Smallpox was successfully eradicated through the efforts of the World Health Organization (WHO) in 1979. 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 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. 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). Over the years, susceptibility to more severe complications was correlated with pre-existing conditions such as atopic dermatitis, immunosuppression (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.

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, 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. 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. ACAM2000 was less neurovirulent than Dryvax in mice and nonhuman primates, provided equivalent immunogenicity in clinical trials, and was licensed in 2007 in the US. However, the overall safety profile of ACAM2000 and other second-generation smallpox vaccines is still comparable to smallpox vaccination during the smallpox eradication campaign. The resulting scar at the site of inoculation, known as the “take,” has been historically accepted as the correlate of protection for smallpox.

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, 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. 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. ACAM2000 was less neurovirulent than Dryvax in mice and nonhuman primates, provided equivalent immunogenicity in clinical trials, and was licensed in 2007 in the US. However, the overall safety profile of ACAM2000 and other second-generation smallpox vaccines is still comparable to smallpox vaccination during the smallpox eradication campaign. The resulting scar at the site of inoculation, known as the “take,” has been historically accepted as the correlate of protection for smallpox.
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 neuroviralent, 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**      |             |            |               |              |
| Immunus/ (MVA)            | Derived from Modified Vaccinia Ankara (MVA) strain S71 and passaged in serum free chicken embryo fibroblasts, licensed in Japan, 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-pox formation clone on chorioallantoic membranes, disruption in the BSR gene, licensed in Japan | Improved safety profile (milder reactions in children and less virulent), “take” (correlate of protection) | Efficacy against smallpox unknown | 21–26 |
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.12 Inoculation with MVA leads to abortive infections in mammals and most mammalian cells, but expression of viral genes still occurs.13 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.14 However, MVA propagation is limited to chick embryo fibroblasts and a few other mammalian cell lines where yield is low,2 but 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, but the deletion of 18 non-essential genes led to the highly attenuated NYVAC strain that is still immunogenic.21,22 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).23 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.24,25 LC16m8 contains a mutation in the BSR gene, which causes the virus to produce smaller plaques and replicate less efficiently in Vero cells,26,27 but unlike MVA, LC16m8 produces a “take” in vaccinees.28,29 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.30

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.31,32 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.33 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.33-35 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 vials and used in a DNA-prime, peptide boost vaccine regimen.39 These fourth-generation vaccines have shown to be protective using animal models, but none are currently being tested in clinical trials.
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, buffaloes), Paste- 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 (WI) | Glycoprotein G | 80 |
| Newcastle disease virus (chickens) | Elthone | Fusion (F) | 81, 82 |
| Leishmaniasis (dogs) | MVA | Trypanosoma cruzi antigen (TRYP), DNA/MVA prime/ boost | 83 |
| Canine distemper virus (dogs) | Copenhagen | Measles virus fusion (F) or hemagglutinin (H) genes | 84 |
| Echinocebus granulosus (marsupial wildlife, possums) | Lister | EGFP (oncosphere-stage antigen) | 85 |
| Rift Valley fever virus (cattle, sheep, zoonotic disease in humans) | Copenhagen | Glycoproteins Gp and Gc | 86 |

Vaccinia Viruses as Vaccine Vectors

The use of VACV as a vector for human vaccines against infections 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. The only Phase III trial to show any evidence of protection against HIV has been the RV144 trial in Thailand. 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%. 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. 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. Lastly, a number of other constructs are being developed and investigated in pre-clinical trials, including vaccines against hepatitis C virus, respiratory syncytial virus, anthrax (with the advantage of being a dual vaccine against anthrax and smallpox), and Nipah virus.

Vaccinia Viruses as Immunotherapeutic Cancer Vectors

The ability of VACV to induce potent immune responses to tumor-associated antigens (TAA) 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. 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...
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 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 human oncofetal antigen 5T4, a placental glycoprotein overexpressed in a number of different cancers (Table 4).62 When administered alone or in conjugation 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 yielding some protection (31.2% efficacy), revitalized the HIV vaccine community.54,57,58

Table 3. Some vaccinia virus–vected 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 adenovirus 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 NVAC-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) Geotax pSA2/07 DNA and MVA/NS1 (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/II 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 | Membrane surface protein 1 (MSP1), or T-cell multiple epitope fused to the thrombospondin domain–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 Mycobacterium bovis bacille Calmette-Guérin (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 |

www.landesbioscience.com Human Vaccines & Immunotherapeutics 965
by some cancers, as well as the immnosuppressive cytokine IL-2 (Table 4).53 Results from a Phase IIb study with non-small-cell lung cancer patients in combination with chemotherapy suggest 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

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 B71, 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 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 ST4 | Alone or in conjunction with other treatments, ST4 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), formerly known as PANVAC | Carcinoembryonic antigen (CEA), MUC-1, and TRICOM | Phase II trials ongoing | 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 vDD CD59 (Western Reserve, TK and VGF) | Cytosine deaminase (CD) and somatostatin receptor (SMR) | Highly selective and oncolytic for tumors with gene-directed enzyme producing 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. Systemic administration of these onco-
lytic 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 addi-
tion, 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 Bioterapeutics], a TK negative VACV expressing GM-CSF. In patients with hepatocellular carcinoma, treatment with JX-594 resulted in antitumor and antivascular activities,69 and on several trial patients with metastatic liver cancer and metastatic melanoma tolerated treatment and anti-tumor effects were observed.70,71 Another example is vvDD-CDSR (Jennerex Bioterapeutics), 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-acti-
vating enzyme cytosome deaminase for therapy with 5-fluorocys-
tosine.72,73 These modifications allow vvDD-CDSR to be highly tumor selective and oncolytic. Finally, GLV-1h68 (Genexis), 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 E1a, E3L, TK, and AS6R genes.74 In pre-clinical trials it showed tumor-specific replica-
tion and solid tumor size reduction.75 In addition, VACVs with tumor selectivity and light-emitting or imaging features (such as VGF and TK) can also be in vivo imaged and detected, and primary and metastatic tumors, as well as to track the therapeutic vector during treatment.75,76

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 clin-
cial trials. Replication-competent VACVs are used for onco-
lytic 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 pursued to make the safety of replication-competent VACV vectors while main-
taining their immunogenicity, such as the deletion of viral genes and expression of cytokines. One approach under develop-
ment to generate next-generation smallpox vaccines that are efficacious and safer is the addition of safety mechanisms to sec-
ond-generation VACV vectors that should allow the conditional replication of the virus and the production of the “tache,” 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 develop-
ment 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 selec-
tivity. The fact that VACV has the capacity to hold at least 25 kb of heterologous DNA while also containing its own TK and VGF genes and expression of cytokines. A new approach under develop-
ment of multi-pathogen, multi-epitope polyvalent vac-
cines. Hence, it seems inevitable that this “old” vaccine will lead to “newer” uses in the near and distant future.

Acknowledgments

The authors would like to thank Caitlin O’Connell for critical review of the manuscript. Work in our laboratory is currently supported by the US Department of Agriculture (USDA), the University of Connecticut Research Foundation (UCRF), and the University of Connecticut Office of Undergraduate Research (OUR).

References

1. Wieland TF. A survey in our time—certification of the global eradication of smallpox. J Infect Dis 1985; 152:463-4; PMID:3983857. http://dx.doi.
org/10.1093/infdis/152.5.463.6
2. Turner E, Henderson DA, Arick J, Jokl L, LaPointe JD. Smallpox and its eradication. Geneva, Switzerland: World Health Organization 1988.
3. Menon B. Poxviruses: the viruses and their replication. In: Fields V, Knipe DM, Howley PM, Eds. Fields’ virology. Philadelphia, PA: Lippincott Williams & Wilkins 2007; 2084-41.
4. Lauer JM, Kukula FL, Nolf JM, Miller JD. Complications of smallpox vaccination 1968: results of ten statewide surveys. J Infect Dis 1975; 132:369-95; PMID:4361849. http://dx.doi.org/10.1093/infdis/132.4.369.
5. Lauer JM, Goldstein J. Evaluation of 21-year risks of smallpox vaccination and policy options. Am J Prev Med 2003; 138:499-515; PMID:12529083.
6. Komp AE, Davis MM, Field GD. Expected adverse events in mass smallpox vaccination campaigns. Emerg Clin North Am 2002; 54:94; PMID:12095216.
7. Strohla KJ, van Ameersberg G, Kansera A, Raumon T, van Lauris RE, Pinnier PH, et al. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. J Virol 2005; 79:7945-52; PMID:15959350. http://dx.doi.org/10.1128/JVI.79.12.7945-51.2005.
8. Greenberg RN, Kennedy JS, Clavien DJ, Flumer EA, Hogan I, Cruz J, et al. Safety and immunogenicity of new cell-cultured smallpox vaccine compared with calf-lymph derived vaccine: a blind, single-centre, ran-
domised controlled trial. Lancet 2005; 365:398-409; PMID:15848456.
9. Wilksa R, Liu J, Nagasaka KC, Mewis G, Coughlin B, Huang PM, et al. Clinical vaccine virus growth in cell culture as a new smallpox vaccine. Nat Med 2005; 11:125-30; PMID:15732065. http://dx.doi.
org/10.1038/nm1206.
10. Haase AT, Caubel JB, Mancini W, Pace J, Johnson CA, Roller M, et al. ACAM2000 (smallpox vaccine) - a multi-center vaccine trial. J Am Coll Surg 2003; 197:555-64; PMID:12835519. http://dx.doi.org/10.1016/S1072-7391(03)00106-9.
11. Maiti A, Steck H, Miller RB, Donn K, Singer H. The smallpox vaccination strain MV/S: market, genetic restrictions, epistatic gain of the parenteral vaccinators and behavior in organisms with a defined defense mechanism (author’s stand). Zentbl Bakteriol 1978; 187:373-80; PMID:419640.
12. Dvorak J, Schaller W, Henn S, Forrer V, Simon G. Highly attenuated modified vaccinia virus Ankara (MVA): the viruses and safety. In: Fields V, Knipe DM, Howley PM, Eds. Fields’ virology. Philadelphia, PA: Lippincott Williams & Wilkins 2007; 2084-41.
S. H. Smith GL. Serological responses in humans to the JKV vaccine. Vaccine 2006; 24:2065-70; PMID:1566575; http://dx.doi.org/10.1016/j.vaccine.2006.03.087.

M. LC16m8: an attenuated smallpox vaccine. Vaccine 2003; 21:181-95; PMID:12620810; http://dx.doi.org/10.1016/S0264-410X(02)00287-3.

Yokote H, et al. Clinical and immunological response to MVA, a recombinant vaccinia virus for smallpox vaccine in mice. Vaccine 2005; 23:1172-84; PMID:16487586; http://dx.doi.org/10.1016/j.vaccine.2004.07.020.

Buchbinder CR, Cohen ML, Xiao Y, Rickert-Homan N, Rizzotto B, Entin-Turk L, et al. A potent subunit-based multicluster vaccine demonstrated to provide sterile immunity and protect against smallpox challenge in mice. Vaccine 2010; 28:3256-7; PMID:20395919; http://dx.doi.org/10.1016/j.vaccine.2010.01.030.

Heinzel S, Bahr R, Stettner U, Eggel B, Krammer F, et al. Genetically engineered poxviruses for subunit vaccine design. J Virol 1996; 70:8343-56; PMID:887317; http://dx.doi.org/10.1128/JVI.70.17.8343-8356.96.

Monis B. Genetically engineered poxviruses for subunit vaccine design, vaccination and safety. Proc Natl Acad Sci USA 1996; 93:11341-8; PMID:8876137; http://dx.doi.org/10.1073/pnas.93.21.11341.

Eytan D. Applications of recombinant vaccinia virus for vaccine development. J Infect Dis 1994; 169:1501-9; PMID:801647; http://dx.doi.org/10.1093/infdis/169.5.1501.

Kanemoto K, Iwamoto N, Taira A, Tsuchida K, Sato S, Shimaoka J, et al. Safety and immunogenicity of a recombinant vaccinia virus vaccine for cattle pox virus infection. Vaccine 2000; 18:3042-6; PMID:10951502; http://dx.doi.org/10.1016/S0264-410X(00)00570-1.

Kanemoto K, Sato S, Taira A, Tsuchida K, Higaki S, Shimaoka J, et al. Evaluation of cattle pox virus recombinant most-protective antigen vaccine. Vaccine 2000; 18:3047-51; PMID:10951506; http://dx.doi.org/10.1016/S0264-410X(00)00571-3.

Kanemoto K, Sato S, Taira A, Tsuchida K, Higaki S, Shimaoka J, et al. Safety and immunogenicity of a recombinant vaccinia virus vaccine for cattle pox virus infection. Vaccine 2000; 18:3042-6; PMID:10951502; http://dx.doi.org/10.1016/S0264-410X(00)00570-1.

Kanemoto K, Sato S, Taira A, Tsuchida K, Higaki S, Shimaoka J, et al. Evaluation of cattle pox virus recombinant most-protective antigen vaccine. Vaccine 2000; 18:3047-51; PMID:10951506; http://dx.doi.org/10.1016/S0264-410X(00)00571-3.

Kanemoto K, Sato S, Taira A, Tsuchida K, Higaki S, Shimaoka J, et al. Safety and immunogenicity of a recombinant vaccinia virus vaccine for cattle pox virus infection. Vaccine 2000; 18:3042-6; PMID:10951502; http://dx.doi.org/10.1016/S0264-410X(00)00570-1.

Kanemoto K, Sato S, Taira A, Tsuchida K, Higaki S, Shimaoka J, et al. Evaluation of cattle pox virus recombinant most-protective antigen vaccine. Vaccine 2000; 18:3047-51; PMID:10951506; http://dx.doi.org/10.1016/S0264-410X(00)00571-3.

Kanemoto K, Sato S, Taira A, Tsuchida K, Higaki S, Shimaoka J, et al. Safety and immunogenicity of a recombinant vaccinia virus vaccine for cattle pox virus infection. Vaccine 2000; 18:3042-6; PMID:10951502; http://dx.doi.org/10.1016/S0264-410X(00)00570-1.
CJI.0b013e3181f5dac7.

Treasure P. Cross-trial analysis of immunologic and M

Amato RJ, Hawkins RE, et al. MVA-5T4-induced D

Carrasquillo JA, Tang N, et al. Oncolytic vaccinia XD, McCart JA, Gorry MC, et al. Oncolytic viro-

Patel RH, Huang T, Luce TC, Bell J, Kill DIH. The targeted oncolytic positive virus JS-594 demonstrates immunogenicity and therapeutic efficacy in patients with hepatic melanoma. Mol Ther 2006; 16:1573-82. PMID:17069796. http://dx.doi.org/10.1038/sj.embor.7400785.

Liu TC, Huang T, Park RH, Bell J, Kill DIH. The targeted oncolytic positive virus JS-594 induces antitumor effects and antitumor immune responses in patients with metastatic melanoma. Cancer Gene Ther 2006; 13:745-53. PMID:16748765. http://dx.doi.org/10.1038/sj.cgt.7701102.

Kaufman HL, Carroll MW, et al. Vaccination of colorectal cancer patients with MVA-5T4: a randomized, double-blind, placebo-controlled phase III study. Clin Cancer Res 2010; 16:5539-47; PMID:20881001; http://dx.doi.org/10.1158/1078-0432.CCR-09-2336.

Mhawech-Fauceglia P, Miller A, et al. Efficacy of recombinant vaccinia virus directing a clade C HIV vaccine directed gene therapy: biodistribution of a thymi- ne light-emitting oncolytic vaccinia virus. Cancer Res 2005; 65:7733-41. PMID:16250034. http://dx.doi.org/10.1158/0008-5472.CAN-05-2717.

Kapostins EA, Kotlarz DR, Zhang X, et al. Vaccinia virus as a vaccine delivery system for mammalian cells. Proc Natl Acad Sci USA 2011; 108:14926-31. PMID:21698785. http://dx.doi.org/10.1073/pnas.1104155108.

Kapostins EA, Kotlarz DR, Zhang X, et al. Evaluating of solid human breast tumors in mice with an immunologically targeted light-emitting oncolytic vaccinia virus armed with a prostate specific antigen gene. Cancer Gene Ther 2008; 15:157-25. PMID:18085424. http://dx.doi.org/10.1177/1078043208089576.

Baker M, Ahmad S, Nilsson C, Francis J, Bevan D, Mohamed H, et al. Vaccinia virus administration into bone in laboratory and in patients with recurrent metastatic bone disease of canine renal cell carcinoma. Vet Pathol 1988; 25:198-201; PMID:3027401. http://dx.doi.org/10.1177/0300985880250203.

Burny A. Newcastle disease virus F glycoprotein protects mice against lethal challenge with respiratory syncytial virus. Science 1985; 227:433-5; PMID:2981435; http://dx.doi.org/10.1126/science.2981435.

McKee M, Yihua T, Moss B. Vaccinia virus recombinant expressing VSV g gene and inhibition of pneumovirus replication in mice and cell line. Science 1985; 227:435-7; PMID:2981436. http://dx.doi.org/10.1126/science.2981436.

Makosinski L, Beale C, Moss B, Yu M. Vaccinia virus expressing VSV g gene protects mice against lethal pneumovirus infection: comparison of vaccinated and unvaccinated mice for survival. Microb Pathog 1989; 7:66-73; PMID:2695165. http://dx.doi.org/10.1016/0882-4010(89)90009-8.

Liu TC, Huang T, Park RH, Bell J, Kill DIH. The targeted oncolytic positive virus JS-594 induces antitumor effects and antitumor immune responses in patients with metastatic melanoma. Cancer Gene Ther 2006; 13:745-53. PMID:16748765. http://dx.doi.org/10.1038/sj.embor.7400785.

Liu TC, Huang T, Park RH, Bell J, Kill DIH. The targeted oncolytic positive virus JS-594 induces antitumor effects and antitumor immune responses in patients with metastatic melanoma. Cancer Gene Ther 2006; 13:745-53. PMID:16748765. http://dx.doi.org/10.1038/sj.embor.7400785.

Hwang T, Moon A, Bollee J, Risik A, Stanford J, Reibock CJ, et al. A mechanical proof-of-concept clinical trial with JS-594, a targeted multi-mechanistic oncolytic positive, in patients with metastatic malig- noma. Mol Ther 2011; 19:1918-23. PMID:21777223. http://dx.doi.org/10.1038/mt.2011.32.

McIntyre M, Ncube S, Skofield D, Rolly RM, Caragolpillai JA, Tang N, et al. Oncolytic vaccinia virus expressing the human immunoreceptor tyrosine-based activation motif (ITAM) mimics XCI in melanoma cell lines. Cancer Gene Ther 2006; 13:1009-16. PMID:16720466. http://dx.doi.org/10.1177/1078043206062257.

Chakravarti I, Kusum MS, Olladore ME, Eric Dong SD, McCartney GA, Jerry GC, et al. Oncolytic virus infections for oncolytic immunotherapy using a replication-selective oncolytic virus armed with a prostate specific antigen gene. Cancer Gene Ther 2008; 15:157-25. PMID:18085424. http://dx.doi.org/10.1177/1078043208089576.

Zhang Q, Yu YA, Wang E, Chen N, Eumane HH, et al. Oncolytic vaccinia virus for cancer therapy in mice with an immunologically targeted light-emitting oncolytic vaccinia virus. Cancer Gene Ther 2007; 14:630-9. PMID:17047208. http://dx.doi.org/10.1177/1078043207079379.

Gonzalez I, Aldinger M, Jopson R, Radulovich, S. Vaccinia virus as a vaccine delivery system for mammalian cells. J Virol 2012; 86:7439-9. PMID:22595676. http://dx.doi.org/10.1128/JVI.05729-12.

Yu SY, Galvan C, Wei C, Chen N, Zhong Q, Dong Y, et al. Reversion of human pancreatic tumor xenografts in mice after a single epitope injection of recombinant vaccinia virus expressing GS-HH. Mol Ther 2009; 17:536-43. PMID:19435219. http://dx.doi.org/10.1038/sj.atv.6302385.

Yu SY, Traveo-Tarrazona Y, Zhang Q, Bell J, Steiner AS, et al. Oncolytic imaging: viruses, vaccine and mammalian cells encoding light-emitting probes recall the locations of primary tumors and metastases in animals. Adv Small Anim Med 2003; 17:76-82. PMID:12869187. http://dx.doi.org/10.1200/JCO.2003.00.803.

Fleer E, Landowski ES. Comparative Results of Newcastle Disease Virus (NDV) Vaccine Uptake by Degal and Dry-Dose Vaccines (Durvet). J Med Vet Med 1964; 251:1-9. PMID:14161226.

Jones L, Greenwold L, Sulic Z, Brown C, Mihalc C, Yihua T. Protection of mice against the pas des potins immum≧ e cancer with a vaccinia virus double recombinant expressing the F and M genes of smallpox virus. Vaccine 1995; 13:961-4. PMID:7622884. http://dx.doi.org/10.1016/0264-410X(95)00084-C.

McIntyre M, Yihua T, Rose JD, Moss B. Vaccinia virus recombinant expressing VSV g gene and inhibition of pneumovirus replication in mice and cell line. Science 1985; 227:435-7; PMID:2981436. http://dx.doi.org/10.1126/science.2981436.

Makosinski L, Beale C, Moss B, Yu M. Vaccinia virus expressing VSV g gene protects mice against lethal pneumovirus infection: comparison of vaccinated and unvaccinated mice for survival. Microb Pathog 1989; 7:66-73; PMID:2695165. http://dx.doi.org/10.1016/0882-4010(89)90009-8.

Liu TC, Huang T, Park RH, Bell J, Kill DIH. The targeted oncolytic positive virus JS-594 induces antitumor effects and antitumor immune responses in patients with metastatic melanoma. Cancer Gene Ther 2006; 13:745-53. PMID:16748765. http://dx.doi.org/10.1038/sj.embor.7400785.

Liu TC, Huang T, Park RH, Bell J, Kill DIH. The targeted oncolytic positive virus JS-594 induces antitumor effects and antitumor immune responses in patients with metastatic melanoma. Cancer Gene Ther 2006; 13:745-53. PMID:16748765. http://dx.doi.org/10.1038/sj.embor.7400785.
92. Goepfert PA, Elango ML, Sun A, Qin L, Cardinale M, Hay CM, et al., National Institute of Allergy and Infectious Diseases HIV Vaccine Trials Network. Phase 1 safety and immunogenicity testing of DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 env-like particles. J Infect Dis 2011; 203:610-9; PMID:21282192; http://dx.doi.org/10.1093/infdis/jiq285.

93. Berthoud TK, Handel M, Lillo PJ, Hvedegaard L, Collins RA, Everly JI, et al. Potential CD8+ T-cell immunogenicity in humans of a novel heterodimeric influenza A vaccine, MVA-NP-M1. Clin Infect Dis 2011; 52:1-7; PMID:21495142; http://dx.doi.org/10.1093/cid/cir117.

94. Cavanaugh B, Ani D, Mehta M, HAE AV, Wrack H, McConkey SJ. Partially randomized, non-blinded trial of DNA and MVA therapeutic vaccine based on hepatitis B virus surface protein for chronic HBV infection. PLoS One 2011; 6:14626; PMID:21347224; http://dx.doi.org/10.1371/journal.pone.0014626.