Alphaviruses in Immunotherapy and Anticancer Therapy

Subjects: Virology

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Alphaviruses have been engineered as expression vectors for vaccine development and gene therapy. Due to the feature of RNA self-replication, alphaviruses can provide exceptional direct cytoplasmic expression of transgenes based on the delivery of recombinant particles, naked or nanoparticle-encapsulated RNA or plasmid-based DNA replicons.

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1. Introduction

During the last decade, immunotherapy has become an attractive alternative for cancer therapy [1]. In this context, viral vectors have also proven useful for immunotherapeutic applications [2]. Alphaviruses have frequently been engineered for the overexpression of suitable antigens and immunostimulatory genes for vaccine development and cancer therapy [3]. Additionally, the expression of cytotoxic and antitumor genes has been used for cancer therapy applications. Semliki Forest virus (SFV) [4], Sindbis virus (SIN) [5] and Venezuelan equine encephalitis virus (VEE) [6] are most commonly used for the engineering of expression systems. Additionally, the naturally occurring oncolytic alphavirus M1 [7] and engineered oncolytic versions based on SFV and SIN vectors [8] have been utilized for cancer therapy. The evaluation of efficacy in appropriate animal models has provided proof of concept before conducting clinical trials.

2. Alphavirus Lifecycle and Expression Vector Systems

Alphaviruses possess an enveloped structure of capsid and spike proteins encapsulating a single-stranded RNA (ssRNA) genome of positive polarity [9]. Upon the infection of host cells, the alphavirus ssRNA is released into the cytoplasm, where translation can immediately occur requiring no delivery of RNA to the nucleus as is the case for other RNA viruses such as the influenza virus and DNA viruses (**Figure 1**). In the cytoplasm, efficient self-replication occurs through a minus-strand RNA template leading to the accumulation of approximately 10^6 copies of subgenomic RNA per cell. Together with the utilization of the highly efficient 26S subgenomic promoter, high-level expression of viral proteins occurs [10]. The RNA self-replication and high-level expression of alphavirus structural proteins generate high-titer virus progeny. Nucleocapsids comprising the capsid protein harboring full-length alphavirus RNA are transported to the cell surface, where the envelope proteins are attached, and mature viral particles are released by budding.

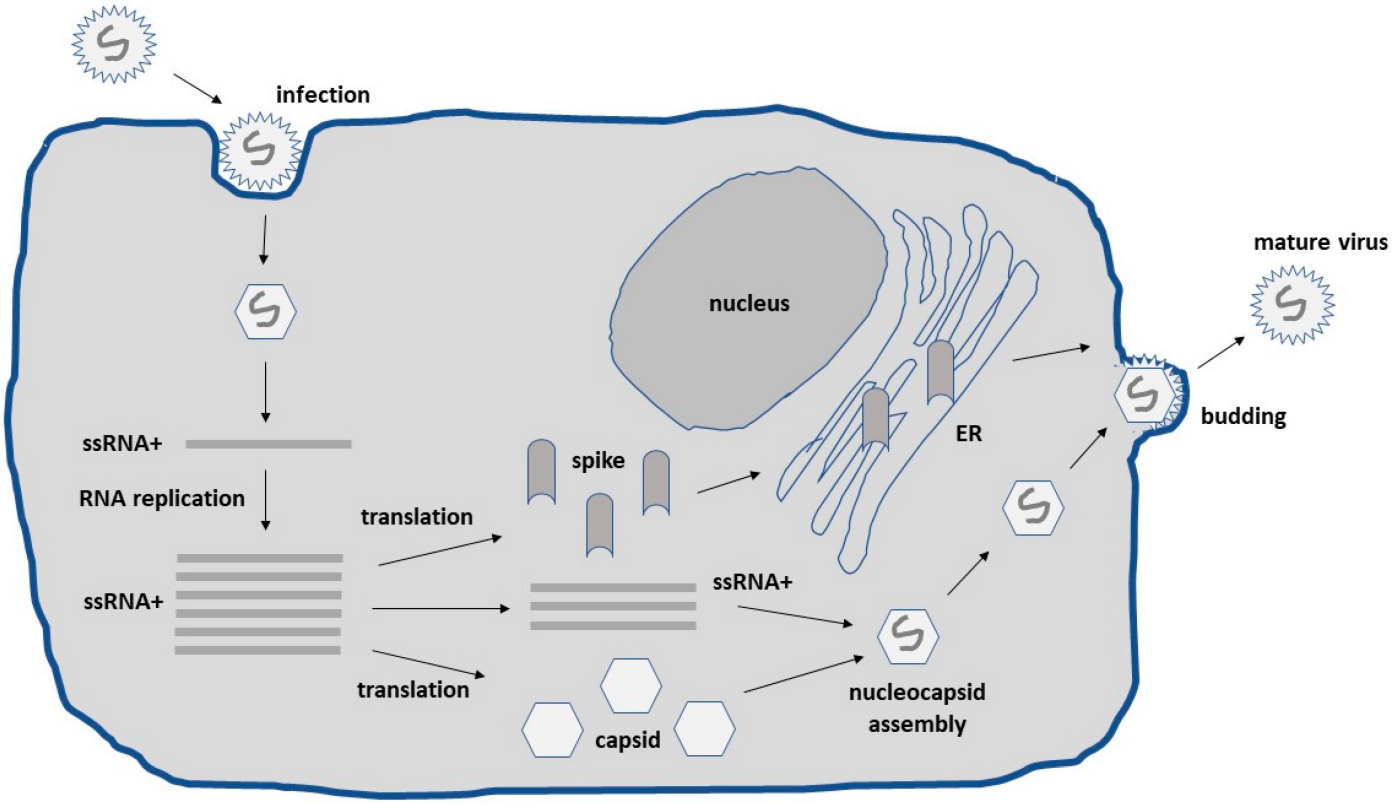
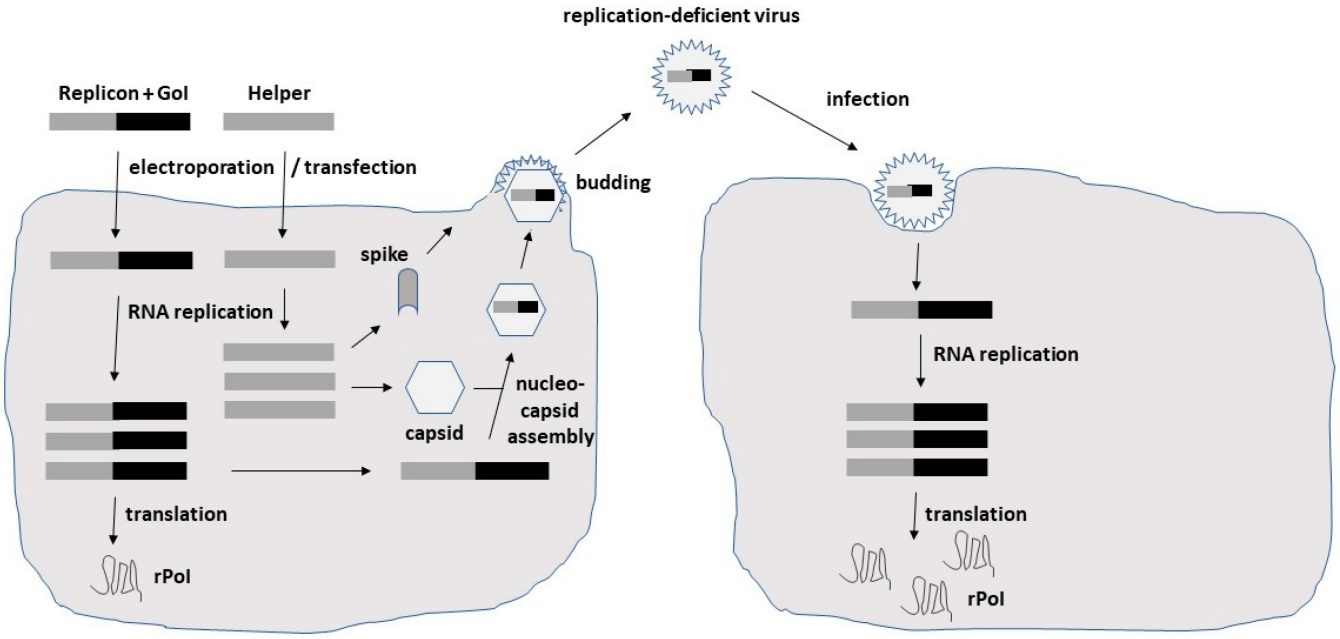


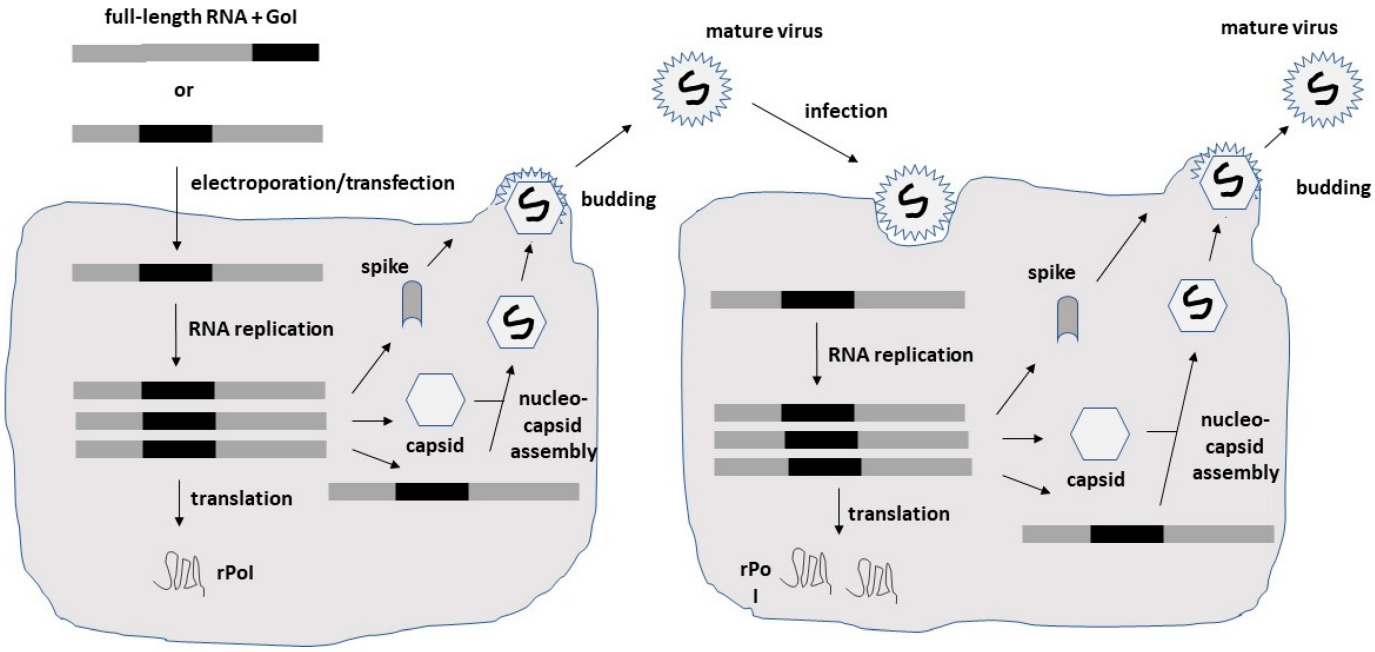
Figure 1. Schematic presentation of the lifecycle of alphaviruses. Alphaviruses infect host cells by endocytosis through endosomal fusion with the plasma membrane. The positive sense ssRNA is released into the cytoplasm for translation of viral proteins and RNA replication. Full-length ssRNA genomes are packaged into nucleocapsids. The alphavirus envelope proteins are transported to the plasma membrane through the endoplasmic reticulum and Golgi. The nucleocapsids are encircled by the envelope proteins at the plasma membrane and released by budding. ER, endoplasmic reticulum.

In the case of expression systems, the focus is on the expression of heterologous genes (**Figure 2**). In the context of replication-deficient alphavirus particles, the structural protein genes have been replaced by the gene of interest (GoI), and a helper vector is engaged in providing the structural proteins in trans (**Figure 2**A). Co-transfection of in vitro transcribed RNA from expression and helper vectors into baby hamster kidney (BHK) cells leads to the production of recombinant particles. As the RNA packing signal is located in one of the genes coding for the non-structural proteins (nsPs), in the nsP2 gene of SFV and nsP1 of SIN [11], uniquely RNA from the expression vector is packaged into viral particles, providing expression of the GoI but not the structural protein genes and thereby, eliminating any production of viral progeny. In contrast, introduction of a second 26S subgenomic promoter and the GoI into the full-length alphavirus RNA genome, either downstream of the nsP or the structural protein genes, generates replication-competent particles capable of both high-level GoI expression and viral progeny production (**Figure 2**B). In addition to the application of recombinant particles, RNA replicons can also be used for GoI expression. As has been demonstrated for the recent BNT162b2 [12] and mRNA-1273 [13] COVID-19 vaccines, RNA-based delivery is highly efficient. However, in contrast to this conventional mRNA approach, alphavirus RNA replicons provide the additional advantage of RNA self-amplification leading to superior expression levels.

Moreover, replacement of the SP6 RNA polymerase promoter by a CMV promoter, DNA replicon vectors for GoI expression (**Figure 2**C) have been engineered for the transfection of cell lines and in vivo administration [14]. The use of DNA replicons eliminates any risk of the production of new virus particles but relies on the less efficient delivery of DNA compared to viral vectors. Moreover, DNA molecules must be delivered to the nucleus for the in vivo transcription of RNA (**Figure 2**C).



(**A**)



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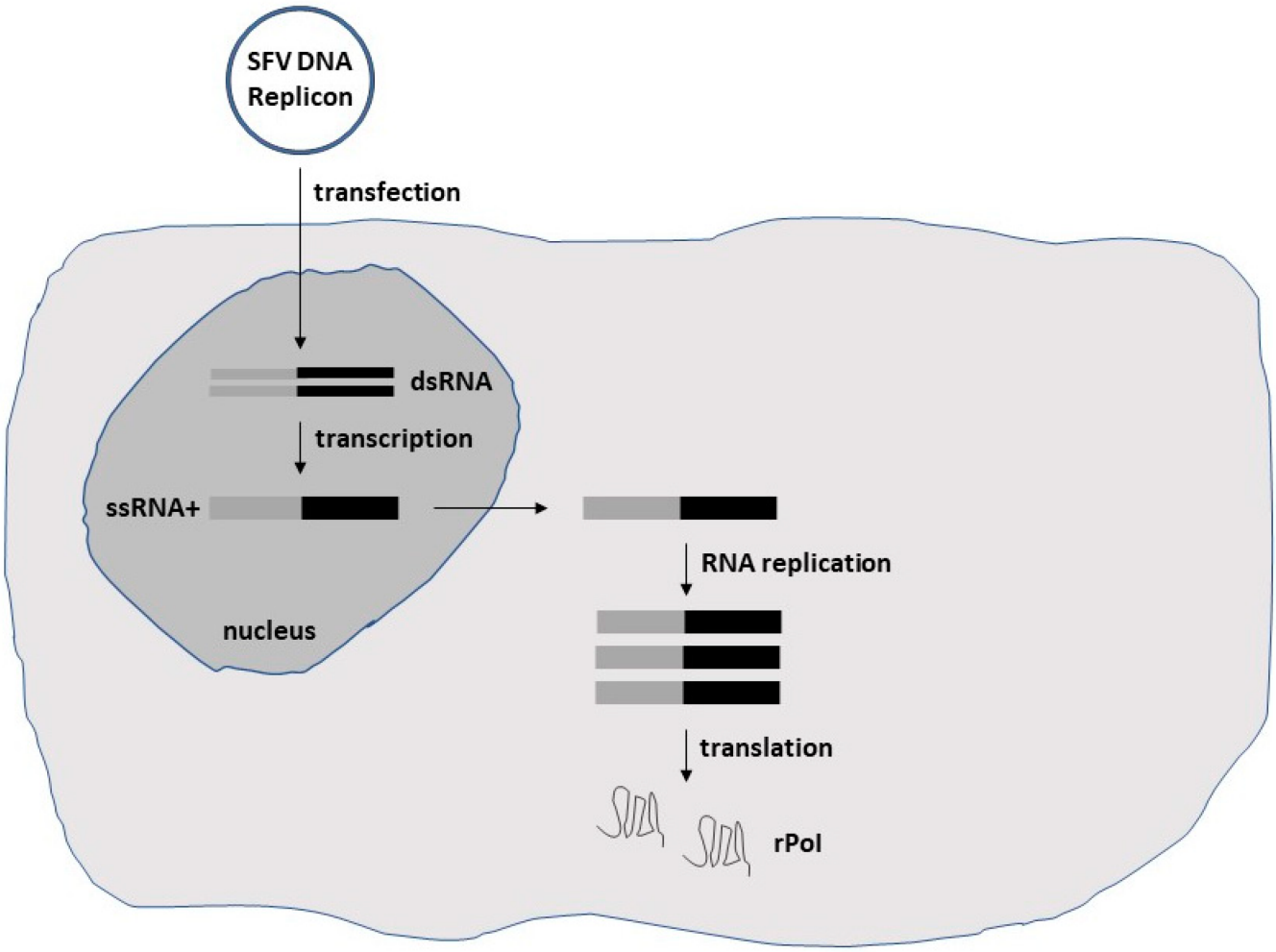

(C)

Figure 2. Schematic presentation of SFV expression systems. (**A**) **Replication-deficient recombinant particles.** In vitro transcribed RNA molecules from the SFV expression vector carrying the non-structural protein (nsP) genes, replicase genes (replicon) and the gene of interest (GoI) and the structural protein genes (capsid, 6K, envelope E1, E2 and E3) from the helper vector are electroporated or transfected into BHK-21 cells. After RNA replication, only the RNA from the expression vector containing the packaging signal is packaged into nucleocapsids and transported to the plasma membrane, where budding of mature viral particles takes place. Although the generated particles are capable of infecting new host cells, no viral progeny is produced due to the absence of the structural protein genes. However, high-level expression of the recombinant protein of interest (rPoI) takes place (**B**) **Replication-competent recombinant particles.** The in vitro transcribed full-length RNA genome with the GoI introduced either downstream of the nsP genes or the structural protein genes is electroporated or transfected into host cells for production of replication-competent viral particles and rPoI expression. (**C**) **DNA replicon vectors.** The replacement of the SP6 RNA polymerase promoter by a CMV promoter upstream of the nsP genes allows for direct transfection of host cells for rPoI expression. DNA replicons in the form of DNA plasmids are transfected into host cells, and DNA replicons are delivered to the nucleus.

Transcribed ssRNA molecules of positive polarity are delivered to the cytoplasm for RNA replication and expression of the rPol.

3. Alphavirus-Based Immunotherapy for Cancer

In the context of cancers, alphaviruses have been frequently used for prophylactic and therapeutic applications. Immunization with alphavirus vectors overexpressing tumor-associated antigens (TAAs) has been a common approach for cancer vaccine development. This approach has been used to provide both prevention against tumor challenges and tumor regression and eradication. Moreover, overexpression of cytotoxic and antitumor genes has been evaluated for cancer therapy. The delivery of immunostimulatory genes from alphavirus vectors has served the means of cancer immunotherapy. Moreover, alphaviruses induce apoptosis through activation of caspases in infected cells [15], which has resulted in tumor regression after administration of alphaviruses carrying no therapeutic genes and has allowed the use of vectors with reporter genes to verify and localize expression in animal tumor models. Finally, engineered or naturally occurring oncolytic alphaviruses have demonstrated tumor cell-specific killing in animal models [16]. Examples of cancer vaccinations, cancer therapy and immunotherapy are given below and summarized in **Table 1**.

Table 1. Examples of alphavirus-based vaccines against cancer.

Cancer	Vector	Finding	Ref
Reporter Genes			
Lung	SFV-EGFP	Tumor regression in mice	[17]
Colon	SIN-LacZ	Complete tumor remission	[18]
	SFV-LacZ RNA	Tumor regression, protection	[19]
TAAs			
Cervical	VEE-HPV-16 E7	Protection against tumor challenges in mice	[20]
	SFVenh-HPV E6-E7	Tumor eradication, long-lasting CTL in mice	[21]
	SFV-sHELP-E7SH	Tumor regression, protection in mice	[22]
	SFV-HPV E6-E7 DNA	85% of immunized mice tumor-free	[23]
	SFVenh-HPV E6-E7	Phase I: Immunogenicity in all patients	[24]
Colon	SFV-VEGFR-2	Inhibition of tumor growth, metastatic spread	[25]

Cancer	Vector	Finding	Ref
	SFV-VEGR-2 + SFV-IL-4	Prolonged survival after coadministration	[25]
	VEE-CEA	Phase I: Ag-specific response, long-term survival	[26]
Pancreatic	VEE-CEA	Phase I: Prolonged survival	[27]
Melanoma	VEE-TRP-2 + DNA	Superior to plasmid DNA vaccine in mice	[28]
	VEE-TRP-2	Humoral immune responses, protection in mice	[29]
	VEE-TRP-2 + CTLA-4 mAbs	Tumor regression in 50% of mice	[30]
	VEE-TRP-2 + GITR mAbs	Tumor regression in 90% of mice	[30]
	SFV-VEGFR-2/IL-12 DNA	Synergistic antitumor activity from combination of	[31]
	+ SFV-Survivin/β-hCG DNA	DNA replicons	
Ovarian	SFV-OVA + VV-OVA	Immune responses, enhanced antitumor activity	[32]
Prostate	VEE-PSMA	Th1-biased immune responses	[33]
	VEE-PSMA	Phase I: Good safety, weak immunogenicity	[34]
	VEE-PSA	PSA-specific Abs, delay in tumor growth	[35]
	VEE-mSTEAP + pcDNA	Prolonged survival, tumor challenge protection	[36]
	VEE-PSCA	Long-term survival of mice	[37]
Cytotoxic and Antitumor Genes			
Glioblastoma	SFV–Endostatin	Tumor growth inhibition, reduced vascularization	[38]
Breast	SIN-HER2/neu DNA	Significant tumor growth inhibition, protection	[39]
	SIN-HER2/neu DNA	80% less DNA needed compared to plasmid DNA	[40]

Cancer	Vector	Finding	Ref
	VEE-HER2/neu ECD/TM	Complete prevention of tumors in mice	[41]
	VEE-HER2/neu ECD/TM	Safe, PR in 1 patient, SD in 2 patients	[42]
Immunostimulation			
Glioblastoma	SFV-IL-18 + rec IL-12	Superior therapeutic effect of combination	[43]
Glioma	SFV-IL-12	70–97% tumor volume reduction in rats	[44]
Brain	SIN-gp100 + SIN-IL-12 DNA	Superior antitumor activity, prolonged survival	[45]
Breast	SFV-IL-12 + LV101	Superior antitumor activity of combination	[46]
Colon	SFVenh-IL-12	Complete tumor regression, long-term survival	[47]
	SFV-IL-12 + anti-PD1	Superior combination therapy in mice	[48]
	VEE-IL-12 + VEE-CEA	Superior combination therapy in mice	[49]
Melanoma	SFV-IL-12 + anti-PD1	Superior combination therapy in mice	[48]
	LSFV-IL-12	Phase I: Good safety and tolerability	[50]
Ovarian	SIN-IL12 + Irinotecan	Long-term survival in 35% of mice	[51]
Oncolytic Viruses			
Glioblastoma	SFV-VA-EGFP	Long-term survival in 16 out of 17 mice	[52]
Prostate	SFV-VA-EGFP	Complete tumor eradication in mice	[53]
Lung	SFV-VA-EGFP	Long-term survival in mice	[8]
Liver	M1	Liver tumor targeting in mice	[54]
Glioma	M1	Replication in tumors	[55]
Bladder	M1	Tumor growth inhibition, prolonged survival	[56]
Breast	M1 + Doxorubicin	Reduced tumor growth in mice	[7]
Pancreatic	M1 + IRE	Tumor growth inhibition, prolonged survival	[57]
Cervical	SIN AR339	Regression of established tumors in mice	[58]

therapy.

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Cancer	Vector	Finding	Ref
Ovarian	SIN AR399	Ascites formation in metastasis mouse model	[59]

Abs, antibodies; anti-PD1, immune checkpoint inhibitor; CEA, carcinoembryonic antigen; CTLA-4, CTL antigen-4; GITR, glucocorticoid-induced tumor necrosis factor; HPV, human papilloma virus; IRE, irreversible electroporation; LV101, *Salmonella typhimurium* AroC strain; mAbs, monoclonal antibodies; mSTEAP, mouse six-transmembrane epithelial antigen of the prostate; OVA, Ovalbumin; PR, partial response; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; rec IL-12, recombinant IL-12; SD, stable disease; SFV, Semliki Forest virus; SIN, Sindbis virus; TRP-2, tyrosine-related protein-2; VEE, Venezuelan equine encephalitis virus; VEGFR-2, vascular endothelial growth factor receptor-2; VV, vaccinia virus.

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