New frontiers in oncolytic viruses: optimizing and selecting for virus strains with improved efficacy

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Abstract: Oncolytic viruses have demonstrated selective replication and killing of tumor cells. Different types of oncolytic viruses – adenoviruses, alphaviruses, herpes simplex viruses, Newcastle disease viruses, rhabdoviruses, Coxsackie viruses, and vaccinia viruses – have been applied as either naturally occurring or engineered vectors. Numerous studies in animal-tumor models have demonstrated substantial tumor regression and prolonged survival rates. Moreover, clinical trials have confirmed good safety profiles and therapeutic efficacy for oncolytic viruses. Most encouragingly, the first cancer gene-therapy drug – Gendicine, based on oncolytic adenovirus type 5 – was approved in China. Likewise, a second-generation oncolytic herpes simplex virus-based drug for the treatment of melanoma has been registered in the US and Europe as talimogene laherparepvec.

Keywords: immunotherapy, viral vectors, clinical trials, drug approval

Introduction

Gene-therapy applications were initiated in the 1990s by utilization of both nonviral and viral delivery vectors.1 Although some progress was seen early on, the whole field, especially the utilization of viral vectors, was severely hampered by some setbacks. Particularly, the death of a young patient treated with adenovirus vectors for the non-life-threatening disease ornithine transcarbamylase2 significantly reduced the interest in gene therapy and slowed its progress. Furthermore, retrovirus vectors used for treatment of children suffering from severe combined immunodeficiency (SCID) showed integration of a therapeutic gene into the LMO2 proto-oncogene region, which triggered leukemia development in some patients.3,4 In hindsight, it is obviously easy to criticize the scientific community for moving too quickly into clinical trials without the proper safety conditions established. The setbacks, however, forced some serious reengineering of viral vectors and clinical protocols to improve delivery and targeting and to meet appropriate safety standards. These modifications include the introduction of elements controlling replication and expression, as well as means of termination of virus propagation by addition of the prodrug ganciclovir after administration of replication-competent Sindbis virus (SINV) carrying a fusion of the herpes simplex virus (HSV) TK gene and the SINV protein nsP3.5 In the long run, vector engineering has significantly improved the properties of second- and third-generation vectors and enabled their safe applications for the treatment of various diseases.6

In this review, the focus is entirely on viral vectors in cancer therapy. One of the key issues from the birth of gene therapy has been delivery, and it remains the talking
point. Intensive vector engineering addressing targeting and delivery by the introduction of target-specific recognition signals and/or delivery-enhancing molecules, such as polymers and liposomes, has contributed to increased efficacy. Furthermore, the design of packaging cell lines has significantly facilitated the utilization of viral vectors for cancer treatment in experimental animal models. An interesting approach comprises employing oncolytic viruses as both naturally occurring and engineered vectors can provide superior therapeutic efficacy, due to their selective tumor cell-killing capacity and potential induction of systemic antitumor immunity. Today, a number of different oncolytic viruses, such as adenoviruses (Ads), HSV, alphaviruses, rhabdoviruses, Newcastle disease virus (NDV), vaccinia viruses (VV), and others have been evaluated for antitumor activity in a number of animal models and in clinical trials.

Viral vectors desirable for therapeutic strategies

Commonly, both nonviral and viral vectors have been applied in cancer therapy. The use of nonviral vectors has mainly been favored by their straightforward application and generally good safety profiles, while the attractive features of viral vectors relate to their ability to provide superior delivery and extreme levels of transgene expression. Viral vectors have in general been characterized by their broad range of host-cell tropism and extreme expression levels of heterologous genes. Transient high-level expression is especially attractive for cancer-therapy applications, as the presence of antitumor and/or toxic products is limited in time. Alternatively, expression vectors comprised of regulation and termination signals have been engineered to restrict vector spread and long-term toxicity. Generally, viral vectors carrying either a DNA or RNA genome can accommodate foreign genetic information of different sizes, depending on which type of viral vector is used. For instance, vectors based on HSV and VV are capable of accommodating more than 30 kb of foreign DNA, whereas most vector systems allow packaging of 6–8 kb of inserts, which is sufficient for covering more or less any therapeutic gene. Only adenoassociated viruses (AAVs) show a somewhat-limited packaging capacity in the range of 4 kb, but even that allows accommodation of a wide range of appropriate therapeutic genes. Both replication-deficient and -competent viral particles have been applied in immunization and therapeutic interventions in animal models. Moreover, alphavirus vectors have been utilized in the form of naked RNA and plasmid DNA for the delivery of therapeutic genetic information for toxic, anticancer, and immunostimulatory genes, as well as for miRNA and shRNA.

Another issue is the potential immunogenicity triggered by the administration of viral vectors. In this context, the original Ad vectors have demonstrated strong immunogenicity, although later-generation versions with gene deletions have proven to be less immunogenic. AA V vectors have also shown strong immunogenicity, especially after virus readministration, which has been circumvented by using different AAV serotypes for subsequent injections.

The discovery of oncolytic viruses, which can provide specific replication in tumor cells and further induce killing without affecting normal cells, has provided attractive alternative opportunities for cancer-therapy applications. In this context, naturally occurring oncolytic viruses and genetically engineered vectors have been subjected to cancer therapy and cancer-vaccine studies in animal models (Table 1), as described in detail herein ("Examples of therapeutic applications of oncolytic viruses" section).

Mechanisms of oncolytic activity

Both natural and engineered oncolytic viruses utilize the general routes of recognition of cell-surface receptors and fusion to the plasma membrane with the special capability of establishing a lytic cycle in malignant cells, while normal tissues remain unaffected. The mechanism of action occurs through RAS-pathway activation or by genetic modifications. In this context, HSV has been demonstrated to replicate only in tumor cells dependent on TK activity. Moreover, in addition to the continuous replication in tumor cells, oncolytic viruses can recruit uninfected cells nearby without resulting in chromosomal integration or causing any major disease. One interesting feature of oncolytic reoviruses, HSV and VV is their ability to induce adaptive immunoresponses, which can contribute indirectly to tumor cell death. Similarly, oncolytic Ads, Coxackie virus (CV) B3, and measles virus (MV) can induce stress of the endoplasmic reticulum, which attracts immune cells and results in immunologic cell death.

Other studies have demonstrated that viral infections of tumors can contribute to the immunosuppressive milieu by inducing immunostimulatory cytokines and chemokines. Although the production of cytokines and chemokines recruits and activates neutrophils, natural killer cells, macrophages, and CD4+ and CD8+ T lymphocytes, contributing to viral clearance, it can also alter immunosuppression and stimulate antitumor responses.
New frontiers in oncolytic viruses

Table 1 Examples of preclinical cancer therapy applications for viral vectors

<table>
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<th>Cancer type</th>
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<th>Delivery</th>
<th>Response</th>
<th>Reference</th>
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Table 1 (Continued)

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Abbreviations: AAV, adenoassociated virus; Ad, adenovirus; AR, androgen receptor; β-gal, β-galactosidase; CAVA21, Coxsackie virus A21; GALV, gibbon ape leukemia virus; GM-CSF, granulocyte-macrophage colony stimulating factor; HSV-TK, herpes simplex virus thymidine kinase; HSV1, herpes simplex virus 1; hTERT, human telomerase reverse transcriptase; IL2, interleukin 2; INOS, inducible nitric oxide synthase; MV, measles virus; NDV, Newcastle disease virus; PEG, polyethylene glycol; RV, retrovirus; scFv, single-chain variable fragment; SFV, Semliki Forest virus; SINV, Sindbis virus; VLPs, virus-like particles; VSV, vesicular stomatitis virus; VV, vaccinia virus; Xp, Xeroderma pigmentosum.

Examples of therapeutic applications of oncolytic viruses

A number of oncolytic viruses have been subjected to studies in animal-tumor models (Table 1) and in a few clinical trials (Table 2). Ads represent the most frequently used viral vectors subjected to cancer therapy. For instance, animal models for ovarian, prostate, gastric, and brain cancer have been established. Related to gastric cancer, expression of RPL23 and p53 from a bicistronic Ad vector provides significantly better tumor-suppression activity in gastric cancer cells and antitumor responses in MKN45 cells compared to administration of the Ad-p53 vector alone. Furthermore, administration of the bicistronic Ad-RPL23/p53 shows survival benefits in a human gastric tumor model. In another approach, oncolytic Ad expressing luciferase (VRX007-Luc) was subjected to intratumoral injections in a Syrian hamster model, which provided similar levels of inhibition of tumor growth, as observed for immunosuppressive and chemotherapeutic agents such as cyclophosphamide. As human telomerase activity is present in more than 85% of primary cancers, the human telomerase reverse transcriptase (hTERT) promoter has been inserted into an attenuated Ad5 vector, resulting in significant tumor regression in an esophageal tumor model. Furthermore, introduction of the bacterial MazF–MazE toxin–antitoxin system into an Ad vector has provided dose-dependent killing of KRAS cells and considerable tumor shrinkage in vivo without displaying...
any side effects. Similarly, an oncolytic Ad vector with a tumor-specific promoter expressing the TRAIL and E1A genes has induced apoptosis in gastric cancer cell lines, inhibition of peritoneal metastasis, and prolonged survival in tumor-bearing mice. In attempts to improve oncolytic inhibition of peritoneal metastasis, and prolonged survival genes has induced apoptosis in gastric cancer cell lines, TRAIL and a tumor-specific promoter expressing the E1A immunogenicity. In this context, formulation of oncolytic has extended the circulation time and reduced vector-based Ads, incorporation of polymers, liposomes, and nanoparticles expressing IL24 (PLC-ZD55-IL24) in BALB/c mice. Intra-liver sequestration and systemic toxicity of oncolytic Ads vectors with polyethylene glycol, lipids, and calcium reduces adsorption of neuroblastoma xenografts. In another approach, the dual-regulated oncolytic Ad wt-E1A(624bp)-TSLC1 targeting the Wnt- and Rb-signaling pathways and carrying the TSLC1 tumor suppressor was engineered. In vivo administration showed efficient inhibition of growth of transplanted tumors of hepatic cancer stem cells and prolonged survival in mice. Oncolytic Ad vectors targeted to the CD133 (prominin 1) cell-surface marker present on cancer stem cells have been developed by Ad-library screening. The engineered vector with the CD133-targeting motif (AdML-TYML) showed

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Abbreviations: Ad, adenovirus; CD, cytosine deaminase; CPlat, carboplatin; CV, Coxsackie virus; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSV-TK, herpes simplex virus thymidine kinase; NDV, Newcastle disease virus; PEG, polyethylene glycol; PSMA, prostate-specific membrane antigen; PSA, PS antigen; SFV, Semliki Forest virus; VEE, Venezuelan equine encephalitis; VV, vaccinia virus.
selective infection and lysis of CD133+ -cultured cells. Nude mice vaccinated with AdML-TYML were protected against challenges with CD133+ colorectal carcinoma (CRC). Moreover, strong antitumor responses were observed in mice with established CD133+ CRC xenografts after intratumoral injections of AdML-TYML. In another study on CRC, oncolytic Ads expressing decorin (DCN), a regulator of cancer development and progression, and the granulocyte-macrophage colony-stimulating factor (GM-CSF) showed significant inhibition of tumor growth and lung metastasis after intratumoral administration in mice with implanted CT26 xenografts.56 Furthermore, multiple protumorigenic pathways were downregulated and antitumor immunoresponses activated.

AAV vectors have also been evaluated in a number of cancer-therapy studies. One issue of concern has been the strong immunogenicity presented by readministration of AAV vectors.18 As a special case due to the immunoprivileged nature of the eye, intravitreal injection of AAV expressing IFN was monitored for bioluminescent reporter-gene expression after intravenous administration.64 The AAV9 vector carrying the NSE promoter showed 100-fold lower expression in the liver. The AAV9-CBA vector targeted astrocytes, neurons, and endothelial cells, while the AAV9-NSE vector provided mainly neuron-specific expression. Moreover, both AAV9-CBA and AVV9-NSE expressing sTRAIL generated slower tumor growth and significantly prolonged survival in mice with intracranial xenografts from glioblastoma patients. Recently, DCN expression from AAV vectors was evaluated in vitro and in vivo.65 It was demonstrated that transduced neuroblastoma cells expressed DCN and systemic administration of AAV-DCN in nude mice promoted intratumoral uptake of paclitaxel.

HSV-based cancer therapy has been verified in a syngenetic C3H squamous-cell carcinoma model using the lytic HSV1 RH2 vector.66 In addition to therapeutic efficacy observed after intratumoral injection, growth in contralateral tumors was also significantly suppressed. In another application, an oncolytic HSV1 vector containing four copies of miR145 targeting the 3 ’-end untranslated region (UTR) of the essential HSV ICP27 gene was able to decrease cell proliferation and prevention of colony formation of non-small-cell lung cancer (NSCLC) cells, which further enhanced cancer-cell killing when combined with radiotherapy.11 It has also been demonstrated that oncolytic HSV vectors expressing NIS increased antitumor activity by concentration of radioactive iodine in human prostate LNCaP cells.67 Moreover, intratumoral injection of HSV-NIS resulted in efficient tumor eradication in nude mice implanted with LNCaP xenografts, and systemic administration provided prolonged survival. Oncolytic HSV vectors have also been tested in syngenetic mouse-rhabdomyosarcoma models in combination therapy with the cell-death-inhibiting ligand PDL1, which might provide a new approach for treatment of childhood soft-tissue sarcomas.68 Moreover, oncolytic HSV2 vectors show significant inhibition of tumor growth and prolonged survival of BALB/c mice with implanted CT26 tumors.69 Additionally, HSV2 replication contributes to reduced myeloid-derived suppressor cells and regulatory T cells in the spleen, which also decreases the number of dendritic cells in tumor-draining lymph nodes.

A glioma-specific HSV1 amplicon virus has been engineered to target tumor cells selectively by replacing the HSV1 ICP4 promoter with the tumor-specific survivin promoter.70 Furthermore, incorporation of 5 miR124 target sequences into the 3’UTR of the ICP4 gene provided translational regulation. The SU4124 HSV1 vector demonstrated enhanced expression of survivin and eIF4E in glioma cells.
and increased expression of miR124 in normal mouse and human brain tissue. Moreover, a strong antitumor effect was observed in a panel of glioma cell lines. Additionally, significantly increased antitumor activity was discovered in mice with human U87 glioma tumors after intratumoral injections.

An interesting observation relates to enhanced replication of oncolytic HSV in glioblastoma after short-term nutritional restriction (fasting).71 Glioblastoma cell lines from human patients subjected to transient fasting for 24 hours increased late HSV expression and improved viral yields. Transient fasting for 48 hours followed by a 24-hour recovery doubled luciferase activity after intratumoral HSV administration in orthotopic glioblastoma xenografts.

Retroviruses have been subjected to a number of cancer-therapy applications, including recombinant bifunctional retrovirus vectors expressing a single-chain variable fragment (scFv) antibody to CEA and the inducible nitric oxide synthase (iNOS) gene.72 SCID mice subcutaneously injected with MKN45 cells expressing CEA showed significant inhibition in tumor growth with 70% reduction in tumor size. The problem of drug resistance has been addressed by demonstrating that retroviruses expressing the TRAIL gene are susceptible to A2780/DDP ovarian cancer cells, which in combination with cisplatin treatment enhanced antitumor activity in nude mice with implanted A2780/DDP xenografts.73 In attempts to improve the safety of retrovirus-based therapy for hematological malignancies, T cells with chimeric antigen receptors have been engineered.74 Additionally, deletion of oncogenes and inactivation of oncogenic signaling pathways have been achieved by introduction of Cas9, zinc finger nucleases (ZFNs), or transcription activator-like effector nucleases (TALENs) into retrovirus vectors.

Replicating retrovirus vectors based on murine leukemia virus and gibbon ape leukemia virus (GALV) have proven effective in tumor killing.75 Comparison of murine leukemia virus and GALV indicated more rapid replication kinetics for the latter in tumors, and in vivo GALV-based suicide-gene therapy demonstrated efficient suppression of HCC-tumor growth. In another study, it was shown that replication competence of retroviruses can provide a powerful tool for generation of novel tumor-specific retrovirus variants, which can be generated by natural selection.76 Moreover, retrovirus vectors are able to integrate stably into the genome of cancer cells, which can contribute to long-lasting therapeutic efficacy, keeping in mind that the integration event is controlled to avoid any unwanted effects, as discussed previously.3

Belonging to the family of retroviruses, lentiviruses have also found a number of applications in cancer therapy. In this context, lentivirus vectors expressing PSCA have been targeted to DC-SIGN-expressing 293T cells and bone marrow-derived dendritic cells, which provided protection against lethal tumor challenges in the TRAMP-C1 syngeneic tumor model and reduced tumor growth in animals with pre-existing tumors.77 In another study, self-inactivated lentivirus vectors expressing human IFNβ achieved 90% transduction efficiency in pancreatic tumor cell lines, leading to inhibition of cell proliferation and induction of cell death.78 Furthermore, progression of pancreatic cancer was prevented for 15 days in mice after administration of lentivirus human IFNβ.

Lentivirus vectors have also been employed in gene silencing. For instance, delivery of lentivirus vectors carrying Wtp53-pPRIME-mi30-shRNA to AFP-positive liver cells resulted in inhibition of proliferation in Hep3B cells and in mice.79 Moreover, lentivirus-based delivery of shRNAs for Livin efficiently induced apoptosis in tumor cells, reduced proliferation of tumors, and contributed to cell-cycle arrest.80 Reduced proliferation and increased apoptosis was also observed in MKN28 gastric cancer cells and in vivo after delivery of lentivirus vectors carrying CDH17 RNAi.81 Related to breast cancer, lentivirus vectors expressing shRNA were used to knock down cyclin Y (CCNY) expression in MCF7 and MDA-MB231 cells, resulting in substantial decrease in cell proliferation and colony formation and inhibition of cancer-cell growth through activation of Bad and GSK3β and cleavage of poly (ADP-ribose) polymerase (PARP) and caspase 3 in a p53-dependent manner.82 Lentivirus vectors have also been applied for targeting MCM7 with shRNAs to suppress the endogenous expression in K562 cells as a novel approach for the treatment of leukemia.83

In attempts to enhance lentivirus gene transfer, nanofibrils have been engineered to provide highly versatile and broad delivery profiles and to facilitate lentivirus concentration.84 Additionally, a platform for insertional mutagenesis was established for lentiviruses to induce HCC efficiently in various mouse models and for the identification of four previously unknown liver cancer-associated genes.85 In another approach, lentivirus vectors were pseudotyped with truncated MV glycoproteins, which provided targeting of lymphocytes and antigen-presenting cells through signaling lymphocyte activation molecule (SLAM) acting as an entry receptor.86 Reporter-gene expression confirmed the targeting, and administration of pseudotyped lenti-MV glycoproteins showed predominant induction of antigen-specific CD8+ T cells and suitability for vaccines eliciting antigen-specific immunoresponses.

Alphaviruses have been subjected to vector engineering, especially for Semliki Forest virus (SFV),87 SINV,88 and Venezuelan equine encephalitis (VEE) virus.89 Applications for cancer therapy have included administration of RNA
replicons, recombinant alphavirus particles, and layered DNA vectors. For instance, immunization of mice with SFV-LacZ RNA demonstrated tumor regression and provided protection against challenges with tumor cells.\(^{90}\) Likewise, SFV EGFP particles subjected to intratumoral injections resulted in tumor regression in immunodeficient mice with human lung carcinoma xenografts.\(^{91}\) Additionally, immunization of mice with SINV plasmid DNA carrying the TRP1 gene showed antitumor activity and immunoprotection in mice.\(^{92}\)

Oncolytic alphaviruses occur naturally and have also been engineered from avirulent SFV strains. High infection rates and lysis of cancer cells were observed for the avirulent SFVA7(74) strain (SFVVA7) and a single intraperitoneal or intravenous injection showed significant tumor regression in SCID mice with established melanomas.\(^{93}\) Similarly, improved survival rates were observed after SFV-VA7-EGFP administration in nude mice with osteosarcoma\(^{94}\) and orthotypic lung-tumor xenografts.\(^{95}\) Furthermore, SFV-VA7-EGFP particles demonstrated efficient replication and killing of two canine-tumor cell lines, and no adverse events occurred in beagle dogs after intravenous administration of 2\(\times\)10\(^8\) particles.\(^{96}\)

Adequate attention has been paid to tumor targeting of alphavirus vectors. In this context, it has been shown that SINV particles possess natural tumor targeting after intraperitoneal administration of mice implanted with tumor xenografts, and subcutaneous SINV-IL12 administration reduced the tumor load to 6.2% of control mice.\(^{97}\) In contrast, studies on SFV particles showed no tumor targeting.\(^{98}\) For this reason, liposome-encapsulated SFV particles were engineered to provide tumor targeting of β-galactosidase after systemic delivery of SFV-LacZ particles in SCID mice.\(^{99}\) Moreover, encapsulated SFV-IL12 particles showed good safety profiles in kidney carcinoma and melanoma patients.\(^{99}\) Another approach comprises engineering six tandem neuron-specific miR124 sequences between the nsP3 and nsP4 genes in the SFV4 genome, which provided glioma targeting and limited spread in the central nervous system (CNS) in BALB/c mice after intraperitoneal delivery of SFV4-miR124 particles.\(^{100}\) Moreover, the naturally occurring oncolytic M1 alphavirus is capable of selective killing of zinc-finger antiviral protein (ZAP)-deficient cancer cells, providing potent oncolytic efficacy and high tumor tropism in vitro and in vivo.\(^{101}\) In another study, the safety of M1 was evaluated in nonhuman primates prior to initiation of clinical trials.\(^{102}\) Five macaques received three rounds of 10\(^9\) pfu of M1 intravenously and were monitored for a number of physiological and biochemical parameters, neutralizing antibodies, and clinical symptoms. No clinical, biochemical, immunological, or medical imaging indicated any evidence of toxicity, suggesting that M1 can be safely used for intravenous administration in cancer patients.

Recently, SFV-VA7 particles were evaluated in human VCaP, LNCaP, and 22Rv1 prostate cancer cell lines and in the nonmalignant RWPE1 prostate epithelial cell line, as well as in subcutaneous and orthotopic mouse LNCaP xenograft models.\(^{103}\) Interestingly, all prostate cancer cell lines, irrespective of their hormone-response status, were efficiently killed by SFVVA7, whereas RWPE1 cells were resistant to SFVVA7, indicating tumor targeting of SFV. This result is in contrast to previous findings of lack of tumor targeting of SFV particles.\(^{98}\) In vivo, a single peritoneal dose of SFVVA7 showed eradication of all subcutaneous and orthotopic LNCaP tumors.

Flaviviruses are enveloped ssRNA viruses engineered for recombinant protein expression and cancer therapy.\(^{104-108}\) For example, Kunjin virus vectors expressing GM-CSF provided cure in more than 50% of mice with established subcutaneous CT26 colon carcinomas after intratumoral administration.\(^{109}\) Moreover, regression of B16-OVA melanoma tumors was obtained after 5 days, with a cure rate of 67%. Subcutaneous administration of Kunjin GM-CSF particles resulted in tumor regression in CT26 lung metastasis in BALB/c mice.

Rhabdoviruses, such as rabies virus\(^{110,111}\) and VSV,\(^{112}\) have been engineered as expression vectors applicable for cancer therapy with a special emphasis on oncolytic VSV vectors.\(^{113}\) The attractive features of VSV comprise high susceptibility of cancer cells, lack of antiviral responses induced by type I IFN, ease of manipulation, replication in the cytoplasm, and no preexisting immunity in humans. VSV particles show superior oncolytic activity in 13 relevant human pancreatic cell lines in comparison to Ads, Sendai virus, and respiratory syncytial virus, although the response is heterogeneous, with some cell lines being resistant to VSV.\(^{114}\) Likewise, strong oncolytic activity was obtained in pancreatic ductal adenocarcinoma cell lines after infection with VSV expressing MUC1, and tumor-growth reduction was achieved in vivo.\(^{115}\) Combination therapy with gemcitabine further enhanced therapeutic efficacy. In another study, VSV demonstrated apoptotic activity in pancreatic ductal adenocarcinoma cell lines.\(^{116}\) However, resistance to apoptosis was seen in three cell lines with high constitutive expression of IFN-stimulated genes. Oncolytic rhabdovirus MG1 particles have shown a strong ability to kill human and canine sarcoma cell lines, and infected more than 80% of human sarcoma tissues tested ex vivo.\(^{117}\) MG1 treatment of sarcoma-bearing mice showed a significant increase in long-lasting cure and also provided...
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Protection against subsequent tumor challenges. VSV vectors have also been tested for replication in cancer cells from ascites. Administration of 10^9 pfu generated a significant inhibition of ascites formation and prolonged survival in mice. Moreover, metabolic adaptive processes in peritoneal carcinoma, including high glycolytic activity and glutamine metabolism, favored VSV replication.

MVs, similar to rhabdoviruses in possessing an enveloped ssRNA genome, have also been subjected to vector development for cancer therapy. Oncolytic Edmonston B (MV Edm) has been employed in studies in xenograft and syngeneic models. For instance, tumor regression was observed in SCID mice implanted with lymphoma xenografts after intratumoral administration of MV Edm. MV-based dual therapy with CEA and thyroidal NIS showed a superior outcome in treatment of mice with implanted SKOV3ip.1 ovarian tumor xenografts in comparison to administration of MV CEA or MV NIS individually. Related to breast cancer, MV-CEA vectors provided significant tumor-growth delay and prolonged survival in an MDA-MB231 mammary-tumor model. Furthermore, intratumoral MV-CEA administration resulted in delayed tumor growth and extended survival in mice with implanted subcutaneous PC3 prostate tumors. Tumor targeting of MV vectors was achieved by introduction of CD46 and SLAM into the hemagglutinin protein combined with the display of a single-chain antibody against EGFR. The retargeted vector showed antitumor activity but no neurotoxicity in MV replication-permissive transgenic mice after systemic administration. Another attempt to target MV vectors involved engineered MV vectors based on the cancer stem-cell marker CD133 (prominin 1). Selective tumor targeting was obtained by replacing the MV hemagglutinin receptor with a CD133-specific scFv. Furthermore, engineered chimera between MV CD133 and VSV showed highly selective elimination of CD133+ cells. The VSV-CD133 vector revealed highly potent oncolytic activity in HCC and prolonged survival of mice after intravenous injection. Moreover, VSV CD133 infected a >10^4-fold larger tumor area in comparison to MV CD133. In another approach, MV vectors based on the MV Schwarz strain encoding a fusion of IL12 and an antibody against PDL1, respectively, resulted in complete remission in 90% of tested mice with established tumors.

NDV is another ssRNA virus, and belongs to the paramyxoviruses. The oncolytic properties of NDV vectors have made them attractive for cancer therapy, which has been confirmed in several animal-tumor models. Comparison of NDV-based expression of IL12 and IL15 indicated lower toxicity of IL15 in mice with implanted melanoma tumors, and intratumoral administration showed efficient suppression of tumor growth for both ND IL12 and NDV IL15. Although not statistically significant, the survival rate was 12.5% higher for NDV IL15. The reverse genetically engineered NDVD90 strain expressing EGFP showed tumor-selective replication, induction of apoptosis in A549 lung cancer cells, and suppression of tumor growth in vivo. Furthermore, a highly virulent NDV strain adapted for replication in HeLa cells promoted upregulation of TRAIL and caspase activation through induction of apoptosis. Interestingly, the NDV Anhinga strain carrying the IL2 gene showed strong inhibition of HCC growth, providing both complete cure and protection against tumor challenges 60 days after immunizations. In another study, administration of the recombinant NDV Anhinga strain expressing TRAIL resulted in efficient HCC suppression without showing any significant toxicity in normal tissue. Related to NDV epidemics in poultry, an oncolytic vector based in the NDV-73T strain with a modified fusion-protein cleavage site and a 198-nucleotide insertion in the HNL intergenic region was engineered, which showed significantly reduced viral gene expression and replication in avian cells, but not in mammalian cells. Moreover, intratumoral and intravenous administration generated selective replication and killing of tumor cells.

A number of Phase I–III clinical trials applying NDV vectors have been conducted for melanomas, glioblastomas, and head and neck cancer, with some encouraging results. For instance, treatment with mesogenic NDV73T resulted in more than 60% 10-year survival in comparison to 6%–33% in the control group. Moreover, the lentogenic NDV Ulster strain demonstrated long-term survival of one glioblastoma patient in comparison to none in the control group. Similarly, the 5-year survival rate was 51% in a Phase II study on head and neck cancer with the NDV-73T strain.

Poxviruses carry a large dsDNA genome with a good packaging capacity of foreign DNA and tumor-selective properties providing cellular destruction by viral replication. Introduction of deletions in the TK and VGF genes allow replication in tumor cells, while normal cells are not affected. Moreover, the modified poxvirus vector enhances immunorecognition of tumors. Furthermore, expression of IL2 or IL12 from a VV vector generates antitumor activity in mice with implanted C6 gliomas. Oncolytic VVs, such as GLV1h68, have lysed human COLO205, HCT15, HCT116, HT29, and SW20 CRC lines efficiently, and significantly inhibited tumor growth and prolonged survival in athymic mice with established colorectal xenografts after a...
single GLV-1h68 intravenous injection.\textsuperscript{142} Likewise, human salivary-gland carcinoma cells were susceptible to GLV1h68, and a single intravenous administration resulted in significant tumor regression.\textsuperscript{143} Genetic modifications made to the VV GLV1h151 vector enhanced cancer specificity, resulting in efficient infection, replication, and killing of several cancer-based (breast, lung, pancreatic, and colorectal) cell lines.\textsuperscript{144} Intravenous injection of VV GLV1h151 confirmed replication in tumors in vivo. The oncolytic GLV1h68 was further evaluated in combination with chemotherapy applying nab-paclitaxel and gemcitabine, which provided enhanced tumor-cell killing in two of four human pancreatic adenocarcinoma cell lines.\textsuperscript{145} The feasibility of chemovirotherapy seemed to be related to efficient viral replication, as the nonresponsive tumor-cell lines showed only low levels of viral replication.

VV vectors have also been applied in tumor-bearing Xeroderma pigmentosum (XP) patients excluded from conventional DNA-damage therapy.\textsuperscript{146} VV vectors demonstrated ten- to 100-fold higher cytotoxicity in tumor-derived cells from XP patients compared to normal control cells, and systemic administration showed long-term tumor regression in XP animal models. The oncolytic GL-ONC1 VV vector was administered intravenously in combination with chemoradiotherapy in patients with primary nonmetastatic head and neck cancers.\textsuperscript{147} The follow-up of patients indicated 1-year progression-free survival and overall survival of 74.4\% and 84.6\%, respectively, which demonstrated the safety and feasibility of GL-ONC1 delivery.

CVs belong to the family of Picornaviridae, with an ssRNA of positive polarity and a nonenveloped structure.\textsuperscript{148} They are known pathogens, and present the leading causes of aseptic meningitis. However, CVA21 has shown promise as an oncolytic vector and demonstrated potential in preclinical cancer models.\textsuperscript{149} In this context, decreased tumor burden has been obtained in tumor models for melanoma,\textsuperscript{150} multiple myeloma,\textsuperscript{151} breast,\textsuperscript{152} prostate,\textsuperscript{153} and lung\textsuperscript{154} cancers. More specifically, multiple-myeloma cell lines have shown high susceptibility to CVA21, resulting in lytic infection. Moreover, when biopsies from patient bone marrow were challenged with CVA21, specific removal of 98.7\% of CD138\textsuperscript{+} plasma cells was obtained with no decrease in the functionality of progenitor cells. For this reason, CVA21 administration might provide an efficient approach for multiple-myeloma treatment prior to transplantation of autologous stem cells. In another approach, a large-scale two-step screening procedure for 28 enteroviral strains was conducted, which identified that CVB3 presented oncolytic activity against nine human NSCLC cell lines. CVB3 induced apoptosis and activated kinase-signaling pathways. Intratumoral CVB3 administration generated substantial tumor regression in mice with established NSCLC tumors. Interestingly, injection of CVB3 into tumors located on the right flank demonstrated replication-competent CVB3 and significant regression in xenografts on the left flank. It was also discovered that intratumoral administration of CVB3 recruited natural killer cells and granulocytes, thereby providing immunostimulatory activity.

**Optimization and selection of oncolytic viruses**

A number of efforts have been made to optimize oncolytic viral vectors. In this context, a CD133-targeting motif (TYML) was introduced into an Ad vector, and provided selective infection and killing of CRC cells and protection against CRC challenges.\textsuperscript{155} Similarly, MV vectors with CD133-specific scF\textsubscript{v} showed potent oncolytic activity and prolonged survival in tumor-bearing mice.\textsuperscript{156} Moreover, dual expression of GM-CSF and decorin (regulator of cancer development and progression) from Ad vectors led to significant inhibition of tumor growth and lung metastasis in vivo.\textsuperscript{66} Also, decorin delivery by AAV promoted intratumoral paclitaxel uptake.\textsuperscript{65}

Another approach consists of gene silencing, of which examples for antitumor activity have been demonstrated for shRNAs for AAV\textsuperscript{66} and miRNAs for HSV1\textsuperscript{70} and lentiviruses.\textsuperscript{79} Furthermore, introduction of six tandem neuron-specific miR124 sequences into the SFV vector has resulted in glioma targeting and only limited spread in the CNS.\textsuperscript{100} Promoter engineering has also allowed enhanced expression targeting, as demonstrated by neuron-specific delivery and 100-fold lower presence in the liver by applying the NSE promoter in AAV9 vectors.\textsuperscript{64} Selective targeting of tumors has also been achieved by replacing the HSV1 ICP4 promoter with the tumor-specific survivin promoter.\textsuperscript{70} Moreover, retroviruses have been subjected to chimeric antigen-receptor engineering to provide safe treatment of hematological malignancies.\textsuperscript{74}

Selection of oncolytic virus strains has also been of great importance. In this context, the naturally occurring M1 alpha-virus has shown potent oncolytic activity and high tumor tropism,\textsuperscript{101} which further demonstrated no evidence of toxicity in macaques and indicated safe intravenous administration in cancer patients.\textsuperscript{102} Similarly, plenty of attention has been paid to reverse engineering of NDV strains, such as NDVD90, which showed tumor-selective replication and decrease in tumor growth.\textsuperscript{131} Similarly, the NDV Anhinga strain has been subjected to preclinical studies for HCC treatment.\textsuperscript{133,134}
Moreover, engineering of the NDV73T strain resulted in reduced viral replication in avian but not mammalian cells. Oncolytic poxvirus strains, such as VV GLV1h68, have also been selected, which have shown antitumor activity for both CRC and salivary-gland carcinoma. Genetic modifications have enhanced cancer specificity for the VV GLV1h151 vector, and when combined with paclitaxel and gemcitabine further increased tumor-cell death.

Effects on tumor vasculature

Oncolytic viruses have also shown selective targeting of tumor vasculature. For instance, VSV caused thrombosis in tumor vessels by selective infection of endothelial cells in the tumor microenvironment. Moreover, HSV and VV are capable of selectively provoking damage to the tumor endothelium. Oncolytic VV vectors have been engineered to express antiangiogenic factors, such as VEGF inhibitors, which leads to suppression of VEGF levels and decrease in perfusion within tumors. Furthermore, oncolytic VV-based targeting of VEGF provided a synergistic antitumor effect with VEGFR tyrosine-kinase inhibitors. This synergism may be caused by off-target inhibition of cellular antiviral defense proteins, such as dsRNA-dependent protein kinases. In the context of the aggressive anaplastic thyroid carcinoma (ATC), oncolytic Ad d1922-947 induced cell death in vitro and tumor regression in animal models. It was also demonstrated that Ad d1922-947 decreased IL8/CXCL8 and MCP-1/CCL2 expression in the 8505-c and BHT101-5 ATC cell lines and reduced IL8 impaired ATC-induced angiogenesis in vivo. Overall, the oncolytic Ad reshaped the protumorigenic ATC microenvironment by modulation of intrinsic cancer-cell factors and immunoresponses.

Clinical trials using oncolytic viruses

The progress made in cancer therapy with oncolytic viruses in preclinical studies has further encouraged the transition into clinical trials. A large number of studies employing, eg, Ad, alphavirus, HSV, reovirus, NDV, MV, and CV vectors have been conducted or are in progress. In this context, the safety, pharmacokinetics, and anticancer activity of an intravesical oncolytic Ad (CG0070) was evaluated in a Phase I trial in patients with refractory primary or metastatic liver cancer. Patients received intravesically either a single or multiple doses of $10^9$, $3 \times 10^9$, and $3 \times 10^{10}$ viral particles, respectively, three times every 28 days or six times weekly. Due to grade 1–2 bladder toxicity, a maximum tolerated dose (MTD) was not reached, although the safety profile was tolerable and anti-bladder cancer activity observed. Ads have also been subjected to several clinical trials in prostate cancer patients, including a replication-selective PSA-targeted oncolytic vector and a replication-competent vector providing double-suicide-gene therapy, which resulted in decreased serum levels of PSA.

NDV has also been subjected to several Phase I–III clinical trials. A 10-year observation of 83 postsurgical patients with stage II malignant melanoma treated with NDV demonstrated no presence of disease in 60%, which is remarkable in comparison to similar studies showing only 5%–33% survival. Moreover, exceptional survival was seen in 21 patients with head and neck cancer and six individuals with cerebral metastases. NDV has also been applied in the treatment of 23 patients with a vaccine consisting of NDV-infected patient-isolated glioblastoma cells followed by $\gamma$-irradiation. The NDV therapy caused no severe side effects and showed longer median progression-free survival (40 weeks for NDV treatment and 26 weeks for controls) and median overall survival (100 weeks for vaccinated and 49 weeks for controls). Furthermore, 91% of the NDV-treated patients survived for 1 year compared to 45% for controls, and long-term survival was 4% and 0 for vaccinated and control patients, respectively. In another study, 20 patients with head and neck squamous-cell carcinoma preconditioned with IL2 were vaccinated with NDV-infected autologous tumor cells, which increased systemic antitumor activity.

The replication-competent NDV PV701 strain was evaluated in a Phase I trial in renal cancer patients. An MTD of $1.2 \times 10^{10}$ pfu/m$^2$ was established, with only flu-like adverse events. Moreover, objective responses were observed and progression-free survival ranged from 4 to 31 months.

Oncolytic VV vectors, such as GL-ONC1, were subjected to a Phase I clinical trial in combination with radio- and chemotherapy in patients with primary nonmetastatic head and neck cancer. Adverse reactions, such as fever, fatigue, rash, nausea, and vomiting, were observed among the 19 patients recruited. The MTD was not reached, but patient follow-up demonstrated 1-year (2-year) progression-free survival and overall survival of 74.4% (64.1%) and 84.6% (69.2%), respectively, indicating safe application of VV in cancer patients undergoing radio- and chemotherapy. In another study, the oncolytic VV vector JX594 was evaluated in patients with refractory primary or metastatic liver cancer. JX594 provided direct oncolysis and overexpression of GM-CSF, thereby stimulating the shutdown of tumor vasculature and antitumor immune responses. Fourteen patients with refractory primary or metastatic liver tumors received one of four doses ($10^8$, $3 \times 10^8$, $10^9$, or $3 \times 10^9$ pfu) every
3 weeks. All patients showed grade I–III flu-like symptoms, four patients presented dose-related thrombocytopenia, and grade III hyperbilirubinemia dose limitations at the highest dose defined the MTD as $10^7$ pfu. Tumor responses were observed in injected and noninjected tumors. Three patients showed partial responses, six presented stable disease, and one demonstrated progressive disease. VVJX594 has been further evaluated in patients with metastatic refractory renal-cell carcinoma. In another approach, a dose-escalating study with intravesical Dryvax VV was conducted in bladder cancer patients. Viral infection was detected in tumor and normal urothelial cells. The study indicated that VV was safely delivered into the bladder. VV vectors have also been applied for vaccination of patients with locally recurrent or progressive prostate cancer. Expression of PSA from a VV vector revealed no dose-limiting toxicity, and intraprostatic administration was safe and elicited significant immunoresponses in the Phase I trial.

Reoviruses have also been subjected to clinical trials. In this context, an oncolytic reovirus (pelareorep) was combined with carboplatin and paclitaxel for the treatment of metastatic pancreatic adenocarcinoma. In the randomized Phase II trial, paclitaxel–carboplatin + pelareorep was compared to paclitaxel–carboplatin, which resulted in no difference in progression-free survival. The presence of pelareorep generated higher levels of 14 proinflammatory plasma cytokines/chemokines. However, although pelareorep delivery was demonstrated to be safe, it did not improve progression-free survival of paclitaxel–carboplatin treatment, but combination with chemotherapy may improve targeting immunosuppressive mediators and enhance oncolytic virotherapy. In another approach, a Phase II study with replication-competent reovirus was conducted in 21 metastatic melanoma patients. Patients treated with $3 \times 10^{10}$ tissue culture infectious dose (TCID)$_{50}$ showed good safety profiles, and productive reovirus replication was observed in biopsies. Furthermore, a Phase I/II dose-escalating combination-therapy study with carboplatin–paclitaxel and an oncolytic reovirus in head and neck cancer patients showed no dose-limiting toxicity, with one patient (3.8%) having a complete response, six patients (23.1%) partial responses, and two patients (7.6%) major clinical responses. Moreover, reoviruses have been subjected to a Phase I study in prostate cancer patients, and demonstrated a good safety profile after repeated intravenous administration and reovirus targeting of metastatic tumors.

The second-generation oncolytic HSV vector M032, which selectively replicates in tumor cells, has been employed for overexpression of IL12 to increase the antitumor effect and provide an antiangiogenic effect to target new-tumor blood-vessel formation. Demonstration in preclinical models has paved the way for initiating a Phase I clinical trial in patients with recurrent progressive glioblastoma multiforme. Furthermore, safety of the HSVG207 vector has been confirmed in preclinical studies in owl monkeys (genus Aotus) and in adult Phase I trials in progressive and recurrent brain tumors. Based on these findings, the design of a Phase I clinical trial to evaluate HSVG207 monotherapy or in combination with radiotherapy has been designed for children with progressive and recurrent supratentorial brain tumors.

Regarding alphaviruses, a Phase I trial has been conducted in patients with metastatic castration-resistant prostate cancer applying VEE particles expressing prostate-specific membrane antigen (PSMA). In the dose-escalating study, patients received five doses of $0.9 \times 10^7$ or $0.36 \times 10^8$ IU of VEE PSMA particles at weeks 1, 4, 7, 10, and 18, followed by another round of administration of the higher dose in six patients. No toxicities were observed in any patient, and VEE PSMA was well tolerated. However, no PSMA-specific cellular immunoresponse was observed, although a PSMA-specific signal was registered by enzyme-linked immunosorbent assay. Although neither clinical benefit nor robust immunomodulation was detected, neutralizing antibodies were produced, which indicated that there is a need for dose optimization. In another Phase I trial, replication-deficient SFV IL12 particles were encapsulated in liposomes and subjected to intravenous administration in terminally ill melanoma and kidney carcinoma patients. No liposome- or virus-related toxicity was observed in any patient. IL12 plasma levels showed a transient five- to tenfold increase, indicating potential immunostimulatory activity. Furthermore, the encapsulation procedure protected the virus from being recognized by the host immune system, allowing repeated administration of SFV IL12 particles.

In the context of CVs, the CVA21 strain has been demonstrated to target ICAM1, which is upregulated in melanoma, NSCLC, and bladder and prostate cancers. In a Phase I/II trial, patients received multiple intravenous doses of the bioselected formulation Cavatak of CVA21, which were well tolerated. Prolonged presence of CVA21 RNA in the serum of some patients suggested that viral replication occurred in tumors. Biopsies from melanoma patients confirmed tumor targeting of CVA21. Moreover, CVA21 seemed to provide increased antitumor activity, which might be further enhanced by combination with immunomodulation.
patients was conducted with CVA21, showing induced immune-cell infiltration in the tumor microenvironment.\textsuperscript{183} Similarly, a Phase IB combination-therapy study with CVA21 and systemic pembrolizumab in 20 advanced-melanoma patients demonstrated a best overall response rate of 60% and stable disease in 27% of the patients.\textsuperscript{184} Treatment tolerability was good, with no dose-limiting toxicity and no grade 3 or higher treatment-related adverse events.

**Conclusion**

In summary, oncolytic viruses, based on engineered vectors or naturally occurring strains, have proven efficient in various preclinical tumor models providing suppression of tumor growth, tumor regression, and in certain cases complete eradication of existing tumors (Table 1). Immunization has also resulted in protection against tumor challenges. The variety of oncolytic viruses (Ad, AAV, HSV, retrovirus, lentivirus, alphavirus, flavivirus, rhabdovirus, MV, NDV, CV, and poxvirus) allows flexibility related to packaging capacity, host range tropism, and mode of expression (duration, chromosomal integration). It can be concluded that no single oncolytic viral vector is universally superior, and for this reason the decision on which vector to use is to a large extent dictated by specific applications and familiarity with each vector system.

Most encouragingly, clinical trials have been conducted or are in progress for most oncolytic vector systems, and have confirmed safe administration in humans (Table 2). Moreover, therapeutic efficacy has also been achieved. Already some time ago, the first drugs based on oncolytic Ads expressing the p53 gene (Gendicine)\textsuperscript{185} and the Ad H101 vector with an E1B55K deletion\textsuperscript{186} were approved in China. Recently, the second-generation oncolytic HSV1 GM-CSF vector was approved for the treatment of melanoma in the US and Europe.\textsuperscript{187,188} A number of other drugs based on oncolytic viruses, such as the oncolytic VV JX594 (pexastimogene devacirepvec) for HCC treatment,\textsuperscript{189} Ad CG0070 expressing cancer will most likely be approved in the near future. Additionally, the third-generation oncolytic HSV1 vector G478, subjected to a Phase II glioblastoma trial, should receive priority reviewing and fast-track drug approval.\textsuperscript{190} Overall, the drug approvals obtained and the ongoing oncolytic virus development bode well for finding safer and more efficacious cancer drugs in the future.

**Disclosure**

The author reports no conflicts of interest in this work.

**References**


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