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A vaccinia virus renaissance

New vaccine and immunotherapeutic uses after smallpox eradication

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Abbreviations: VACV, vaccinia virus; MVA, Modified Vaccinia Ankara; IMV, intracellular mature virus; EEV, extracellular enveloped virus; TAA, tumor-associated antigen; TK, thymidine kinase; FDA, Food and Drug Administration

In 1796, Edward Jenner introduced the concept of vaccination with cowpox virus, an Orthopoxvirus within the family Poxviridae that elicits cross protective immunity against related orthopoxviruses, including smallpox virus (variola virus). Over time, vaccinia virus (VACV) replaced cowpox virus as the smallpox vaccine, and vaccination efforts eventually led to the global eradication of smallpox in 1979. VACV has many characteristics that make it an excellent vaccine and that were crucial for the successful eradication of smallpox, including (1) its exceptional thermal stability (a very important but uncommon characteristic in live vaccines), (2) its ability to elicit strong humoral and cell-mediated immune responses, (3) the fact that it is easy to propagate and (4) that it is not oncogenic, given that VACV replication occurs exclusively within the host cell cytoplasm and there is no evidence that the viral genome integrates into the host genome. Since the eradication of smallpox, VACV has experienced a renaissance of interest as a viral vector for the development of recombinant vaccines, immunotherapies and oncolytic therapies, as well as the development of next-generation smallpox vaccines. This revival is mainly due to the successful use and extensive characterization of VACV as a vaccine during the smallpox eradication campaign, along with the ability to genetically manipulate its large dsDNA genome while retaining infectivity and immunogenicity, its wide mammalian host range, and its natural tropism for tumor cells that allows its use as an oncolytic vector. This review provides an overview of new uses of VACV that are currently being explored for the development of vaccines, immunotherapeutics and oncolytic virotherapies.

Next-Generation Smallpox Vaccines

Smallpox was successfully eradicated through the efforts of the World Health Organization (WHO) in 1979.[1,2] However, in the last decade there has been a renewed interest in the development of next-generation smallpox vaccines due to the threat of bioterrorism and the possible emergence of other orthopoxviruses (such as monkeypox virus) as significant human pathogens. Although the smallpox vaccines used during the smallpox eradication campaign (now called first-generation vaccines) were very efficacious, they were typically propagated in the skin of calves[2] under conditions that did not adhere to good manufacturing practices (GMP). VACV strains widely used during the smallpox eradication campaign included the New York City Board of Health (NYCBH), Lister and EM-63 (**Table 1**). These live vaccines were commonly administered by scarification with a bifurcated needle, leading to a cutaneous reaction due to local virus replication. The resulting scar at the site of inoculation, known as the "take," has been historically accepted as the correlate of protection for smallpox.[1-3] However, these first-generation smallpox vaccines were associated with a number of adverse reactions ranging from mild (e.g., malaise, mild rash and fever) to severe (e.g., eczema vaccinatum, progressive vaccinia and post-vaccinial encephalitis).[2,4] Over the years, susceptibility to more severe complications was correlated with pre-existing conditions such as atopic dermatitis, immunosupression (e.g., due to HIV/AIDS and immunosuppressive therapy), and cardiac disease. Individuals with such conditions, or with contacts that have such conditions, are currently contraindicated for smallpox vaccination.[5,6]

A number of second-generation vaccines have been developed focusing on sterile cell culture techniques for vaccine propagation (**Table 1**). For example, the Elstree-BN vaccine developed by Bavarian Nordic was grown in chick embryo fibroblasts,[7] and the cell-cultured smallpox vaccine (CCSV) developed by DynPort Vaccine Company was derived from the NYCBH strain grown on normal diploid MRC-5 human lung cell cultures.[8] Similarly, Acambis (now part of Sanofi Pasteur) isolated a single clone derived from the Dryvax vaccine that was grown in Vero cells and named ACAM2000.[9] ACAM2000 was less neurovirulent than Dryvax in mice and nonhuman primates,[10] provided equivalent immunogenicity in clinical trials,[11] and was licensed in 2007 in the US.[12] However, the overall safety profile of ACAM2000 and other second-generation smallpox vaccines is still comparable to

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Table 1. Some first and next-generation smallpox vaccines

Vaccine (parental strain)	Description	Advantages	Disadvantages	Reference(s)
First generation				
Dryvax (NYCBH)	Propagated in calf skin, used in the US eradication campaign, replaced by ACAM2000 in 2007	Well characterized, low pathogenicity, "take" (correlate of protection)	Adverse reactions ranging from mild to severe	2, 78
Lister	Propagated mainly in calf skin, widely used in the eradication campaigns in UK, Africa, Asia, Oceania	Well characterized, moderate pathogenicity, "take" (correlate of protection)	Adverse reactions ranging from mild to severe	2
EM-63	Propagated in calf skin, used in the former Soviet Union	Well characterized, low pathogenicity, "take" (correlate of protection)	Adverse reactions ranging from mild to severe	2
Second generation				
ACAM2000 (Dryvax)	Single clone derived from Dryvax and propagated in Vero cells, FDA licensed and part of the US Strategic National Stockpile	Improved manufacturing (produced under GMP), less neurovirulent, immunologically non-inferior to Dryvax in clinical trials, "take" (correlate of protection)	Similar safety profile as Dryvax (adverse reactions ranging from mild to severe)	9–12
Elstree-BN (Lister)	Derived from the Lister/Elstree-RIVM strain and passaged in chicken embryo fibroblasts, phase I clinical trials completed	Improved manufacturing, "take" (correlate of protection)	Adverse reactions ranging from mild to severe	7, 54
CCSV (NYCBH)	Cell-Culture Smallpox Vaccine (CCSV) propagated in MRC-5 cells, Phase I clinical trials completed	Improved manufacturing, "take" (correlate of protection)	Adverse reactions ranging from mild to severe	8, 54
Third Generation				
Imvamune® (MVA)	Derived from Modified Vaccinia Ankara (MVA) strain 571 and passaged in serum free chicken embryo fibroblasts, replication defective in most mammalian cells, under fast track status at the FDA (phase III trials)	Improved safety profile, extensive clinical testing	Efficacy against smallpox unknown, boosting may be required for protection, no observable "take"	13, 14, 16, 17, 54
NYVAC (Copenhagen)	Derived from Copenhagen strain by deletion of 18 nonessential genes leading to a high degree of attenuation and reduced ability to grown in human cells	Improved safety profile	Efficacy against smallpox unknown, induces lower antibody responses in humans, boosting may be required for protection, no observable "take"	18–20
LC16m8 (Lister)	Derived from Lister strain by passage in rabbit kidney cells at low temperature and selection of small pock formation clone on chorioallantoic membranes, disruption in the B5R gene, licensed in Japan	Improved safety profile (milder reactions in children and less virulent), "take" (correlate of protection)	Efficacy against smallpox unknown	21–26

Table 1. Some first and next-generation smallpox vaccines (continued)

Vaccine (parental strain)	Description	Advantages	Disadvantages	Reference(s)
Fourth generation				
DNA vaccines	Single or combination of VACV IMV specific genes (A27, D8L, F9L, H3L, L1R), EEV specific genes (A33R, A56R, B5R), core antigen (A4L), or variola virus gene counterparts	Improved safety profile, protection in animal models	No clinical trials, boosting may be required for protection, no observable "take"	27–30
Protein (subunit) vaccines	Single or combination of VACV IMV specific genes (A27, H3L, L1R) and EEV specific genes (A33R, B5R)	Improved safety profile, protection in animal models	No clinical trials, boosting may be required for protection, no observable "take"	31–33
T-cell epitope vaccines	Multi-T-cell epitope vaccine based on antigenic sequences and DNA-prime, peptide-boost	Improved safety profile, protection in mice	No clinical trials, boosting may be required for protection, no observable "take"	34

the first-generation vaccines such as Dryvax, with similar rates of complications.[11]

A number of highly attenuated strains of VACV have been developed and are now being considered as safer smallpox vaccine alternatives, called third-generation smallpox vaccines (**Table 1**). One example is Modified Vaccinia Ankara (MVA), that was developed by passage of VACV strain Ankara over 570 times in chick embryo fibroblasts.[13] Inoculation with MVA leads to abortive infections in mammals and most mammalian cells, but expression of viral genes still occurs.[14] This highly attenuated VACV strain has been extensively tested in humans and has sparked considerable interest because it has been demonstrated to be extremely safe.[15] However, MVA propagation is limited to chick embryo fibroblasts and a few other mammalian cell lines where yield is low,[14] and the immunogenicity is not as robust compared with previous smallpox vaccine generations. For example, immune responses in phase I and II clinical trials to an MVA-based vaccine developed by Bavarian Nordic (Imvamune®) are dose-dependent and can require two immunizations to achieve immune responses similar to first-generation vaccines.[16,17]

Genetic manipulation of the VACV genome has also played a role in the development of highly attenuated VACV strains. The Copenhagen strain of VACV was considered to have higher pathogenicity,[2] but the deletion of 18 non-essential genes led to the highly attenuated NYVAC strain that is still immunogenic.[18,19] However, NYVAC induces lower antibody responses in humans when compared with Dryvax or Lister first-generation vaccine strains, and it does not induce anti-A27 antibodies that are seen in the immune response to first-generation vaccines and can neutralize intracellular mature virus (IMV).[20] Two major disadvantages of highly attenuated VACV strains such as MVA and NYVAC is that they do not produce a "take" in vaccinees and their efficacy against smallpox was never determined.

An additional third-generation vaccine is LC16m8, an attenuated VACV derived from the Lister strain that has an excellent safety profile.[21-23] LC16m8 contains a mutation in the B5R gene, which causes the virus to produce smaller plaques and replicate less efficiently in Vero cells,[24,25] but unlike MVA, LC16m8 produces a "take" in vaccinees.[22,23] This vaccine was licensed in Japan and used in the 1970s eradication campaign, but since smallpox was no longer endemic at that time, its efficacy against smallpox is currently unknown. A disadvantage that must be considered is that LC16m8 does not induce neutralizing antibodies against the B5 protein, the main target for extracellular enveloped virus (EEV) neutralizing antibodies.[26]

A number of different approaches are being used for the development of the so-called fourth-generation smallpox vaccines that eliminate the possibility of the VACV vector to cause adverse events or revert to a more pathogenic phenotype (**Table 1**). These include the development of subunit and DNA vaccines typically composed of VACV (or variola virus counterpart) membrane proteins that elicit neutralizing antibodies against the IMV and EEV forms of the virus.[27-33] A particularly new approach is the use of conserved and immunogenic multi-T-cell epitopes that are used in a DNA-prime, peptide boost vaccine regimen.[34] These fourth-generation vaccines have shown to be protective using animal models, but none are currently being tested in clinical trials, and to be efficacious against smallpox they will likely require booster immunizations.

Vaccinia Viruses as Animal Vaccine Vectors

The successful use and extensive characterization of VACV during the smallpox eradication campaign, along with the ability to genetically manipulate its large dsDNA genome while retaining infectivity, its heat stability and low cost of production, makes VACV particularly attractive for the development of animal vaccines.[35,36] A large number of antigens from animal pathogens have been expressed in VACV, and the majority elicit protective immune responses (examples shown in **Table 2**). The most successful recombinant VACV vaccine has been the oral vaccine for sylvatic rabies (Raboral V-RG®) that expresses the rabies glycoprotein (G).[37,38] This recombinant virus is packaged into vaccine baits that are distributed in areas affected by rabies. The heat

Table 2. Some vaccinia virus-vectored animal vaccines

Pathogen (Species)	Parental VACV Strain	Protein(s) Expressed /Comments	Reference(s)
Rabies virus (foxes, raccoons, skunks, coyotes)	Copenhagen	Glycoprotein G, licensed in the US as Raboral V-RG®	37–39
Rinderpest virus (ruminants, cattle, buffalo), Peste-des-petits-ruminants virus (goats and sheep)	NYCBH, Copenhagen	Rinderpest virus fusion (F) and hemagglutinin (H) genes	40, 41, 79
Vesicular stomatitis virus (horses, cattle, pigs)	Western Reserve (WR)	Glycoprotein G	80
Newcastle disease virus (chickens)	Elstree	Fusion (F)	81, 82
Leishmania (dogs)	MVA	Tryparedoxin peroxidase (TRYP), DNA/MVA prime/boost	83
Canine distemper virus (dogs)	Copenhagen	Measles virus fusion (F) or hemagglutinin (H) genes	84
Echinococcus granulosus (marsupial wildlife, possums)	Lister	Eg95 (oncosphere-stage antigen)	85
Rift Valley fever virus (cattle, sheep, zoonotic disease in humans)	Copenhagen	Glycoproteins Gn and Gc	86

stability of the V-RG vaccine proved to be extremely helpful in sylvatic rabies vaccination programs, which have led to better control of rabies in Europe and North America.[39] Several recombinant VACV vaccines engineered to express the fusion and hemagglutinin glycoproteins of rinderpest virus have also been developed,[40,41] providing sterilizing immunity to cattle challenged with virulent rinderpest virus over one year after vaccination.[41] Recombinant VACVs expressing heterologous viral antigens have also been protective against peste-des-petits-ruminants virus, vesicular stomatitis virus, Newcastle disease virus, canine distemper virus, and Rift Valley fever virus in a variety of animal species (reviewed in **Table 2**).

Vaccinia Viruses as Human Vaccine Vectors

The use of VACV as a vector for human vaccines against infections agents has also been extensively investigated (**Table 3**), with most of the effort focused on the development of vaccines against HIV. Initially, replication-competent VACVs were typically employed, but more recent efforts center on the use of replication-defective poxviruses due to safety concerns. In addition, replication-defective poxvirus vectors, including MVA, NYVAC and avipoxviruses (such as canarypox virus and fowlpox virus), offer the advantage of allowing multiple booster immunizations even in subjects with pre-existing immunity.[42] The only Phase III trial to show any evidence of protection against HIV has been the RV144 trial in Thailand.[43] The RV144 vaccine regimen consisted of four priming injections of a recombinant canarypox expressing HIV-1 Gag, protease and Env, followed by two booster immunizations with a recombinant Env subunit vaccine. The trial involved 16,402 subjects and the estimated vaccine efficacy (prevention of HIV-1 infection) was 31.2%.[43] The results from the RV144 Thai trial, albeit modest, reinvigorated the HIV vaccine community and their interest in poxvirus-vectored HIV vaccines. A number of phase I and II HIV clinical trials, usually in the form of a DNA prime and recombinant MVA or NYVAC boost, have shown that these vaccine regimens are safe, immunogenic, and have the potential to improve the efficacy obtained with the RV144 Thai trial (**Table 3**).

Recombinant MVA has also been extensively used alone or in prime-boost strategies in vaccine clinical trials for other viral diseases such as influenza and hepatitis B, as well as bacterial and parasitic diseases such as tuberculosis and malaria (**Table 3**). Three malaria phase I trials with recombinant chimpanzee adenovirus (ChAd63) and MVA expressing different *Plasmodium falciparum* antigens have shown that this prime-boost strategy is safe and immunogenic.[44-46] Likewise, phase I trials with an MVA vector expressing the *Mycobacterium tuberculosis* 85A antigen, aimed to serve as a booster immunization after bacille Calmette-Guerin (BCG) vaccination, showed that the vaccine can induce potent Th1 responses.[47] Lastly, a number of other constructs are being developed and investigated in pre-clinical trials, including vaccines against hepatitis C virus,[48] respiratory syncytial virus,[49] anthrax (with the advantage of being a dual vaccine against anthrax and smallpox),[50] and Nipah virus.[51]

Vaccinia Viruses as Immunotherapeutic Cancer Vectors

The ability of VACV to induce potent immune responses to tumor-associated antigens (TAAs) expressed in its genome has been employed for the development of immunotherapies for cancer (**Table 4**). One example is PROSTVAC® (Bavarian Nordic), a therapeutic cancer vaccine for prostate cancer that consists of a replication-competent VACV prime followed by multiple replication-defective avian poxvirus (fowlpox) boosts.[52,53] Both poxviruses express a modified prostate-specific antigen (PSA), along with three T-cell costimulatory molecules termed TRICOM (B7.1, ICAM-1 and LFA-3). The immune response to the altered PSA (with a single amino acid change in an HLA-A2 epitope) is aimed to target cancerous prostate cells, while the costimulatory molecules increase the immunogenicity of the constructs. This treatment has been studied in phase II clinical trials in patients

Table 3. Some vaccinia virus-vectored human vaccines

Pathogen (disease)	Vaccine name (VACV parental strain)	Protein(s) expressed	Comments	Reference(s)
HIV-1 (AIDS)	Sanofi Pasteur ALVAC-HIV (canarypox) prime and VaxGen AIDSVAX subunit boost	ALVAC-HIV: HIV-1 Gag and PR B; Env E AIDSVAX: HIV-1 Env B/E	Used canarypox (an avian poxvirus), first phase III HIV vaccine clinical trial (RV144) that yielded some protection (31.2% efficacy), revitalized the HIV vaccine community	43
HIV-1 (AIDS)	DNA prime and MVA-CMDR (MVA) boost	DNA: HIV-1 Env A/B/C, Rev B, RT B, Gag A/B MVA-CMDR: HIV-1 Env E, Gag/Pol A	Phase I/II trials completed, safe and immunogenic with T-cell and antibody responses	87
HIV-1 (AIDS)	DNA prime and NYVAC-C (Copenhagen) boost	HIV-1 Gag, Pol, Nef, Env C	Phase II trials completed, safe and immunogenic with T-cell and antibody responses (non-neutralizing)	54, 88–90
HIV-1 (AIDS)	MVA-B (MVA)	HIV-1 Env, Gag, Pol, Nef B	Phase I trial completed, safe, immunogenic with T-cell and antibody responses	91
HIV-1 (AIDS)	GeoVax pGA2/JS7 DNA and MVA/HIV62 (MVA) boost	DNA (complex): HIV-1 Gag, PR, RT, Env, Tat, Rev, and Vpu MVA: HIV-1 Gag, PR, RT, and Env	Produce non-infectious virus-like particles (VLPs), Phase I trial completed, Phase IIa ongoing, trial with inclusion of GM-CSF as an adjuvant started in 2012	54, 92
Plasmodium falciparum (Malaria)	Chimpanzee Adenovirus (ChAd63) prime and MVA boost	Merozoite surface protein 1 (MSP1), or T-cell multiple epitope fused to the thrombospondin-related adhesion protein (ME-TRAP), or apical membrane antigen 1 (AMA1)	Three phase I trials suggest that the chimpanzee adenovirus prime / MVA boost strategy is safe and highly immunogenic	44–46
Mycobacterium tuberculosis (Tuberculosis)	Aeras MVA85A (MVA)	Highly conserved *M. tuberculosis* antigen 85A	Aim is to boost immunity induced by the current tuberculosis vaccine *M. bovis* bacille Calmette-Guerin (BCG), phase I trial completed and vaccine induced potent Th1 cell responses, phase II trials ongoing	47
Influenza virus (Influenza)	MVA-NP+M1 (MVA)	Influenza A Nucleoprotein (NP) and Matrix protein 1 (M1)	Phase I trials concluded, safe and immunogenic (induces high T-cell responses)	93
Hepatitis B virus (HBV)	DNA prime and MVA.HBs (MVA) boost	HBV S antigen (HBsAg) genotype D	Therapeutic vaccine trial resulted in variable immune responses that did not control HBV infection	94

with metastatic castration-resistant prostate cancer, where treatment was well tolerated, death rate was reduced by 44%, and the median overall survival was 8.5 mo longer than in patients receiving control vectors.[52,53] This increase in overall survival was noticeably superior to that afforded by the currently approved treatment (docetaxel, 2–3 mo increase in overall survival), and Phase III clinical trials are ongoing.[52,54] Bavarian Nordic is also performing clinical trials with an MVA vector (MVA-BN® PRO) expressing two prostate TAAs, PSA along with prostatic acid phosphatase (PAP),[54,55] and with MVA-BN® HER2, an MVA vector expressing the TAA human epidermal growth factor receptor 2 (HER2) to treat breast cancer.[54,56]

TroVax® (Oxford BioMedica) is an MVA vector that expresses the human oncofetal antigen 5T4, a placental glycoprotein overexpressed in a number of different cancers (**Table 4**).[57] When administered alone or in conjuction with other treatments (e.g., chemotherapy and cytokines), Trovax® mounted high anti-5T4 immune responses that were associated with increased overall survival.[54,57-61] Poxvirus vectors expressing another TAA, the cancer-testis NY-ESO-1 antigen, were tested in phase II clinical trials in a VACV prime followed by fowlpox boost regimen with melanoma and ovarian cancer patients that were at risk for recurrence or progression after completion of their primary therapy.[54,62] The trials provided evidence for an extended overall survival and progression-free survival in patients that developed detectable anti-NY-ESO-1 immune responses.[62]

TG4010 (Transgene SA) is an MVA vector that expresses mucin-1 (MUC-1), a TAA expressed by glandular epithelia and

Table 4. Some vaccinia virus vectors used for cancer immunotherapy

Cancer	Therapy name (VACV parental strain)	Protein(s) expressed	Comments	Reference(s)
Prostate	Bavarian Nordic PROSTVAC-V (NYCBH) prime and PROSTVAC-F (fowlpox) boosters	PSA and TRICOM (T-cell costimulatory molecules B7.1, ICAM-1, and LFA-3)	Phase II trials showed an enhanced median overall survival in patients with metastatic castration-resistant prostate cancer, currently in Phase III trials	52–54
Prostate	Bavarian Nordic MVA-BN® PRO (MVA)	PSA and PAP	Under phase I/II trials, additional studies targeting antigens to exosomes show improved efficacy	54, 55
Various such as colorectal, renal, ovarian, fallopian, peritoneal, prostate	Oxford BioMedica TroVax® (MVA)	Human oncofetal antigen 5T4	Alone or in conjunction with other treatments, 5T4 antibody responses correlated with increased survival, phase II and III ongoing/completed	54, 57–61
Breast	Bavarian Nordic MVA-BN® HER2 (MVA-BN)	Human epidermal growth factor receptor 2 (HER2)	Phase I completed	54, 56
Lung carcinoma, solid tumors	Transgene TG4010 (MVA)	Mucin-1 (MUC-1) and IL-2	Phase I completed, II/III ongoing	54, 63, 64
Ovarian, melanoma	VACV NY-ESO-1 (NYCBH) prime and fowlpox NY-ESO-1 boosters	NY-ESO-1 (cancer/testis antigen)	Phase II trials completed	54, 62
Breast, lung, ovarian	Bavarian Nordic CV-301 (MVA-BN), formally known as PANVAC	Carcinoembryonic antigen (CEA), MUC-1, and TRICOM	Phase II trials ongoing	54, 65

by some cancers, as well as the immostimulatory cytokine IL-2 (**Table 4**).[63] Results from a Phase IIb study with non-small-cell lung cancer patients in combination with chemotherapy suggests that TG4010 has beneficial effects and additional trials are ongoing.[54,64] Finally, CV-301 (Bavarian Nordic), formerly known as PANVAC, is an MVA vector that in addition to MUC-1, also expresses carcinoembryonic antigen (CEA) and the TRICOM set of T-cell costimulatory molecules, having a potential effect on multiple cancers.[65] A recent pilot study in patients with metastatic breast and ovarian cancer suggests that CV-301 may be beneficial to patients and a phase II study is underway.[54,65]

Vaccinia Viruses as Oncolytic Cancer Therapy Vectors

Oncolytic viruses are promising new therapies for the clinical treatment of cancers. As a replication-competent virus, VACV displays a natural tumor tropism and kills cancer cells through apoptosis and other mechanisms.[66] Moreover, recombinant VACVs in which the vaccinia growth factor (VGF) and thymidine kinase (TK) genes have been deleted acquire enhanced tumor selectivity (tropism), likely because actively dividing cancer cells

Table 5. Some vaccinia virus vectors used for oncolytic cancer therapy

Cancer	Therapy Name (VACV Parental Strain)	Protein(s) Expressed	Comments	Reference(s)
Various such as melanoma, colorectal, liver, lung, renal, squamous cell, head and neck	Jennerex Biotherapeutics JX-594 (Dryvax, TK^-)	GM-CSF and β-galactosidase	Antitumor and antivascular activities, phase I and II trials ongoing/completed	54, 69–71
Various such as melanoma, breast, liver, colorectal	Jennerex vvDD-CDSR (Western Reserve, TK^- and VGF^-)	Cytosine deaminase (CD) and somatostatin receptor (SMR)	Highly selective and oncolytic for tumors with gene-directed enzyme prodrug therapy using 5-fluorocytosine, SMR gene is used for molecular imaging, phase I trials ongoing	54, 72, 73
Advanced solid tumors, head and neck, peritoneal	Genelux GL-ONC1 (Lister, $F14.5L^-$, TK^-, and $A56R^-$)	Renilla luciferase-GFP fusion, β-galactosidase, β-glucuronidase	Tumor specific replication and solid tumor size reduction in pre-clinical trials, phase I and II trials ongoing	54, 74–76

have high metabolic rates that make VGF and TK expression by VACV dispensable.[67,68] Systemic administration of these oncolytic VACV vectors (e.g., intravenously) targets both tumors and metastases, and current clinical trials show that VACV vectors can be an effective oncolytic cancer therapy (**Table 5**). In addition, recombinant VACVs expressing host immunomodulating genes such as human GM-CSF and other cytokines, anti-angiogenic agents and extracellular matrix genes also show enhanced tumor selectivity and oncolytic effects.[66] For example, JX-594 (Jennerex Biotherapeutics) is a TK negative VACV expressing GM-CSF. In patients with hepatocellular carcinoma, treatment with JX-594 resulted in antitumor and antivascular activities,[69] and in clinical trials patients with metastatic liver cancer and metastatic melanoma tolerated treatment and anti-tumor effects were observed.[70,71] Another example is vvDD-CDSR (Jennerex Biotherapeutics), a TK and VGF negative vector engineered to express a reporting gene (somatostatin receptor) for molecular in vivo imaging after systemic delivery, as well as the prodrug-activating enzyme cytosine deaminase for therapy with 5-fluorocytosine.[72,73] These modifications allow vvDD-CDSR to be highly tumor selective and oncolytic. Finally, GL-ONC1 (Genelux), also known as GLV-1h68, is a light-emitting oncolytic VACV expressing a luciferase-green fluorescent protein (GFP) fusion gene and containing deletions in the F14.5L, TK, and A56R genes.[74] In pre-clinical trials it showed tumor-specific replication and solid tumor size reduction.[74-76] In addition, VACVs with tumor selectivity and light-emitting or imaging features (such as expression of somatostatin receptor and ^{111}In-pentreteodide treatment) can be innovative and helpful tools to non-invasively detect and locate primary and metastatic tumors, as well as to track the therapeutic vector during treatment.[72,77]

Future Perspectives

Since the eradication of smallpox more than 30 y ago, VACV has experienced a renaissance of interest as a viral vector for the development of recombinant vaccines, immunotherapies and oncolytic therapies, as well as the development of next-generation smallpox vaccines. This renewed interest in both replication-competent and replication-defective VACVs has driven a number of vaccine and therapeutic candidates to clinical trials. Replication-competent VACVs are used for oncolytic therapy, and as live vaccines they elicit superior immune responses, but safety is a concern due to potential adverse events. Conversely, replication-defective VACVs are not as immunogenic as their replication-competent counterparts, but are extremely safe and offer the capacity to be administered multiple times with minimal interference from pre-existing immunity. A number of approaches have been used to enhance the safety of replication-competent VACV vectors while maintaining their immunogenicity, such as the deletion of viral genes and expression of cytokines. A new approach under development to generate next-generation smallpox vaccines that are efficacious and safer is the addition of safety mechanisms to second-generation VACV vectors that should allow the conditional replication of the virus and the production of the "take," the correlate of protection against smallpox (Hagen CJ, Titong A and Verardi PH, unpublished data). With these built-in safety systems, VACV replication can be controlled so that adverse events in vaccinees and contacts can be treated with antibiotics. These new technologies can also be applied for the development of VACV vectors for human vaccines and therapies with enhanced safety features.

A range of potential new uses is under development in areas such as tumor imaging and enhanced oncolytic and tumor selectivity. The fact that VACV has the capacity to hold at least 25 kb of heterologous DNA while retaining infectivity also enables the development of multi-pathogen, multi-epitope polyvalent vaccines. Hence, it seems inevitable that this "old" vaccine will lead to "newer" uses in the near and distant future.

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