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Review Article

Viral Vector-Based Cancer Immunotherapy

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Abstract

Cancer immunotherapy has encountered substantial progress during the last decade. Particularly, the development of novel viral and non-viral vectors has contributed to the development of more efficient subunit vaccines. A large number of viral vectors including adenoviruses, adeno-associated viruses, alphaviruses, flaviviruses, herpes simplex viruses, retro- and lentiviruses, measles virus, rhabdoviruses and vaccinia virus have been engineered. Immunization with RNA, especially self-replicative RNA replicons, plasmid DNA and recombinant viral particles and oncolytic viruses have elicited strong antibody responses in different animal models. Virus-based delivery of tumor antigens, immunostimulatory molecules such as cytokines, and antibodies has provided prophylactic protection against tumor challenges in mice and also generated therapeutic efficacy in tumor-bearing animals. Several clinical trials have been conducted and the first herpes simplex virus-based vaccine for treatment of melanoma has been approved. In this review, cancer immunotherapy applications of various viral vectors are discussed.

Keywords: Virus; Viral vector; Cancer; Immunotherapy

Introduction

The history of cancer immunotherapy can be dated back to the discovery by William B Coley in 1891 of tumor shrinkage after injection of bacteria into inoperable bone sarcoma [1]. As cancer cells are recognized by the immune system, enhanced stimulation can be achieved by immunization with Tumor-Associated Antigens (TAAs) and other immunostimulatory molecules. Furthermore, immunization with immunosuppressive cytokines and growth factors may trigger tumor growth inhibition.

Although significant progress has been achieved in cancer therapy, cancer deaths still represent a large fraction of mortality [2]. The importance of the immune system in cancer prevention and therapy has received much attention lately. New insights into the mechanisms of the immune system including antigen presentation by Dendritic Cells (DCs), T-cell activation and macrophage polarization have contributed to the development of innovative therapeutics with the potential of medical breakthroughs [3]. In attempts to target cancer cells several immunotherapeutic approaches have been evaluated including cancer vaccines, oncolytic viruses, immune checkpoint antagonists, stimulatory agonists and different forms of cellular therapies. One fundamental part of immunotherapy is for the immune system to selectively recognize tumor cells from normal cells as lack of antigenicity of cancer cells has been discovered due to mutated antigens or their deficient presentation [4]. Because successful cancer immunization depends on the efficiency of antigen uptake as well as the presentation by DCs to tumor-specific $CD8^+$ and $CD4^+$ T-cells the choice of target antigen is crucial. Moreover, induction of productive inflammation necessary for the promotion of effective immune responses in cancer patients is compromised by deficiencies of the immune system and tumor-derived immunosuppressive or anti-inflammatory signals. Therefore, it is essential to boost the general immune response or to decrease the immune suppression in the tumor environment [5]. Approaches to address these shortcomings have included the administration of cytokines, immune growth factors and adjuvants. However, systemic delivery of cytokines has resulted in adverse events due to prolonged use and it would be advantageous to provide direct intratumoral administration of cytokines or develop strategies for restoring the cytokine imbalance. In this context, viral vectors offer an attractive alternative to achieve local transient expression of antigens and immunostimulatory molecules.

Viral vectors are known for their efficient delivery and high transgene expression *in vitro* and *in vivo* allowing a low-toxicity option to enhance tumor antigenicity, immunogenicity of antigen-specific vaccines and modulation of the tumor microenvironment [6]. A number of different viral vectors have been evaluated in cancer immunotherapy. For instance, adenoviruses, Adeno-Associated Virus (AAV), alphaviruses, flaviviruses, Herpes Simplex Virus (HSV), lentiviruses, measles virus, rhabdoviruses, retroviruses and Vaccinia Virus (VV) have been utilized in various immunization studies in animal models and in a few clinical trials. Viral vectors have been applied in different forms such as replication-deficient and –proficient recombinant particles, oncolytic viruses, RNA replicons and DNA plasmids. In this review, examples are presented of applications of different viral vectors in cancer immunotherapy targeting TAAs, immunomodulating cytokines and combination therapies (Table 1).

Viral vector development

The essential requirements for immunotherapy is to transiently stimulate the host immune response machinery without a lasting elevated presence of cytokines and other immunomodulators as that will lead to serious adverse events. In this context, many viral vectors have proven attractive and due to their different properties related to genome composition, life-cycle and host cell susceptibility, a number of types of expression vectors and packaging systems has been engineered. Each viral vector systems including some applications is

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Table 1: Examples of viral vector-based tumor immunotherapy.

Cancer/Target	Virus	Delivery	Response	Ref
Bile duct				
Ruc-GFP	VV	V	tumor growth suppression	[159]
Bladder				
CD40L	RV	V	antitumor response	[125]
Brain				
p53 + GM-CSF	Adenovirus	VLPs	antitumor activity	[25]
Alphastatin	HIV	VLPs	tumor growth inhibition	[129]
CD46/SLAM/EGFR	MV	VLPs	prolonged survival	[143]
IL-12	SFV	VLPs	prolonged survival	[60]
IL-12	SFV	VLPs + DCs	tumor protection	[62]
Breast				
CD40L	AAV	VLPs	human DC activation	[41,42]
CEA	MV	VLPs	prolonged survival	[142]
Her2/neu	Adenovirus	VLPs	therapeutic effect	[27]
Her2/neu	SIN	DNA	prolonged survival	[72]
MGBA	Adenovirus	VLPs + DCs	antitumor immunity	[28]
neu	AAV	VLPs	prolonged survival	[43]
neu	VEE	VLPs	antitumor response	[73]
VEGF Ab	VV	VLPs	tumor regression	[157]
Cervix				
HPV E6/E7	Adenovirus	VLPs	tumor growth delay	[27]
HPV E6/E7	SFV	VLPs	tumor eradication	[75]
HPV E7	KUN	VLPs	tumor protection	[108]
HPV L1	AAV	VLPs	pseudovirus protection	[38]
Colon				
Anti-DR5	AAV	VLPs	suppression of tumor growth	[39]
GM-CSF	KUN	VLPs	tumor regression	[107]
IL-12	SFV	VLPs	antitumor response	[63]
IL-18	SFV	VLPs	tumor regression	[68]
LacZ	SFV	RNA	tumor protection	[59]
Gastric				
CEA scFV-iNOS	RV	V	tumor growth inhibition	[122]
Leukemia				
IFNβ-NIS	VSV	VLPs	tumor response	[152]
Liver				
Anti-DR5	AAV	VLPs	suppression of tumor growth	[39]
B7.1-Angiostatin	AAV	VLPs	protection in mice	[42]
IL-12	SFV	VLPs	antitumor response	[63,65]
mir30-shRNA	HIV	VLPs	tumor growth inhibition	[132]
Lung				
CD40L	AAV	VLPs	tumor growth inhibition	[40]
HPV E6/E7	SFV	VLPs + Sun + Rad	tumor-free survival	[92]
IL-12	SFV	VLPs	antitumor response	[63]
IL-12	SIN	VLPs	tumor targeting, regression	[66]
Livin shRNA	HIV	VLPs	tumor growth suppression	[133]
miR-145	HSV	V	reduced cell proliferation	[113]

Osteopontin	HIV	VLPs	tumor growth inhibition	[135]
VEGF	AAV	VLPs	inhibition of metastases	[37]
Lymphoma				
LacZ	MV	VLPs	tumor regression	[140]
Melanoma				
GM-CSF	KUN	VLPs	tumor regression	[107]
gp100	VSV	OV + ACT	tumor regression	[153]
HSV-1 d106S	HSV	V + pDCs	cytotoxic effect	[116]
IL-12	SFV	VLPs	antitumor response	[63]
MHC-1	Adenovirus	VLPs	antitumor activity	[26]
Mpt	KUN	VLPs, RNA, DNA	tumor protection	[109]
OVA-Epi	YFV	VLPs	tumor protection	[110]
TRP-2	VEE	VLPs	antitumor response	[70]
VEGF-IL-12 + Sur-hCG	SFV	DNA	tumor growth inhibition	[91]
Ovarian				
CCL19/XCL1	Adenovirus	VLPs	tumor suppression	[16-18]
CEA + NIS	MV	VLPs	therapeutic effect	[141]
GM-CSF	SFV	VLPs	tumor cytotoxicity	[69]
GM-CSF	HSV	V	prolonged survival	[118]
TRAIL	RV	V + cisplatin	antitumor activity	[123]
Pancreatic				
IFNβ	HIV	VLPs	tumor growth inhibition	[130]
Matrix protein	VSV	VLPs	apoptotic response	[151]
Prostate				
4-1BBL	Adenovirus	VLPs + DCs	tumor growth inhibition	[19]
CEA	MV	VLPs	prolonged survival	[139]
FasL	RV	V	cytotoxic effect	[127]
NIS	HSV	OV	tumor eradication	[115]
NIS	VV	V + Rad	prolonged survival	[159]
OVA	AAV	VLPs	tumor growth inhibition	[44]
PSCA	VEE	VLPs	tumor regression	[84,85]
PSCA	HIV	VLPs + DCs	tumor protection	[131]
PSMA	Adenovirus	VLPs + DCs	CTL response in humans	[21]
PSMA	VEE	VLPs	tumor response	[80]
PSMA	VEE	VLPs	neutralizing antibodies	[160]
STEAP	Ad + MVA	VLPs	tumor protection	[22]
STEAP	VEE	VLPs	tumor response	[83]
Renal				
Endostatin	RV	V	tumor growth inhibition	[124]
Retinoblstoma				
IFN-β	AAV	VLPs	antitumor response	[36]
Skin				
HSV-1 R2	HSV	V	antitumor activity	[114]
Thyroid				
GM-CSF/IL-12	RV	V	tumor regression	[126]

AAV: Adeno-Associated Virus; Ad: Adenovirus; DCs: Dendritic Cells; EGFR: Epidermal Growth Factor Receptor; HPV: Human Papilloma Virus; HSV: Herpes Simplex Virus; iNOS: inducible Nitric Oxide Synthase; KUN: Kunjin virus; Mpt: Mouse polytope; MV: Measles Virus; NIS: Sodium Iodide Symporter; OV: Oncolytic Virus; OVA-Epi: Ovalbumin Epitope; pDCs: plasmacytoid Dendritic Cells; RV: Retrovirus; Ruc-GFP: Renilla luciferase-Green Fluorescent Protein; SFV: Semliki Forest Virus; SIN: Sindbis virus; SLAM: Signaling Lymphocytic Activation Molecule; Sun: Sunitib; Sur: Survivin: VEE: Venezuelan Equine Encephalitis virus; VLPs: Virus-Like Particles; YFV: Yellow Fever Virus; V: Virus.

described in more detail with a special emphasis on alphaviruses.

Adenoviruses

The most commonly used viral vectors are based on adenoviruses [7]. Double-stranded DNA (dsDNA) adenoviruses have been frequently used for recombinant protein expression in mammalian cells [8] and for applications in gene therapy [9]. During the years adenovirus vectors have seen a multitude of improvements including engineering of gutless vectors with deletions in the E1-4 genes to reduce the vector toxicity and increase packaging capacity [10]. Moreover, second and third generation adenovirus vectors have demonstrated better biosafety profiles and capsid-modified vectors provide improved vascular gene transfer and reduced pre-existing immunity in humans [11]. Adenoviruses have commonly been subjected to several preclinical and clinical trials [12,13]. Engineering of packaging cell lines for adenovirus have significantly facilitated virus production [14]. Recently, engineering of an HEK293 packaging cell line overexpressing the adenovirus 5 precursor terminal protein resulted in accelerated recombinant adenovirus packaging and amplification [15].

Adenovirus vectors have been subjected to virotherapy in relation to the treatment of ovarian cancer [16]. For instance, recombinant fiber-mutant (integrin-targeting RGD) adenovirus vectors expressing the chemokine CCL19 or XCL1 showed improved transduction of OV-HM ovarian carcinoma cells [17]. Tumor-suppressive activity was discovered for CCL19 in B6C3F1 mice after transduction of OV-HM cells whereas no antitumor effect was seen for XCL1. In another study, adenovirus-based expression of the mouse CC chemokine ILC/CCL27 and CX (3) C chemokine fractalkine CX (3) CL1 was evaluated in immunocompetent mice [18]. Only Ad-RGD-mILC administration managed to suppress tumor growth, which was T-cell dependent and involved both $CD4^+$ and $CD8^+$ T-cells. Adenovirus vectors have also been evaluated in animal models and in early stage clinical trials in patients with hormone-refractory prostate cancer [19]. DCs transduced with adenovirus expressing 4-1BBL increased the percentage of $CD3^+CD56^+$ cells in Cytokine-Induced Killer (CIKs) cells. Moreover, co-culturing of CIKs with adenovirus-transduced DCs enhanced IL-21 and IFN-γ secretion and reduced TGF-β production. The cytotoxicity of CIKs against prostate cancer was also enhanced by Ad-4-1BBL transduced DCs, which provide tumor growth inhibition and prolonged survival. In another study, adenoviruses expressing Prostate Specific Membrane Antigen (PSMA), 4-1BLL and GFP were transfected into DCs derived from peripheral blood cells of healthy volunteers [20]. Mature DCs transfected with Ad-PSMA/4-1BLL showed high levels of IL-12 secretion and induced CTLs to stimulate and enhance the killing effect of PSMA-specific effector cells [21]. Furthermore, strong sustained antigen-specific $CD8^+$ T-cell responses were obtained in C57BL/6 and BALB/c mice after administration of simian adenovirus ChAdOx1 and modified vaccinia Ankara virus MVA encoding the Six Transmembrane Epithelial Antigen of the Prostate 1 (STEAP1) [22]. Disappointingly, the high vaccine immunogenicity only provided low protection against tumor challenges, but a combination with the PD-1 blocking antibody significantly improved survival rates and tumor eradication. Adenovirus vectors have also been subjected to brain tumor models and especially for glioblastoma multiforme [23,24]. Adenovirus vectors expressing human wild-type p53, Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) and B7-1 (BB-102) genes [25] have demonstrated antitumor activity *in vitro*. NOD/SCID mice vaccinated with inactivated U251 glioma cells transduced with Ad-BB-102 were challenged with live U251 glioma cells showed reduced tumor growth. Furthermore, immunized animals showed significantly increased total T-cell numbers, total T-cell proportion, $CD4^+$ and $CD8^+$ T-cell proportion in the spleen. Related to melanoma, oncolytic adenoviruses were applied for tumor-specific Major Histocompatibility Class I (MHC-1) peptide engineering on the viral surface, which demonstrated superior antitumor activity and increased percentage of antitumor $CD8^+$ T-cells and mature epitope-specific DCs [26]. The PeptiCRAd vector loaded with Tyrosinase-Related Protein 2 (TRP-2) and human gp100 substantially reduced the growth of primary-treated tumors and secondary-untreated melanomas. Moreover, PeptiCRAd-GM-CSF targeting the human Melanoma-Associated antigen A1 (MAGE-A1) eradicated established tumors in humanized mice bearing human melanomas. Tumor-Associated Antigens (TAAs) such as Her2/neu and the tumor virus antigen HPV-16 E6/E7 have been evaluated in breast and cervical tumor models, respectively [27]. In this context, immunostimulatory proteins such as recombinant antibodies (αCTLA-4, αCD137, αCD3), cytokines and chemokines (IL-15, LIGHT, mda-7) and co-stimulatory ligands (CD80) have been expressed from adenovirus vectors. However, no significant therapeutic effect was obtained unless systemic T-regulatory T-cell (Treg) co-administration of anti-CD25 mAb in the breast cancer model or low-dose cyclophosphamide in the cervical cancer model took place. In the cervical cancer model induction of a systemic antitumor immune response delayed the growth of distant tumors. Related to breast cancer, the novel breast cancer-associated antigen Mammaglobin A (MGBA) was introduced into a replication-deficient adenovirus vector and DCs isolated from healthy female volunteers were transduced and subjected to stimulation of $CD8^+$ CTLs *in vitro* and co-cultured with cancer cell lines [28]. Infection with Ad-MGBA improved DC maturation and enhanced IL-12 secretion. However, the secretion of IL-10 was down-regulated. The Ad-MGBA-infected DCs induced antitumor immunity against MGBA (+) breast cancers.

Adeno-Associated Virus (AAV)

AAV belongs to the *Parvoviridae* family and possesses a single-stranded DNA (ssDNA) genome with a fairly small packaging capacity of foreign DNA [29]. The vectors are replication-deficient and require an adenovirus helper plasmid for high titer virus production [30]. A modified system to improve the purity of AAV production was established by the application of a mini-Ad helper and completely removed infectious adenovirus particles. Moreover, new helper plasmids expressing the AAV rep and cap genes and the adenovirus E2A, VA1 and E4 genes resulted in 80-fold yields in HEK293 cells and absence of infectious adenovirus [31]. Features such as lack of pathogenicity and toxicity and the ability to transduce both dividing and non-dividing cells have made AAV attractive [32]. AAV vectors have the capability of integrating into the host cell DNA genome, where it remains latent [33]. Isolation of AAV-cellular junction DNA sequences demonstrated a specific integration site in the human chromosome 19, which should favor long-term gene therapy approaches, but not necessarily immunotherapy applications, where short-term high-level expression is desirable. AAV vectors

have demonstrated good safety profiles in clinical trials. However, the immune responses triggered by AAV might be of concern for repeated administration although it can be addressed to some extent by using different AAV serotypes [34,35].

AAV vectors have been applied for a number of cancer therapy studies. For instance, intravitreal administration of an AAV vector carrying the interferon-β (IFN-β) gene generated a potent anti-tumor response in an orthotopic xenograft model of retinoblastoma [36]. Expression of Vascular Endothelial Growth Factor (VEGF) from AAV serotype 2 vectors prevented pulmonary metastases of 4T1 tumors after a single intravenous injection of BALB/c mice [37]. Related to cervical cancer, AAV vectors expressing the Human Papillomavirus (HPV) type 16 L1 protein were subjected to intransal administration in rhesus macaques [38]. After an initial immunization with AAV5-L1 a boost with AAV9-L1 elicited higher antibody titers than a single immunization with AAV5-L1. The generated antibodies were able to neutralize HPV-16 pseudoviruses for at least 7 months. In another study, AAV vectors were applied for the expression of the anti-DR5 (death receptor 5) mouse-human chimeric antibody (Adximab) [39]. Adximab was secreted and efficiently bound DR5 and induced apoptosis in various tumor cells. When nude mice with human liver and colon cancer xenografts were subjected to a single intramuscular injection significant suppression of tumor growth was observed. Additionally, different serotypes of conventional AAV and self-complimentary AAV (scAAV) expressing the CD40 ligand (CD40L) evaluated in A549 lung cancer cells indicated that the transduction efficiency of serotype AAV2/5 was higher than for other tested serotypes [40]. Moreover, the scAAV2/5 was significantly more efficient than the conventional AAVs. *In vivo*, scAAV2/5-CD40L generated significant growth inhibition of transplanted A549 lung tumors in BALB/c nude mice. Similarly, AAV-CD40L has been applied for the generation of CD40L-transgenic breast tumor cells, which specifically activated immature human DCs [41]. Moreover, when MM157, MM231 and MCF7 breast tumor cell lines were transduced with AAV-EGFP variable transduction (14-93%) was observed [42]. Treatment of tumor cells with epirubicin or carboplatin significantly increased the transgene expression. In another study, AAV-based angiostatin therapy was combined with expression of the T-cell co-stimulator B7.1 from AAV vectors [42]. Transduction of EL-4 liver tumor cells resulted in high expression levels of B7.1 on the surface of 80% of cells. Mice vaccinated with AAV-B7.1 transduced tumor cells rejected further challenges with tumor cells and resisted a secondary challenge with unmodified parental cells. However, immunized mice very sensitive to a heavy burden of EL-4 cells, whereas intraportal injection with AAV-angiostatin provided protection of mice previously vaccinated with AAV-B7.1. AAV5 and 6 vectors expressing a truncated neu-oncogene were subjected to a single oral dose in a neu-positive murine TUBO breast cancer model, which significantly improved survival compared to intramuscular administration [43]. Moreover, the survival time was longer for mice immunized with AAV6-neu than AAV5-neu. The tumor protection lasted in 80% of the animals for 320 days. Recently, a novel capsid-optimized AAV6 vector expressing Ovalbumin (OVA) showed increased transduction efficiency in bone marrow-derived DCs [44]. Intramuscular administration of the mutant AAV6-OVA vector elicited a strong specific T-cell activation in peripheral blood compared to the wild type AAV6. Furthermore, subcutaneous tumor growth was suppressed in mice immunized with the mutant AAV6 vector expressing Prostatic Acid Phosphatase (PAP).

Alphaviruses

Alphaviruses are single-stranded RNA (ssRNA) viruses with a positive polarity belonging to the *Togaviridae* family [45]. Although generally considered as mild pathogens, some epidemics have recently been caused by alphaviruses, particularly Chikungunya virus (CHIK) [46,47]. However, alphavirus vectors utilized for cancer immunotherapy have been based on virulent strains. Mainly three alphaviruses, Semliki Forest Virus (SFV) [48], Sindbis virus (SIN) [49] and Venezuelan Equine Encephalitis Virus (VEE) [50] have been engineered as vectors for recombinant protein expression. A number of topologically different recombinant proteins have been expressed at high levels, particularly from SFV vectors [48,51]. The packaging capacity of alphavirus vectors is reasonably good, in the range of 8-10 kb, but engineering of an SFV vector with a mutated capsid protein generates virus particles of 210 nm accommodating up to 18 kb of foreign RNA in comparison to the wild type size of 60 nm [52]. In contrast to many other virus-based expression systems, alphaviruses can provide delivery of recombinant particles, RNA replicons and layered DNA/RNA plasmid vectors [53,54]. Especially, application of recombinant particles generated with second generation helper vectors [55] or split helper systems [56,57] and RNA replicons provide high biosafety standards as no integration into the host genome will occur. Moreover, the replication-deficient particles disintegrate and the viral RNA is degraded within 5-7 days *in vivo* [58].

Alphavirus vectors have been subjected to numerous preclinical studies related to cancer immunotherapy [53]. For instance, administration of SFV RNA replicons expressing the LacZ reporter gene provided mice protection against tumor challenges after a single intramuscular injection with 1 μg of RNA [59]. Moreover, alphavirus vectors have been applied for expression of cytokines in various cancer therapy studies. In this context, mice bearing 203-gliomas were administered SFV-LacZ, retrovirus DFG-IL12 and SFV-IL-12 particles, which due to the SFV induced apoptotic death of glioma cells prolonged survival of those animals receiving SFV-IL-12 [60]. In another study, DCs isolated from the bone marrow were infected with SFV-B16 and SFV-203 particles were administered to mice bearing 203 tumor xenografts [61]. SFV immunizations induced apoptosis in DCs, which facilitated the uptake of apoptotic cells by other DCs, provided protection against tumor challenges and prolonged survival of mice with established tumors. Similarly, DCs isolated from bone marrow were pulsed with SFV-LacZ and SFV-IL-12 and subjected to treatment of mice with B16 brain tumors [62]. The outcome was prolonged survival of animals with established tumors. Moreover, SFV-IL-12 particles have shown induced therapeutic antitumor responses in mouse lung and colon cancer, woodchuck hepatocellular carcinoma, melanoma [63] and mastocytoma [64]. In another approach SFV-IL-12 particle administration was compared to liver-specific inducible IL-12 expression from plasmid DNA (pTonL2(T)-mIL12) [65]. Growth arrest was detected in most tumors after SFV-IL-12 immunization resulting in 100% survival rate, which was not the case for the plasmid DNA-based treatment. Other IFNγ inducing cytokines such as IL-15 and IL-18 have been evaluated for potential antitumor activity in colon and ovarian cancer models [66-68]. For instance, when the SFV10-E vector providing 10-fold higher

expression levels compared to the conventional SFV vector was used for overexpression of IL-18 therapeutic efficacy including tumor eradication was obtained in BALB/c mice with subcutaneous K-BALB and CT26 tumors [68]. Another immunostimulatory cytokine, the GM-CSF has been expressed from SFV and evaluated in a mouse ovarian tumor model [69]. Intraperitoneal injection increased the number of macrophages and neutrophils, but the observed tumor growth inhibition was modest with no benefit related to survival.

Several TAAs have been evaluated in alphavirus-based vaccine studies. For instance, VEE particles have been used for the expression of the melanoma antigens tyrosinase related protein (TRP-1, TRP-2), gp100 and Tyrosinase (Tyr) in tumor models [70,71]. VEE-TRP-2 presented a durable antitumor effect in a B16 melanoma model. Compared to co-immunization with VEE-gp100 and VEE-Tyr, vaccination with VEE-TYR-2 alone was significantly more efficient [70]. Comparative immunization studies with plasmid DNA and VEE particles expressing Tyr in mice demonstrated that even if T-cell responses were detected from both vaccination strategies, only VEE particles managed to significantly delay tumor growth [71]. Related to breast cancer, both SIN DNA plasmids and VEE particles have been subjected to immunization studies for the tyrosine kinase receptor Her2/neu [72,73]. Mice were injected with the breast cancer cell line A2L2 either into the mammary fat pad as a model of solid tumor growth or intravenously as a model of lung metastasis and immunized with SIN plasmid DNA or an adenovirus vector expressing the Her2/neu gene for prophylactic and therapeutic aims [72]. Significant tumor growth inhibition was observed when the immunization took place before the tumor challenge with A2L2 cells. However, no protection was obtained when the vaccination took place two days after the tumor challenge. On the other hand, a therapeutic prime-boost protocol for the SIN-neu DNA plasmid followed by Ad-neu injection significantly prolonged the overall survival of mice injected intravenously with A2L2 tumor cells. Another approach involved VEE-neu particles administered to a rat mammary tumor model [73]. Effective antitumor immunity was obtained and cure was achieved in 36% of rats with pre-existing tumors. Furthermore, DCs transduced with VEE-neu particles provided high level of transgene expression, DC maturation and secretion of pro-inflammatory cytokines [74]. Robust neu-specific $CD8^+$ T-cell and anti-neu IgG responses were observed after immunization with VEE-infected DCs expressing a truncated form of neu. A single vaccination was sufficient to induce the regression of large established tumors.

Examples of cervical cancer immunotherapy with alphavirus vectors include immunization with SFV vectors expressing HPV type 16 E6/E7 proteins [75,76]. Vaccination with SFV vectors carrying the E6 and E7 induced a strong HPV-specific CTL response and eradicated HPV-transformed tumors. Compared to an adenovirus vector expressing the E6/7 fusion protein the SFV-based immunization provided a stronger prime-boost CTL response and better therapeutic efficacy [77]. Tattoo injection (intradermal) of SFV-E6/7 particles showed similar or higher levels of immune response compared to intramuscular administration [78]. Related to cancer immunotherapy for prostate cancer, specific antigens such as the PSMA, the STEAP and the PSCA have been targeted [79]. Subcutaneous administration of VEE-PSMA particles showed strong cellular and humoral immune responses in mice [80]. As no relevant animal tumor models exist for PSMA, the efficacy of VEE-PSMA immunizations was evaluated in a clinical trial [81]. Recently, VEE particles derived from the TC-83 replicon were utilized for the expression of PSA for immunization in HLA-DR transgenic mice to evaluate cellular and humoral immune responses [82]. The outcome was strong PSA-specific $CD8^+$ T-cell and Th1-type antibody responses and eradication of PSA-expressing tumor cells. Moreover, in the prostate cancer DR2bxPSA F1 mouse model a significant delay in tumor growth was observed. The predominantly prostate-specific STEAP antigen [83] expressed from VEE vectors was assessed for both prophylactic and therapeutic efficacy. A significantly prolonged overall survival was observed in TRAMPC-2 prostate tumor-bearing mice pre-immunized with VEE-STEAP particles. In the context of therapeutic efficacy, VEE-STEAP particles were co-administered with STEAP expressed from plasmid DNA, which resulted in a short but statistically significant delay in tumor growth. As PSCA is up-regulated in localized and metastatic tumors, it represented a better target for therapy. Therefore, prophylactic immunization with plasmid PSCA-cDNA followed by VEE-PSCA administration resulted in s specific immune response and tumor challenge protection in 90% of vaccinated TRAMP mice [84,85].

Various combination therapies involving TAAs, cytokines and drugs have proven successful. For instance, the efficacy of SFV-based HPV vaccines was improved by co-administration of SFV-IL-12 particles [86]. IL-12 stimulated antigen-specific CTL responses and enhanced antitumor activity of SFV-HPV16-E6/7 immunized mice at low doses. However, the increase in dose did neither improve immune responses nor tumor regression. Another example is the combination therapy of SFV-IL-12 particles with anti-CD137 mAbs [87]. Intratumoral co-administration of viral particles and mAbs inhibited tumor growth in a syngeneic TC-1 lung carcinoma model. In another approach, DCs transduced with SFV-IL-12 particles were combined with systemic administration of IL-18, which resulted in prolonged survival of B16 tumor-bearing mice [88]. Specific antitumor CTL responses were induced and protection against challenges with cancer cells was obtained after co-expression of the human melanoma-associated antigen gp100 and IL-18 from SIN DNA vectors in mice with established B16-hgp100 tumors [89]. Alphaviruses have also been combined with cancer drugs. A significant reduction in tumor growth was detected when doxorubicin or paclitaxel was administered prior to immunization with SIN DNA plasmids expressing neu [90]. In contrast, alphavirus or doxorubicin administration alone did not have the favorable effect. Additionally, SFV DNA vectors expressing VEGFR2 domains and IL-12 co-immunized with another SFV DNA plasmid expressing survivin and β-hCG antigens elicited strong humoral and cellular immune responses against survivin, β-hCG and VEGFR-2 in immunized mice [91]. Compared to single immunizations with either of the SFV DNA plasmids, the co-administration inhibited tumor growth and prolonged survival in a B16 melanoma mouse model. Similarly, immunization with SFV HPV E6/7 combined with sunitib and a single low-dose irradiation enhanced the intratumoral ratio of antitumor effector cells to myeloid-derived suppressor cells [92]. Moreover, enhanced antitumor efficacy and 100% tumor-free survival was achieved after triple treatment of tumor-bearing mice.

Flaviviruses

Flaviviruses are positive-sense ssRNA viruses of which several such as Dengue virus and most recently Zika virus have demonstrated pathogenicity causing encephalitis [93] and microcephaly [94]. Several flaviviruses such as Kunjin Virus (KUN) [95], West Nile virus [96,97], Yellow fever virus [98,99], Dengue virus [100,101] and Tick-borne encephalitis virus [102,103] have been engineered for the development of vectors for DNA, RNA and recombinant particle delivery. The most frequently used KUN vectors have the flanking regions of the first 20 codons of the Core protein (C20) and the last 22 codons of the Envelope protein (E22) for the insertion of heterologous genes in frame with the rest of the KUN polyprotein [104]. Expression initially generates fusion proteins with the KUN polyprotein, which then are processed and also cleaved off from KUN sequences through the introduction of flanking FMDV-2A protease sequences [105]. KUN particle production has been facilitated by the engineering of the tetracycline-inducible BHK packaging cell line for transfection of replicon RNA [106].

KUN vectors have been subjected to cancer immunotherapy. For instance, non-cytopathic KUN RNA replicon vectors were applied for GM-CSF expression by intratumoral injections into mice bearing subcutaneous CT26 colon carcinoma and B16-OVA melanomas [107]. More than 50% of established CT26 and B16-OVA tumors were cured and the regression of CT26 tumors correlated with the induction of anti-cancer $CD8^+$ T-cells. Regression of CT26 lung metastases was also observed. Due to the difficulty of curing these aggressive tumors, the KUN vector approach looks promising. KUN vectors have furthermore been subjected to immunization studies for cervical cancer [108]. Introduction of a CTL epitope of the HPV16 E7 protein into a poly-epitope induced E7-directed T-cell responses and provided protection against challenges with an E7-expressing epithelial tumor in mice. Comparison studies indicated that KUN particles were more effective than naked replicon RNA or plasmid DNA. In another study, a model immunogen was delivered from KUN vectors as naked RNA, DNA plasmids and as recombinant particles [109]. A single vaccination with any of the KUN vectors was able to induce similar $CD8^+$ T-cell responses as obtained for VV. In comparison to conventional DNA vaccines, which required 100 μg DNA, only 0.1 μg of KUN DNA was needed to elicit $CD8^+$ T-cell responses. Immunization with naked replicon RNA provided protection against challenges with B16 tumor cells in mice.

Yellow fever virus vectors have also been evaluated for the expression of a cytotoxic T-lymphocyte epitope (SIINFEKL) derived from chicken ovalbumin [110]. Mice inoculated with recombinant yellow fever virus particles elicited SIINFEKL-specific $CD8^+$ lymphocytes and provided protection against challenges with lethal doses of malignant melanoma cells expressing ovalbumin.

Herpes simplex virus

The genome of Herpes Simplex Virus (HSV) is linear double-stranded DNA (dsDNA) containing approximately 80 genes [111]. HSV has received reasonable attention and has been considered as an attractive alternative for gene therapy applications due to the large packaging capacity of foreign DNA, attenuated oncolytic activity and establishment of life-long latent infection in neurons [112].

The neurovirulent HSV-1 RH2 vector, which has demonstrated lytic capability in Squamous Cell Carcinoma (SCC) cells, was examined in a syngeneic C3H mouse model [113]. Evaluation studies in the bilateral SCCVII tumor model demonstrated antitumor activity and suppression of tumor growth. Furthermore, the growth of contralateral tumors was also significantly inhibited. In another study, four copies of miR-145 target sequences were introduced into the 3'-untranslated region of ICP27, an essential HSV-1 gene for evaluation of target specificity and toxicity in normal and lung cancer cells *in vitro* [114]. The miR-145 expression was higher in normal cells than in Non-Small Cell Lung Cancer (NSCLC) cells resulting in selectively reduced cell proliferation and prevention of colony formation in NSCLC cells. Combination of HSV-based therapy with radiotherapy resulted in significantly increased killing of cancer cells. An oncolytic HSV vector containing the human Sodium Iodide Symporter (NIS) was engineered to facilitate non-invasive monitoring *in vivo* of the spread of HSV and to improve antitumor activity [115]. Human LNCaP prostate cancer cells infected with oHSV-NIS efficiently concentrated radioactive iodine *in vitro* and *in vivo*, which led to efficient eradication of LNCaP xenografts in nude mice. Systemic administration of oHSV-NIS provided prolonged survival and the therapeutic effect could be further enhanced by ^{131}iodine administration. In another approach the replication-deficient HSV-1 d106S strain was studied in tumoricidal plasmacytoid DCs (pDCs) co-cultured with 11 melanoma cell lines [116]. A strong cytotoxic effect, similar to the one induced by natural killer cells, was observed. Apoptotic and necrotic cell death was induced in most melanoma cell lines. Furthermore, similar responses were observed in three leukemia cell lines, indicating that the antitumor response was of a general nature.

The oncolytic HSV-2 was demonstrated to attract active migration of adoptively transferred T-cells after intratumoral administration [117]. Moreover, T-cells persisted significantly longer and generated significantly higher levels of CXCL9, CXCL10 and other chemokines. In combination with adoptive T-cell therapy, the HSV-based approach could be potentially attractive for treating solid tumors. In relation to ovarian cancer, HSV amplicons expressing GM-CSF and HF10, a highly attenuated HSV-1 strain functioning as a helper virus, prolonged survival and decreased intraperitoneal dissemination [118]. However, a stronger effect was observed for the HSV-mGM-CSF amplicon. Increased $CD4^+$ T-cell concentrations were detected in the spleen. Again stronger immune responses were seen after mGM-CSF amplicon administration in comparison to HF10 in the spleen.

Retroviruses

Retroviruses are double-stranded RNA (dsRNA) viruses, which have been frequently used for gene therapy applications and have provided the first success in treatment of Severe Combined Immune Disease (SCID) in children [119]. However, random integration of therapeutic genes into oncogene regions caused leukemia in retrovirus-treated patients [120,121]. Engineering of recombinant bi-functional retrovirus Vectors, which displayed an scFV antibody to CEA and expressed the inducible Nitric Oxide Synthase (iNOS) gene, addressed the problem and resulted in a significant inhibition of MKN-45 tumor growth [122]. In another approach, retrovirus vectors expressing the TNF-Related Apoptosis Inducing Ligand (TRAIL) gene efficiently transduced drug-resistant A2780/DDP ovarian

carcinoma cells *in vitro* [123]. Exposure of retrovirus-transduced cells to cisplatin enhanced the antitumor activity *in vitro* and in nude mice with A2780/DPP tumors. In the context of renal cancer, retroviral vectors carrying the Endostatin (ES) gene were subjected to transduction of NIH/3T3 cells [124]. Significant tumor growth inhibition was observed in SCID mice bearing CaKi-1 derived tumors after subcutaneous injection of ES-transduced cells. Furthermore, the microvascular density was significantly reduced and the intratumoral necrotic area showed a 23-fold increase. Retrovirus vectors expressing CD40L were applied for the transduction of the MBT2 mouse bladder cell line [125]. Mouse bone marrow-derived DCs co-cultured with the retrovirus transduced cells (MBT2-CD4) produced 8-fold increase in IL-12 levels. Vaccination with MBT2-CD40L cells induced antitumor responses against parental tumors, but the immunization was not sufficient to induce tumor regression of existing tumors. In a comparison between retrovirus- and adenovirus-based vectors, the delivery of IL-12 and/or GM-CSF was evaluated in the FRTL-Tc rat model for thyroid cancer [126]. Subcutaneous administration of FRTL-Tc cells transduced with IL-12 or GN-CSF expressing retrovirus resulted in significantly smaller tumors, but showed little effect on distant tumors. Similar results were obtained for adenovirus delivery indicating that neither vector provided any systemic vaccine effect. An oncoretroviral vector for the overexpression of the antitumorogenic T-cell FasL or the non-cleavable ncFasL was engineered [127]. Co-stimulation of T-cells expressing FasL, ncFasL and ncFasL/c-FLIP all mediated cytotoxicity in RM-1, LNCaP and TRAMP-C1 prostate cancer cell lines. Furthermore, comparison of oncoretrovirus delivery to radiation, mixantrone or docetaxel therapy showed that Fas and H-2(b) expression was up-regulated after each treatment. A retrovirus-based transduction method has been developed for the robust expansion of genetically modified Natural Killer (NK) cells [128]. This resulted in strong activation of NK cells by the combination of IL-15 and K-562 feeder cells.

Lentiviruses

Although lentiviruses belong to the family of retroviruses they possess a distinguished feature being capable of transducing both dividing and non-dividing cells. This has favored the utilization of lentiviruses instead of classic retroviruses. However, a drawback is that most lentivirus expression systems are based on HIV, which obviously has required substantial engineering to ensure proper biosafety levels.

Lentivirus vectors have been subjected to several applications in cancer therapy. In this context, a lentivirus vector expressing alphastatin, a 24 amino acid fragment of human fibrinogen, stably infected HUVEC cells and sustained alphastatin secretion, which contributed to the inhibitory effect of HUVEC cell migration and differentiation [129]. Expression of alphastatin decreased tumor vascularization substantially and also inhibited tumor growth. Furthermore, alphastatin showed inhibition of the initial stages of angiogenesis by inducing VEGF and bFGF. A self-inactivated lentivirus vector has been utilized for high efficiency transduction of pancreatic cancer cell lines [130]. Lentivirus-based expression of hIFNβ in pancreatic cell lines demonstrated inhibition of cell proliferation and induced cell death by apoptosis. In mice, HIV-1-hIFNβ administration prevented pancreatic cancer progression for 15 days and induced tumor regression in 50% of the animals. In another study, lentivirus vectors expressing PSCA (DCLV) were transduced into DCs for immunization of C57BL/6 mice in a prostate cancer model [131]. DCLV-PSCA preferentially delivered the PSCA antigen gene to DC-SIGN-expressing 293T cells and bone marrow-derived DCs (BMDCs). Immunization studies in C57BL/6 mice generated robust PSCA-specific $CD8^+$ and $CD4^+$ T-cell responses. Furthermore, immunization with DCLV-PSCA provided protection against tumor challenges in the TRAMP-C1 synergetic tumor model. The vaccination was also able to inhibit growth of tumor metastases in a PSCA-expressing B16-F10 model.

In another approach, the anti-alpha fetoprotein scFV-directed lentivirus vector has been applied for transduction of the Wtp53-pPRIME-mir30-shRNA gene into liver cancer cells [132]. Lentivirus transduction demonstrated efficient inhibition of the proliferation of Hep3B cells. Immunization of BALB/c nude mice bearing subcutaneous human hepatocellular carcinoma (HCC) showed a clear impact on tumor growth, apoptosis and micro-vessel formation. Another example of lentivirus-based delivery of shRNA comprises the silencing of livin, a member of the family of inhibitors of apoptosis protein (IAP) [133]. Livin silencing has previously been shown to promote apoptosis in lung cancer cells [134]. BALB/c mice with xenografts derived from the SPC-A-1 lung adenocarcinoma cell line were immunized with a lentivirus-based livin shRNA vector, which resulted in down-regulation of livin expression, induction of tumor cell apoptosis, reduction in tumor cell proliferation and suppression of tumor growth. Furthermore, livin silencing induced a G0/G1-phase cell cycle arrest and cyclin D1 down-regulation, which are the key regulators of the G0/G1- to S-phase transition. Interestingly, lentiviruses expressing a triple mutant of osteopontin (OPN) have been subjected to oral delivery using a nose-only-inhalation chamber in a K-ras (LA1) lung cancer model [135]. Aerosol-delivered lenti-OPN TM showed inhibition of lung tumorigenesis, inhibition of OPN-mediated Akt signaling pathway and enhanced apoptosis in the lungs.

Measles virus

Measles viruses (MV) possess an enveloped negative-sense ssRNA virus belonging to the *Paramyxoviridae* family residing in humans with no known animal reservoirs [136]. Expression vectors have been engineered for MV including replicating MV, which can be rescued from cloned DNA constructs [137]. Application of reverse genetics has enabled the rescue of recombinant MV in an HEK293 helper cell line [138]. In this case, the genes of interest have been introduced between the Phosphoprotein (P) and the Matrix protein (M) or between the Hemagglutinin (HA) and the large protein (L), respectively, in the measles virus genome. The HEK 293 helper cell line is transfected with recombinant MV constructs and a plasmid expressing the MV polymerase L gene prior to transfer of syncytia to Vero cell cultures after three days. Finally, MV particles are harvested at 80-90% cytopathicity.

The attenuated oncolytic Edmonston-B (MV-Ed) strain of MV has been subjected to several cancer vaccine studies [139]. The MV-Ed strain can selectively infect and replicate in tumor cells. Intratumoral injection of MV-Ed virus containing the LacZ gene (MVLacZ) induced regression of large established human lymphoma xenografts in SCID mice [140]. Intravenous immunization with

MVLacZ also resulted in significant decrease in tumor progression and it was further confirmed that MV replication occurs within tumors. In another approach, immunization of mice with SKOV3ip.1 ovarian xenografts with two MV vectors expressing CEA and NIS demonstrated therapeutic efficacy [141]. The combination therapy was superior to immunization with MV-CEA or MV-NIS alone. In another study, MV-CEA delivery demonstrated a substantial delay in tumor growth and provided prolonged survival of mice bearing MDA-MB-231 mammary tumors [142]. Similarly, intratumoral injection of MV-CEA resulted in delay of tumor growth and superior survival in a subcutaneous PC-3 prostate xenograft model [139]. In attempts to improve delivery and efficacy, the CD46 and the signaling lymphocytic activation molecule (SLAM) sequences in the hemagglutinin protein combined with a single-chain antibody against the Epidermal Growth Factor Receptor (EGFR) were introduced into the MV vector, which significantly increased tumor regression and prolonged survival in EGFR-expressing gliomas [143].

Rhabdoviruses

The *Rhabdoviridae* family comprises enveloped, bullet-shaped viruses with a ssRNA genome of negative polarity [144]. Vectors for the expression of heterologous proteins have been engineered for both rabies virus (RABV) [145] and Vesicular Stomatitis Virus (VSV) [146,147]. Based on recombinant VV, a reverse genetics approach, where insertion of the VSV N, P and L genes downstream of a T7 promoter and an Internal Ribosome Entry Site (IRES), has been established for the recovery of VSV particles [147].

A VV-free system has been established for RABV [145]. In an interesting approach, chimeric Virus-Like Particles (VLPs) were generated by grafting VSV glycoprotein (VSV-G) on the surface of SFV pseudo particles to provide high safety standards as VSV-G does not share any homology with the SFV genome [148]. It was achieved by expressing the VSV-G protein *in trans* from a mutated SFV 26S promoter.

As VSV vectors have demonstrated no pre-existing immunity in humans, they have become attractive for cancer therapy applications [149]. In this context, an oncolytic VSV demonstrated superiority in comparison to conditionally replicative adenovirus, Sendai virus and RSV vectors in pancreatic cancer cell lines [150]. Among the thirteen aggressive Pancreatic Ductal Adenocarcinoma (PDAC) cell lines tested VSV managed to kill those that were resistant to other viruses. However, some PDAC cell lines were resistant although low levels of very early VSV RNA synthesis occurred. In a related study, 10 human PDAC cell lines were transduced with VSV vectors expressing the wild type matrix protein or the ΔM51 mutant [151]. VSV vectors showed activation of both extrinsic and intrinsic apoptosis pathways and VSV-ΔM51 primarily targeted the type II extrinsic pathway. Robust apoptotic responses were detected in cell lines with defective IFN signaling. However, resistance to apoptosis was discovered in cells lines constitutively expressing high levels of IFN-Stimulated Genes (ISGs). Furthermore, syngeneic Acute Myeloid Leukemia (AML) tumors responded to intravenous therapy with VSV vectors expressing IFNβ and NIS in a dose-dependent manner [152]. Oncolytic virotherapy applying VSV vectors expressing the melanocyte antigen glycoprotein 100 (gp100) has been combined with Adoptive Cell Transfer (ACT) in a mouse melanoma model [153]. Tumor regression and antitumor immunity was observed and the tumor response was associated with *in vivo* T-cell persistence.

Vaccinia viruses

VV is a large enveloped virus with a 190 kb dsDNA genome belonging to the family of poxviruses [154]. Originally, VV expression vectors were engineered with a bacteriophage T7 RNA polymerase promoter and the LacZ and chloramphenicol acetyltransferase genes inserted for recombinant protein expression [155]. VV vectors comprise features such as packaging of more than 20 kb foreign inserts, a wide host range and high expression levels of heterologous proteins.

Oncolytic VVs have been subjected to pre-clinical studies in feline mammary carcinoma [156]. Systemic administration of tumor-bearing mice with VV expressing the VEGF single chain antibody GLAF-2 resulted in a significant inhibition of tumor growth. Furthermore, treatment with VV caused a drastic reduction in intratumoral VEGF levels and inhibition of angiogenesis. Engineering of a melanogenic VV-based system provides the diagnostic potential of Magnetic Resonance (MRI) and opto-acoustic deep tissue imaging. However, melanin overexpression leads to attenuated virus replication. Therefore, a novel recombinant VV strain (rVACV) expressing the key enzyme of melanogenesis (tyrosinase) under the control of an inducible promoter was engineered, which generated melanin after addition of doxycycline in two tumor xenograft mouse models [157]. This approach facilitated MRI signal opto-acoustic tomography enhancement as well as the oncolytic potential. The therapeutic potential of VV vectors expressing NIS was evaluated in combination with radiotherapy in prostate cancer models [158]. *In vitro*, NIS expression, cellular radio iodide uptake and apoptotic cell death was analyzed in PC3, DU145, LNCaP and WPMY-1 human prostate cells lines indicating that the apoptotic cell killing was dose- and time-dependent. NIS gene expression was functional and mediated radio iodide uptake. *In vivo*, addition of radio iodide to VV-NIS infected tumors resulted in superior tumor growth restriction and prolonged survival compared to single agent therapy in the TRAMP mouse model. Oncolytic VV (VACV GLV-1h68) vectors have also been subjected to studies in human Cholangiocarcinoma (CC) cell lines (KMC-1, KMBC and KMCH-1) and in subcutaneous flank xenografts in nude mice [159]. VACV GLV-1h68 efficiently infected all tested CC cell lines and showed significant suppression of tumor growth *in vivo*.

Clinical trials

Viral vector-based immunization also involves a number of clinical trials and only some cases will be discussed here. In this context, recombinant VEE-based replicon particles expressing PSMA were subjected to a phase I dose-escalation clinical trial in patients with castration resistant metastatic prostate cancer [160]. The patients were treated with up to five doses of either 0.9×10^7 IU or 10^8 IU. No toxicity was associated with the vaccinations, but no PSMA-specific cellular immune responses were detected and only weak signals were observed by ELISA for the lower dose. Similar results were obtained for the higher dose. Although neither clinical benefit nor robust immune signals were achieved, neutralizing antibodies were produced in both cohorts indicating that dose optimization is necessary.

A phase I-II clinical trial was conducted with an adenovirus vector expressing CEA (Ad5-[E1-, E2b-]-CEA (6D) in metastatic colorectal cancer (mCRC) [161]. The immunization elicited cytotoxic T-cell responses and activated $CD4^+$ and $CD8^+$ T-cells. The overall survival was prolonged by 20% (median survival 11 months) during long-term follow-up with no reported adverse events.

Patients with taxol and platinum-refractory recurrent ovarian cancer and normal CEA levels have been subjected to intraperitoneal immunizations with MV-CEA in a phase I clinical trial [162]. No dose-limiting toxicity was detected in 21 patients receiving doses of 10^3-$10^9 TCID_{50}$ every four weeks for up to six cycles. A dose-dependent increase in CEA levels in the peritoneal fluid was observed. Tumor biopsies showed overexpression of the measles receptor CD46 in 13 out 15 patients. Dose-dependent disease stabilization was achieved in 14 out of 21 patients with a median duration of 92.5 days (range 54-277 days). The median survival of patients was 12.15 months (range, 1.3-38.4 months), which compared favorably to an expected median survival of 6 months in this patient population.

The modified VV Ankara TG4010 expressing mucin-1 (MUC-1) and IL-2 was utilized for immunizations in a phase IIb/III randomized, double-blind, placebo-controlled clinical trial in patients with stage IV Non-Small Cell Lung Cancer (NSCLC) without a known activating EGFR mutation and with MUC1 expression in at least 50% of tumor cells [163]. The patients received subcutaneous administration of 10^8 PFU of TG4010 or placebo weekly for six weeks and then every 3 weeks up to progression. In the TG4010 group (111 patients) the median progression-free survival was 5.9 months (95% CI 5.4-6.7) and in the placebo group (111 patients) 5.1 months (95% CI 4.2-5.9). No serious adverse event was related to the TG4010 treatment. The TG4010 immunization in combination with first-line chemotherapy seemed to improve progression-free survival in comparison to placebo plus chemotherapy.

Finally, replication-competent HSV vectors have been applied in phase I-III human clinical trials in glioblastoma and melanoma patients, where GM-CSF treatment displayed efficacy [164]. It was recently approved by the FDA for use in standard patient care [165]. Also replication-deficient HSV vectors have been subjected to phase I-II clinical trials for disorders such as pain, neuropathy and neurodegenerative conditions [166].

Conclusion

In summary, a large variety of viral vectors have been employed for immunization studies in animal tumor models. In most cases, strong humoral and cellular immune responses have been detected in vaccination approaches against TAAs, immunostimulatory and antibodies. Moreover, combination of viral vector immunizations with drug molecules and irradiation has proven successful. Several studies have demonstrated protection of immunized animals against challenges with appropriate tumor cells. The repertoire of disease indications is also wide covering more or less all types of cancers (Table 1). It is hard to pick a certain viral vector system in place of another. However, the RNA viruses possessing self-replicative RNA immunostimulatory cytokines and DNA vaccinations with SIN vectors have indicated that 100- to 1000-fold lower doses are required in comparison to immunizations with conventional plasmid DNA [167].

Viral vector-based clinical trials conducted for different cancer indications have generated some positive results. Although the immune responses have been relatively modest in some cases, the efficacy can most likely be improved by vector engineering and dose optimization. The good news is, however, that the first HSV-based cancer immunotherapy was approved by the FDA in October 2015, which encourages further efforts in the vaccine field.

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