

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

Kenneth Lundstrom

PanTherapeutics, Lutry, Switzerland

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 down 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

Correspondence: Kenneth Lundstrom
PanTherapeutics, 49 Route de Lavaux,
Lutry CH1095, Switzerland
Tel +41 79 776 6351
Email lundstromkenneth@gmail.com

point.¹ 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 occurring⁷ and engineered⁸ vectors can provide superior therapeutic efficacy, due to their selective tumor cell-killing capacity and potential induction of systemic antitumor immunity.⁹ Today, a number of different oncolytic viruses, such as adenoviruses (Ads),¹⁰ HSV,¹¹ alphaviruses, rhabdoviruses,¹² Newcastle disease virus (NDV),¹³ vaccinia viruses (VVs),¹⁴ 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.¹ 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.¹⁵ Transient high-level expression is especially attractive for cancer-therapy applications, as the presence of anti-tumor 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.¹⁵ 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.¹⁵ 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 shRNA¹⁶.

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.¹⁷ AAV vectors have also shown strong immunogenicity, especially after virus readministration, which has been circumvented by using different AAV serotypes for subsequent injections.¹⁸

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.^{19,20} The mechanism of action occurs through RAS-pathway activation or by genetic modifications.^{21,22} In this context, HSV has been demonstrated to replicate only in tumor cells dependent on TK activity.²³ 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.²⁴ One interesting feature of oncolytic reoviruses,²⁵ HSV,²⁶ and VV²⁷ is their ability to induce adaptive immunoresponses, which can contribute indirectly to tumor cell death. Similarly, oncolytic Ads,²⁸ Coxsackie virus (CV) B3,²⁹ and measles virus (MV)³⁰ 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.^{31–33} 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.^{34–36} Moreover, various

Table 1 Examples of preclinical cancer therapy applications for viral vectors

Cancer type	Target	Delivery	Response	Reference	
Brain	SLAM, EGFR	MV	Tumor regression	125	
	sTRAIL	AAV9 CBA and NSE promoters	Slower tumor growth	64	
	miR124	SFV-miRT124	Prolonged survival	100	
	IL2, IL12	VV	Antitumor response	141	
	shRNA MYCN	Ad	Induction of apoptosis	162	
	Decorin	AAV	Promotion of paclitaxel uptake	65	
Breast	HSV1	HSV1, survivin promoter	Selective tumor targeting	70, 71	
	CEA	MV	Tumor-growth delay	123	
	Neu	AAV5, AAV6	Long-term survival	62	
	CCNY	Lentivirus	Reduced cell proliferation	82	
	Coxsackie virus	CVA21	Reduced tumor burden in mice	152	
Colon	MazF-MazE	Ad	Tumor regression	49	
	HSV2	HSV2	Prolonged survival	69	
	β -gal	SFV RNA	Tumor protection	90	
	GM-CSF	Kunjin VLPs	Tumor regression	109	
	VV	VVGLVh168	Prolonged survival	142	
	CD133	Ad CD133-TYML	Antitumor response	55	
	IL12-PDL1	MV Schwarz	Complete remission	129	
Esophageal	hTERT	Ad5	Tumor regression	48	
Gastric	RPL23, p53	Ad	Prolonged survival	46	
	TRAIL, EI	Ad	Prolonged survival	50	
	iNOS + CEA scF _v	RV	Tumor-growth inhibition	73	
	RNAi CDH17	Lentivirus	Decreased tumorigenicity	81	
Leukemia	shRNA MCM7	Lentivirus	Antitumor activity	83	
Liver	IL24	Ad + PEG, lipid, calcium phosphate	Tumor targeting	52	
	IL24, Bcl3 IAP	AAV	Tumor suppression	60	
	HSV-TK	AAV, albumin promoter	Selective tumor killing	61	
	shRNA miR30 IL2	HIV	Inhibition of proliferation	79	
	M1	M1	Oncolytic activity	101	
	IL2	NDV Anhinga	Cure, tumor protection	133	
	TRAIL	NDV Anhinga	Tumor suppression	134	
	TSCLI	Ad-Wnt-E1A-(δ 24bp)-TSLC1	Prolonged survival	54	
	MLV, GALV	RV, MLV, GALV	Suppression of tumor growth	75	
	Wtp53-mi30-shRNA	Lentivirus	Inhibition of cell proliferation	179	
	CD133	MV VSV	Prolonged survival	126	
	Lung	Decorin, GM-CSF	Ad	Inhibition of lung metastasis	56
		VEGF	AAV2	Prevention of metastasis	58
		miR145	HSV1	Reduced cell proliferation	11
shRNA livin		HIV1	Reduced tumor proliferation	80	
EGFP		SFV VLPs	Tumor regression	91	
EGFP		SFVVA7	Prolonged survival	95	
EGFP		NDVD90	Tumor-selective replication	131	
Coxsackie virus		CVA21	Reduced tumor burden in mice	154	
MV Edmonston		MV Edmonston	Tumor regression	121	
Lymphoma		TRP1	SINV DNA	Tumor protection	92
	SFVVA7	SFVVA7	Tumor regression	93	
	GM-CSF	Kunjin VLPs	Tumor regression	109	
	IL15, IL12	NDV	Tumor-growth suppression	130	
	NDV	NDV73T	Improved survival in patients	136	
	Coxsackie virus	CVA21	Reduced tumor burden in mice	150	
Myeloma	Coxsackie virus	CVA21	Reduced tumor burden in mice	151	
	CEA, NIS	MV	Tumor regression	122	
Ovarian	EI	Ad hTERT	Antitumor response	44	
	TRAIL	RV + cisplatin	Antitumor response	73	
	EGFP	SFV-VA7	Prolonged survival	94	

(Continued)

Table 1 (Continued)

Cancer type	Target	Delivery	Response	Reference
Pancreatic	Matrix protein	VSV VLPs	Tumor regression	114
	MUC1	VSV VLPs	Tumor-growth reduction	115
	IFN β	Lentivirus	Prevention of cancer progression	78
	VV	VVGLV1h68 + paclitaxel	Tumor killing	145
	IL12	SINV	Reduced tumor load	97
Prostate	CEA	MV	Tumor-growth delay	124
	AR siRNA	AAV	Tumor suppression	63
	NIS	HSV	Tumor eradication	67
	PSCA	HIVI	Tumor protection	77
	β -gal	SFV + liposomes, PEG	Tumor targeting	99
	SFV-VA7	SFV-VA7	Tumor targeting	103
	Coxsackie virus	CVA21	Reduced tumor burden in mice	153
Retinoblastoma	IFN β	AAV	Antitumor response	57
Salivary gland	VV	VVGLV1h68	Tumor regression	139
Sarcoma	HSV1	HSV1 + PD1	Therapeutic efficacy	68
	MGI	Rhabdovirus MGI	Long-lasting cure	117
Skin	HSV1	HSV1 RH2	Tumor regression	66
	VV	VV	Long-term regression in Xp model	146

Abbreviations: AAV, adenoassociated virus; Ad, adenovirus; AR, androgen receptor; β -gal, β -galactosidase; CVA21, 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; scF_v, 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*.

antiviral immunoresponses have been shown to contribute to the anticancer activity of oncolytic vesicular stomatitis virus (VSV), Maraba virus, VV, HSV, and reovirus by inducing IFN1, leading to the secretion of several immunostimulatory cytokines and chemokines, such as tumor necrosis factor (TNF) and TRAIL.³⁷ Similarly, expression of proinflammatory genes, such as IL12 or IL18, from oncolytic HSV³⁸ and Ad³⁹ vectors has enhanced tumor-specific immunity. Moreover, coexpression of IL12 and CCL2 from an oncolytic HSV vector accelerates the recruitment of activated macrophages and T cells without affecting virus replication, albeit providing improved survival rates.⁴⁰

An interesting finding relates to enhanced antitumor activity in the presence of preexisting antiviral immunity. While improved survival has been obtained in immunocompetent tumor models, the same phenomenon is not present in immunosuppressed mice.⁴¹ In contrast, innate immune cells are capable of rapid clearance of replicating oncolytic HSV particles, which presents a significant limitation of oncolytic virotherapy.⁴² Furthermore, it was discovered in a Phase IB clinical trial with the oncolytic HSV1-derived γ ,34.5-deleted G207 vector that a stronger inflammatory response and IFN-stimulated gene expression were detected in long-term survivors compared to nonresponders.⁴³ In summary, four phases contribute to oncolytic virotherapy: direct cellular lysis, cytokine-induced apoptosis, innate immune-cell cytotoxicity, and antigen-specific adaptive T-cell killing.

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,⁴⁴ prostate,⁴⁵ gastric,⁴⁶ and brain cancer⁴⁷ 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.⁴⁶ 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.⁴⁸ 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

Table 2 Examples of clinical cancer therapy applications for viral vectors

Cancer type	Target	Delivery	Response	Reference
Bladder	GM-CSF	Ad CG0070	Good tolerance, antitumor activity	165
	GM-CSF	Ad CG0070	Close to approval	191
	VV	Dryvax VV	Safe delivery in Phase I	171
Brain	IL12	HSV1	Phase I design	178
	HSV + radiation	HSV1 G207	Phase I safety	179
	HSV	HSV1 G207	Phase IB, antitumor activity	180
	HSV + radiation	HSV1 G207	Phase I design	43
	HSV1	HSV1 G47 δ	Fast-track approval	190
	NDV	NDV Ulster	Long-term survival in patients	137
Head and neck	NDV	NDV73T	Improved survival rate in patients	138
	VV	VV GL-ONC1	Improved survival in patients	147
	p53	Ad	Approved drug	185
	p53	Ad E1B55K deletion	Approved drug	185
	Reolysin + paclitaxel/CPlat	Reovirus	No toxicity in Phase I/II	176
	Pelareorep	Reovirus	Close to drug approval	192
Kidney	IL12	SFV + liposomes, PEG	Tumor targeting, clinical safety	99
	NDV	PV701	Objective responses in Phase I	168
	VV	VVJX594	Phase I evaluation	170
Liver	VV	VVJX594	Close to drug approval	189
Melanoma	IL12	SFV + liposomes, PEG	Tumor targeting, clinical safety	99
	NDV	NDV73T	Improved survival in patients	136
	GM-CSF	HSV1	Approved drug	187
	Reovirus	Reovirus	Safe delivery, Phase II	175
	CV	CVA21	Antitumor activity in Phase I/II	182
	CV	CVA21	Immunoresponse in Phase II	183
	CV + pembrolizumab	CVA21	Response in Phase IB	184
	Reolysin + paclitaxel/CPlat	Reovirus	Safe delivery, Phase II	174
Pancreatic	Adenovirus	CG7870	Decreased serum PSA in Phase I	166
	CD/HSV-TK	Adenovirus	Decreased serum PSA in Phase I	167
	PSMA	VEE	Neutralizing antibodies in Phase I	181
	Pelareorep	Reovirus	Repeated delivery in Phase I	173
	PSA	VV	Immunoresponse in Phase I	172

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.

any side effects.⁴⁹ 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.⁵⁰ In attempts to improve oncolytic Ads, incorporation of polymers, liposomes, and nanoparticles has extended the circulation time and reduced vector-based immunogenicity.⁵¹ In this context, formulation of oncolytic vectors with polyethylene glycol, lipids, and calcium reduces liver sequestration and systemic toxicity of oncolytic Ads expressing IL24 (PLC-ZD55-IL24) in BALB/c mice.⁵² Intravenous injection demonstrates efficient targeting of Huh7 tumors, with no observed toxicity.

Related to neuroblastoma, multidrug resistance has been a major issue hindering successful chemotherapy. It has triggered the engineering of an oncolytic Ad vector carrying shRNAs against the *MYCN* oncogene (ZD55-shMYCN),

which correlates with the expression of the protein MRP.⁵³ ZD55-shRNA-based downregulation of *MYCN* inhibited tumor-cell proliferation and induced apoptosis in neuroblastoma cells. Furthermore, ZD55-shRNA was capable of resensitizing doxorubicin-resistant cells to doxorubicin and resulted in reduced proliferation, increased apoptosis, and inhibited cell migration, which reduced the in vivo growth rate of neuroblastoma xenografts. In another approach, the dual-regulated oncolytic Ad wnt-E1A(δ 24bp)-TSLC1 targeting the Wnt- and Rb-signaling pathways and carrying the TSLC1 tumor suppressor was engineered.⁵⁴ 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.⁵⁵ The engineered vector with the CD133-targeting motif (AdML-TYML) showed

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 β has provided a strong antitumor effect in a preclinical retinoblastoma model without any issues of readministration.⁵⁷

Despite immunogenicity issues, AAV2-based expression of VEGF generates prevention of pulmonary metastases in mice with implanted 4T1 tumors.⁵⁸ In another approach, AAV3 targets hepatoblastoma and hepatocellular carcinoma (HCC) cell lines efficiently by using hepatocyte growth factor receptor (HGFR) as a cellular coreceptor.⁵⁹ Furthermore, AAV vectors have been used in combination therapy of the p53-independent Bcl3-insensitive apoptotic protein and IL24 in HepG2 cells and nude mice *in vivo*.⁶⁰ In another study on HCC, HSV-TK expression driven by the albumin promoter and human alpha-fetoprotein (AFP) enhancer from AAV showed selective killing of AFP-positive HCC cells, but not nonhepatocyte tumor cells or AFP- or albumin-negative hepatic tumor cells.⁶¹ In the context of oral administration, AAV5 and AAV6 serotypes expressing a truncated form of the *Neu* oncogene have shown significantly improved survival and long-lasting protection in 80% of mice implanted with Neu-positive TUBO breast tumors.⁶²

AAV vectors have also been applied in gene silencing. In this context, two unique shRNAs induced apoptotic cell death in androgen receptor-positive prostate cancer cells and suppressed tumor growth after intratumoral injection of mice with implanted xenografts from either androgen-responsive or castration-resistant prostate cancer cells.⁶³ Furthermore, tail-vein injections provided xenograft elimination within 10 days. Engineering of the ubiquitous chicken beta actin (CBA) and neuron-specific enolase (NSE) promoters into an AAV9 vector 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 AAV9-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 syngenic 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 syngenic 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 (scF_v) 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 synergic tumor model and reduced tumor growth in animals with pre-existing tumors.⁷⁷ In another study, self-inactivated lentivirus vectors expressing human IFN β 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 IFN β .

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 GSK3 β 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 osteosarcoma⁹⁴ 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 2×10^5 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

protection against subsequent tumor challenges. VSV vectors have also been tested for replication in cancer cells from ascites.¹¹⁸ Administration of 10^8 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.^{119,120} 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.¹²¹ 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.¹²² Related to breast cancer, MV-CEA vectors provided significant tumor-growth delay and prolonged survival in an MDA-MB231 mammary-tumor model.¹²³ Furthermore, intratumoral MV-CEA administration resulted in delayed tumor growth and extended survival in mice with implanted subcutaneous PC3 prostate tumors.¹²⁴ 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.¹²⁵ 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).¹²⁶ Selective tumor targeting was obtained by replacing the MV hemagglutinin receptor with a CD133-specific scF_v. 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.¹²⁷

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.^{128,129} 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 NDV IL12 and NDV IL15.¹³⁰ 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.¹³¹ Furthermore, a highly virulent NDV strain adapted for replication in HeLa cells promoted upregulation of TRAIL and caspase activation through induction of apoptosis.¹³² 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.¹³³ 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.¹³⁴ 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.¹³⁵ 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,¹³⁶ glioblastomas,¹³⁷ and head and neck cancer,¹³⁸ 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.¹³⁶ Moreover, the lentogenic NDV Ulster strain demonstrated long-term survival of one glioblastoma patient in comparison to none in the control group.¹³⁷ Similarly, the 5-year survival rate was 51% in a Phase II study on head and neck cancer with the NDV-73T strain.¹³⁸

Poxviruses carry a large dsDNA genome with a good packaging capacity of foreign DNA and tumor-selective properties providing cellular destruction by viral replication.¹³⁹ Introduction of deletions in the *TK* and *VGF* genes allow replication in tumor cells, while normal cells are not affected.¹⁴⁰ 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.¹⁴¹ 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.¹⁴² Likewise, human salivary-gland carcinoma cells were susceptible to GLV1h68, and a single intravenous administration resulted in significant tumor regression.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.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.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-damaging therapy.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.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.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.¹⁴⁹ In this context, decreased tumor burden has been obtained in tumor models for melanoma,¹⁵⁰ multiple myeloma,¹⁵¹ breast,¹⁵² prostate,¹⁵³ and lung¹⁵⁴ 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⁺ 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.⁵⁵ Similarly, MV vectors with CD133-specific scF_v showed potent oncolytic activity and prolonged survival in tumor-bearing mice.¹²⁶ 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.⁵⁶ Also, decorin delivery by AAV promoted intratumoral paclitaxel uptake.⁶⁵

Another approach consists of gene silencing, of which examples for antitumor activity have been demonstrated for shRNAs for AAV⁶³ and miRNAs for HSV1⁷⁰ and lentiviruses.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.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.⁶⁴ Selective targeting of tumors has also been achieved by replacing the HSV1 ICP4 promoter with the tumor-specific survivin promoter.⁷⁰ Moreover, retroviruses have been subjected to chimeric antigen-receptor engineering to provide safe treatment of hematological malignancies.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,¹⁰¹ which further demonstrated no evidence of toxicity in macaques and indicated safe intravenous administration in cancer patients.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.¹³¹ Similarly, the NDV Anhinga strain has been subjected to preclinical studies for HCC treatment.^{133,134}

Moreover, engineering of the NDV73T strain resulted in reduced viral replication in avian but not mammalian cells.¹³⁵ Oncolytic poxvirus strains, such as VV GLV1h68, have also been selected, which have shown antitumor activity for both CRC¹⁴² and salivary-gland carcinoma.¹⁴³ Genetic modifications have enhanced cancer specificity for the VV GLV1h151 vector,¹⁴⁴ and when combined with paclitaxel and gemcitabine further increased tumor-cell death.¹⁴⁵

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.¹⁵⁵ Moreover, HSV and VV are capable of selectively provoking damage to the tumor endothelium.^{156,157} 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.^{158,159} Furthermore, oncolytic VV-based targeting of VEGF provided a synergistic antitumor effect with VEGFR tyrosine-kinase inhibitors.¹⁶⁰ This synergism may be caused by off-target inhibition of cellular antiviral defense proteins, such as dsRNA-dependent protein kinases.^{161,162} 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.¹⁶³ 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.¹⁶⁴ In this context, the safety, pharmacokinetics, and anticancer activity of an intravesical oncolytic Ad (CG0070) was evaluated in a Phase I trial in 35 patients with non-muscle-invasive bladder cancer.¹⁶⁵ Patients received intravesically either a single or multiple doses of 10^{12} , 3×10^{12} , 1×10^{13} and 3×10^{13} 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 vector¹⁶⁶ and a replication-competent vector providing double-suicide-gene therapy,¹⁶⁷ which resulted in decreased serum levels of PSA.

NDV has also been subjected to several Phase I–III clinical trials.^{136–138} 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 γ -irradiation.¹³⁷ 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.¹³⁸ The replication-competent NDV PV701 strain was evaluated in a Phase I trial in renal cancer patients.¹⁶⁸ An MTD of 1.2×10^{10} pfu/m² 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.¹⁴⁷ 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.¹⁶⁹ JX594 provided direct oncolysis and overexpression of GM-CSF, thereby stimulating the shutdown of tumor vasculature and antitumor immunoresponses. Fourteen patients with refractory primary or metastatic liver tumors received one of four doses (10^8 , 3×10^8 , 10^9 , or 3×10^9 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 10^9 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.¹⁷⁰ In another approach, a dose-escalating study with intravesical Dryvax VV was conducted in bladder cancer patients.¹⁷¹ 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.¹⁷² 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.^{173,174} In this context, an oncolytic reovirus (pelareorep) was combined with carboplatin and paclitaxel for the treatment of metastatic pancreatic adenocarcinoma.¹⁷⁴ 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.¹⁷⁵ Patients treated with 3×10^{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.¹⁷⁶ 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.¹⁷³

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.¹⁷⁷ 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*)¹⁷⁸ and in adult Phase I trials in progressive and recurrent brain tumors.^{179,180} 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 malignant supratentorial brain tumors.⁴³

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).¹⁸¹ In the dose-escalating study, patients received five doses of 0.9×10^7 or 0.36×10^8 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 immunosignaling 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.⁹⁹ 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.¹⁸² In a Phase I/II trial, patients received multiple intravenous doses of the bioselected formulation Cavatak of CVA21, which were well tolerated.¹⁸² 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 immunosuppression blockade. Additionally, a Phase II clinical trial in advanced-melanoma

patients was conducted with CVA21, showing induced immune-cell infiltration in the tumor microenvironment.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.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)¹⁸⁵ and the Ad H101 vector with an *E1B55K* deletion¹⁸⁶ 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.^{187,188} A number of other drugs based on oncolytic viruses, such as the oncolytic VV JX594 (pexastimogene devacirepvec) for HCC treatment,¹⁸⁹ Ad CG0070 expressing GM-CSF for bladder cancer,¹⁹⁰ and pelareorep (Reolysin) based on a wild-type variant of reovirus for head and neck cancer¹⁹¹ will most likely be approved in the near future. Additionally, the third-generation oncolytic HSV1 vector G47δ, subjected to a Phase II glioblastoma trial, should receive priority reviewing and fast-track drug approval.¹⁹² 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.

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