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Oncolytic Poxviruses

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Abstract

Current standard treatments of cancer can prolong survival of many cancer patients but usually do not effectively cure the disease. Oncolytic virotherapy is an emerging therapeutic for the treatment of cancer that exploits replication-competent viruses to selectively infect and destroy cancerous cells while sparing normal cells and tissues. Clinical and/or preclinical studies on oncolytic viruses have revealed that the candidate viruses being tested in trials are remarkably safe and offer potential for treating many classes of currently incurable cancers. Among these candidates are vaccinia and myxoma viruses, which belong to the family Poxviridae and possess promising oncolytic features. This article describes poxviruses that are being developed for oncolytic virotherapy and summarizes the outcomes of both clinical and preclinical studies. Additionally, studies demonstrating superior efficacy when poxvirus oncolytic virotherapy is combined with conventional therapies are described.

Keywords

vaccinia virus; myxoma virus; virus tropism; oncolytic; virotherapeutics; virotherapy

INTRODUCTION

Oncolytic virotherapy, which exploits replication-competent viruses to selectively infect and destroy cancerous cells while sparing normal cells and tissues, is an emerging therapeutic for the treatment of cancer. Current standard treatments of cancer include surgery (if a tumor is resectable), radiotherapy, chemotherapy, thermotherapy, and biological/immunological therapy. Although available treatments prolong survival of cancer patients, and certain pediatric cancers are now considered curable, many common adult cancers remain essentially incurable. Therefore, more efficacious targeted therapies for the treatment of diverse cancers are much needed. Oncolytic viruses are under intensive clinical development because they can selectively target cancerous cells and tissues without causing significant adverse events in the cancer patient. Additionally, they possess the intrinsic ability to self-amplify in cancerous tissues and spread within tumor beds in a fashion that can stimulate more effective antitumor immune responses. Clinical and/or preclinical studies have

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revealed that candidate oncolytic viruses are remarkably safe and offer potential for treating many classes of currently intractable cancers.

Several candidate oncolytic viruses, or their attenuated versions, are being currently tested; these include members of Rhabdoviridae (e.g., vesicular stomatitis virus, Maraba virus), Poxviridae [e.g., vaccinia virus (VV), myxoma virus (MYXV)], Adenoviridae (e.g., adenovirus serotype 5), Herpesviridae (e.g., herpes simplex virus 1), Picornaviridae (e.g., coxsackievirus, poliovirus), Reoviridae (reovirus) and Paramyxoviridae (e.g., measles virus, Newcastle disease virus). The best candidates are those that also display positive synergistic cancer-specific cytotoxicity with more conventional therapeutic approaches, such as chemotherapy, small-molecule cell cycle inhibitors, radiation therapy, and antiangiogenesis agents. Furthermore, many oncolytic viruses have also been engineered to express transgenes to enhance their antitumor potency, immunoreactivity, and/or cancer specificity. The topic of oncolytic virotherapy, and the status of current clinical trials, has been extensively reviewed elsewhere (1–10). This review focuses on the promising oncolytic features of poxviruses, particularly VV and MYXV, that are currently being tested for their oncolytic potential in clinical trials and/or preclinical models.

BACKGROUND ON POXVIRUSES

Poxviruses, which belong to the family Poxviridae, are large enveloped viruses with a linear double-stranded DNA genome (11). Their genomes vary from 130 to 375 kbp and encode 150 to 300 or more genes. The central genomic region includes genes that are highly conserved among poxviruses, whereas the terminal regions tend to encode viral factors that are immunomodulatory or subvert host self-defense mechanisms such as innate antiviral responses (11). Poxviruses replicate entirely in the cytoplasm of an infected cell, in discrete perinuclear structures known as viral factories. The virions encapsidate numerous viral enzymes, including DNA polymerase, early transcription factors, DNA-dependent RNA polymerase, and capping enzymes needed to initiate replication and viral gene expression in the cytoplasm (11). There are two infectious forms of poxviruses: the intracellular mature virion (IMV) and the extracellular enveloped virion (EEV) that is released from an infected cell (12, 13). The details of poxvirus replication have been reviewed elsewhere (11, 14). The specific cellular receptor or receptors utilized by either form have not been identified, but given the wide tropism of poxviruses for most cultured mammalian cells, it is believed that poxviruses attach to cell surface determinants that are ubiquitously expressed on most mammalian cells (11, 14). Indeed, the IMV form of VV utilizes cell surface heparan sulfate and/or the extracellular matrix protein laminin as a cellular attachment factor (15–18). Poxvirion attachment to the cell surface allows the viral entry/fusion complex on the IMV membrane to fuse with the cellular plasma membrane, thereby releasing the virion core into the cytoplasm to initiate the viral replication cycle (19). Additionally, VV can enter target mammalian cells via macropinocytosis followed by fusion of the virion membrane with internal endosomal membranes and/or via direct fusion with the cell surface membrane (20–22). Importantly, poxviruses can bind and enter most mammalian cell types in a species-independent fashion, but what occurs next depends on whether the virus replication cycle can proceed all the way to making progeny virus (i.e., permissive infection) or aborts due to an incompatibility with the specific cell or the induction of robust antiviral responses (i.e.,

restrictive or nonpermissive infection) (11, 14). Furthermore, cells that are progressing rapidly through their cell cycle, such as many cancer cells, tend to be more permissive for poxvirus infections than comparable somatic cells that are quiescent (nondividing).

VV, which belongs to the genus *Orthopoxvirus*, is the prototypical poxvirus and is well known for its use as a live-attenuated vaccine for the global eradication of smallpox (11). Infected cells first produce IMVs in the cytoplasm, and these further acquire two additional membrane envelopes derived from the *trans* Golgi network or early endosome to become wrapped virions (11, 23–25). The wrapped virions are then transported to the cell surface via microtubules and fuse with the plasma membrane (26, 27); the resulting EEVs contain one additional membrane envelope. IMVs are important for spread between hosts, and EEVs promote the dissemination of virus within the host (28, 29). EEVs are more resistant than IMVs to neutralizing antibodies and are not inactivated by the complement system (30–32). Stock preparations of poxviruses used for oncolytic virotherapy are mostly composed of IMVs, but following permissive replication within tumor tissues, both IMV and EEV forms of the progeny virus are produced.

Advantages of poxviruses as oncolytic virotherapeutics include safety in humans, ease of production of high-titer stocks, stability of virus preparations, and feasibility of genetic manipulation for transgene expression (33). In addition, poxvirus replication takes place entirely in the cytoplasm of an infected cell, so viral genome integration into host chromosomes does not occur. Poxviruses are highly immunogenic, with a robust capacity to costimulate acquired antitumor immunity following replication within tumor tissues. Several effective antiviral agents are either licensed or under late-phase clinical development, in case of adverse events or rare cases of excessive virus replication in specific patients (34). Four poxviruses from three different genera have been investigated for oncolytic potential: VV (*Orthopoxvirus*), MYXV (*Leporipoxvirus*), racoonpox virus (*Orthopoxvirus*), and yaba-like disease virus (*Yatapoxvirus*) (35–38). All of these viruses undergo productive lytic replication in most human cancer cells. Of these, VV and MYXV have been most extensively tested and are the focus of this review.

POXVIRUS TROPISM

The specificity of certain nonpoxvirus oncolytic viruses for cancerous cells comes from the unique cell surface receptors required for virus entry that can be overexpressed on these cells. For example, the measles virus Edmonston strain and some adenovirus species can infect cells expressing CD46 in a receptor density dependent manner (5). However, for many other oncolytic viruses, including poxviruses, cellular tropism instead is mediated more by the aberrant signaling environments within cancer cells. For example, primary somatic cells display a rapid antiviral response to infection that can retard or abort viral replication, whereas almost all cancer cells are generally less able to induce the normal portfolio of innate antiviral responses. Poxviruses are believed to exert their cancer cell specificity downstream from binding and entry; normal cells tend to resist infection prior to the generation of progeny virus, whereas cancer cells are more apt to be fully permissive. Furthermore, preferential expansion of permissive virus replication within tumors can lead

to elevated immunoreactivity that, in addition to clearing the virus, can produce novel forms of acquired antitumor immune responses that mediate regression of uninfected cancer cells.

VV is the best-studied poxvirus and has been widely used in vaccine platforms (39–41). It is being developed as an oncolytic virotherapeutic agent for various types of neoplasms, including hepatocellular carcinoma, melanoma, pediatric solid tumors, and lung cancer (38, 42–46). The origins of VV are obscured by history; the virus currently has no known host species but exhibits a wide tropism for many classes of normal and cancerous mammalian cells in culture. The three strains of VV currently in clinical trials for oncolytic virotherapeutics have been modified for further attenuation and/or enhanced selectivity for replication in cancerous cells. All three oncolytic strain platforms have the viral thymidine kinase (TK) gene deleted (Table 1). TK is an essential enzyme for the pyrimidine synthesis pathway; viral TK gene deletion thus results in preferential replication in cells with high intracellular nucleotide pools, thereby increasing the selectivity of the virus for rapidly dividing cancerous cells (47). In one study, the extent of JX-594 replication correlated with cellular TK levels, and the reduction of endogenous TK levels in HeLa cells using a lentivirus expressing TK-specific short hairpin RNA decreased progeny virus production (45). Additionally, JX-594 replication was dependent on cellular epidermal growth factor receptor (EGFR)/Ras/mitogen-activated protein kinase (MAPK) pathway signaling and was suppressed by an inhibitor specific for extracellular signal–regulated kinase (45). In the VV double deletion (vvDD) platform (Table 1), the vaccinia growth factor (VGF) gene is also deleted for further attenuation and greater dependence on the cell cycling status of the target cells. VGF is expressed early during VV infection and is secreted as a ligand of EGFR to stimulate the proliferation of adjacent quiescent cells; it thus significantly impacts the spread of VV within normal tissues (48, 49). In mice, and presumably humans as well, the vvDD platform is less pathogenic than the parental Western Reserve strain of VV. Yet, this decrease in virulence does not affect its ability to replicate and effectively kill target human tumor cells, indicating that vvDD retains substantial oncolytic efficacy (50). The VV strains that have been or are now being tested in various stages of human clinical trials are summarized in Table 2.

Another poxvirus with promising oncolytic potential is MYXV, a member of the *Leporipoxvirus* genus (51, 52). Unlike VV, MYXV has an extremely rabbit-restricted host range in nature and is pathogenic only to European rabbits (*Oryctolagus cuniculus*), where it causes the rabbit-specific disease myxomatosis (53, 54). MYXV does not cause any detectable disease in other host species tested, including humans and even highly immunocompromised mice, indicating that it should be an extremely safe oncolytic virotherapeutic agent in cancer patients (53–56). Despite its extremely narrow host range, MYXV can productively infect and kill various nonrabbit cancerous cells both in vitro and in vivo (35). Its permissiveness in human cancer cells is largely based on multiple dysregulated intracellular pathways found in transformed cells, such as (*a*) failure to induce synergistic interferon and tumor necrosis factor antiviral responses, which efficiently abort MYXV replication in normal primary human fibroblasts (57, 58); (*b*) upregulation of activated Akt, which enhances MYXV infection (59, 60); and (*c*) defects in tumor-suppressor pathways that preferentially support MYXV infection, including mutations in

p53, ataxia telangiectasia (ATM), and retinoblastoma protein (Rb) (61). To date, the oncolytic potential of MYXV has been tested only in preclinical animal models for various types of cancer, including hematological malignancies such as acute myeloid leukemia and multiple myeloma and solid tumors such as pancreatic cancer, glioma, and melanoma. Chan et al. (35) recently summarized the results of these studies.

VACCINIA VIRUS ONCOLYTIC VIROTHERAPY

Besides engineering VV strains to maximize preferential replication within cancerous cells, investigators have also modified the virus platform to express various anticancer transgenes. Such transgenes may encode cytokines to enhance the immune stimulatory effects of the virus, agents to disrupt tumor vasculature or the extracellular matrix that hinders virus spread, prodrug-converting enzymes that generate antimetabolites within the cancerous cells, and/or bioactive molecules to detect therapeutic effects using a safe and noninvasive imaging method (43). The first VV tested in phase I clinical trials, pexastimogene devacirepvec (Pexa-Vec; JX-594), is a derivative of the Wyeth vaccine strain; it is being developed by Jennerex Biotherapeutics (United States) and Transgene (France). Pexa-Vec is not only TK^-; it is also armed with an immunomodulatory gene, encoding granulocyte monocyte colony-stimulating factor (GM-CSF), to stimulate the immunotherapeutic effects of the oncolytic virus (62). Mastrangelo et al. (63) first tested Pexa-Vec as a gene therapy platform to deliver GM-CSF intratumorally in patients with surgically incurable cutaneous melanoma. These authors found that multiple intratumoral injection of Pexa-Vec was safe, that the function of GM-CSF was maintained, and that effective tumor regression was induced (63). More recently, completed phase I and II clinical trials on patients with solid tumors, advanced hepatocellular carcinoma, or melanoma have demonstrated that Pexa-Vec can be successfully delivered either intravenously—even in the presence of neutralizing antibodies from prior smallpox vaccination—or intratumorally (64–67). The ability to effectively deliver VV to tumors even in smallpox-vaccinated patients was somewhat unexpected and suggests that the virus dosages that produced therapeutic effects were likely in excess of the levels that could be effectively inactivated by the patient sera. Pexa-Vec was demonstrated to replicate, even after as many as nine cycles of administration, despite the high neutralizing antibody titers (66). The virus was well tolerated up to a dose of 2×10^9 PFU (intravenous) or 1×10^9 PFU (intratumoral). Dose-dependent therapeutic effects were observed; these were correlated with increased survival. Intravenous administration led to the delivery of virus to metastasized tumors, where it replicated and expressed its transgenes in a dose-dependent manner without affecting normal tissues, because the virus preferentially replicates in dividing cancerous cells (64). Intratumoral delivery resulted in shedding of infectious viruses into the bloodstream that reached distant uninjected tumor sites (64, 67). Pexa-Vec achieves its efficacy through direct oncolysis, abetted by induced antitumor immunity augmented by GM-CSF, and through the antivascular effects of the virus in tumor beds (68). However, a recent randomized phase II trial with Pexa-Vec against hepatocarcinoma failed to reach significant survival benefit over blinded controls, possibly due to the late stage of disease in patients entered into this trial. This finding highlights a critical issue for oncolytic virus trials: Early-stage clinical trials tend to be conducted in patients with late-stage cancers who were admitted into the trial only after failing state-of-

the art therapies. These patients likely have reduced immunocompetency against their resultant tumors, which have been selected for resistance to the previous rounds of chemotherapy. Because oncolytic virotherapy is now thought to function optimally when the virus replication within tumor tissues stimulates a broader and more reactive antitumor immune response profile, possibly including improved levels of both cellular and humoral immune responses, future trials should focus on patients at the beginning of their therapeutic regimens, when their immunocompetency is highest.

Another strain of VV that is being tested in clinical trials and has been extensively studied in preclinical animal models is GL-ONC1 (GLV-1h68); it is being developed by Genelux Corporation (Table 1) (69). GLV-1h68 is attenuated by deletion of *F14.5L*, *J2R* (the viral TK gene), and *A56R* (the hemagglutinin gene) (69). GLV1h68 does not cause any body-weight loss even in immunodeficient nude mice and is much less toxic than the parental Lister strain virus, yet it still exhibits selective tumor targeting and even higher oncolytic efficacy than the parental Lister strain virus (69). GLV-1h68 causes efficient tumor regression and/or eradication in nude mice bearing various types of transplanted human tumors including colorectal cancer, prostate cancer, and salivary gland carcinoma (Table 3). Infiltration and activation of innate immune cells at tumor sites are believed to play a role in complete tumor elimination in those models, indicating that virotherapy-induced immune activation provides superior tumor regression efficacy. Currently, patients with solid tumors, peritoneal carcinomatosis, head and neck cancer, or lung cancer are being recruited for phase I–II clinical studies with GLV-1h68 (Table 2).

A third strain of VV that is being tested in clinical and preclinical studies is vvDD, which has both the TK and VGF genes deleted in order to increase its replication selectivity for cancerous cells; this strain replicates more selectively in cancer cells with preexisting EGFR/Ras pathway activation (Table 1) (50). It was recently shown that in immunodeficient mice bearing pediatric solid tumors, such as sarcomas or neuroblastoma, vvDD inhibits tumor growth and prolonged survival, indicating efficient oncolytic activity (70). A phase I clinical trial is underway to test vvDD on patients with solid tumors (Table 2).

Modified Vaccinia Viruses with Enhanced Oncolytic Efficacy

GLV-1h68 has been further modified to express various therapeutic agents with potential for improved anticancer properties. The tumor selectivity, toxicity, and oncolytic activities of these recombinant constructs were compared with those of the parental virus in preclinical mouse models bearing various types of tumors. For example, glioblastoma multiforme (GBM) is the most common and most aggressive malignant primary brain tumor in humans and generally comes with a poor prognosis. Temozolomide improves survival, but the development of resistant cell populations quickly renders the treatment ineffective. Evidence suggests that bone morphogenetic proteins (BMPs), which belong to the TGF-β superfamily of proteins, play a role in regulating cancer; in the context of GBM, BMPs can induce rapid tumor regression. BMP-4 has been tested as a differentiation agent to control colon cancer in mice, indicating the potential of exogenous BMPs to treat GBM and colon cancer (71, 72). GLV-1h285, which overexpresses BMP-4, was constructed and tested in immunocompromised mice bearing either a low or a high burden of human GBM cancer

stem cells (73). Compared with the parental GLV-1h68, intracranially administered GLV-1h285 replicated better, resulting in significantly enhanced tumor regression (73). Furthermore, no tumor recurrence was observed in mice receiving GLV-1h285 treatment, most likely due to the differentiation of cancer stem cells induced by virally expressed BMP-4, which subsequently facilitates VV replication and also possibly depletes the cancer stem cell pool (73). These findings suggest that GLV-1h285 possesses enhanced oncolytic virotherapeutic potential against GBM in preclinical models.

The FDA-approved drug bevacizumab is used in combination with chemotherapy to treat metastatic colorectal cancer and most forms of metastatic non–small cell lung cancer. Bevacizumab binds human vascular endothelial growth factor (VEGF), thereby inhibiting the formation of tumor vasculature. Patients with high tumor levels of VEGF have a less favorable prognosis for treatment outcome. Therefore, extensive studies to inhibit tumor vascularization have been conducted. A recombinant GLV-1h108 bearing the GLAF-1 single-chain antibody gene, which expresses an immunoglobulin-derived protein that binds to both human and murine VEGF, was constructed and tested to determine whether localized delivery of anti-VEGF antibody from recombinant VV had combined oncolytic virotherapy and immunotherapy effects in mice bearing human lung tumor xenografts (74). A single intravenous injection of GLV-1h108 resulted in the production of functional single-chain antibody in tumor xenografts (74). Importantly, tumor regression was more pronounced in mice treated with GLV-1h108 than in mice receiving the parental GLV-1h68, indicating enhanced therapeutic effects (74). Although the mechanism by which anti-VEGF single-chain antibody provides a superior therapeutic benefit is unclear, these data offer promising evidence that oncolytic poxviruses could be used for tumor-specific delivery of antibody-based drug therapy.

Malignant pleural effusion (MPE) is a complication associated with various cancer types, including advanced lung cancer, breast cancer, and lymphomas. Currently, there is no treatment for MPE; only palliative therapies are available. The development of MPE depends on invasion of the pleura and expression of VEGF by cancer cells. The oncolytic efficacy of GLV-1h68 and GLV-1h108 was compared in mice subcutaneously implanted with lung adenocarcinoma (75). Intravenous injection of GLV-1h108 resulted in significantly increased tumor regression compared with injection of the parental GLV-1h68, indicating enhanced oncolytic efficiency (75). Moreover, treatment with GLV-1h108 effectively controlled the formation of malignant effusions (75). It is believed that in addition to mediating VEGF inhibition, VV contributes to the resolution of MPE by inducing an inflammatory response to the viral infection within the tumor bed.

The cell cycle regulator cell division cycle 6 (Cdc6) plays an important role in the assembly of prereplicative complexes at the origin of replication and is essential for the initiation of DNA replication in dividing eukaryotic cells. Mutations in the Walker A motif of the yeast, mammalian, or *Xenopus* version of Cdc6 caused failure in the assembly of prereplicative complexes, which subsequently impaired initiation of DNA replication. Therefore, a recombinant GLV-1h237 expressing a Walker A motif–mutant Cdc6 was constructed and tested in an immunocompromised mouse model bearing human breast cancer (76). GLV-1h237 exhibited superior antitumor activity compared with the parental GLV-1h68,

with potential to interfere in host cancer cell DNA synthesis (76). Combining oncolytic virotherapy with agents that interfere with host cell DNA synthesis thus constitutes another promising approach to enhance oncolytic efficacy.

Recombinant human erythropoietin (rhEPO) is a glycoprotein hormone that regulates red blood cell formation and is used to treat cancer-related anemia (77, 78). Several clinical studies have suggested that rhEPO also promotes tumor growth (79, 80). In some animal experiments, rhEPO was shown to have antiapoptotic effects, induce angiogenesis, and promote tumor growth (81, 82). However, other animal studies showed no evidence of such deleterious effects (83–85). A recombinant GLV-1h210 expressing hEPO was constructed, and the effects of intratumorally expressed hEPO on tumor growth were examined in xenografted mice bearing human lung cancer (86). Insertion of the hEPO gene did not compromise virus replication (86). Intravenous injection of GLV-1h210 resulted in tumor-specific production and secretion of functional hEPO, which significantly increased red blood cell numbers and hemoglobin levels (86). Mice receiving GLV-1h210 exhibited enhanced tumor regression compared with mice injected with GLV-1h68 (86). The enhanced efficacy of GLV-1h210 most likely resulted from localized hEPO expression enlarging tumor vessels and thereby facilitating virus spread within the tumor (86). Therefore, virus-mediated expression of hEPO within the tumor microenvironment not only improved the oncolytic efficacy of the virus but also alleviated cancer-related anemia.

Modified Vaccinia Viruses with Enhanced Virus Spread

The efficacy of oncolytic viruses can, in theory, be improved by enhancing their cell-to-cell spread, which can be achieved by modifying the virus either to produce more progeny EEVs or to express a transgene-based protein that disrupts the tissue extracellular matrix. A recombinant vvDD that expresses a mutated version of A34 has been constructed and tested for oncolytic potential; in vitro analysis showed that vvDD-A34R produced more EEVs and total infectious progeny virus than the vvDD parent virus, resulting in a higher cytotoxicity in cultured cancer cells (87). Its oncolytic activity was further compared with that of the parental virus in immunocompetent mice bearing peritoneal carcinomatosis. Compared with vvDD infection, vvDD-A34R infection resulted in more efficient remote virus spread and higher replication levels, prolonging survival and enhancing antitumor effects (87). The oncolytic efficacy of vvDD and vvDD-A34R was tested in both immunologically naive and preimmunized mice bearing peritoneal carcinomatosis, using intraperitoneal injection of either purified (i.e., naked) virus or syngeneic cancer cells preinfected ex vivo with virus. Carrier cell–based virus delivery was more efficient than naked virus injection in preimmunized mice (87). In terms of oncolytic efficacy, vvDD-A34R displayed higher replication levels than vvDD in tumor nodules, leading to long-term disease regression in 70% of the mice (87). Therefore, vvDD-A34 Rissuperior to the parent vvDD as an oncolytic agent for the treatment of peritoneal carcinomatosis in preclinical models.

To enhance spread by disrupting the extracellular matrix, which can hinder virus spread within tumor beds, a recombinant GLV-1h255 expressing matrix metallopeptidase 9 (MMP-9) was constructed and its oncolytic activities were tested in nude mice bearing human PC-3 prostate cancer xenografts (88). Compared with GLV-1h68 treatment,

GLV-1h255 treatment led to significant overexpression of intratumoral MMP-9, followed by a decrease in collagen IV content in the area of infection within tumors (88). High GLV-1h255 virus titers, which led to enhanced tumor regression, were found in the tumors (88). This study indicates that an engineered VV can be used to modify physical barriers to virus dissemination within the tumor microenvironment as a means to enhance the spread of the oncolytic virus, thereby increasing the oncolytic efficiency of the virus platform.

Modified Vaccinia Viruses for Noninvasive Imaging

Direct, noninvasive imaging is exceedingly useful for monitoring the therapeutic effects of oncolytic viruses in real time in tumor-bearing test animals or patients. Therefore, VV constructs have been engineered to express reporter genes that allow for monitoring of viral replication by noninvasive imaging. A vvDD with the somatostatin receptor (SR) gene inserted under control of a VV promoter is now being tested in a phase I clinical trial (89). Expression of SR by the replicating virus allows investigators to monitor tumor-specific expansion of vvDD in situ through the use of SR-mediated uptake of radiotracers (89).

Recently, oncolytic virus constructs expressing the human sodium iodide symporter (hNIS) gene have been generated and tested to assess whether viral hNIS expression can provide a convenient noninvasive, safe, and painless imaging method to monitor in real time the replication of an oncolytic virus, its localization in tumors, and its effectiveness as an oncolytic virotherapeutic agent. hNIS is a transmembrane glycoprotein that mediates uptake of iodine into follicular cells of the thyroid gland, which is the first step in the synthesis of thyroid hormone, and it has traditionally been exploited to treat and image thyroid cancer. The uptake of intravenously injected radioiodine by hNIS allows for deep-tissue imaging via ^{124}I positron emission tomography (PET) or ^{131}I single-photon emission computed tomography (SPECT) (90). A recombinant VV GLV-1h153 expressing hNIS under the control of a virus promoter was constructed and tested for oncolytic potential and suitability for imaging in mice bearing various types of tumors (91–94). hNIS expressed by GLV-1h153 mediated specific uptake of radiolabeled iodine by cancer cells, facilitating whole-body PET imaging (93). Additionally, GLV-1h153 maintained its oncolytic activities: GLV-1h153 treatment resulted in the expected level of tumor regression and prolonged survival (94). Importantly, GLV-1h153-infected tumors were detected via ^{124}I PET and ^{99m}Tc scintigraphy 1 week after systemic GLV-1h153 administration, demonstrating that GLV-1h153 not only retains oncolytic efficacy but also supports noninvasive imaging of the tumor localization and therapeutic effects of the virus (92, 94).

Triple-negative breast cancers (TNBCs) have a more aggressive tumor biology than non-TNBCs. The current available standard treatment for TNBCs is combined chemotherapy, but this is not effective when the disease relapses. The oncolytic efficacy of GLV-1h153 has been tested in nude mice bearing metastatic TNBCs (92, 93). GLV-1h153 treatment resulted in tumor necrosis with no evidence of viable breast cancer cells (95). This study was the first report to describe the use of a novel oncolytic VV in preventing or treating metastatic TNBCs.

Surgery is the current definitive treatment for early-stage breast cancer. Current methods for intraoperative assessment of tumor margins have some technical and practical limitations,

and the rate of positive margins after surgery is therefore higher than desired. GLV-1h153 was tested for its suitability to detect positive margins in nude mice bearing mammary fat pad tumors (92). Administration of GLV-1h153 into the surgical wound after resection of 90% of tumor allowed positive surgical margins to be identified via PET scanning as early as 6 h after radiotracer injection (92). Most importantly, GLV-1h153 effectively prevented the progression of residual tumor at surgical margins, such that 50% of GLV-1h153-treated animals had complete regression of residual tumors (92). Thus, localized injection of GLV-1h153 provides an efficient method not only to identify positive margins postsurgery but also to control or completely eliminate localized residual tumors.

Anaplastic thyroid carcinoma (ATC) (96), the most aggressive type of thyroid cancer, is resistant to radiotherapy—an effective current treatment for thyroid cancer—due to the intrinsic loss of hNIS expression. The oncolytic potential of GLV-1h153 was tested in ATC cell lines and in nude mice bearing human ATC xenografts in the hind leg (93). Infection of ATC cell lines with GLV-1h153 resulted in hNIS protein expression in ATC cells, leading to efficient hNIS-specific radioactive iodine uptake by cells (93). Additionally, intratumoral administration of GLV-1h153 allowed for visualization of tumor localization using scintigraphic imaging via ^{99m}Tc pertechnetate scintigraphy (93). Importantly, reconstituting hNIS through expression by GLV-1h153 may provide a way to sensitize ATC cells for radiation therapy.

MYXOMA VIRUS ONCOLYTIC VIROTHERAPY

MYXV, a rabbit-specific poxvirus, has been extensively tested for its safety and oncolytic potential in both immunocompetent and immunocompromised murine models bearing various types of solid tumors, including glioblastoma, medulloblastoma, melanoma, and pancreatic cancer (35, 97–99). Similar to VV, the cellular tropism of MYXV to cancerous cells is usually not mediated at the cell surface receptor level, although some examples of human cells that do not bind the virus (such as normal $CD34^+$ hematopoietic stem cells) have been reported (16, 100). Recent studies have shown that ex vivo MYXV infection of human patient bone marrow or peripheral blood mononuclear cell samples selectively eliminates contaminating acute myeloid leukemia or multiple myeloma cells from the specimen without affecting the ability of the resident normal $CD34^+$ stem and progenitor cells to engraft immunodeficient NOD/scid/IL2Rγ-knockout (NSG) mice (100, 101). Therefore, MYXV is currently being developed as an ex vivo purging agent to delete contaminating cancerous cells from cancer patient autologous bone marrow transplant specimens prior to reinfusion of the autograft back into the patient following myeloablative chemotherapy.

The safety and oncolytic potential of MYXV were first demonstrated in a xenograft model of human glioblastoma in immunocompromised mice (102). The MYXV strain that has been extensively tested in preclinical animal models is derived from a Lausanne strain engineered to express a fluorescent protein gene inserted at the intergenic region between the M135R and M136R genes. Intracranial injection of MYXV into either nude or immunocompetent mice is not toxic, demonstrating that MYXV is safe even in severely immunocompromised hosts (102).

MYXV can productively infect various human cancer cell lines (35). The virus downregulates class I major histocompatibility complex (MHC) expression on the surface of infected cells (103); a recent study demonstrated this effect in infected glioma cells in vivo (104). This downregulation led to increased natural killer (NK) cell–mediated recognition and efficient killing of infected glioma cells (104). Thus, MYXV infection not only leads to the direct killing of cancer cells but also promotes early immune cell–mediated antitumor responses.

Modified Myxoma Virus for Further Attenuation

MYXV also encodes multiple immunomodulatory proteins involved in subverting the host immune system and other antiviral responses (54, 97, 105, 106). Some of these effectors are rabbit specific, whereas others operate in a pan-species fashion dependent on the extent of host immune target conservation. A panel of MYXV constructs was created with targeted deletions of individual immunomodulatory protein–encoding genes, and the functions of those proteins have been studied (107). Some of these mutant recombinant viruses are host-range restricted (i.e., they have lost the ability to infect certain types of cultured cells) but retain the ability to infect and kill cancer cells in vitro (107, 108). For example, the recombinant M135R-knockout MYXV (vMyx-M135KO-gfp) is more attenuated than wild-type MYXV in rabbits (109). Although vMyx-M135KO-gfp was nonpathogenic to its natural rabbit hosts, it can efficiently infect and kill human cancer cells in vitro (108) and in vivo (110). Indeed, vMyx-M135KO-gfp exhibits improved oncolysis against human glioma cells in vitro, indicating that it is attenuated but retains full oncolytic activities (108). Currently, the M135-knockout MYXV is being developed for human clinical trials because this construct is fully oncolytic against human cancer cells but cannot induce myxomatosis in rabbits; thus, vMyx-M135KO-gfp is completely nonpathogenic for all known vertebrate hosts.

Modified Myxoma Virus for Enhanced Virus Spread

Several preclinical studies have demonstrated that MYXV is a potentially effective virotherapeutic for xenografted human gliomas (102, 111). However, in a murine model bearing transplanted human gliomas on both sides of the brain, single intratumoral injection of MYXV could eliminate the tumor mass only in the hemisphere in which MYXV was administered, indicating that the virus did not efficiently spread to the tumors in the contralateral hemisphere (102). The VV F11 protein promotes virus exit from an infected cell by inhibiting Rho signaling, which maintains the integrity of the cortical actin layer (112). Irwin & Evans (112) showed that a recombinant MYXV expressing the VV F11L gene (MYXV-F11L) replicates more efficiently than wild-type MYXV in monkey or rabbit cell lines. In a variety of human cancerous cell lines, MYXV-F11L replicates more efficiently than control viruses (113). In the human MDA-MB-231 tumor-bearing xenotransplanted mouse model, survival was prolonged when MYXV-F11L was administered. To test whether MYXV-F11L facilitated virus spread, investigators administered either wild-type or MYXV-F11L virus into the tumor on one side of mice bearing bilateral MDA-MB-231 tumors in opposite mammary fat pads (113). Uninjected tumor volume was significantly reduced in mice injected with MYXV-F11L compared with mice that received wild-type MYXV (113). Thus, the spread of MYXV from tumor to tumor

can indeed be enhanced by expressing the VV-F11L gene. It is unknown whether this improved virus spread would be accompanied by increased antitumor immune responses in an immunocompetent host.

Myxoma Virotherapy of Stem Cell Transplants Ex Vivo Can Prevent Graft-Versus-Host Disease

Unexpectedly, during studies on the ability of ex vivo MYXV virotherapy to eliminate cancer cells from autologous stem cell transplant specimens by pretreatment with virus 1 h prior to transplant, the posttransplant mortality was greatly reduced in NSG mice engrafted with human bone marrow samples pretreated with MYXV compared with control engrafted mice (114). The control mice were subsequently shown to have died from acute graft-versus-host disease caused by donor $CD3^+$ T cells, whereas the ex vivo pretreatment of engrafted bone marrow with MYXV prevented the disease (114). Therefore, it is possible that ex vivo MYXV pretreatment not only deletes contaminating cancer cells in autologous stem cell transplants but also could reduce the severity of posttransplant graft-versus-host disease in allogeneic donor stem cell transplants.

COMBINATION OF ONCOLYTIC POXVIRUSES WITH OTHER THERAPIES

Most cancer treatment regimens rely on combinations of multiple agents. Indeed, several studies have shown a superior efficacy when virotherapy is combined with traditional therapies (111, 115–117). However, the order of administration can be critically important given that some drug therapies inhibit poxvirus replication. In one clinical study, three patients with hepatocellular carcinoma received sequential JX-594 intratumoral injection followed by standard sorafenib treatment (115). Sorafenib is an oral small-molecule multikinase inhibitor with antiproliferative and antiangiogenic effects in humans and mice. In previous clinical studies, patients treated with sorafenib exhibited rapid and marked tumor necrosis, and 60% of patients had progressive disease (118, 119). In the more recent study, JX-594 treatment alone did not induce tumor necrosis; however, when sorafenib treatment was initiated after JX-594 treatment, all three intrahepatic tumors became significantly necrotic and no progressive disease was observed (115). JX-594 may therefore sensitize hepatocellular carcinoma tumors to sorafenib and potentially to other VEGF receptor (VEGFR) inhibitors. The mechanism(s) remain(s) to be determined, and a randomized controlled trial of sorafenib alone versus JX-594 followed by sorafenib should be conducted for validation.

Similarly, a renal cell cancer patient with a life expectancy of <6 months received four injections of JX-594 into liver metastases, followed by a full dose of sunitinib (115). Sunitinib is a small-molecule inhibitor of VEGFR. The patient exhibited a complete whole-body tumor response and remains alive and disease free more than 4 years after treatment initiation (115). Thus, enhanced cancer regression can be achieved by sequentially combining a virotherapeutic agent with a conventional drug therapy. These findings also indicate the importance of randomized and blinded clinical trials to determine whether combined therapy provides an enhanced result compared with either modality alone.

Furthermore, the effects of VV or MYXV virotherapy combined with conventional radiotherapy, chemotherapy, or immunotherapy have been studied in preclinical immunocompetent and immunocompromised mouse models for various types of cancer (65, 94, 111, 116, 120–127). The strains tested for combined therapy and the study outcomes are summarized in Table 4. One study indicated that preirradiation of tumors allowed preferential VV replication, leading to enhanced oncolytic efficacy (120). Taken together, these studies show that combining VV or MYXV with drug treatment, radiation, or chemotherapy enhances their oncolytic efficacy, demonstrating that it is worthwhile to expand testing of combined therapies in clinical trials (65, 74, 111, 116, 120–123, 127). Moreover, several derivatives of GLV-1h68 were constructed for transgene expression to make conventional therapies more effective. For example, GLV-1h324 expressing melanin was constructed, and its oncolytic activities were tested (128). GLV-1h324 retained its oncolytic activity even though higher amounts of melanin seemed to inhibit viral replication at later time points during infection. But melanin did not significantly influence virus-induced cancer cell lysis (128). The study showed that not only was GLV-1h324 still actively oncolytic, but it also facilitated deep-tissue optoacoustic imaging and magnetic resonance imaging (128). Additionally, when energy was transferred specifically to melanin by using a near-infrared laser to produce thermal energy, the thermal energy eventually heated melanin-producing cells to temperatures that caused protein denaturing and cell death (128). Therefore, melanin is suitable for targeted laser-induced thermotherapy. Importantly, combining GLV-1h324 with laser-induced thermotherapy enhanced tumor regression.

Various malignant melanoma cell lines were tested for their permissiveness to GLV-1h68, and melanoma cells harboring BRAF mutants were least sensitive to GLV-1h68 infection (122). In vitro combination of GLV-1h68 with radiation led to greater cytotoxicity only in melanoma cells harboring (V600D/E)-BRAF mutations, indicating a strong synergistic effect between GLV-1h68 and radiation (122). In vivo analysis supported the previous findings that only the combined treatment delayed tumor growth and prolonged the survival of nude mice bearing BRAF-mutant tumors. Further, combined treatment was shown to induce enhanced apoptosis, which was due not to increased viral replication (122) but to the inhibition of GLV-1h68-induced JNK phosphorylation by radiation. Silencing of the JNK gene in combination with GLV-1h68 enhanced cell death only in BRAF-mutant melanoma cells (122). This study provides a strong rationale for combining GLV-1h68 with radiotherapy in patients bearing BRAF-mutant tumors.

IMMUNOLOGIC BARRIERS TO POXVIRUSES AS ONCOLYTIC THERAPEUTIC AGENTS

A key challenge is to develop the most optimal and efficient delivery system for oncolytic viruses, allowing access to more tumor sites. For example, although intravenous JX-549 produced oncolytic antitumor effects in hepatocarcinoma patients, such effects were seen only in patients receiving the highest tolerated dosage that exhibited minimal adverse events (10^9 infectious units per dose) (64). Because intravenously delivered IMVs can be neutralized by preexisting antibodies in vaccinated patients and are cleared nonspecifically by the liver and spleen before the bulk of input virus reaches enough of the target tumor

sites, virus must be administered at a much higher dose for optimal effectiveness. Even in the absence of the preexisting antiviral immune responses, induced neutralizing antibodies and antiviral cellular responses are mounted upon multiple administrations of the same virus, such that virus administered later in the disease course is less effective. However, VV and MYXV belong to different poxvirus genera; hence, acquired immunity to one virus does not block infection by the other. Thus, administration of a second oncolytic poxvirus after the first virus has been cleared by acquired immune responses should allow for longer-term delivery of therapeutic viruses in an individual patient. Cellular carriers infected ex vivo with virus prior to infusion have been tested as another potential means to circumvent the virus delivery problem (129–131). For example, adipose-derived stem cells (ADSCs) can be productively infected ex vivo by several oncolytic viruses (132). Multiple administration of ADSCs loaded with MYXV led to long-term survival of mice bearing gliomas, indicating that ADSCs may be suitable for use as MYXV-delivery vehicles to tumor sites (133). As another example, cytokine-induced killer (CIK) cells bear phenotypic markers of NK and T cells, express the receptor NK group 2D (NKG2D), and are not MHC restricted (134). They mediate killing of tumor cells by recognizing a class of stress-associated ligands expressed on tumor cell surfaces (134). By 72 h after intravenous delivery, CIK cells are localized primarily at the tumor site. Additionally, CIK cells can be useful carrier vehicles to deliver oncolytic VV to tumors, including for the treatment of minimal residual disease and in the face of antiviral immunity (135).

The development of specific immunomodulatory drugs to intercept immune suppressor cells (e.g., Tregs, myeloid suppressor cells) that prevent full immune responses against a developing cancer is now generating substantial excitement. The tenet of this strategy is that patient immune responses against tumor antigens are often actively blocked by the combined activities of immune suppressor cells. New classes of immunomodulatory reagents targeted against these cells, such as anti-CTLA4, anti-PD1, and anti-PD-1L, are being developed to break this immune tolerance and show considerable clinical promise for reactivating dormant or anergic immune responses to cancer (96, 136, 137). Because the most successful results from oncolytic virotherapy trials have been in patients (called elite responders) in whom tumor regression continues long after the therapeutic virus has been cleared (10), combination of these immune checkpoint inhibitor drugs with oncolytic virotherapy—at earlier times after cancer diagnosis, when the patient's immune system is most functional—should be tested in future clinical trials.

FUTURE PERSPECTIVES

Clinical and preclinical studies have demonstrated that poxviruses can serve as effective oncolytic virotherapeutic agents for various types of cancer. Although VV is currently in clinical trials, MYXV's effectiveness as an oncolytic virotherapeutic agent has been demonstrated only in preclinical animal models. Thus, properly controlled human clinical studies must now be conducted to investigate MYXV's oncolytic potential in cancer patients. Several preclinical studies have shown that both VV and MYXV can be very effective against cancers for which there are no current therapies, such as gliomas and pancreatic cancer. Direct comparison of their oncolytic potential also has indicated that these two viruses differ in their potencies against specific types of cancer (16, 138). For example,

MYXV is a better candidate for an ex vivo purging agent to selectively eliminate contaminating multiple myeloma cells from patient bone marrow transplant samples (16), whereas VV is more potent in controlling the growth of certain head and neck squamous cell carcinoma cell lines in vitro (138). Therefore, although both viruses exhibit impressive oncolytic potential, their efficacies may be quite different for specific types of cancer targets. Controlled human clinical trials will be critical to determine whether one virus is clinically superior to the other for specific cancer indications.

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Glossary

Vaccinia virus (VV)	the prototypical poxvirus; belongs to the genus *Orthopoxvirus* and is well known for its use as a live-attenuated smallpox vaccine
Myxoma virus (MYXV)	a member of the *Leporipoxvirus* genus that is pathogenic only to European rabbits
Intracellular mature virions (IMVs)	the first form of progeny infectious virus produced within the cytoplasm of a poxvirus-infected cell
Extracellular enveloped virions (EEVs)	progeny infectious viruses released from a VV-infected cell that are responsible for long-range dissemination of virus
Thymidine kinase (TK)	an essential enzyme for pyrimidine synthesis; deletion of the poxviral TK gene increases the virus's selectivity for rapidly dividing cancerous cells
VV double deletion (vvDD)	vaccinia Western Reserve strain derivative that has the growth factor gene deleted and expresses SR for monitoring of tumor-specific expansion
In vivo	experimentation conducted in live animals
Pexa-Vec	Wyeth vaccine strain derivative with the TK gene deleted and the GM-CSF gene added to stimulate the virus's immunotherapeutic effects
Granulocyte monocyte colony-stimulating factor (GM-CSF)	a cytokine that enhances immune-stimulatory effects
GL-ONC1	vaccinia Lister strain derivative attenuated by deletion of *F14.5L*, *J2R* (the viral TK gene), and *A56R* (the hemagglutinin gene)

Bone morphogenetic proteins (BMPs)	TGF-β superfamily members that help regulate cancer in the context of glioblastoma multiforme and induce rapid tumor regression
Vascular endothelial growth factor (VEGF)	a signaling protein that promotes vasculogenesis and angiogenesis
Recombinant human erythropoietin (rhEPO)	a glycoprotein hormone that regulates red blood cell formation and is used to treat cancer-related anemia
Ex vivo	experimentation conducted on primary cells harvested from humans or animals
Matrix metallopeptidase 9 (MMP-9)	a type IV collagenase involved in the breakdown of extracellular matrix
Somatostatin receptor (SR)	SR-mediated uptake of radiotracers by oncolytic virus–expressed SR allows monitoring of tumor-specific virus expansion
In situ	monitoring of phenomena, such as therapeutic effects, in living organisms
Human sodium iodide symporter (hNIS)	a transmembrane glycoprotein that mediates iodine uptake; uptake of intravenous radioiodine by oncolytic virus–expressed NIS allows for deep-tissue imaging
vMyx-M135KO-gfp	myxoma Lausanne strain derivative that has the M135 gene deleted and expresses green fluorescent protein; nonpathogenic even to European rabbits
Adipose-derived stem cells (ADSCs)	currently being tested as cellular carriers to facilitate virus delivery to cancer tissues

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Table 1

Summary of poxviruses being developed for oncolytic virotherapy

Poxvirus	Genetic strain	Features
Pexa-Vec (JX-594)	VV Wyeth	TK^-; GM-CSF and lac-Z genes inserted
GL-ONC1 (GLV-1h68)	VV Lister	$F14.5L^-$, TK^-, $A56R^-$; Renilla luciferase-GFP, lac-Z, and gusA genes inserted
GLV-1h99	VV Lister	GLV-1h68 expressing human norepinephrine transporter
GLV-1h108	VV Lister	GLV-1h68 expressing GLAF-1
GLV-1h153	VV Lister	GLV-1h68 expressing hNIS
GLV-1h210	VV Lister	GLV-1h68 expressing hEPO
GLV-1h237	VV Lister	GLV-1h68 expressing Walker A motif mutant of Cdc6
GLV-1h255	VV Lister	GLV-1h68 expressing MMP-9
GLV-1h285	VV Lister	$F14.5L^-$, TK^-, $A56R^-$; Renilla luciferase-GFP, BMP-4, turboRFP genes inserted
GLV-1h324	VV Lister	GLV-1h68 expressing melanin
vvDD-CDSR	VV Western Reserve	TK^-, VGF^-; lac-Z, cytosine deaminase, and SR genes inserted
vvDD	VV Western Reserve	TK^-, VGF^-; cytosine deaminase gene inserted
vvDD-A34R	VV Western Reserve	vvDD expressing A34R K151E mutation
vvDD-SR-RFP	VV Western Reserve	TK^-, VGF^-; RFP gene inserted
OVV-CXCR4-A-Fc	VV Western Reserve	TK^-, VGF^-; CXCR4 antagonist gene inserted
vMyx-gfp(tdTr)	MYXV Lausanne	MYXV expressing a reporter GFP (or tdTomato)
MYXV-F11L	MYXV Lausanne	MYXV expressing VV F11L gene
vMyx-M135KO-gfp	MYXV Lausanne	MYXV with M135 gene deletion, expressing GFP

Abbreviations: VV, vaccinia virus; MYXV, myxoma virus.

Table 2

Summary of vaccinia virus oncolytic virotherapy clinical trials

Poxvirus	Tumor type(s)	Administration route	Phase	Status and outcomes	National Clinical Trial (NCT) ID
Pexa-Vec (JX-594)	CRC	IV	1	Ongoing, not recruiting	NCT01469611
	HCC (sorafenib-naive)	IV	2a	Ongoing, not recruiting	NCT01636284
	CRC	IV	1b	Ongoing, not recruiting	NCT01380600
	Solid tumors	IV	1	Less new tumor outgrowth in patients receiving high dose of virus (64)	NCT00625456
	HCC	IT	1	Maximum tolerated dose determined to be 1×10^9 PFU; virus spread to distant uninjected tumors (67)	NCT00629759
	Pediatric solid tumors	IT	1	Ongoing, not recruiting	NCT01169584
	Melanoma	IT	1	Low-dose injected up to 9 doses; virus spread to distant uninjected tumors (66)	NCT00429312
	HCC	IT	2	Low- versus high-dose; dose-dependent survival; systemic virus spread (65)	NCT00554372
	HCC	IV	2b	Enrollment completed	NCT01387555
	CRC	IV	1/2a	Recruiting	NCT01394939
	Peritoneal carcinomatosis of ovarian cancer origin	IV	2	Not yet open for recruitment	NCT02017678
GL-ONC1 (GLV-1h68)	Solid tumors	IV	1	Recruiting	NCT00794131
	Peritoneal carcinomatosis	IP	1/2	Recruiting	NCT01443260
	Head and neck cancer	IV	1	Recruiting	NCT01584284
	Lung cancer	Intrapleural	1	Recruiting	NCT01766739
vvDD-CDSR	Solid tumors	IT/IV	1	Ongoing, not recruiting	NCT00574977

Abbreviations: CRC, colorectal carcinoma; HCC, hepatocarcinoma; IP, intraperitoneal; IT, intratumoral; IV, intravenous.

Table 3

Summary of the oncolytic poxviruses studied in preclinical animal models

Virus name	Tumor type(s)	Administration route	Outcomes	Reference(s)
vvDD	Pediatric solid tumors	IV	Inhibited tumor growth; prolonged survival	70
vvDD-A34R	M38 peritoneal carcinoma	IP	More efficient remote virus spread; enhanced efficacy and host survival	87
OVV-CXCR4-A-Fc	Murine 4T1 metastatic or orthotopic breast cancer	IV	Inhibited primary tumor growth and metastatic lesion formation; disrupted tumor vasculature	139
GLV-1h68	Colorectal	IV	Inhibited tumor growth; elicited inflammation-mediated innate immune response	140
	Breast cancer stem-like cells PC-3 prostate cancer	Retro-orbital	Efficient replication in cancer stem-like cells	141
		IV	Primary tumor regression; strong inflammatory responses	142
	Hepatocellular carcinoma	IV	Reduced primary tumor size; strong inflammatory responses	143
	Bone and soft-tissue sarcoma cells	IT	Near-complete tumor regression without any observed toxicity	144
	Salivary gland carcinoma	IT	Efficient tumor regression without toxicity	145
	Squamous cell carcinoma	IT	Near-complete tumor regression without any observed toxicity	146
GLV-1h99	Pancreatic cancer/pleural mesothelioma	IT/intrapleural	Efficient tumor elimination; specific uptake of radiotracer by hNET; allowed deep-tissue imaging using PET	147, 148
GLV-1h108	A549 lung cancer	IV	Significant enhanced efficacy comparison with GLV-1h68	74
	Lung cancer	IV	Enhanced tumor regression	75
GLV-1h120	A549 lung cancer	IV	Enhanced oncolytic activity; alleviated cancer-related anemia	86
GLV-1h153	Metastatic or orthotopic triple-negative breast cancer	IV/IT	Tumor regression; facilitated deep-tissue imaging to identify positive surgical margins	92, 95
	Pleural mesothelioma	Intrapleural	Reduced tumor burden; improved survival; facilitated monitoring by ^{131}I-SPECT/CT	91
	Pancreatic cancer	IV/IT	Tumor regression; NIS-specific radiouptake by cells; facilitated PET imaging	90, 94
	Anaplastic thyroid carcinoma	IT	Oncolytic; potential sensitizer for radiation therapy; facilitated imaging	93
GLV-1h237	Breast cancer	IV	Enhanced antitumor activity; potential to interfere with host cell DNA synthesis	76
GLV-1h255	PC-3 prostate cancer	IT	Enhanced tumor regression; facilitated virus spread within tumors by degrading collagen IV	88

Virus name	Tumor type(s)	Administration route	Outcomes	Reference(s)
GLV-1h285	Glioblastoma primary cancer stem cell lines	Intracranial	Superior tumor growth control; no recurrence	73
GLV-1h324	A549 lung cancer	IT	Maintained oncolytic activity; facilitated deep-tissue imaging; provided suitable target for laser-induced thermotherapy	128
vMyx-gfp(tdTr)	Acute myeloid leukemia	Ex vivo[a]	90% of mice free of human acute myeloid leukemia in bone marrow	101
	Multiple myeloma	Ex vivo[a]	All mice free of human multiple myeloma in bone marrow	100
	Pancreatic cancer	IP	Reduced tumor burden; prolonged survival	116
	Gliomas	IT	92% of mice free of disease	102
	Rhabdoid tumors in brain or hind flank	IT	Reduced tumor size	149
	Medulloblastoma	IT	Prolonged survival	123
	Murine melanoma	IT	Reduced tumor size	110
		IV	Decreased development of lung metastasis	110
		Ex vivo[a]	Prevented tumor implantation	110
MYXV-Fl1L	Breast cancer	IT	Enhanced virus spread to uninjected contralateral tumor	113

Abbreviations: IP, intraperitoneal; IT, intratumoral; IV, intravenous.

[a]Ex vivo treatment of transplant sample with myxoma virus prior to engraftment.

Table 4

Summary of combination therapies with oncolytic poxviruses tested in clinical or preclinical studies

Poxvirus	Tumor type(s)	Combination therapy	Status	Outcomes	Reference
GLV-1h68	Pancreatic tumors	Bevacizumab	Preclinical	Enhanced tumor regression, followed by complete regression	74
		Cisplatin	Preclinical	Accelerated tumor regression with complete tumor regression in 88% of mice	150
		Gemcitabine	Preclinical	Accelerated tumor regression; inhibited tumor growth	150
	BRAF mutant melanoma	Radiation	Preclinical	Delayed tumor growth; prolonged survival; increased cytotoxicity and apoptosis	122
	Glioma	Nonionizing radiation[a]	Preclinical	Preferential virus replication in preirradiated tumors	120
	Breast cancer	β-gal-activatable prodrug	Preclinical	Beneficial effects on tumor regression	124
LIVP 1.1.1	Glioma	Nonionizing radiation[a]	Preclinical	Preferential virus replication in preirradiated tumors	120
GLV-1h324	Solid tumors	Laser-induced thermotherapy	Preclinical	Enhanced tumor regression	128
GLV-1h164	Glioma	Radiation	Preclinical	Increased reduction in intratumoral VEGF levels and tumor vessel numbers	121
GLV-1h153	Pancreatic cancer	Radioiodine	Preclinical	Successful radiouptake; enhanced tumor killing	90
GLV-1h90	Prostate cancer	Chemotherapy	Preclinical	Enhanced antitumor effect of the virus; reduced chemotherapy-induced side effects	125
Pexa-Vec (JX-594)	Hepatocellular carcinoma	Sorafenib[b]	Preclinical	Prolonged time-to-tumor progression	115
	Melanoma	Sorafenib[c]	Preclinical	Enhanced reduction of lung tumors	115
	Hepatocellular carcinoma	Sorafenib[b]	Clinical	Rapid tumor necrosis; no progressive disease	115
	Renal cell cancer	Sunitinib[b]	Clinical	Whole-body tumor response; free of disease for more than 4 years	115
vMyx-gfp	Late-stage metastatic pancreatic carcinomatosis	Gemcitabine[b]	Preclinical	Enhanced survival	116
	Gliomas	Rapamycin[b]	Preclinical	Prolonged survival	111
	Medulloblastoma	Rapamycin[b]	Preclinical	Long-term survival	123
	Human brain tumor–initiating cells	Rapamycin[d]	Preclinical	Prolonged survival	127

[a] Preirradiation of tumors prior to virus administration.

[b] Virus administration followed by drug.

[c] Simultaneous administration of virus and drug.

[d] Drug treatment 1 day prior to virus administration.