 toxicities (fever and lymphopenia) and there were no grade 4 toxicities.[52] Conley et al. reported the results of a first-in-human phase I trial using AdAPT-001 demonstrating the safety and tolerability of intratumoral AdAPT-001 therapy at doses up to 1e12 viral particles (vp).[53]

In the five trials testing OV therapy in the pediatric setting, treatment was overall well tolerated though treatment durations were often shorter due to rapid disease progression. HSV1716 was the first oncolytic HSV-1 evaluated in children using both intratumoral and intravenous routes of administration, both showing good tolerability. Almost all enrolled pediatric patients were HSV-1 seronegative reflecting the relative lack of exposure to many common viral pathogens among children. Moreno and colleagues recently reported the first study evaluating intratumoral T-VEC in children.[31] The median duration of T-VEC treatment was just 5.1 weeks before disease progression necessitated treatment termination. There was one reported observation of injection site ulceration that tested positive for HSV-1. In another study, three of three pediatric patients who received oncolytic vaccinia virus JX-594 at the top dose developed skin pustular lesions within a week after treatment that was assumed to be vaccinia-associated pox lesions that resolved within 3–4 weeks.[29] These observations indicate children may be at higher risk of developing symptoms of pathogenic virus infection, though symptoms were generally mild and resolved spontaneously. In a trial evaluating intravenous therapy with Reolysin in children, there were two reported grade 5 serious adverse events (SAEs) within 3 weeks of treatment. While these SAEs were likely due to rapidly progressing metastatic disease, possible relationship to OV therapy could not be ruled out.[30]

These results collectively demonstrate the overall favorable safety profile of OV therapies in patients with advanced sarcoma. OV combination therapy regimens were also found to be well tolerated and there was no reported exacerbation of expected adverse events associated with standard chemo-, immuno-, or RT protocols. These findings are reported with the caveat that most patients received intratumoral therapy, and the safety profile of intravenously administered OVs would very likely differ significantly. OV therapy may be more challenging in pediatric cancer patients, with early results suggesting an increased risk of complications compounded by the aggressive nature of pediatric tumors (particularly in the relapsed/refractory setting), and potential for pathogenicity stemming from relative naivety of the immune system in children (potentially further suppressed by prior cytotoxic chemotherapy protocols).

Table 3. Engineered oncolytic viruses tested for sarcoma therapy and summary of the modification(s) to the respective viruses

Viral platform	Oncolytic virus	Notable modification	Intended impact
Vaccinia virus	JX-594	• Thymidine kinase deletion • Human GM-CSF gene insertion • LacZ gene insertion	• Tumor selective replication • Promotes induces antitumoral immune response • Serves as a marker (β-galactosidase)
HSV-1	HSV1716	• Deletion ICP34.5 • Maintains thymidine kinase expression	• Removes neurovirulence and improves tumor selective replication • Susceptible to acyclovir treatment
	T-VEC	• ICP34.5 deletion • ICP47 deletion • Insertion of GM-CSF	• Removes neurovirulence and improves tumor selective replication • Promotes antigen presentation to MHC class I and II • Induces antitumor immune responses
Adenovirus (Group C)	ONYX-015	• Modifications to eliminate E1B-55kd expression	• Inhibition of virus replication in cells with normal p53 function. Selective replication in cells that lack normal p53 function (tumor cells)
	H103	• E1B-55kDa deletion • HSP70 gene insertion	• Enhances tumor selectivity (as above) • Chaperone promotes tumor antigen presentation to activate antitumor immune response
	Telomelysin	• Replacement of E1B gene transcriptional element with an internal ribosomal entry site (IRES) • Human telomerase reverse transcriptase gene (hTERT) promoter driven viral gene expression	• Increases tumor selectivity and specificity to enhance antitumor efficacy and diminish toxicity to normal tissue
	AdApt-001	• "Minimal modification" - 50 bp deletion in E1A gene promoter • TGF-β trap transgene insertion	• Enhance tumor selectivity without attenuating virus replication • Sequesters TGFβ1 and TGFβ3 from initiating TGFβ signaling cascade in targeted cells

TUMOR EFFICACY

Thirteen of 194 (7.7%) patients with sarcoma had an objective radiologic response (RECIST criteria) following OV therapy. Given that 37% of OV-treated sarcoma patients were enrolled in phase I dose-escalation studies, it is likely that many patients received a subtherapeutic OV dose, contributing to the modest response rates. In all reviewed phase I studies, OVs were found to be well tolerated at all doses tested with no maximum tolerated dose (MTD) reached. Factors such as the limits of GMP manufacturing can be a barrier to further dose escalation. For example, the first-in-human BETA PRIME trial (NCT04673942) tested the oncolytic adenovirus AdAPT-001 at three dose levels ranging from 2.5e11 to 1e12 vp with no dose-limiting toxicities reported and the MTD was not reached. Tumor shrinkage was observed in two of three patients treated at the top dose in the dose-escalation phase. This study included an expansion cohort at the top dose showing partial responses (PR >30% tumor reduction) in three of 15 evaluable patients that included the one patient who responded to OV monotherapy, a patient with chordoma who had a partial response in just the injected lesion following intratumoral AdAPT-001 therapy. It should be noted that additional sarcoma patients (leiomyosarcoma and chordoma) had prolonged disease stabilization resulting in a clinical benefit rate (PR or prolonged disease stabilization) of 37%. Larger cohorts of sarcoma patients were included in phase I/II and phase II trials, all using combination therapy regimens. In the phase I/II trial of ONYX-015 combined with full-dose mitomycin, doxorubicin, and cisplatin chemotherapy, one patient with malignant peripheral nerve sheath tumor (MPNST) had 70% reduction in the maximal dimension of the injected lesion, and reduction of 50% and 75% in two uninjected lesions. This response lasted 11 months. No correlation could be made between viral replication and treatment response.[43] Phase II evaluation of oncolytic vaccinia, JX-594, combined with low-dose cyclophosphamide in patients diagnosed with advanced soft tissue sarcoma showed no clinical benefit, with all patients progressing within 6 months.[52] While not included in formal analysis, a recent study reported no overall clinical benefit of adding avelumab (a PD-L1 inhibitor) to JX-594 and cyclophosphamide combination therapy, though a partial response was observed in a single patient with angiosarcoma.[27]

In the phase II study described by Kelly et al. assessing intratumoral T-VEC in combination with pembrolizumab, 20 patients with 11 different sarcoma subtypes were enrolled. Sixty percent of patients had received three or more prior lines of therapy, including five (25%) who received prior CPI therapy. Seven (of 20) patients with five subtypes of soft tissue sarcoma had partial response (ORR 35%), with a median time to response of 14.4 weeks (range 6.6–31.9 weeks) and median duration of response of 56.1 weeks (range 49.4–87 weeks). The histologic subtypes are noted in Table 4. Of note, responses were observed in two of the five patients who had progressing disease while on CPI therapy administered immediately prior to enrollment (angiosarcoma and epithelioid sarcoma).[51] The

Table 4. Clinical trials that utilized a combination therapy regimen

Year published	References	Oncolytic virus	Combination used in treatment arm	Total sarcomas in treatment arm (number of subtypes)	Sarcoma subtypes with objective radiologic response (number of patients)
2005	Galanis et al.[43]	ONYX-015	Mitomycin, doxorubicin, cisplatin	6 (4)	MPNST (1)
2012	Karapanagiotou et al.[85]	Reolysin	Carboplatin and Paclitaxel	1 (1)	
2014	Burke et al.[28]	NTX-010	Cyclophosphamide (Part B of trial)	9 (1)	
2015	Kolb et al.[30]	Reolysin	Cyclophosphamide (Dose level 3)	6 (NR)	
2020	Kelly et al.[51]	T-VEC	Pembrolizumab	20 (11)	Angiosarcoma (2) Epithelioid sarcoma (1) Myxofibrosarcoma (1) Sarcoma unclassified (1) UPS (2)
2021	Monga et al.[35]	T-VEC	Radiation	30 (10)	Myxoid liposarcoma (1)
2022	Toulmonde et al.[52]	JX-594	Metronomic (low dose) Cyclophosphamide	15 (6)	
2023	Chawla et al.[36]	T-VEC	Trabectedin and Nivolumab	50 (15)	Leiomyosarcoma (1) Liposarcoma (1) UPS (1)

Table indicates the number of patients with sarcomas, number and histologic subtypes of patients that had an objective radiologic response.
MPNST, malignant peripheral nerve sheath tumor, NR, not reported, UPS, undifferentiated pleomorphic sarcoma.

TNT regimen (T-VEC, nivolumab, trabectedin) administered in heavily pre-treated patients resulted in tumor shrinkage in six of 39 evaluable patients, with three confirmed PRs in patients with leiomyosarcoma, UPS, and liposarcoma (ORR 7.7%). Despite the modest response rate (notably lower than that achieved with T-VEC plus pembrolizumab), a disease control rate of 85% was achieved resulting in a median progression-free survival (PFS) of 7.8 months, nearly double the median PFS of 4.1 months achieved with trabectedin alone.[4] New and more potent oncolytic HSV-1 vectors have been developed and entered the clinic. RP1 is one such potency-enhanced oncolytic HSV-1 being tested in combination with nivolumab in the phase I/II IGNYTE trial in patients with skin cancer. Preliminary results show good tolerability and objective (partial or complete) responses in four of six patients with angiosarcoma in the non-melanoma skin cancer cohort (not included in formal analysis).[54]

In the phase Ib/II clinical trial conducted by Monga et al., the investigators evaluated the impact of weekly intratumoral T-VEC in combination with standard of care external beam radiation therapy (EBRT) in patients with operable soft tissue sarcomas of the extremities and trunk.[35] Following confirmation of the safety of the weekly T-VEC dosing with radiation, they sought to determine if the combination could improve the pathologic near complete response rate (near-PCR defined as pathologic tumor necrosis $\geq$95%) from the historic 8%-10%–25%. In this neoadjuvant trial of patients with operable sarcomas, only one patient with myxoid liposarcoma had partial response by RECIST 1.1 criteria. However, seven patients of 29 (24%) achieved near-pCR: three patients with myxoid liposarcoma and four with UPS.[35] Near pathologic complete response to preoperative treatment has proven to be predictive of outcome in osteosarcoma, but though suggestive, does not similarly correlate in patients with soft tissue sarcoma.[55,56]

No objective clinical responses were reported in pediatric patients with heavily pre-treated sarcoma who received an investigational OV therapy. Given the aggressive nature of pediatric malignancies, this is not unexpected, particularly in the relapsed/refractory setting. In the absence of objective responses, duration of disease stabilization was used as marker of clinical utility. HSV1716 therapy resulted in stable disease in two patients (Ewing sarcoma and chondrosarcoma) following intravenous treatment,[32] and in two patients (rhabdomyosarcoma and chondrosarcoma) following intratumoral treatment (radiologic assessment done 4 weeks post therapy).[33] Disease stabilization in the patient with Ewing sarcoma was durable and this patient was alive more than 2 years after treatment. In the first assessment of intratumoral T-VEC in children, two of the three patients with stable disease had sarcoma diagnoses (soft tissue sarcoma not specified, and rhabdomyosarcoma) with treatment ongoing at 38 and 40 weeks post start of treatment. Intratumoral JX-594 therapy resulted in stable disease in the injected lesion in one patient with Ewing sarcoma, but progression of uninjected lesions.[29] Intravenous therapy with oncolytic Seneca Valley virus (NTX-010) and reovirus (Reolysin) resulted in stable disease outcomes in six of 12 patients (including one patient with alveolar rhabdomyosarcoma) and in three of 24 evaluable patients respectively. The treatment of relapsed/refractory pediatric sarcoma remains a formidable challenge and early results with OV therapy, while showing anecdotal clinical benefit, have not demonstrated compelling efficacy in this setting.

CORRELATIVE STUDIES

Given the heterogeneity of sarcomas, correlative studies have the potential to identify common biomarkers associated with response to OV and combination therapy approaches. Serologic studies for neutralizing antibodies were reported in the trials evaluating ONYX-015, HSV1716 (Seprehvir), and Reolysin, overall showing

an increase in antiviral antibodies following intratumoral or intravenous administration of virotherapies in patients with various malignancies including sarcoma. Anti-adenoviral antibodies were evident at baseline in two of four patients and increased or were present in all patients following administration of ONYX-015.[43,44] Antibody levels were not reported following intratumoral AdAPT-001 adenoviral therapy, but serum analysis showed detectable increase in viral genomes in serum 4 or 8 days post-treatment in five patients indicating virus replication and entry into circulation.

In the phase I study testing intratumoral or intravenous HSV1716 therapy in young cancer patients, all but one (of 18) were negative for HSV1 antibodies at baseline. Following intratumoral HSV1716 administration, five of eight patients who were negative for HSV1 antibodies at baseline seroconverted.[33] All evaluable patients seroconverted after receiving intravenous HSV1716 therapy.[32] Similarly, in a phase I trial evaluating Reolysin in children and young adults (up to 21 years old), 18 sarcoma cases were included out of 29 total. Nine of 24 (38%) evaluable patients had detectable anti-reovirus antibodies at baseline. All 24 evaluable patients had an increase in their antiviral antibody levels following their first treatment, with no difference in peak viremia irrespective of baseline antibody status.[30] These results indicate seroconversion following intratumoral or intravenous administration of OVs, but also suggest that pediatric patients are less likely to have been exposed to common human pathogens that form the backbone of several clinical stage OVs.

Tissue sampling and analysis was conducted in several studies. No research biopsies were performed on pediatric patients. Galanis and colleagues performed baseline biopsies to confirm diagnoses and evaluate p53 and MDM2 status in their trial evaluating ONYX-015 in patients with advanced sarcomas. Repeat biopsy on day 5 of cycle 1 was conducted to perform *in situ* hybridization (ISH) for adenoviral DNA. Two of six patients had detectable adenoviral DNA by ISH in their tumor tissue but not surrounding normal tissue, which the authors concluded was suggestive of tumor-specific viral replication.[43] In their follow-up study to determine the safety of intravenous administration of Seprehvir (HSV1716), Streby and colleagues amended their protocol to allow for a day 7 post intravenous administration biopsy in patients older than 17 years. This was a positron emission tomography (PET) imaging-guided biopsy to detect intratumoral HSV-1 by PCR and immuno-histochemistry. Two patients with chondrosarcoma and osteosarcoma, respectively, had tissue sampling with no evidence of HSV-1, with no analysis of immune infiltrate.[32]

More recent phase II studies incorporated immunologic analyses on tumor specimens. In the trial combining T-VEC with pembrolizumab in patients with locally advanced/metastatic sarcomas, pre- and post-treatment biopsies were conducted to quantify the tumor-infiltrating lymphocyte (TIL score) and characterize infiltrating lymphocyte immune biomarker expression. Adequate specimens for analysis were obtained in 16 patients with pretreatment and 14 patients with post-treatment biopsies. Six of 11 patients with paired evaluable tumor samples converted from PD-L1 negative to positive following treatment. PD-L1 conversion did not appear to correlate with response. Kelly and colleagues noted the mean TIL score was higher among responders (TIL score = 3) compared with patients with treatment-refractory disease (TIL score = 2). More importantly, they found that in the 12 patients with assessable tumor pairs, the responsive patients had CD3+/CD8+ TIL clusters and aggregates at the infiltrating edge of the tumor at baseline that increased with treatment. In stark contrast, non-responders had minimal infiltrates in their pre- and post-treatment samples, and virtually no evidence of TIL clusters or aggregates.[51] Histopathologic and molecular profiling of tumors from selected patients treated with the TNT regimen showed tumor necrosis and increased TILs in one patient with UPS, and a post-treatment reduction in malignant cells and a corresponding increase in immunologic signatures in the TME in a rhabdomyosarcoma tumor specimen from a patient that had sustained disease remission (lasting >2.5 years). An ongoing trial testing RP1, a potency-enhanced oncolytic HSV-1, combined with nivolumab in non-melanoma skin cancers also included responding angiosarcoma patients. Correlative analyses of paired biopsies revealed increased T cell and inflammation associated gene expression signatures post-treatment, though sarcoma-specific results were not discussed.[54] Toulmonde et al. conducted their evaluation of the immune response to JX-594 through proteomic analysis of paired plasma samples prior to and following administration of the oncolytic virus, on cycle 1 days 8 and 22 respectively. They found significant elevation of pro-inflammatory markers, such as the chemokine CXCL10 and soluble CD8 antigen suggesting lymphocyte activation. Of note, they also found significant upregulation of immunosuppressive cytokines TGF-β and IL18,[52] providing a rationale for adding avelumab (PD-L1 agonist) to JX-594 and cyclophosphamide combination therapy in a cohort of patients with advanced soft tissue sarcoma (NCT02630368). This study built in findings published by Petitprez et al. using the presence of intratumoral tertiary lymphoid structures as a biomarker of tumor immunogenicity. Analysis of matched (pre- and post-treatment) biopsy specimens showed an increase in CD8+ TILs in 10 of 11 patients, with the most pronounced increase observed in a patient with angiosarcoma who had a partial response,[13] The only neoadjuvant OV study included in this report was conducted by Monga and colleagues, combining T-VEC with EBRT in patients with extremity and trunk sarcomas. Analyses on pre-treatment biopsies and post-treatment resection specimen, as well as comparison with historic institutional controls treated with radiation only found substantial increases in the CD3-, CD4-, and CD8-expressing T cells, and to a lesser extent CD56+ (Natural Killer) cells, following treatment with T-VEC and radiation.[35]

EXPANDED THERAPY ACCESS PROGRAM

We identified seven additional studies that included the experience of patients with sarcoma treated on the Advanced Therapy Access Program (ATAP), regulated by the Finnish Medicines Agency (Fimea).[40,57–64] These were not evaluated in detail in our report. The advantages and challenges of this access program have been highlighted.[65] The adenoviral constructs and sarcoma subtypes

Table 5. Summary of the sarcoma patients treated on the Finnish Advanced Therapy Access Program (ATAP)

Primary author	Adenovirus	Route of administration	Total number of patients	Sarcoma subtypes (total number)
Cerullo	Ad5-D24-GMCSF	4/5ths dose IT (or IC) followed by 1/5th dose IV	20	Leiomyosarcoma (1) Synovial sarcoma (1)
Nokisalmi	ICOVIR-7	IT or IC	21	Leiomyosarcoma (1)
Koski	Ad5/3-D24-GMCSF	4/5ths dose IT (or IC) followed by 1/5th dose IV	21	Sarcoma NOS (1) Chondrosarcoma (1) Synovial sarcoma (1)
Pesonen	Ad5-RGD-D24-GMCSF	4/5ths dose IT (or IC) followed by 1/5th dose IV	7	Liposarcoma (1)
	Ad5-D24-RGD		9	Synovial sarcoma (1)
Hemminki	Ad5/3-E2F-Δ24-GMCSF (CGTG-602)	4/5ths dose IT (or IC) followed by 1/5th dose IV	13	Sarcoma NOS (1) Fibrosarcoma (1)

IC, intracoronary; IT, intratumoral; IV, intravenous; NOS, = not otherwise specified.

treated are summarized in Table 5. Two of the reports evaluating either the immunologic effects of low-dose cyclophosphamide or antiviral and antitumor immunity had overlapping patients and are not presented in the table.[61,62] As with the clinical trials, adenoviruses were well tolerated with adverse effects similar to the previously referenced trials evaluating replication-competent OVs. Most sarcoma patients did not display evidence of RECIST criteria radiologic responses, whether alone or in combination with chemotherapy (cyclophosphamide). In the study reported by Hemminki et al., there were two patients with sarcoma who received low doses of cyclophosphamide and temozolomide to reduce their regulatory T cells and enhance autophagy, respectively. One of these patients, a 50-year-old female with fibrosarcoma (identified as S354), had complete metabolic response by PET-computed tomography (CT) and 76% reduction in tumor volume in 6 months, and was stable after 9 months.[63] While patient S354 did not have tissue sampling, a patient with ovarian cancer who had a minor metabolic response on PET-CT had pre- and post-treatment biopsies that showed significant increases in the levels of CD3, CD4, and CD8 T cells above baseline.[63]

STRATEGIES TO ENHANCE THERAPEUTIC EFFICACY IN SARCOMA

Challenges facing the translation of OVs and strategies to bypass these limitations have been well described. These have consistently included, but are not limited to, overcoming suboptimal delivery and host-mediated immune clearance, enhancing oncolytic efficacy through improving viral replication and augmenting their immunogenic potential.[66–68] Sarcomas pose additional unique hurdles: the vast heterogeneity of the disease group coupled with the rarity of individual subtypes has consistently hampered new drug development. Lumped approaches to drug development have been suboptimal and have hindered rather than helped advance the success of sarcoma clinical trials. The pivotal ANNOUNCE trial that failed to confirm the superiority of olaratumab when paired with doxorubicin over doxorubicin alone, allowed nearly 30 sarcoma subtypes.[69] In contrast, over the past decade sequential regulatory body approvals for pazopanib, trabectedin, eribulin, and tazemetostat have been gained in patients with advanced sarcomas. The keys to the success of these trials included narrowing of the eligibility criteria and enrollment of subtype-specific cohorts.[4,6,70,71]

The tally of just 13 responses in the 194 sarcoma patients reviewed who received OV therapy in a clinical trial setting may not inspire confidence in this approach for sarcoma. However, like the disease, the details matter. OV monotherapy was studied in several phase I studies in patients with advanced solid tumors that sporadically included some sarcoma patients, providing limited description of the sarcoma subtypes and did not include a statistically relevant cohort of sarcoma patients. In fact, 12 of the 13 objective clinical responses were achieved in the context of a prospectively planned sarcoma cohort where patients received OV therapy in combination with either chemotherapy (n = 1), radiotherapy (n = 1), CPI therapy (n = 7), or combination chemo- and CPI therapy (n = 3). The highest response rate was achieved with T-VEC and pembrolizumab in a cohort of patients with locally advanced or metastatic sarcoma, with clinical responses observed in seven of 20 (35%) patients enrolled and a median duration of response of 56 weeks. This was an impressive (albeit preliminary) outcome in a cohort of previously treated sarcoma patients, made more notable by the observation of response in two patients whose disease was progressing on prior CPI therapy. This is a rare demonstration of a combination therapy that can re-sensitize tumors to checkpoint blockade, a growing need as CPIs are increasingly used to treat various malignancies.

The observation of clinical responses mainly in the context of intratumoral OV combination therapy brings to light several important factors that will be critical to driving successful clinical outcomes in sarcoma. First, the biological activity of OVs may be best leveraged in the context of combination therapy in sarcoma. Most clinical stage OVs are being tested in combination with CPIs, though combinations with other immunotherapies (e.g., CAR-T cells) are also being explored.[72,73] A second consideration is that the use of intratumoral OVs is limited to readily accessible injectable lesions. Sarcomas frequently metastasize to the lung, and pulmonary metastatic disease is the main cause of death for patients. While Kelly et al. reported remission of both injected and distant noninjected lesions in three

patients indicative of systemic antitumor immune response following T-VEC and pembrolizumab therapy, such abscopal is relatively uncommon clinically. This leads to our third consideration that as the field of OV therapy evolves, it is becoming apparent that this early generation of clinical stage OVs were safe but lack efficacy. The next generation of OV therapies that are entering the clinic are selected and/or engineered to be more potent and more immunogenic,[68] while others are being developed for safe systemic therapy to reach sites of metastatic disease in patients with advanced malignancies.[74] Advancing new, more potent OVs, testing novel combination therapy strategies and optimizing dose regimens for sarcoma treatment is dependent upon preclinical evaluation in clinically relevant sarcoma models. Morton et al. evaluated NTX-010 in 23 cell lines and 36 xenograft models, and consistent *in vitro* and *in vivo* activity was displayed in neuroblastoma and rhabdomyosarcoma,[41] providing a basis for phase I evaluation of NTX-010 in children with relapsed/refractory solid tumors dominated by neuroblastoma sarcoma diagnoses. Makielski et al. reported the safety and clinical benefit of intravenous therapy with a clinical stage recombinant oncolytic vesicular stomatitis virus (VSV) in a veterinary trial in companion dogs with naturally occurring osteosarcoma, providing the basis for future studies in sarcoma.[75] Notably, canine sarcomas may more closely resemble pediatric sarcomas based on similarities in clinical presentation and overlapping gene expression profiles.[76,77] Translational studies in naturally occurring canine sarcoma can guide development of novel OV therapies for sarcoma.[78] Continued advances in the availability of clinically relevant sarcoma models will aid preclinical screening and optimization of the OV therapy and increase the likelihood of successful human translation.[79]

As new OV platforms are translated and tested clinically, correlative studies should be employed to monitor the immunomodulatory effects of OV therapy (and combinations thereof). Several clinical OV studies in sarcoma included correlates to detect presence of virus in tumors and neutralizing antibodies. These rarely correlated with clinical response. Kelly et al. highlighted the importance of both baseline presence and increase in number of CD3+/CD8+ TIL clusters in patients who responded to treatment with T-VEC plus pembrolizumab, irrespective of their sarcoma subtype. Trials conducted in the neoadjuvant setting (prior to standard of care surgical tumor resection) offer several advantages.[35,75,78] They facilitate evaluation of OV therapies in earlier stage (operable) disease, providing the potential to alter the disease course in a setting where the role of chemotherapy remains controversial. More importantly, they provide a unique opportunity to perform robust corollary analysis on larger tissue volumes to assess the biological effects of OV therapy on heterogeneous sarcoma tumors. Assessment of immune correlates, particularly in early-stage clinical trials, will allow identification of biomarkers that are potential surrogates of early clinical activity that can guide optimal design of OVs and combination therapy regimens. Monga and colleagues also provide strength to the observation that traditional parameters to assess radiologic response may not be the most suitable for assessing the impact of OVs on a given tumor.[35] While there was only one patient who achieved a radiologic PR, seven of 29 evaluable patients had near complete pathologic response to the combination of preoperative T-VEC and external beam radiation. Therefore, while clinical response is commonly a primary benchmark for therapeutic efficacy, in the context of the aggressive nature of relapsed or refractory sarcomas (particularly in pediatric patients), alternative clinical endpoints such as pathologic response or durable disease stabilization also are indicative of clinical benefit for patients with advanced sarcoma.

Conclusions

Replication-competent OVs represent an alternative and underutilized tool capable of unlocking the potential of immune-based approaches for the treatment of patients with sarcoma. From the limited findings from clinical use of OVs for sarcoma therapy, this modality may be best utilized in a combinatorial approach. Given the heterogeneity of this disease group, greater preclinical screening in clinically relevant sarcoma models is warranted, to increase the likelihood of successful human translation. It is important to note that most virotherapy approaches being investigated for sarcoma treatment to date have been intratumorally administered. Therefore, they need to be sufficiently immunogenic to elicit abscopal antitumor immune responses to eliminate disseminated disease in patients with metastatic sarcoma. It is critical that future development of OVs in patients with sarcoma include strains engineered for systemic delivery to maximize the ability to treat patients with disseminated disease. In addition, future prospective trials should ensure they incorporate corollary studies for rigorous interrogation of the impact of the viruses on the sarcoma immune microenvironment to facilitate the development of predictive biomarkers and guide combinatorial strategies.

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AUTHOR CONTRIBUTIONS

The conception, design, data review, interpretation, and writing of this manuscript were equally performed by S.I.R. and S.N.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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