Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER FIVE

Modified Vaccinia Virus Ankara: History, Value in Basic Research, and Current Perspectives for Vaccine Development

A. Volz, G. Sutter[1]
German Center for Infection Research (DZIF), Institute for Infectious Diseases and Zoonoses, LMU University of Munich, Munich, Germany
[1]Corresponding author: e-mail address: gerd.sutter@lmu.de

Contents

Abstract

Safety tested Modified Vaccinia virus Ankara (MVA) is licensed as third-generation vaccine against smallpox and serves as a potent vector system for development of new candidate vaccines against infectious diseases and cancer. Historically, MVA was developed by serial tissue culture passage in primary chicken cells of vaccinia virus strain Ankara, and clinically used to avoid the undesirable side effects of conventional smallpox vaccination. Adapted to growth in avian cells MVA lost the ability to replicate in mammalian hosts and lacks many of the genes orthopoxviruses use to conquer their

Advances in Virus Research, Volume 97
ISSN 0065-3527
http://dx.doi.org/10.1016/bs.aivir.2016.07.001

host (cell) environment. As a biologically well-characterized mutant virus, MVA facilitates fundamental research to elucidate the functions of poxvirus host-interaction factors. As extremely safe viral vectors MVA vaccines have been found immunogenic and protective in various preclinical infection models. Multiple recombinant MVA currently undergo clinical testing for vaccination against human immunodeficiency viruses, *Mycobacterium tuberculosis* or *Plasmodium falciparum*. The versatility of the MVA vector vaccine platform is readily demonstrated by the swift development of experimental vaccines for immunization against emerging infections such as the Middle East Respiratory Syndrome. Recent advances include promising results from the clinical testing of recombinant MVA-producing antigens of highly pathogenic avian influenza virus H5N1 or Ebola virus. This review summarizes our current knowledge about MVA as a unique strain of vaccinia virus, and discusses the prospects of exploiting this virus as research tool in poxvirus biology or as safe viral vector vaccine to challenge existing and future bottlenecks in vaccinology.

1. INTRODUCTION

Poxviruses engineered to express foreign gene products are established tools for the development of novel vaccines and therapeutics in biomedical research. Large packaging capacity for heterologous DNA, strict virus-specific control of recombinant gene expression, lack of virus persistence in the host, immunogenicity and efficacy as vaccine, and ease of vector and vaccine production were important contributors to this success story. Concerns about the safety of conventional vaccinia viruses as smallpox vaccine have been addressed by the study of replication defective viruses unable to produce infectious progeny in human cells. Today, the highly attenuated vaccinia virus strain MVA can be considered as one of the vaccine viruses of choice in preclinical and clinical research. MVA is replication-deficient in cells of mammalian origin and fails to produce many of the virulence factors encoded by conventional vaccinia virus. Because of its safety for the general environment MVA can be handled under conditions of biosafety level 1 (BSL-1). Nonreplicating MVA can enter any target cell and activate its molecular life cycle to express all classes of viral and recombinant genes. Therefore, recombinant MVA have been established as an extremely safe and efficient viral vector system for basic research and for the development of vaccines suitable for industrial scale production. Here, we review the development of MVA as product from serial tissue culture passage in chicken embryo fibroblasts (CEF), its key biological properties, and recent accomplishments in vaccine research using recombinant MVA.

2. HISTORY AND DEVELOPMENT OF MVA

2.1 Ancestry and Generation by Serial Passage

Modified Vaccinia virus Ankara (MVA) was developed by serial passage in chicken fibroblast tissue culture to serve as safer vaccine during the last years of the WHO smallpox eradication campaign. Its ancestor virus is the vaccinia virus strain Ankara which was originally propagated on the skin of calves and donkeys at the Turkish vaccine institute in Ankara for smallpox vaccine production. In 1953, the vaccinia virus strain Ankara was brought to Munich and added to the strain collection of the Institute for Infectious Diseases and Tropical Medicine at the University of Munich. Herrlich and Mayr cultivated the virus on the chorioallantois membranes (CAM) of embryonated chicken eggs and therefore named it as Chorioallantois Vaccinia virus Ankara (CVA) (Herrlich and Mayr, 1954). At the Bayerische Landesimpfanstalt München (Bavarian State Institute for Vaccines), CVA was grown on the skin of calves to manufacture smallpox vaccine for the vaccination campaigns in Munich in 1954/1955. In addition, at the University of Munich, CVA was tested in passage experiments in various tissue cultures to study the genetic stability and the evolution of orthopoxviruses. Mayr and Munz (1964) reported that 371 passages of CVA in primary CEF had resulted in the development of an infection phenotype with restricted host (cell) tropism and it was discussed that similar biological properties were known from poxviruses that are highly adapted to specific hosts, e.g., variola virus (VARV) to humans or fowlpox virus to chicken. Successive passage of vaccinia virus in minced chicken embryo tissue had been described as successful strategy for in vitro amplification of the smallpox vaccine virus in a culture system (Rivers and Ward, 1931, 1933). The serial passage of CVA in CEF was further continued by Anton Mayr and colleagues and, in 1968 after the 516th passage on CEF, the virus was renamed Modifiziertes Vakziniavirus Ankara (MVA) and provided to the Bavarian State Institute for Vaccines to test its suitability for smallpox vaccine production (Stickl and Hochstein-Mintzel, 1971).

2.2 Early Characterization of Biological Properties

Phenotypic changes relating to the repeated passage of the CVA virus in CEF cultures were first observed upon infection of the embryonated egg.

For many years, CAM inoculations were the gold-standard experimental system for the phenotypic study of various poxviruses (Goodpasture et al., 1931, 1932; Mayr et al., 1955). MVA infection is characterized by the formation of small proliferative lesions on the CAM. In contrast, considerably larger CAM lesions with variable size areas of central necrosis are typically found with CVA or other conventional vaccinia viruses (Herrlich and Mayr, 1954). Interestingly, the CAM lesions of MVA were noted to closely resemble those induced by variola or fowlpox viruses following egg inoculation (Mayr and Munz, 1964; Stickl and Hochstein-Mintzel, 1971). In addition, it was observed early on that MVA had lost the capacity of vaccinia virus to cause prominent cytopathic effects and/or to form plaques in first-generation tissue cultures such as CEF, primary bovine, or porcine kidney cells, or human HeLa cells (Mayr and Munz, 1964). The most characteristic changes in the in vivo behavior of the virus were reported from experimental inoculations of rabbits (German Great White) (Mayr et al., 1975; Stickl and Hochstein-Mintzel, 1971). Intradermal infections or cutaneous infections by scarification with conventional vaccinia virus (VACV) result in the formation of typical skin lesions. Such lesions were totally absent following inoculation with MVA suggesting a substantial loss of virulence upon in vivo infection. These data were confirmed by the finding that newborn (strain NMRI; 1–3 days old) or adult mice (12–15 g) survived intracerebral inoculations of MVA at doses that resulted in 100% mortality following CVA infection (Mayr et al., 1975; Stickl and Hochstein-Mintzel, 1971). The inability of MVA to induce primary reactions with pock lesions forming upon intradermal or cutaneous inoculation was also confirmed in the cynomolgus monkey model (*Macacus fascicularis*). Macaques tolerated intracranial inoculations with MVA without obvious adverse effects, whereas animals injected with CVA developed severe systemic disease. Moreover, intradermal or intramuscular vaccination with about 2×10^5 infectious units (IU) of MVA vaccine protected macaques from severe disease following intravenous challenge with VARV strain Madras 1965 (Mayr et al., 1975; Stickl and Hochstein-Mintzel, 1971). These data from early preclinical characterization in laboratory animals already suggested that MVA had maintained immunogenicity as vaccine but demonstrated a dramatic loss of virulence. Upon the 516th CEF passage the virus was renamed MVA and transferred to the Bavarian State Vaccine Institute in Munich for evaluation as safer smallpox vaccine in clinical trials.

2.3 Early Use as Safe Vaccine Against Smallpox

The first MVA vaccine preparation to be tested in humans was produced in CEF cultures and contained 10^6 IU MVA/mL (Stickl and Hochstein-Mintzel, 1971). In first attempts, the use of this vaccine by scarification failed to induce any kind of skin reactions and the application by intracutaneous inoculation (0.2 mL vaccine suspension containing 2×10^5 IU MVA) was chosen for first clinical testing and primary vaccination of 107 individuals aged 2–38 years (Stickl and Hochstein-Mintzel, 1971). After 4–6 days, minor local reactions developed at the site of injection with redness and swelling of the skin of the forearm. However, the investigators observed no pock lesions or other symptoms normally associated with smallpox vaccination and none of the 107 individuals developed fever (body temperature $\geq$38 °C). Interestingly, it was noted that the MVA application failed to induce circulation antibodies that inhibited the hemagglutination by VACV. Thus, the efficacy of immunization was tested with secondary vaccination by scarification using VACV strain Elstree in 64 of the 107 individuals that had received MVA for primary vaccination. Skin reactions typical for follow-up smallpox vaccinations were noted in 62 of 64 patients and suggested a successful primary immunization with the MVA vaccine. These data supported further clinical development of MVA as smallpox vaccine and, following the testing in more than 7000 patients (Stickl et al., 1974), the Bavarian State Vaccine Institute in Munich obtained the first marketing authorization for MVA as primary prevaccine against smallpox in Germany in 1977 (Paul-Ehrlich-Institut, 31.01.1977). Until 1980, the MVA smallpox vaccine was given to more than 120,000 humans without documentation of severe adverse events otherwise associated with the use of conventional VACV vaccines (Mahnel and Mayr, 1994). Immunizations with this first licensed MVA vaccine stopped with the end of the smallpox vaccination program in Germany.

2.4 The Genome of MVA

Only a few years later, targeted genetic modification of the vaccinia virus genome became possible and the concept to generate recombinant viruses for gene expression or vaccination also restored interest in MVA. Restriction mapping of the MVA genome was based on the clonal virus isolate F6 from the 572nd CEF passage of MVA and revealed alterations in the MVA genome that are the likely genetic basis for attenuation and growth

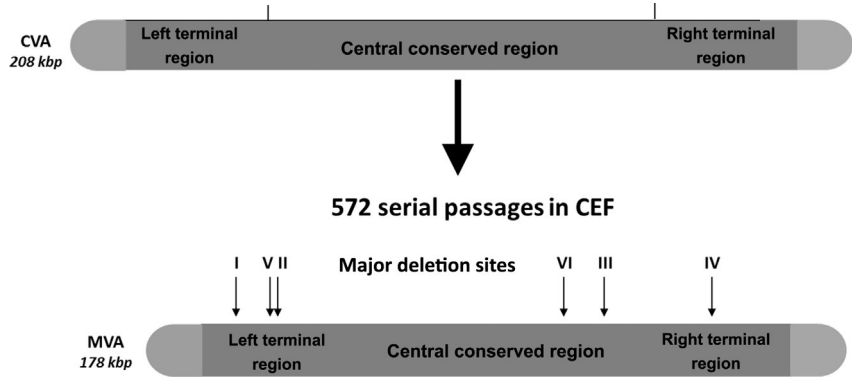

Fig. 1 Generation of Modified Vaccinia virus Ankara (MVA) from ancestor virus Chorioallantois Vaccinia virus Ankara (CVA): Continuous serial passages of CVA in CEF resulted in significant loss of genetic information (~30 kb) in the genome of MVA. as demonstrated by the occurrence of by six major deletions (I–VI) relative to the CVA genome.

restriction (Meyer et al., 1991). Comparison to the genome maps of CVA ancestor viruses revealed that the MVA genome harbors large deletions and mutations affecting many genes with functions in virus–host interaction (Fig. 1). First important observations included (i) the failure to rescue productive MVA growth in cells of human origin by restoring the VACV host range gene K1L despite presence of the second VACV host range gene *C7L* with the MVA genome; (ii) the absence of the gene for the major VACV A-type inclusion body protein and the lack of this protein among the polypeptides made in MVA-infected cells; (iii) the demonstration that the coding sequences of the VACV hemagglutinin (HA) started right adjacent to deletion III within the MVA genome which a few years later allowed to identify the truncation of the HA promoter sequence explaining for the HA-negative phenotype of MVA and the inability to detect HA-specific antibodies upon MVA immunization (Antoine et al., 1996). Notably, these early findings were further complemented by the elucidation of the full-length sequence of the MVA genome (Antoine et al., 1998) and the discovery that MVA lacks important immunomodulatory genes (Blanchard et al., 1998).

2.5 First Recombinant MVA

The highly attenuated phenotype of the virus also encouraged the evaluation of MVA as an expression vector. Yet, MVA cannot productively replicate in most cells of mammalian origin and high-level expression of recombinant

genes was doubtful because other host range mutants of VACV are inhibited already early in their life cycle (Drillien et al., 1978, 1981). The coding sequences for the *Escherichia coli* enzymes β-galactosidase and guanine-phosphoribosyl-transferase served as the first heterologous genes to be inserted and expressed at the site of deletion III in the MVA genome (Sutter and Moss, 1992). Surprisingly, the production of early and late viral proteins turned out to be unimpaired in MVA-infected human cells (Fig. 2). Indeed, the unique ability to efficiently express viral and recombinant genes supported its general application as exceptionally safe viral vector. To ascertain whether MVA vectors would be applicable for immunization experiments with recombinant antigens, a first MVA vector vaccine was constructed and tested that simultaneously expressed the HA and nucleoprotein (NP) genes of influenza A virus (Sutter et al., 1994). The recombinant

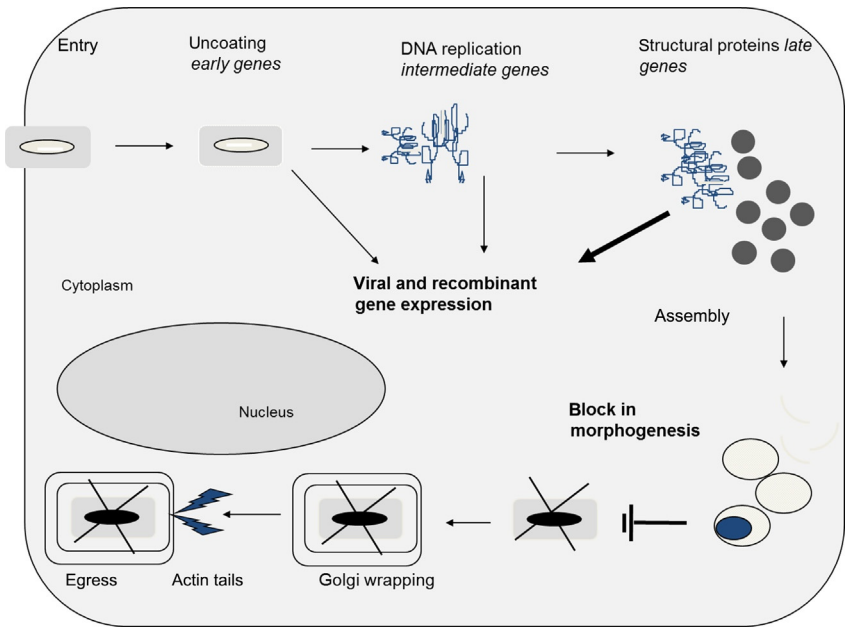

Fig. 2 Schematic representation of the nonpermissive life cycle of MVA in cells of mammalian origin. While MVA does not productively replicate in most mammalian cells, it can efficiently enter any cell and start its cascade-like life cycle resulting in unimpaired expression of viral early and intermediate genes, synthesis of viral genomic DNA, and the abundant expression of viral late genes. Thus, foreign and MVA proteins are efficiently produced and the block of the MVA life cycle occurs at the step of virion assembly resulting in assembly of immature virus particles that are not released from the infected cell.

MVA was found to be immunogenic, since vaccination of mice by various routes resulted in high levels of serum antibodies that inhibited hemagglutination by influenza A virus. Moreover, the vector vaccine also elicited strong cytotoxic T cell responses directed to both influenza virus proteins. Importantly, animals could be completely protected against a lethal respiratory tract influenza challenge following single inoculations with relatively low doses of the recombinant MVA vaccine. To generate these first MVA vectors, the foreign gene sequences were targeted precisely to the site of the naturally existing deletion III in the MVA genome. This strategy in designing the vector was to avoid unnecessary changes in the genotype and phenotype of the resulting recombinant MVA. The development of other recombinant MVA vaccines with heterologous genes from simian immunodeficiency virus (SIV) or parainfluenza virus 3 inserted in deletion III rapidly followed (Hirsch et al., 1996; Wyatt et al., 1996). Also, the natural deletion II within the MVA genome was successfully used as insertion site to express recombinant bacteriophage T7 RNA polymerase (Sutter et al., 1995). In addition, the thymidine kinase locus, a well-exploited insertion site in replication-competent VACV vectors, likewise, served to generate recombinant MVA delivering antigens of HIV-1 or *Plasmodium berghei* (Hanke et al., 1998; Schneider et al., 1998).

3. MVA AS TOOL FOR RESEARCH IN POXVIRUS BIOLOGY

Poxviruses including the prototype orthopoxvirus VACV are excellent models to study virus–host interactions. VACV can efficiently antagonize the activity of interferons (IFNs), cytokines, chemokines, and innate immune signaling (for review, see Smith et al., 2013). Hereby, VACV exploits the expression of soluble-binding proteins and receptor antagonists. Other viral immune evasion proteins work intracellularly to inhibit apoptosis or to interfere with host signaling pathways activating antiviral immune mechanisms. Notably, of the many VACV genes involved in immune evasion, most are inactivated or truncated in the MVA genome (Antoine et al., 1998). Interestingly, early studies by Mayr and coworkers had suggested distinct immune stimulatory activities associated with experimental MVA inoculations (Mayr et al., 1975). E.g., intraperitoneal application of virus to mice enhanced the in vivo clearance of carbon marker particles from the blood of the animals suggesting an increased phagocytic activity of immune cells 2 days after MVA inoculation. In addition, intranasal delivery of MVA to rabbits resulted within hours in the induction of "serum

interferons" that efficiently inhibited sindbis virus replication in an in vitro infection assay. In the more recent past, various studies further elucidated the mechanisms of MVA-mediated induction of type I IFNs following in vitro and in vivo infections (Blanchard et al., 1998; Dai et al., 2014; Delaloye et al., 2009; Ishii et al., 2006; Waibler et al., 2007, 2009). Indeed, the lack of many immune evasion factors and the failure of MVA infection to interfere early with key host (cell) defence mechanisms may explain the severe growth restriction of MVA, its high attenuation upon in vivo infection and its immunogenicity when used as vaccine. Moreover, due to the extensive loss of genetic information MVA may serve as a particularly useful tool to study VACV host-regulatory genes. Obviously, there are several pathways of the host (cell) defence that are targeted by multiple VACV proteins and the phenotype of a VACV single gene mutant may be masked by the complementary function of (an)other viral gene(s) (Dobson and Tscharke, 2015). There are three obvious strategies using MVA to elucidate on functions of selected VACV host-interaction factors: (i) the few remaining regulatory genes in the MVA genome can be targeted for inactivation, (ii) candidate VACV genes missing in MVA are reinserted in the genome to rescue a host-interaction phenotype, or (iii) a combination of these latter approaches may serve to investigate putatively complementary gene functions. In the following we describe some principles learnt by MVA research that may help to better understand the regulation of virus–host interactions and likely influence safety and immunogenicity of VACV-based vaccines and therapeutics (Fig. 3).

3.1 MVA Genes Regulating the Host Cell Tropism

One most striking feature of MVA is its inability to replicate in most cells of mammalian origin (Carroll and Moss, 1997; Drexler et al., 1998; Meyer et al., 1991). In contrast, wild-type VACV has a broad cellular host range and productively infects various cell substrates (Drillien et al., 1978). It is noteworthy that poxvirus infections do not rely on the availability of specific cellular receptors but the viruses can efficiently bind to and enter many different cells from diverse animal species. After entry, however, the success of VACV replication depends on the functional activity of a subset of viral genes, the so-called "host range" genes (for review, see Bratke et al., 2013; Haller et al., 2014; McFadden, 2005). These viral genes encode regulatory proteins that control the intracellular host defence, e.g. by manipulation of the sensing of viral pathogen associated molecular patterns, the

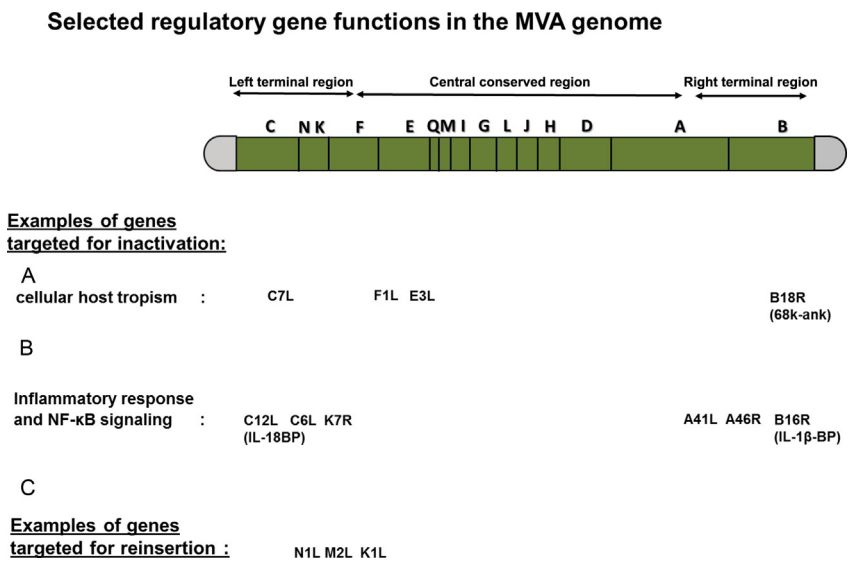

Fig. 3 Selected host-regulatory genes functionally maintained in the MVA genome: a schematic representation of the MVA genome is shown on the *top*. (A) Viral genes regulating the intracellular host tropism (host range genes) include the open reading frames C7L, F1L, E3L, and B18R. (B) Viral inhibitors of host inflammatory responses and host NF-kB signaling are highlighted by the MVA regulatory genes C12L, C6L, K7R, A41L, A46R, and B16R. (C) VACV N1L, M2L, and K1L genes are deleted or truncated in the MVA genome. Functional repair of these genes in the MVA genome may serve as example for the use of MVA in research addressing the regulation of VACV immune evasion or host tropism.

signal transduction, the cell cycle, or the onset of programmed cell death. Some eminent viral regulators of intracellular host tropism are still functional in the MVA genome and include the proteins C7, K3, E3, F1, and B18 (68K) (following the nomenclature as established for the genome of VACV strain Copenhagen; Goebel et al., 1990).

The VACV genes *K1L* and *C7L* (encoding the VACV proteins K1 and C7) are known to control virus replication in mammalian cells and both gene functions need to be inactivated to restrict the growth of wild-type VACV (Gillard et al., 1986; Perkus et al., 1990). Already early work by Drillien and coworkers suggested that the replication defect of a VACV host range mutant (in the absence of *K1L* and *C7L*) is established at the level of viral gene expression (Drillien et al., 1981). However, the severe growth defect of the highly attenuated MVA is not rescued when its genome is engineered to contain functional copies of both *K1L* and *C7L* suggesting

that additional viral factor(s) await discovery (Backes et al., 2010; Meyer et al., 1991; Sutter et al., 1994). However, a *C7L*-deleted MVA lacks late gene expression in human and murine cells and induces phosphorylation of eukaryotic translation initiation factor 2α (eIF2α). The deficiency of late gene expression and the phosphorylation of eIF2α in the absence of C7 can be prevented by K1 as shown by the reinsertion of the *K1L* gene into the genome of *C7L*-deleted MVA (Backes et al., 2010).The complementary function of *K1L* and *C7L* is intriguing because the two genes and encoded proteins are unrelated in sequence. Interestingly, recent studies identified the human genes SAMD9 (sterile alpha motif domain-containing 9) and WDR6 (tryptophan-aspartic acid repeat 6) as host restriction factors for poxviruses and as targets of C7 and K1 (Liu and McFadden, 2015; Sivan et al., 2015). To date, rather little is known about the molecular functions of SAMD9 and WDR6, but it can be assumed that they act in (an) innate antiviral defense pathway(s) awaiting further elucidation.

The viral proteins K3 and E3 (encoded by *K3L* and *E3L* genes) are well-known intracellular inhibitors of IFN-induced antiviral activities of the host cell (Chang et al., 1992; Davies et al., 1992). K3 has homology to the alpha subunit of eIF2α and serves as a pseudo-substrate for double-stranded RNA (dsRNA)-activated protein kinase (PKR) to prevent virus-induced phosphorylation of eIF2α by PKR. In turn, E3 can sequester dsRNA to inhibit the stimulation of PKR and to prevent activation of 2′-5′ oligoadenylate synthase and the endoribonuclease RNaseL. In addition, E3 has been described to independently inhibit the phosphorylation of the transcription factors IRF-3 and IRF-7 and thus to block the production of type I IFNs (Smith et al., 2001; Xiang et al., 2002). These IFNs evasion mechanisms provided by K3 and E3 substantially contribute to the broad cellular host range of VACV and numerous host range phenotypes of VACV mutants lacking *E3L* or *K3L* genes have been found (Werden et al., 2008). Interestingly, CEF-adapted MVA has maintained fully active *E3L* and *K3L* sequences. Indeed, a functional *E3L* gene is required for MVA replication in CEF where E3 functions as an inhibitor of apoptosis and/or IFN induction to allow for unimpaired late protein synthesis (Hornemann et al., 2003). In human HeLa, HaCat or 293T cells studies with an *E3L*-deleted MVA mutant (MVA-ΔE3L) revealed that E3 is also essential to secure viral intermediate and late protein synthesis by counteracting host cell-specific activation levels of antiviral pathways PKR or RNaseL (Ludwig et al., 2005, 2006). MVA-ΔE3L also increased the type I IFN and/or chemokine production when compared to wild-type MVA in infections of primary murine

fibroblasts or murine bone marrow-derived dendritic cells which is in agreement with the known activation of transcription factors IRF-3 and IRF-7 in the absence of E3 (Dai et al., 2014; Ishii et al., 2006). Likewise, apoptosis induction in MVA-ΔE3L infected murine fibroblasts required the proapoptotic cellular BH3-only protein Noxa which is well in line with the IRF-3/IFN-beta-mediated activation of Noxa (Delaloye et al., 2009; Fischer et al., 2005).

Cell death by apoptosis is an important defence mechanism to protect the host against viral infection. In turn, poxviruses have evolved specific viral proteins to inhibit the onset of programmed cell death and these regulatory proteins are relevant determinants of virus tropism at the cellular level (Taylor and Barry, 2006). The VACV F1 protein (encoding gene *F1L*) is an antiapoptotic protein with Bcl-2-like structure proposed to block apoptosis by binding to the proapoptotic family protein Bak (Postigo et al., 2006; Wasilenko et al., 2003, 2005). *F1L* is also conserved in the MVA genome and *F1L*-deficient MVA (MVA-ΔF1L) induces enhanced apoptosis in HeLa cells and in mouse embryonic fibroblasts (Fischer et al., 2005). Additional work with MVA-ΔF1L demonstrated that triggering of apoptosis predominantly requires the induction of the pro-apoptotic BH3-only protein Noxa which then activates proapoptotic Bak (Ferrer et al., 2011). Hereby, activation of Noxa was linked to the recognition of viral RNA and the upregulation of type I IFN signaling. Thus, the Noxa-dependent induction of apoptosis observed upon infections with MVA-ΔE3L (Fischer et al., 2005) may be explained by the failure to sequester viral RNA in the absence of E3 resulting in strong activation of pro-apoptotic Noxa (Fig. 4). Ankyrin repeat (ANK) motifs are found in many poxvirus regulatory proteins that determine the cellular host tropism and/or counteract antiviral host responses controlled by NF-kB signaling (Herbert et al., 2015). Otherwise ANK motifs are described important for many protein–protein interactions and cellular ANK proteins are involved in diverse regulatory tasks including cellular transcription, cell cycle control, or cellular differentiation (Mosavi et al., 2004). The only ANK-containing protein encoded by the MVA genome is the 68-kDa Ankyrin-like protein (68k-ank; homologue to the VACV Cop *B18R* gene product). In addition to ANKs, 68k-ank contains an F-box-like PRANC (pox protein repeats of Ankyrin C-terminal) domain (Mercer et al., 2005). MVA 68k-ank binds to cellular Skp1a and forms a Cullin-1-based SCF ubiquitin ligase complex in an F-box-dependent manner (Sperling et al., 2008). A 68k-ank-deficient MVA mutant shows reduced transcription of intermediate and late viral

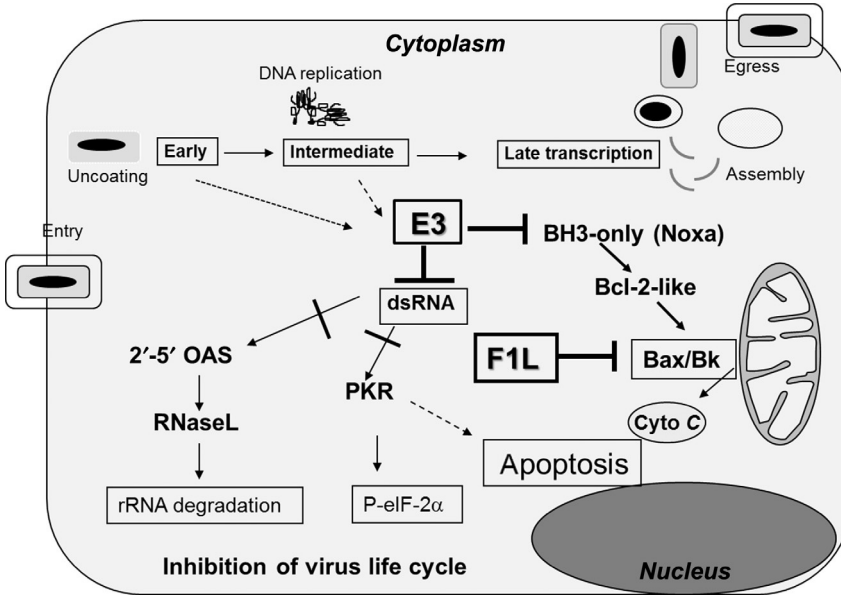

Fig. 4 Role of the MVA regulatory proteins E3 and F1 within the intracellular virus life cycle: E3 can efficiently sequester dsRNA produced during infection to counteract the activation of the antiviral host enzymes OAS (2′-5′ oligoadenylate synthetase)/RNaseL (Ribonuclease L) or PKR (Protein kinase RNA-activated). Additional functions of the E3 protein are to prevent the activation of the host proapoptotic protein Noxa and to block the phosphorylation of the transcription factors IRF-3 and IRF-7 inhibiting the production of type I interferons. The MVA protein F1 can bind or prevent the activation of proapoptotic host proteins Bax/Bak or Noxa and acts as efficient inhibitor of cytochrome *C* release (Cyto *C*) and apoptosis induction at the level of host cell mitochondria.

genes and suffers from a drastically impaired late protein synthesis in human and murine cells (Sperling et al., 2009). Thus, 68k-ank is essential for the completion of the MVA molecular life cycle. Interestingly, the F-box domain of 68k-ank is dispensable for rescue of the 68k-ank mutant phenotype suggesting multiple activities of this host-regulatory MVA protein.

MVA is intensely used as vector vaccine being tested against a variety of infectious diseases (see Section 4) and some cancers. However, we still know very little about the influence on MVA vaccine immunogenicity and vaccine efficacy with regard to these MVA proteins regulating the host cell tropism. Indeed, modulating the functional activity of these proteins could result in quite different outcomes. In one scenario, the inactivation of these regulators may further enhance MVA-mediated activation of the innate immune system and may lead to improved protective immunity; another possible scenario is reduced MVA vaccine efficacy because

the deletion of some of these genes (e.g., *E3L*, *C7L*, *68k-ank*) may drastically impair viral replication, the levels of gene expression and MVA-mediated antigen synthesis

3.2 MVA Genes Regulating Inflammatory Response and Immunogenicity

In addition to regulatory genes targeting the host tropism, the MVA genome also contains a number of genes with known immunomodulatory functions. One interesting example is gene *B16R* encoding the VACV interleukin-1β receptor protein (IL-1βR). This viral cytokine-binding protein has high-specific affinity for IL-1β (Alcami and Smith, 1992; Spriggs et al., 1992) and is thought to play an important role in the regulation of inflammatory responses following VACV infection (Alcamí and Smith, 1996). Synthesis of IL-1βR could be demonstrated in various MVA-infected cultured cells (Blanchard et al., 1998; Zimmerling et al., 2013). Interestingly, primary murine myeloid dendritic cells are important IL-1β producer cells upon MVA infection but free IL-1β can be detected only in the absence of IL-1βR using the deletion mutant MVA-ΔIL-1βR. Immunizations with this MVA deletion mutant led to significantly enhanced virus-specific CD8+ T-cell responses and increased protective capacity against lethal challenge infection with virulent VACV strain Western Reserve (WR) (Staib et al., 2005). In addition, the gene sequence encoding the VACV interleukin-18 (IL-18) binding protein (IL-18BP; *C12L* gene) is also functionally retained in the MVA genome (Smith et al., 2000). VACV IL-18BP binds soluble IL-18 and prevents it to reach its cellular receptor targeting the proinflammatory and antiviral function(s) of this cytokine (Born et al., 2000; Calderara et al., 2001; Symons et al., 2002). The possibility to enhance vaccine immunogenicity by inactivation of the IL-18BP gene in MVA was investigated in two previous vaccination studies in mice. Cottingham and coworkers found no significant difference in VACV-specific T cell responses when comparing vaccines based on MVA mutated in *C12L* or wild-type MVA (Cottingham et al., 2008). A second study demonstrated functional activity of the MVA IL-18BP and immunizations with the MVA deletion mutant increased VACV epitope-specific CD8+ and CD4+ T-cell responses and protective capacity against a VACV challenge infection (Falivene et al., 2012). To diminish the inflammatory host response, another strategy of VACV is the production of secreted viral chemokine receptor proteins which prevent the chemokine-mediated recruitment of leukocytes (Fig. 5) (Alcamí et al., 1998; Graham et al., 1997; Ng et al., 2003). Interestingly, MVA has lost

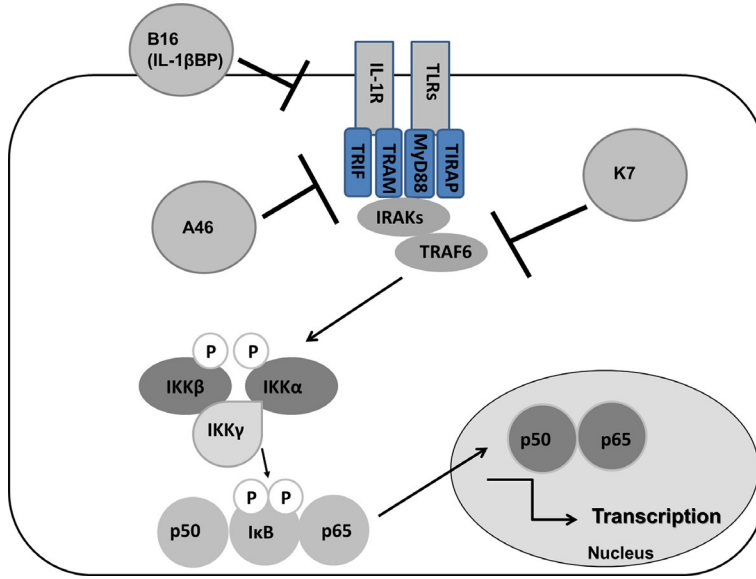

Fig. 5 Remaining MVA inhibitory proteins targeting the innate NF-kB signaling pathway by MVA regulatory proteins: *IRAKs*, interleukin-1R-associated kinases; *TRAF*, TIR-domain-containing adapter-inducing interferon-β; *IKK*, inhibitor of nuclear factor kappa-B kinase; *NF-kB*, nuclear factor kappa-B; *TRIF*, TIR-domain-containing adapter-inducing interferon-β; *TRAM*, TRIF-related adaptor molecule; *TIRAP*, Toll-interleukin 1 receptor (TIR) domain-containing adapter protein; *MyD88*, myeloid differentiation primary response gene 88; *IL-1R*, interleukin-1 receptor; *TLRs*, Toll-like receptors.

much of this VACV immune evasion activity. MVA efficiently induces chemotaxis and triggers the rapid immigration of leukocytes to the site of in vivo inoculation (Lehmann et al., 2009). Yet, MVA still produces the secreted protein A41 which binds chemokines with relatively low affinity suggesting another functional mechanism than just blocking the binding of chemokines to their cellular receptors (Bahar et al., 2008). Moreover, deletion of the *A41L* gene in MVA improved the CD8+ T-cell immunogenicity and the protective capacity of vaccination (Clark et al., 2006).

VACV encodes an impressive variety of intracellular virus proteins that can inhibit the host signaling pathways for NF-kB and IRF-3 (for review, see Smith et al., 2013). Together these proteins serve to dampen the innate host response by blocking the induction of type I IFNs, chemokines, and proinflammatory cytokines. MVA fails to functionally produce a substantial number of these inhibitory proteins including A52, B14, C4, C16, K1, M2, and N1. Indeed, MVA infection induces the activation of NF-kB (Oie and Pickup, 2001) and the reinsertion of the original VACV gene sequences into

the MVA genome allowed to further elucidate the function of K1 and M2 as NF-kB inhibitors (Hinthong et al., 2008; Shisler and Jin, 2004). Other inhibitory genes, such as *C6L*, *K7R*, *A46R*, and *A49R*, are fully conserved in MVA. Interestingly, the removal of *C6L*, *K7R*, or *A46R* from the MVA genome is reported to contribute to higher frequencies of antigen-specific CD8+ and CD4+ T cells, enhanced polyfunctionality of T cells and higher antigen-specific antibody titers when tested in recombinant MVA-producing HIV candidate antigens (Garber et al., 2009; García-Arriaza et al., 2011, 2013). A similar improved immunogenicity is also described for a recombinant MVA-HIV virus deleted in the *N2L* gene (García-Arriaza et al., 2014). These data suggest the functional activity of MVA N2 despite the fact that the MVA gene harbors an in-frame deletion causing the loss of five amino acids (aa 31–35). VACV N2 is an interesting regulatory protein because it acts as inhibitor of IRF-3 activity within the nucleus of infected cells (Fig. 6) (Ferguson et al., 2013).

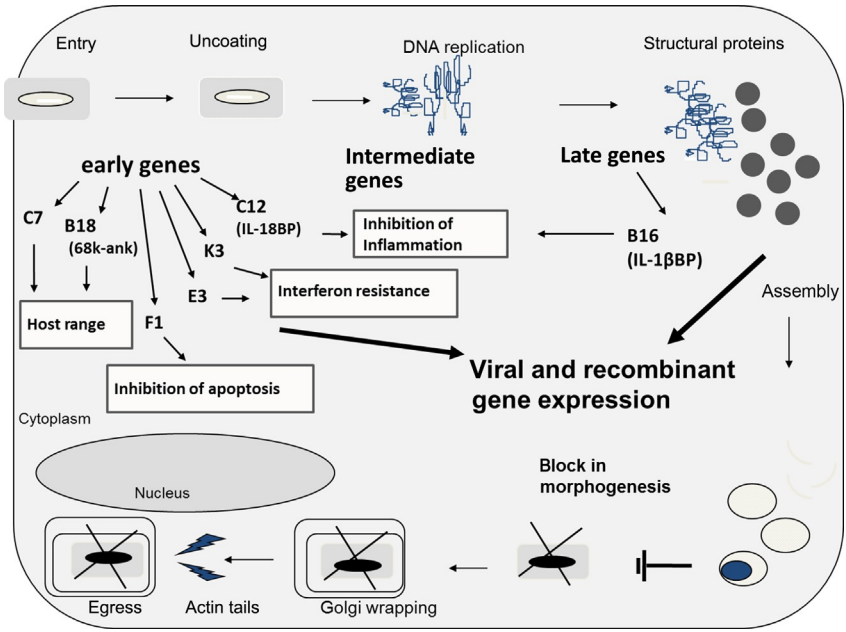

Fig. 6 Schematic overview depicting the synthesis of MVA regulatory proteins involved in modulation of the host response: Most of these host-regulatory proteins are produced early during MVA infection associated with the rapid intracellular activation of innate host responses including the induction of apoptosis, interferons and interferon stimulated gene products or inflammatory cytokines. Interestingly, a few viral inhibitory proteins, such as the viral receptor of IL-1β (B16), are produced at late times of infection suggesting a predominantly extracellular function of these inhibitors of the host response.

4. RECOMBINANT MVA VACCINES AGAINST INFECTIOUS DISEASES

4.1 Vector Technologies and Requirements for Clinical Use

Shortly after the eradication of smallpox, VACV acquired a new mission as eukaryotic cloning vector for the expression of heterologous genes. Homologous recombination that frequently occurs between VACV genomes within an infected cell was discovered to allow for efficient insertion of foreign DNA into the VACV genome (Mackett et al., 1982; Panicali and Paoletti, 1982; Wittek and Moss, 1980; Wittek et al., 1980). This technology enabled the development of new recombinant vaccines that could be beneficial to decrease the impact of various infectious diseases. Over the years replication-competent recombinant VACV has been successfully introduced for use in veterinary medicine as accomplished with the recombinant VACV vaccines against rabies (Blancou et al., 1986; Esposito et al., 1987; Kieny et al., 1984). The application of recombinant VACV for vaccination of humans was lagging behind restricted due to the well-known side effects of VACV smallpox vaccines. In these circumstances the availability of replication-deficient VACV such as NYVAC or MVA has spurred the establishment of exceptionally safe next-generation poxviral vectors (Sutter and Moss, 1992; Tartaglia, 1992). The technology for recombinant MVA has been adapted from VACV. The still most frequently and effectively practiced strategy to generate recombinant poxviruses employs homologous DNA recombination in infected cells, a relatively frequent event during VACV genome replication (0.1%) (Mackett et al., 1984; Nakano et al., 1982). Recombination is typically directed by a gene transfer plasmid. For transcriptional control of foreign genes, various virus-specific promoters are used to achieve moderate to strong expression during the whole virus replication cycle (Baldick et al., 1992; Chakrabarti et al., 1985; Davison and Moss, 1989; Di Pilato et al., 2013, 2015; Wennier et al., 2013; Wyatt et al., 1996). Usually, the plasmid carries the specific expression unit with a virus-specific promoter next to the multiple cloning site for insertion of various foreign gene sequences and selectable markers to facilitate the clonal isolation of recombinant MVA (Mackett et al., 1984; Staib et al., 2004). The foreign gene sequences and the marker gene are flanked on both sides by genomic MVA sequences that direct the recombination of the expression cassette to favored loci in nonessential regions of the MVA genome. In MVA, the sites of major deletions are suited for the insertion

of foreign gene sequences without affecting essential regions in the MVA genome (Sutter and Moss, 1992; Sutter et al., 1995). Until today, deletion site III serves as one of the most frequently used insertion loci (Kreijtz et al., 2014; Song et al., 2013; Volz et al., 2016). In addition, standard insertion sites used in conventional VACV, the thymidine kinase gene locus (*J2R*) and the HA gene locus (*A56R*), were readily established for the construction of recombinant MVA (Antoine et al., 1996; Schneider et al., 1998). Moreover, due to the high fidelity of homologous recombination also small non-essential regions between MVA genes can be used to introduce heterologous DNA for vector construction (Wyatt et al., 2009). Multiple insertion sites may allow for integration of various expression units for foreign genes in the same MVA genome when opting for multivalent recombinant vaccines. For the generation of recombinant MVA, cells are infected with MVA and simultaneously transfected with the respective MVA transfer plasmid to allow for homologous recombination (Kremer et al., 2012b; Staib et al., 2004). Recombinant MVA viruses are clonally isolated in repetitive cell culture passages screening for specific selection markers. Different protocols have been established to allow differentiation between wild-type and recombinant MVA. Very first approaches took advantage of specific enzymes that allow for color discrimination. Here, the transfer vector contains an antibiotic selection marker or a reporter gene allowing the screening due to a change in phenotype such as coexpression of the *E. coli* β-galactosidase and β-glucuronidase (Carroll and Moss, 1995; Chakrabarti et al., 1985). Among the coexpressed antibiotic resistance markers, the *E. coli gpt* gene encoding the enzyme xanthine-guanine-phosphoribosyltransferase is frequently used for purification of recombinant viruses by dominant positive selection for resistance against mycophenolic acid (Falkner and Moss, 1988; Isaacs et al., 1990). Staining procedures require additional time of tissue culture, supplementation of agar overlays, and the use of chromogenic substrates and antibiotics. Complementation of a defect in virus production is a faster and more convenient method to obtain recombinant MVA viruses. A first growth selection protocol was initiated using the VACV host range gene *K1L* to rescue recombinant MVA replication in rabbit kidney RK-13 cells (Staib et al., 2000). Blasco and Moss had introduced selection for VACV plaque formation through coinsertion of the F13L gene which was adapted to isolate MVA vector viruses (Blasco and Moss, 1995; Sánchez-Puig and Blasco, 2005). Moreover, MVA mutant virus and a matching complementing cell line enables for growth selection based on the essential *D4R* gene function (Ricci et al., 2011). Up to now, the

generation of recombinant MVA viruses is based on well-established techniques for isolation of clonal viruses that include the use of serum-free media and marker-free recombinant viruses. Specific regulatory guidelines help to supervise the generation of recombinant vector vaccines suitable for applications in humans (EMA, 2010). Yet, in consequence, generation of recombinant MVA is to a certain extent more limited to the use of some preferred methods (Kremer et al., 2012b). E.g., one preferred scheme is the isolation of recombinant MVA through screening for transient cosynthesis of marker proteins without enzyme activity and not related to an antibiotic or chemotherapeutic resistance phenotype. Fluorescence proteins such as green or red fluorescent proteins are conveniently used as well-characterized inert marker proteins. Alternative procedures to engineer poxvirus genomes have been pioneered more recently. The entire VACV and MVA genomes were cloned as bacterial artificial chromosomes (BAC), which can be engineered in *E. coli* by homologous recombination with bacteriophage lambda-derived enzymes (Cottingham et al., 2008; Domi and Moss, 2002, 2005). The modified BAC clones can be used to produce pure recombinant poxvirus in mammalian cells with the initial assistance of a helper virus but without further requirements for plaque purification. Another up-to-date method, yet to be adapted to the construction of recombinant MVA, utilizes the CRISPR-Cas9 system to insert alternative gene sequences into the VACV genome (Yuan et al., 2015). Importantly, independent of the methodology used for generation of a recombinant MVA virus any new vector virus has to be thoroughly quality controlled for genetic identity, purity, genetic stability, recombinant gene expression, and growth characteristics (Kremer et al., 2012b). Typically, the in vitro characterization already starts during the process of clonal isolation of MVA vector viruses by plaque purification. A collection of straightforward methodologies has been established to confirm genomic identity and the correct insertion of the target gene sequence within the MVA genome using finger-print PCRs to assess the insertion sites and the six naturally occurring deletion sites. Levels and kinetics of recombinant protein synthesis, the stability, or the posttranslational modification of the foreign target proteins are typically monitored using standardized in vitro infection experiments and antigen-specific immune detection assays (e.g., Western blotting). In this context, it is also important to analyze the growth capacities of recombinant MVA. The well-known replication deficiency of MVA in mammalian cells is a key biological characteristic of MVA and allows the genetically modified virus to be handled in Germany under conditions of BSL-1 with minimal potential biohazard to

laboratory personnel, clinicians, patients, and the general environment (Bundesamt für Vebraucherschutz und Lebensmittelsicherheit, 2002). Therefore, new recombinant MVA are routinely tested for their growth characteristics in cells of human origin, preferably in cells with normal differentiation such as HaCat cells (Boukamp et al., 1988). This experimental assay serves to exclude that the target gene sequence inserted within the MVA genome influences the growth behavior and biological safety of MVA. Further growth analyses must confirm the replicative capacity of new recombinant MVA in cell cultures suitable for industrial scale production. In 2010, the European Medicines Agency (EMA) has released a guideline on quality, nonclinical, and clinical aspects of live recombinant viral vectored vaccines in Europe (EMA, 2010). At present, primary CEF remains the major cell substrate matching the requirements for the generation and manufacturing of recombinant MVA vector vaccines for clinical evaluation in humans. Several vaccine producers established methodologies under Good Manufacturing Practice (GMP) to allow for large-scale serum-free preparation and cultivation of primary CEF for MVA amplification. However, primary CEF are considered a demanding cell substrate for optimizing industrial virus productions. Thus, in the last few years, various efforts have been made to establish alternative production cell lines for MVA apart from the primary CEF. MVA can be efficiently replicated in different continuous avian cell lines based on chicken embryo, duck embryo, or quail embryo tissues using chemically defined media and bioreactor processes to replace primary CEF (Jordan et al., 2011; Lohr et al., 2009). Moreover, it is encouraging that some of these designer cell lines have already been used to manufacture first candidate vaccines being approved for clinical evaluation (Genzel, 2015).

4.2 Vector Vaccines Against Influenza

In medical research and development ongoing efforts focus on the study of candidate vaccines against infectious diseases that are more "complicated" to prevent, e.g., those caused by newly emerging pathogens such as severe acute respiratory syndrome coronavirus, West Nile virus (WNV), or avian influenza virus. For decades, the availability of a safe and efficient vaccine against different influenza viruses has been one of the biggest demands of public health systems. Until today, infection with seasonal influenza virus kills about 250,000–500,000 people every year. Here, the main risk groups comprise the elderly, immunocompromised and children, with the highest

incidence of fatal outcome (Loubet et al., 2016). Moreover, in addition to the circulating seasonal influenza viruses, there is a continuous threat of new pandemic influenza viruses that might arise from pigs or birds (Fouchier et al., 2013; Herfst et al., 2012). During the last century, there have been four pandemics that together caused more than 50 million deaths (Russell et al., 2012). Hence, it is not surprising that influenza was the target of the first recombinant MVA vaccine. This vector virus was designed to codeliver the influenza virus A/PR/8/34 (H1N1) antigens HA, and NP (MVA-HA-NP) (Sutter et al., 1994). BALB/c mice immunized with MVA-HA-NP mounted efficient levels of HA-specific antibodies as well HA- and NP-specific CD8+ T cells. A single intramuscular immunization was sufficient to protect these mice against a lethal respiratory challenge with influenza virus A/PR/8/34. Subsequently, MVA-HA-NP was shown to induce mucosal immunity following oral immunization and to allow for partial protection against a heterologous influenza virus subtype H3N2 challenge infection (Bender et al., 1996). After these results from testing a first recombinant MVA-expressing influenza virus antigens, it took sometime to encourage a multitude of other experiments targeting influenza in veterinary as well as human medicine. Breathnach and coworkers characterized a recombinant MVA-producing HA or NP from equine influenza A virus H3N8. Vaccination of ponies with MVA-HA induced robust levels of antibodies and protected against challenge with influenza virus H3N8 A/equine/Kentucky/1/81. Also MVA-NP-vaccinated ponies did not show severe clinical symptoms upon influenza virus challenge infection suggesting the induction of cellular immune responses with protective capacity (Breathnach et al., 2006). Because of the zoonotic transmission and the pandemic potential of various highly pathogenic avian influenza viruses of the H5 subtype, the development of H5-specific vaccines for humans is considered a priority since 1997 (de Jong et al., 1997; Yang et al., 2015). Vaccine development is complicated by antigenically different clades of influenza viruses within the H5 subtype. In this context, Kreijtz and coworkers evaluated recombinant MVA expressing the HA genes of the two different influenza H5N1 viruses A/Vietnam/1194/04 and A/HK/156/97 (MVA-HA-VN/04, MVA-HA-HK/97) (Kreijtz et al., 2007, 2009a,b). Vaccination with either of the recombinant MVA efficiently induced antibody responses against the homologous influenza virus. Moreover, immunization with MVA-HA-VN/04 (clade 1) was able to activate antibodies not only against the homologous virus but also to A/HK/156/97 (clade 0) and to a lesser extent to A/Indonesia/5/05 (clade 2.1). This potent activation of

influenza virus-neutralizing H5-specific antibodies correlated with protective efficacy against homologous and heterologous challenge infection (Kreijtz et al., 2007). Interestingly, the exceptional immunogenicity of the HA antigen of influenza A/Vietnam/2004 was confirmed in another study comparing recombinant MVA vaccines expressing HA antigens from various influenza A viruses H5N1 (clades 0, 1, 2.1, 2.2, 2.3) (Hessel et al., 2011). MVA-HA-VN/04 also proved immunogenic and protective when tested as single-shot vaccine in chickens against challenge with the highly pathogenic avian influenza virus A/duck/Vietnam/TG24-01/05 (Veits et al., 2008).

In cynomolgus macaques, prime-boost immunization with MVA-HA-VN/04 was able to protect the animals against homologous virus A/Vietnam/1194/04 and infection with clade 2.1 influenza virus A/Indonesia/5/05. The absence of virus infected cells in the lungs and the lack of fever and severe interstitial pneumonia highlighted the induction of solid protective immunity in vaccinated animals (Kreijtz et al., 2009b). Results from further evaluation in mice also demonstrated the efficacy of MVA-HA-VN/04 vaccines in dose-sparing and single-shot immunizations. Here, minimal requirements for induction of protection against homologous and heterologous influenza virus challenge infections include a single immunization with 10^6 pfu (plaque-forming units) MVA-HA-VN/04 or prime-boost immunizations with 10^4 pfu MVA-HA-VN/04. Of note, in some experiments, protection was observed in the absence of detectable HA-specific antibodies. These data look very encouraging with regard to pandemic risks where a vaccine has to protect rapidly and at minimal doses (Kreijtz et al., 2009a). In this context recombinant MVA expressing the HA from pandemic H1N1 A/California/04/2009 turned out to be highly immunogenic and protective when tested in the mouse model and ferrets (Hessel et al., 2010; Kreijtz et al., 2010). In ferrets, prime-boost vaccination with MVA-HA-CA/09 efficiently activated virus-specific antibodies, reduced the clinical signs after challenge infection with A/Netherlands/602/2009, and protected against severe histopathological changes in the lungs. Two immunizations also significantly lowered the presence of infectious virus in the upper and lower respiratory tract (Kreijtz et al., 2010). Due to these promising results from in vivo preclinical evaluation, a first-in-man phase I/IIa clinical study of MVA-HA-VN/04 has been facilitated (Kreijtz et al., 2014). The vaccine was safely tolerated and serious side effects were not observed. Furthermore, this candidate vaccine proved to be

immunogenic in all individuals enrolled in this study. MVA-HA-VN/04 efficiently activated antibodies cross-reacting with homologous and heterologous H5 subtype influenza viruses and with the recently emerging highly pathogenic avian influenza virus subtype H5N8 (De Vries et al., 2015). Of note, prime-boost applications via the intramuscular route induced levels of influenza H5N1-specific antibodies that raise expectations for protective capacity. Interestingly, booster vaccinations allowed for a remarkable enhancement of H5N1 specific antibodies when given 12 months after primary immunization. These results firstly describe a major benefit of booster vaccinations with recombinant MVA using an extended time period between primary and secondary immunization (Kreijtz et al., 2014). Future clinical studies using similar regimens might help to develop new vaccination strategies. Overall, the induction of robust levels of influenza HA-specific antibodies can be expected to afford cross-protective immunity to viruses of the same influenza virus subtypes. In addition, MVA-mediated delivery or codelivery of influenza virus T cell antigens is being considered to possibly confer broader protection against various subtypes (for review, see Altenburg et al., 2014). Probably, most extensively tested as T cell vaccine is a recombinant MVA vaccine expressing the influenza virus NP and Matrix 1 (M1) proteins (MVA-NP+M1; Lambe et al., 2013; Mullarkey et al., 2013). This candidate vaccine was also shown to induce influenza-specific CD8+ T cells in phase I/IIa clinical studies and to protect humans from an experimental influenza challenge infection (Berthoud et al., 2011; Lillie et al., 2012). Finally, aiming on broadly protective "universal" influenza virus vaccines Kamlangdee and coworkers tested an innovative recombinant MVA expressing an in silico generated synthetic H5 antigen representing a mosaic sequence of more than 2100 H5N1 viruses (Kamlangdee et al., 2014). Interestingly, this candidate vaccine protected mice against H5N1 viruses from all clades but also against infection with influenza virus A/PR/8/34 (H1N1). Taken together these highly encouraging data support the clinical evaluation of existing MVA candidate vaccines and the further development of novel recombinant MVA against influenza.

4.3 Vector Vaccines Against AIDS, Tuberculosis, and Malaria

A safe and effective human immunodeficiency virus (HIV) vaccine is urgently needed to control the worldwide HIV epidemic. However, the development of a vaccine against AIDS represents a substantial scientific

challenge related to HIV antigenic variability, the lacking understanding of immune correlates for protection, limitations of available animal models, and the enormous constraints associated with the probable need for multiple large-scale clinical trials in different parts of the world (for review, see Excler et al., 2014). Moreover, the fragile immune system of HIV-infected individuals sets high standards to candidate vaccine safety. In the recent past, highly attenuated poxviruses continued to play a major role in the international search for an AIDS vaccine also taking advantage of established technologies for vector vaccine production at industrial scale. Different recombinant MVA-expressing HIV proteins have undergone preclinical and clinical testing for the activation of protective immune responses against AIDS often in combination with DNA-based and/or adenoviral vector vaccines (for review, see Iyer and Amara, 2014; Ondondo, 2014). Recombinant MVA vaccines targeting different HIV-1 subtypes continued to prove safe and immunogenic in additional clinical studies (Goepfert et al., 2014; Joachim et al., 2015; Munseri et al., 2015; Nilsson et al., 2015). Important new findings included data for the induction of high levels of antibody-dependent cellular cytotoxicity-mediating antibodies and months durability of the vaccine-induced HIV Env-specific antibody responses. In this context, a necessary target antigen comprises the immunodeficiency virus envelope (Env) protein, as it could be shown to elicit antibody responses with enhanced protective capacity in nonhuman primate chimeric simian/human immunodeficiency virus (SHIV) or SIV challenge infection models (Barouch and Michael, 2014; Barouch and Picker, 2014; Roederer et al., 2014). Recent approaches are working on the induction of broadly neutralizing antibodies at the mucosal site of viral entry based on Env-specific vaccines. Other important HIV immunogens delivered by MVA candidate vaccines include Gag, Pol, and Nef proteins targeting the induction of HIV-specific CD4+ and CD8+ T cells (Gómez et al., 2012). In addition, new synthetic HIV-specific "mosaic" immunogens are under evaluation as improved antigens for induction of CD8+ T cells (Ondondo et al., 2016). The large number of different HIV candidate vaccine necessitates the development of preclinical model systems to evaluate and select the most promising vaccine candidates. In many preclinical experiments, varying degree of protection against homologous immunodeficiency virus infection has been found, predominantly depending on the challenge virus and/or the animal model used for evaluation. However, HIV has an extraordinary genetic diversity and the "Holy Grail" AIDS vaccine would have to cross-protect against different HIV clades. A major scientific challenge is now to find

appropriate antigens or epitopes that elicit a cross-protective immune response. For sometime, induction of cellular immunity was the primary focus of HIV vaccine development but the generation of broadly neutralizing antibodies is also believed to be indispensable (Douek et al., 2006). Previous data from two studies in the macaque model showed that booster vaccinations with oligomeric or native Env proteins enhance Env-binding and virus-neutralizing antibody responses primed by recombinant MVA vaccines, and suggest that such antibodies are indeed likely to play a role in vaccine-induced protection (Earl et al., 2002). Currently, SIV or SHIV challenge infections in different nonhuman primates are considered to be the most appropriate animal models to test for immunogenicity and efficacy. These models very closely mimic the pathogenesis of HIV infections in humans concerning viremia, progressive depletion of CD4+ T cells, and the clinical manifestation of AIDS (Van Rompay, 2012). The thorough characterization of recombinant MVA candidate vaccines in the SIV model continues to further elucidate on the protective capacity of SIV antigen-specific immune responses (Chamcha et al., 2016; Iyer et al., 2015; Kwa et al., 2015; Valentin et al., 2014). Thus, these nonhuman primate models might allow for the generation of proof-of-concept data on antiviral immunity that effectively inhibits immunodeficiency virus replication and disease development.

In addition, MVA vector vaccines have proven to be excellent candidates for vaccine development against other infectious diseases with global impact such as tuberculosis and malaria (for review McShane and Williams, 2014). The incidence of disease caused by *Mycobacterium tuberculosis* is steadily increasing often on the basis of poverty-impaired health services, widespread HIV infection, or the emergence of resistant *M. tuberculosis*. In recent efforts to elicit more potent antimycobacterial immunity, MVA vector viruses served to identify new promising target antigens and resulted in the development of first subunit vaccines entering clinical testing (McShane et al., 2004; Sheehan et al., 2015). Here, the conserved mycobacterial antigen 85A turned out to be a promising immunogen for induction of antigen-specific T cells against *M. tuberculosis*. A recombinant MVA candidate vaccine-expressing 85A under transcriptional control of the VACV early/late promoter P7.5 (MVA85A) has been extensively tested in preclinical models and phase I to phase IIb clinical studies. Vaccination of BALB/c mice with MVA85A induced both CD4+ and CD8+ T-cell responses and conferred protection against challenge with *M. tuberculosis* (McShane et al., 2002). Importantly, in an approach to efficiently trigger

activation of 85A-specific cellular immune responses by vaccination, recombinant MVA85A has been successfully evaluated for boosting the immunogenic effects of a *Mycobacterium bovis* BCG primary immunization. Preclinical studies in different animal models, including mice, guinea pigs, nonhuman primates, and cattle, confirmed the protective efficacy of the heterologous BCG prime-MVA85A boost vaccination. In these experiments protection was associated with a reduction of bacterial loads in the lungs rather than with sterilizing immunity (Goonetilleke et al., 2003; Williams et al., 2005a,b). Unfortunately, when testing BCG-MVA85A immunizations in a large phase IIb clinical trial in children in Africa there was no improvement compared to the conventional BCG only immunization schedule (Tameris et al., 2013). MVA85A was well tolerated and immunogenic as shown by the induction of 85A antigen-specific CD4+ T cells. However, the immunizations demonstrated no significant efficacy against tuberculosis or *M. tuberculosis* infection. Nevertheless, the study provides an important and encouraging pool of safety data for a recombinant MVA candidate vaccine tested in more than 1300 infants. Overall, the results obtained from the clinical testing of MVA85A support the development of improved recombinant MVA85A candidate vaccines against tuberculosis but also including additional antigens of *M. tuberculosis*.

In 2013, there were more than 500,000 estimated deaths and about 200 million clinical illnesses due to malaria, the majority in central and southern Africa. Thus, an effective vaccine against malaria is urgently required (Hoffman et al., 2015). A variety of antigens from *Plasmodium falciparum* has been expressed and tested with recombinant VACV. The pathogenesis of malaria involves a complex life cycle including different blood and non-blood life stages of the parasites in the human host as well as in the mosquito host. A major challenge is the choice of optimal target antigens mediating protective immune responses against the multiple phases of malaria. Desirable targets should result in an efficient activation of humoral immunity as well as malaria-specific CD8+ T cells. In a landmark study, Schneider and coworkers had evaluated recombinant MVA vaccines in a mouse malaria model using *P. berghei*. The study demonstrated efficient induction of malaria-specific T cells that could be further enhanced by using DNA prime in combination with recombinant MVA as boosting agent. Of note, this study demonstrated a need for antigen-specific CD8+ T cells to induce protection against *P. berghei* sporozoite challenge infection (Schneider et al., 1998). First clinical trials have been initiated using recombinant MVA expressing the important malaria liver stage antigens TRAP/CSP alone

or in a heterologous prime-boost schedule in combination with adenoviral vectors (Gilbert et al., 2002). Further improvement of this antigen has been achieved by fusion of TRAP with a peptide sequence encompassing a Plasmodium-specific multiple epitope (ME-TRAP) (Gilbert et al., 1997). In detail, Moorthy and coworkers generated a single polypeptide ME-TRAP of 789 amino acids which combines a multiple epitope string (ME) consisting of 14 CD8+ T-cell epitopes with three CD4+ T-cell epitopes from tetanus toxin, BCG, and Pf-circumsporozoite protein (PfCSP) and two B-cell epitopes within the established TRAP antigen. Here, DNA prime and recombinant MVA boost schedules have been evaluated for safety and immunogenicity in a phase I/II clinical trial in humans. No severe adverse effects were observed after application of MVA-ME-TRAP alone or in combination with DNA prime vaccination (Moorthy et al., 2003). In following clinical evaluations, immunogenicity and protection has been assessed in African adults in a malaria-endemic area. Heterologous regimens using DNA prime and MVA boosting proved to be more immunogenic compared to homologous application of either vaccine used alone. However, despite measurable immunogenicity, robust protection against challenge with *P. falciparum* could not be detected (Moorthy et al., 2003, 2004). Another advancement was based on studies that tested different combinations of primary immunizations with recombinant fowlpox and recombinant MVA vaccines (Anderson et al., 2004). Next steps included the clinical evaluation of these malaria vaccine candidates in heterologous prime-boost vaccinations. Here, the booster immunizations resulted in an efficient activation of malaria-specific CD8+ T cells in adults and children (Vuola et al., 2005; Webster et al., 2005). However, efficacy testing in field studies in endemic areas failed to robustly protect against malaria infection at various stages (Bejon et al., 2006). Another innovative approach of heterologous prime-boost vaccination has been conducted by Rodriguez and colleagues in the murine *P. berghei* infection model. The alternative usage of porcine parvovirus-like particles delivering CS protein peptide sequences resulted in efficient priming of protective CD8+ T-cell responses following booster vaccination with recombinant MVA expressing the CS antigen (Rodríguez et al., 2012). More recently another heterologous prime-boost vaccination schedule has been thoroughly evaluated using chimpanzee-adenovirus vectors for priming and recombinant MVA for booster vaccinations (for review, see Sebastian and Gilbert, 2016). This combination of vector immunization induced high frequencies of ME-TRAP-specific T-cell responses in humans as a promising approach

to robustly protect against *P. falciparum* (Ewer et al., 2013). Moreover, a recent study suggested the feasibility of developing malaria transmission-blocking vector vaccines to target *P. falciparum* within the mosquito. Antibodies with high-level transmission-blocking activity could be elicited upon prime-boost immunizations of mice with recombinant chimpanzee adenovirus and MVA-expressing candidate malaria antigens Pfs230-C and Pfs25 (Kapulu et al., 2015). Thus, future approaches in the development of MVA vector vaccines against malaria aim at the induction of a more balanced immunity based on both efficient humoral and cellular immune responses. Yet, complex clinical phase II/III studies in humans in endemic areas will be needed to evaluate the efficacy of these new promising approaches.

4.4 MVA Vector Vaccines Against Emerging Infections

Newly emerging pathogens represent another global public health risk as they can suddenly and unexpectedly arise from a previously unknown ecological niche, in most cases an animal reservoir. Thus, major concerns are zoonotic infections that are transmitted from animals to humans, which, when sufficiently adapted to the human host, may rapidly spread in the human population (Kuiken et al., 2011; Reusken et al., 2016; Steffen et al., 2012). During the past 20 years, public health systems had been confronted with a multitude of new pathogens each demonstrating a different scenario of emergence. In this context, the year 1999 with the sudden occurrence of West Nile fever in the Western hemisphere (New York City, USA) is often marked as the beginning of a new era of epidemics (for review, see Suthar et al., 2013). WNV is a member of the genus flaviviruses and, as an arbovirus, continuously circulates between different mosquito species and birds as the major animal virus reservoirs. Through the bite of an infected mosquito, WNV can be transmitted to mammalian hosts, primarily to humans or horses, causing the so-called West Nile Fever sometimes resulting in neuroinvasive disease with the potential for severe outcomes especially in elderly and immunocompromised humans. The virus had first been isolated in 1937 from a febrile woman in the West Nile District in Uganda (Goldblum et al., 1954). Since then it was observed to be or become endemic in regions of Europe, Africa, and Asia periodically causing WNV outbreaks in humans and/or horses. In 1999, the virus was introduced into the New York City District of Queens, supposedly by an infected mosquito or bird (Lanciotti et al., 1999; Murray et al., 2010; Nash et al., 2001). Thus,

WNV had relocated to a new, naïve population facilitating its impressive spread across the North American continent leading to a total of about 41,762 human infections and 1765 deaths between 1999 and 2014 (http://www.cdc.gov/westnile/statsmaps/). Concurrently, the virus also increasingly emerges in European countries, resulting in outbreaks of severe disease in horses and humans. Thus, a safe and effective WNV vaccine for humans is urgently needed in particular to protect at-risk populations. A recent study tested different recombinant MVA vaccines delivering the WNV envelope protein (WNV-E) and fulfilling all the principal requirements to proceed to clinical testing in humans. Vaccine immunogenicity, induction of neutralizing antibodies and E-specific CD8+ T-cell responses, and the capacity to protect against lethal challenge infections were evaluated in different mouse models. The MVA–WNV candidate vaccines allowed to compare the performance of WNV-E antigens expressed in four different conformations. In MVA-prM/ME, WNV-E is produced together with the WNV membrane protein to induce the synthesis and the release of virus like particles (VLPs) upon MVA infection. MVA-E_{sol} produces a soluble version of WNV-E that is secreted from infected cells. MVA-E_{TMV} and MVA-E_{TMC} prominently expose WNV-E antigens on the cell surface by providing heterologous transmembrane domains derived from either VACV (TMV, transmembrane domain vaccinia virus) or chikungunya virus (TMC, transmembrane domain chikungunya virus). Upon prime-boost vaccinations in BALB/c mice, all four MVA–WNV candidate vaccines elicited circulating serum antibodies binding to recombinant WNV-E protein and neutralizing WNV in tissue culture infections In addition, immunizations in HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout mice efficiently induced WNV-E-specific CD8+ T-cell responses. Finally, the MVA–WNV candidate vaccines protected C57BL/6 mice against challenge infections with lineage 1 and lineage 2 WNV and activated cross-neutralizing antibodies. Thus, further studies are warranted to evaluate these recombinant MVA–WNV vaccines in other preclinical models in an effort to select and develop an MVA–WNV candidate vaccine for clinical testing in humans (Volz et al., 2016).

Recently, the family *Coronaviridae* provided different new pathogens suddenly arising from an ecological niche. Since 2003 two novel beta coronaviruses have been introduced into human populations causing acute atypical necrotizing pneumonia, the so-called severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) (Graham et al., 2013). In early 2003, the SARS

epidemic initially occurred in Southern China and rapidly spread to more than 37 countries causing 8096 infections and 774 deaths. Bat species and civet cats were identified as animal reservoirs transmitting the virus to humans and/or other animals. The abrupt emergence of the SARS-CoV in China in November 2002 and its worldwide distribution until the end of 2003 is representative for the scenario of a pandemic public health emergency. Luckily, further spread of the disease could be prevented and no further infections with SARS-CoV were detected since early 2004. The successful containment of SARS was likely due to multiple causes, e.g., the relative ease of clinical case isolation and the persistence of the pathogen in an animal reservoir rarely enabling transmission to humans. However, the lessons learned from SARS include the need for the timely development of specific vaccines to counteract such an outbreak scenario with high morbidity and mortality rates and the lack of any specific treatment option. The most effective approach to deal with emerging pathogens is vaccination. MVA with its excellent safety profile and well-established vector production platform holds great potential to rapidly develop new vaccines against such emerging pathogens ready to use in an immediate public health response. In an ideal scenario, MVA emergency vaccines against selected pathogens of risk are being developed and undergo preclinical and phase I/II clinical evaluations already in preepidemic times. In the case of a disease outbreak, these recombinant MVA candidate vaccines could be immediately tested in efficacy studies when used to vaccinate people of special risk in endemic areas. This concept already spurred the development of a first set of recombinant MVA candidate vaccines against highly pathogenic avian influenza viruses and the recently emerging MERS-CoV as current examples of new zoonotic agents. MERS-CoV was first described in September 2012. In contrast to experiences with SARS in the epidemic of 2002/2003, the MERS-CoV continues to cause disease in humans in the fifth year after its first appearance in 2012. At present, WHO reports a total of 1782 laboratory confirmed cases including 634 deaths (http://www.who.int/emergencies/mers-cov/en/). The epidemiology of human MERS-CoV infections centers in the Middle East, e.g., in Qatar, Saudi Arabia, Jordan, and United Arab Emirates. Sporadically, MERS-CoV infections are also spread to other countries in Europe, North-America, and Asia due to transmission by travelers infected in the Middle East. Raj and coworkers identified the human cell surface amino peptidase dipeptidyl 4 or CD26 as functional receptor of MERS-CoV-mediating entry into the cell (Raj et al., 2013). By now dromedary camels are known and generally accepted to be the critical animal reservoir

responsible for spreading the virus to humans (Haagmans et al., 2014; Memish et al., 2014; Meyer et al., 2014; Raj et al., 2014). These primary zoonotic infections can result in interfamilial or health care related secondary transmissions. The elderly and immunocompromised persons are among the people of risk to suffer most from severe and lethal MERS-CoV infections. Other individuals at risk are health care workers and people with close contact to camels. All these groups are considered relevant target populations for potential MERS-CoV vaccines. So far, there is no licensed vaccine against MERS-CoV or SARS-CoV available. Different approaches have been undertaken to develop effective means to prevent or cure these new infectious diseases. Here, MVA has been tested as viral vector vaccine against both beta coronaviruses. The envelope spike (S) protein has been proven to be a major target of SARS-CoV-neutralizing antibodies (He et al., 2004; Sui et al., 2004). Indeed, a recombinant MVA producing the SARS-CoV S antigen was evaluated in different animal models and demonstrated the induction of SARS-CoV-specific immune responses, including SARS-CoV-neutralizing antibodies and S-antigen-specific T cells in mice, rabbits, and rhesus macaques (Bisht et al., 2004; Chen et al., 2005). Prime boost immunization effectively inhibited SARS-CoV replication in cynomolgous monkeys after respiratory SARS-CoV challenge infection suggesting the induction of protective immunity. In the case of the MERS-CoV, a recombinant MVA expressing the S protein of MERS-CoV (MVA-MERS-S) was generated rapidly after the discovery of MERS in 2012 (Song et al., 2013). Here, the MERS-CoV-S-encoding sequences were introduced into the deletion site III of the MVA genome. Preclinical evaluation of MVA-MERS-S candidate vaccine confirmed the induction of MERS-CoV-neutralizing antibodies and MERS-S epitope-specific CD8+ T cells in BALB/c mice comparing different dosages and application routes (Volz et al., 2015). Moreover, preclinical testing in a special mouse model allowed for the first demonstration of the protective capacity of this MERS candidate vaccine. After adenovirus-mediated transduction with the human dipeptidylpeptidase 4 receptor, BALB/c mice are susceptible for respiratory MERS-CoV challenge infections and the monitoring of virus loads allows to determine the efficacy of experimental immunization (Zhao et al., 2014). As the virus is assumed to persist in dromedary camels, further preclinical analysis in dromedary camels demonstrated immunogenicity and protective efficacy of MVA-MERS-S (Haagmans et al., 2016). Simultaneous immunizations by the intranasal and intramuscular routes resulted in efficient induction of virus-neutralizing antibodies in serum and nasal secretions of

vaccinated animals. Contrary to the mock vaccinated control animals, camels vaccinated with MVA-MERS-S revealed a significant reduction of excreted infectious virus and viral RNA transcripts after MERS-CoV challenge infection. In addition, vaccination with MVA-MERS-S also induced the activation of orthopoxvirus-specific antibodies that readily cross-neutralized camelpox virus. Camelpox virus causes severe systemic disease in camels with case fatality rates as high as 28%. Clinical disease is characterized by papules and pustules that initially appear at the primary site of infection. This is then followed by the development of generalized rash and fever between day 9 and 11 postinfection. Young camels are more susceptible to severe clinical disease. The activation of camelpox virus-neutralizing antibodies suggested the potential dual use of this candidate MERS-CoV vaccine in dromedaries to efficiently protect against MERS-CoV and camelpox virus infection (Haagmans et al., 2016). These data further support the general safety and efficacy of the MVA-MERS-S candidate vaccine and introduce the possibility for application as veterinary vaccine with important implications to the One Health concept. Vaccination of dromedary camels in areas endemic for MERS-CoV could reduce the burden of virus excretions from the animal reservoir and thus inhibit the transmission of MERS-CoV to human populations. In addition, it is important to characterize the MVA-MERS-S candidate vaccine in humans and efforts are ongoing to start a first-in-man clinical evaluation of MVA-MERS-S as soon as possible. In this first clinical phase I/II testing, safety and immunogenicity of the MVA-MERS-S candidate vaccine will be analyzed in healthy volunteers in Germany. Results from these phase I/II clinical evaluations are prerequisite for testing the vaccine in larger phase II studies in endemic countries. In addition, the availability of an investigational drug batch of MVA-MERS-S might allow for application of as emergency vaccine in the case of a suddenly occurring outbreak scenario, when the virus is rapidly transmitted and spread throughout a new geographic area. Exemplary for this was the introduction of MERS-CoV to South Korea on 20 May 2015 (Min et al., 2016). A 68-year-old man returning from the Middle East had been diagnosed with MERS 9 days after he initially visited the hospital for medical help. In the hospital further spread of MERS-CoV occurred by transmission to several health care workers and other patients. In this outbreak, a total of 186 individuals have been infected, with a total of 36 deaths. In this context, the government of the Republic of Korea began to implement intense case and contact management activities that in the end stopped the epidemic of MERS-CoV in a nonendemic region. However, the

sudden and rapid spread of MERS-CoV in South Korea again highlighted the global risks of newly emerging pathogens that might unexpectedly threaten a naïve population (Jeong-Sun et al., 2015; Kim et al., 2015).

Another very recent example for the sudden reemergence of a highly infectious pathogen is the unprecedented Ebola virus epidemic in West Africa starting in 2013 and continuing for over 2 years. During this devastating and most widespread Ebola virus outbreak, the virus had caused more than 28,000 disease cases and 11,325 deaths mainly geographically linked to the Africa but some cases were also diagnosed in travelers from Africa to the United States, Germany, France, Spain, and Great Britain (de La Vega et al., 2015; Quaglio et al., 2016). Initially, this large epidemic began in the village of Meliandou, Guéckédou Prefecture, Guinea in the end of 2013. Most likely bats transmitted the virus to humans followed by massive spread of the disease to other villages. Moreover, the virus causing this outbreak was identified to be the most notorious member of the genus Ebolavirus and the species Zaire Ebolavirus (EBOV) (Baize et al., 2014). In consequence, the epidemic in West Africa was associated with high morbidity and mortality rates that enabled an undamped transmission and perpetuation of the virus in the population for a rather long time period (Gire et al., 2014). This had not been observed for Ebolavirus outbreaks in the past. So far, there are no efficient therapeutics available (Choi and Croyle, 2013) and there are also no vaccines licensed to protect against Ebolavirus. However, since this very recent epidemic, research activities to develop and evaluate candidate vaccines against Ebolavirus have been intensified. Most promising candidates already advanced to clinical evaluation in humans, and include a recombinant Vesicular Stomatitis Virus (VSV) delivering the Ebolavirus Zaire glycoprotein (VSV-EBOV) and a chimpanzee-adenovirus (ChAd3)-Zaire Ebola virus (ZEBOV) also expressing the Ebolavirus Zaire glycoprotein as target antigen. With regard to the ChAd3-Zaire, the strategy is to use a heterologous prime-boost immunization schedule, boosting with an appropriate recombinant MVA-expressing Ebolavirus Zaire glycoprotein (Ewer et al., 2016; Tapia et al., 2016). This strategy was supported by data from the preclinical evaluation of a ChAd3-EBOV candidate vaccine in nonhuman primates. Here, prime-boost vaccination with the ChAd3-EBOV vaccine alone did not confer protective immunity over a period of several months. However, robust protection against lethal challenge with EBOV could be obtained following booster vaccination with a recombinant MVA-EBOV candidate vaccine coexpressing the Ebolavirus Zaire and Sudan glycoproteins under the control of the P7.5 vaccinia virus early promoter (Stanley et al., 2014). The approach of using a

ChAd3-EBOV prime and MVA-EBOV boost vaccination has been further developed by the engineering of a multivalent recombinant MVA coproducing the ZEBOV and Sudan Ebola virus glycoproteins and other filovirus antigens (MVA-BN-Filo). A first phase Ib clinical study demonstrated safety, tolerability, and immunogenicity of the ChAd3-EBOV prime and MVA-BN-Filo booster immunizations in adults in the United States and in Mali. Without any observation of severe side effects the combined ChAd3-EBOV and MVA-BN-Filo vaccinations proved to be highly immunogenic as it was shown by the activation of EBOV-specific antibodies and CD4+ and CD8+ T-cell responses (Tapia et al., 2016). Ewer and colleagues investigated another heterologous prime-boost vaccination schedule in 60 healthy adult volunteers in Oxford, United Kingdom, using a ChAd3 vector vaccine and a monovalent recombinant MVA encoding the surface glycoprotein of ZEBOV. Again, ChAd3 immunization boosted with MVA-elicited B-cell and T-cell immune responses to ZEBOV that were clearly superior to those induced by the ChAd3 vector vaccine alone (Ewer et al., 2016). Nevertheless, a heterologous vaccination strategy with two different viral vector vaccines is a complex regimen to be applied in large field studies or in the case of emergency vaccination when whole populations have to be protected rapidly. Thus, it should be interesting to evaluate the efficacy of a prime-boost vaccination scheme based on MVA-EBOV only. In summary, the highly versatile and safe MVA vector platform should be particularly useful to effectively control newly emerging or reemerging infectious diseases. The vector system may be readily exploited in a plug-and-play generic approach for the rapid generation of vaccine candidates suitable for rapid emergency immunization and, simultaneously, the clinical-stage development of a new licensable product.

5. MVA AS THIRD-GENERATION VACCINE AGAINST SMALLPOX

Eradication of human smallpox has been achieved by prophylactic use of VACV to immunize humans, the only host reservoir of the causative agent VARV. However, other members of the genus Orthopoxvirus can cause zoonotic infections (Kroon et al., 2011; McCollum and Damon, 2014; Vora et al., 2015). Moreover, recent fears that monkeypox virus (MPXV) or VARV could be used as biological weapons have renewed the interest in safe vaccines against VARV or other zoonotic orthopoxviruses (Moss, 2011). MVA holds great promise for worldwide use as

third-generation smallpox vaccine due to its well-established characteristics concerning safety and immunogenicity (Drexler et al., 2003; Jones et al., 2016; Knitlova et al., 2014; Meseda et al., 2016; Tree et al., 2016; Wyatt et al., 2004). In 2013, an MVA vaccine has been licensed in Europe for active immunization against smallpox in adults and for use in situations where it is considered necessary to protect against smallpox in accordance with official recommendations (European Medicines Agency, 2013). Data from multiple phase II clinical studies have confirmed the safety and immunogenicity of the MVA vaccine in patient populations considered at risk for conventional smallpox vaccination including individuals with atopic dermatitis or HIV infection (Greenberg et al., 2015; Overton et al., 2015; von Sonnenburg et al., 2014). In addition, MVA has been successfully tested for cardiac safety in a large phase II clinical trial (Zitzmann-Roth et al., 2015). This is important because vaccines based on replication-competent VACV strains are associated with a high incidence of myo-/pericarditis, a severe cardiac complication. Additional recent data from clinical studies addressed the meta-analysis of MVA-induced immune responses in various patient populations and the comparative evaluation of inoculation routes or improved formulations of the MVA vaccine (Frey et al., 2015; Troy et al., 2015). Today, with the eradication of smallpox, we lack an established human disease representing the pathogenesis of a systemic orthopoxvirus infection in humans. Therefore, analyzing the protective efficacy of orthopoxvirus-specific vaccines is not straightforward. Preclinical evaluations in animal models are required to test vaccine-mediated protection in relation to antigen-specific responses, and animal models must mimic the target poxvirus diseases in humans as closely as possible. VARV infection in cynomolgus macaques can result in a lethal disease with similarities to smallpox (Jahrling et al., 2004; Wahl-Jensen et al., 2011). However, VARV is a biosafety level 4 agent and the handling with this virus is highly restricted. Thus, several other animal models using the orthopoxviruses cowpox virus (CPXV), MPXV, ectromelia virus (ECTV), and VACV have been developed (Chapman et al., 2010; Esteban and Buller, 2005). MVA, as safe smallpox vaccine, has been tested in these different infection models. MVA vaccination by the intramuscular or subcutaneous route protected mice against severe respiratory challenge infection with CPXV or VACV-WR (Coulibaly et al., 2005; Drexler et al., 2003). Of note, MVA could also protect mice lacking several components of the immune system, resembling high-risk groups in the population that would require an alternative to the standard smallpox vaccine (Wyatt et al., 2004). In nonhuman primates,

the challenge infection with MPXV is the most appropriate model to evaluate the preclinical efficacy of new candidate smallpox vaccines. Here, a standard dosage of 10^8 pfu MVA robustly protected cynomolgous monkeys against intravenous or intratracheal MPXV challenges (Earl et al., 2004; Stittelaar et al., 2005). In this context Edghill-Smith and coworkers demonstrated the essential need of antibodies for protection against fatal monkeypox disease (Edghill-Smith et al., 2005). One major limitation in these orthopoxvirus infection models, using VACV, MPXV, or CPXV to test protective capacity of vaccination, is the high amounts of infectious virus ($>10^6$ pfu) required to induce lethal disease. In contrast, very few infectious particles of VARV could generate fatal smallpox disease in naive humans. To overcome this shortcoming, the ECTV infection of mice can serve as an additional highly appropriate model for the reassessment of the correlates of protective immunity (for review Sigal, 2016). ECTV, the causative agent of mousepox, is a natural mouse pathogen causing a classical systemic poxvirus disease (Esteban and Buller, 2005). Very low amounts of ECTV are sufficient for infection and induction of lethal mousepox disease in susceptible mouse strains. After an asymptomatic incubation period of 6–8 days following intranasal infection, mousepox disease starts in the respiratory tract followed by a systemic virus spread to internal organs such as liver and spleen (Paran et al., 2009; Parker et al., 2009). Specific signs of illness are characterized by severe bronchopneumonia and hepatitis. Mice that survive this systemic phase of severe disease develop a characteristic pustular rash on the skin very reminiscent to that seen in human smallpox. The ECTV-mouse model offers the opportunity to study an orthopoxvirus pathogen in an experimentally easily accessible natural host. ECTV is highly adapted to the mouse immune system and this allows to thoroughly analyze the mechanisms of viral immune evasion and vaccine-induced immune protection. Several studies evaluated MVA vaccine-mediated protection against lethal mousepox disease in more detail. Coulibaly and coworkers demonstrated the usefulness of respiratory ECTV challenge infections as improved model system for efficacy testing. Here, intramuscular immunization with a single dose of 10^6 pfu MVA prevented severe disease and death in mice challenged 3 weeks after vaccination. A single dose of 10^7 robustly protected against any signs of illness and disease after the respiratory mousepox challenge. However, after vaccination with 10^6 or 10^7 pfu VACV Wyeth, all mice suffered from severe lethal disease (Coulibaly et al., 2005). In the CPXV challenge model, these different candidate vaccines were equally protective underlining the impact of choosing a specific challenge virus.

This study demonstrates the need for data from various preclinical models as key component in developing next-generation orthopoxvirus-specific vaccines for application in humans. In the case of an emergency with newly arising highly pathogenic orthopoxviruses, e.g., because of unintentional or intentional release of VARV, vaccination protocols for rapid induction of protective immunity are urgently needed (Reardon, 2014; Sasse and Gelderblom, 2015). For smallpox vaccination there are historical reports that VACV application in a time window of up to 4 days after exposure with VARV may be protective. Analysis of MVA as emergency vaccine confirmed short-term protective capacity of vaccination in mice, latest when applied at the day of the lethal respiratory challenge with VACV strain WR. Postexposure prophylaxis could not be achieved in the VACV-WR model, independent of dosage and application route (Staib et al., 2006). However, when testing emergency vaccination in the ECTV-mouse model, a standard dosage of 10^8 pfu MVA robustly protected against lethal respiratory mousepox infection up to 2 days before the challenge. Even more, in this natural virus–host system, 10^8 pfu MVA also prevented death and severe disease when applied up to 4 days after the lethal challenge. However, postexposure vaccination could not inhibit the onset of sign of disease including respiratory symptoms and body weight loss (Paran et al., 2009). In a follow-up study, Kremer and colleagues analyzed the immune components mediating this rapid protection in more detail (Kremer et al., 2012a). Analysis of MVA-induced protection in mice with defined deficiency in the innate or adaptive immunity identified CD4+ T cells to be essentially required to allow for MVA-induced CD8+ T-cell expansion. Interestingly, selected components of the innate immune system and B cell-mediated responses were fully dispensable for prevention of fatal disease by immunization given 2 days before challenge. Analyzing protective capacity of MVA immunization in RAG −/− mice that lack T cells and B cells, these mice could not be protected against the lethal ECTV challenge infection. These results underlined the prerequisite of adaptive cellular immunity for mediating a rapid protection with perforin-mediated cytotoxicity proven to be a key immune mechanism. In the case of emergency, when rapid induction of protective immunity by vaccination is desirable for prevention of morbidity and mortality, the instant activation of protective virus-specific immunity by single-shot vaccination, would be ideal. Moreover, the possibilities of dose-sparing immunization regimens would increase the numbers of people that can be vaccinated. Volz and coworkers assessed the minimal requirements for the induction of protective immunity

against lethal ECTV challenge infection 2 days after immunization with MVA (Volz et al., 2014). C57BL/6 mice had been intramuscularly vaccinated with tenfold increasing doses of MVA starting with 10^3 up to 10^8 pfu, the MVA standard dosage. Interestingly, a minimal amount of 10^5 pfu fully protected the mice against the lethal respiratory challenge infection with ECTV. Moreover, inoculations with 10^4 pfu MVA were still sufficient to prevent death of all vaccinated animals but could not protect against the induction of mousepox disease. Analysis of immune responses again identified CD8+ T cells as the key components mediating the rapid protection in the low dose immunization model. Moreover, MVA immunization at low dosage also protected IFNAR −/− mice, indicating efficient activation of cellular immunity even in the absence of type I IFN signaling. When monitoring for virus-specific CD8+ T-cell responses in mice vaccinated with the minimal protective dose of MVA, we found significantly enhanced levels of antigen-specific T cells in animals that were MVA vaccinated and ECTV challenged compared to mice that were only vaccinated. The initial priming of naïve CD8+ T cells by MVA immunization appears to be highly efficient and, even at low doses, mediates a rapid in vivo burst of pathogen-specific CD8+ T cells upon challenge. These findings define striking requirements for protective emergency immunization against severe systemic infections with orthopoxviruses. These data are of important practical relevance for public health, as producing sufficient amounts of vaccine is expected to be a major challenge should an outbreak occur. Moreover, prevention of other infections may require similar immune mechanisms to elicit rapid protective immunity; hence, MVA could be an extremely useful vaccine for delivering heterologous T cell antigens, particularly for infectious diseases that fit a scenario of emergency vaccination. Thus, studies evaluating recombinant MVA candidate vaccines as emergency vaccines might be promising for the development of new prophylactic or therapeutic approaches.

6. CONCLUSIONS

Today, almost 40 years after its first licensing in Germany, MVA is well established as safety-tested, immunogenic, and efficacious third-generation smallpox vaccine. In 2013, the European Medicines Agency and Canada Health granted the marketing authorization of an MVA vaccine to immunize against VARV infection, in the absence of human smallpox or naturally occurring VARV (European Medicines Agency, 2013). Thus,

efficacy data needed to be generated from animal models of orthopoxvirus infections considered to be representative for human smallpox. A similar licensing process by the US Food and Drug Administration is ongoing and appears to be at an advanced stage. Despite the eradication of VARV more than three decades ago, these efforts are still important mostly due to the threat of VARV being—accidently or intentionally—released into unprotected human populations. Moreover, the use of a licensed MVA vaccine should be ideal to protect individuals at risk—including laboratory workers—against other zoonotic orthopoxviruses such as CPXV and MPXV that continue to cycle in rodent reservoirs and can cause disease in humans.

In addition, during 25 years, MVA has been continuously improved as an extremely safe and efficient viral vector system for the synthesis of high levels of foreign proteins in nonpermissive human cells. At the moment, recombinant MVA viruses expressing various heterologous antigens are among the most promising vector candidates to develop innovative vaccination strategies to protect against complex infections such as AIDS, tuberculosis, or malaria, or against rare but threatening emerging diseases. Desirable common characteristics of MVA as viral vector vaccine include the genetic stability, the well-established production procedures and the general safety for the environment. Results coming from clinical testing of various recombinant MVA vaccines further emphasize its excellent safety record and its immunogenicity as vaccine in humans. In the context of recent clinical findings, it is noteworthy that repeated vaccinations with the same recombinant MVA resulted in substantial booster induction of antigen-specific antibodies even in the presence of high levels of MVA-specific immunity. These results together with other promising data gained from the combined application of MVA with various other viral vector platforms greatly enhance the general acceptance of viral vectors as next-generation candidate vaccines. In addition to its promising characteristics concerning immunogenicity, the nonreplicating recombinant MVA vaccines are also well positioned to satisfy very stringent requirements for a broad safety in various settings. MVA vaccines appear highly suitable for use in immunocompromised individuals and in the elderly representing important target populations in ever-ageing populations in the industrialized world. At the same time, recombinant MVA should be also safe to immunize persons with severe comorbidities, e.g., HIV infection, tuberculosis, or malaria, as it is often seen in developing nations. New vaccines that rapidly protect against threatening emerging pathogens are urgently needed. This it is further highlighted by the

WHO-list of the "top most wanted" emerging diseases likely to cause major epidemics. Here, the longstanding experience with the MVA vector vaccine platform in preclinical and clinical research should lead to important contributions to the development of protective vaccination strategies against newly emerging pathogens.

Finally, the exciting results from ongoing fundamental research on the biology of MVA spur the possibility to even improve the efficacy of future MVA vaccines. Here, an exemplary area of research targets the still unknown functions of host-regulatory genes remaining conserved in the MVA genome. Indeed, modulating the functional activity of these regulatory MVA proteins could be beneficial in enhancing the immunogenicity of MVA vaccines and activate innate and/or adaptive immune responses to heterologous antigens.

REFERENCES

Alcami, A., Smith, G.L., 1992. A soluble receptor for interleukin-1β encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. Cell 71, 153.

Alcamí, A., Smith, G.L., 1996. Soluble interferon-γ receptors encoded by poxviruses. Comp. Immunol. Microbiol. Infect. Dis. 19, 305.

Alcamí, A., Symons, J.A., Collins, P.D., Williams, T.J., Smith, G.L., 1998. Blockade of chemokine activity by a soluble chemokine binding protein from vaccinia virus. J. Immunol. 160, 624.

Altenburg, A.F., Kreijtz, J.H.C.M., de Vries, R.D., Song, F., Fux, R., Rimmelzwaan, G.F., Sutter, G., Volz, A., 2014. Modified vaccinia virus Ankara (MVA) as production platform for vaccines against influenza and other viral respiratory diseases. Viruses 6, 2735.

Anderson, R.J., Hannan, C.M., Gilbert, S.C., Laidlaw, S.M., Sheu, E.G., Korten, S., Sinden, R., Butcher, G.A., Skinner, M.A., Hill, A.V.S., 2004. Enhanced CD8+ T cell immune responses and protection elicited against plasmodium berghei malaria by prime boost immunization regimens using a novel attenuated fowlpox virus. J. Immunol. 172, 3094.

Antoine, G., Scheiflinger, F., Holzer, G., Langmann, T., Falkner, F.G., Dorner, F., 1996. Characterization of the vaccinia MVA hemagglutinin gene locus and its evaluation as an insertion site for foreign genes. Gene 24, 43.

Antoine, G., Scheiflinger, F., Dorner, F., Falkner, F.G., 1998. The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses. Virology 244, 365.

Backes, S., Sperling, K.M., Zwilling, J., Gasteiger, G., Ludwig, H., Kremmer, E., Schwantes, A., Staib, C., Sutter, G., 2010. Viral host-range factor C7 or K1 is essential for modified vaccinia virus Ankara late gene expression in human and murine cells, irrespective of their capacity to inhibit protein kinase R-mediated phosphorylation of eukaryotic translation initiation factor 2α. J. Gen. Virol. 91, 470.

Bahar, M.W., Kenyon, J.C., Putz, M.M., Abrescia, N.G.A., Pease, J.E., Wise, E.L., Stuart, D.I., Smith, G.L., Grimes, J.M., 2008. Structure and function of A41, a vaccinia virus chemokine binding protein. PLoS Pathog. 4, e5.

Baize, S., Pannetier, D., Oestereich, L., Rieger, T., Koivogui, L., Magassouba, N.F., Soropogui, B., Sow, M.S., Keïta, S., De Clerck, H., Tiffany, A., Dominguez, G.,

Loua, M., Traoré, A., Kolié, M., Malano, E.R., Heleze, E., Bocquin, A., Mély, S., Raoul, H., Caro, V., Cadar, D., Gabriel, M., Pahlmann, M., Tappe, D., Schmidt-Chanasit, J., Impouma, B., Diallo, A.K., Formenty, P., Van Herp, M., Günther, S., 2014. Emergence of Zaire Ebola virus disease in guinea. N. Engl. J. Med. 371, 1418.

Baldick, C.J., Keck, J.G., Moss, B., 1992. Mutational analysis of the core, spacer, and initiator regions of vaccinia virus intermediate-class promoters. J. Virol. 66, 4710.

Barouch, D.H., Michael, N.L., 2014. Accelerating HIV-1 vaccine efficacy trials. Cell 159, 969.

Barouch, D.H., Picker, L.J., 2014. Novel vaccine vectors for HIV-1. Nat. Rev. Microbiol. 12, 765.

Bejon, P., Mwacharo, J., Kai, O., Mwangi, T., Milligan, P., Todryk, S., Keating, S., Lang, T., Lowe, B., Gikonyo, C., Molyneux, C., Fegan, G., Gilbert, S.C., Peshu, N., Marsh, K., Hill, A.V.S., 2006. A phase 2b randomised trial of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP among children in Kenya. PLoS Clin. Trials 1, e29.

Bender, B.S., Rowe, C.A., Taylor, S.F., Wyatt, L.S., Moss, B., Small, P.A., 1996. Oral immunization with a replication-deficient recombinant vaccinia virus protects mice against influenza. J. Virol. 70, 6418.

Berthoud, T.K., Hamill, M., Lillie, P.J., Hwenda, L., Collins, K.A., Ewer, K.J., Milicic, A., Poyntz, H.C., Lambe, T., Fletcher, H.A., Hill, A.V.S., Gilbert, S.C., 2011. Potent CD8 (+) T-cell immunogenicity in humans of a novel heterosubtypic influenza a vaccine, MVA−NP+M1. Clin. Infect. Dis. 52, 1.

Bisht, H., Roberts, A., Vogel, L., Bukreyev, A., Collins, P.L., Murphy, B.R., Subbarao, K., Moss, B., 2004. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. Proc. Natl. Acad. Sci. U.S.A. 101, 6641.

Blanchard, T.J., Alcami, A., Andrea, P., Smith, G.L., 1998. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. J. Gen. Virol. 79, 1159.

Blancou, J., Kieny, M.P., Lathe, R., Lecocq, J.P., Pastore, P.P., Soulebot, J.P., Desmettre, P., 1986. Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. Nature 322, 373.

Blasco, R., Moss, B., 1995. Selection of recombinant vaccinia viruses on the basis of plaque formation. Gene 158, 157.

Born, T.L., Smith, D.E., Garka, K.E., Renshaw, B.R., Bertles, J.S., Sims, J.E., 2000. Identification and characterization of two members of a novel class of the interleukin-1 receptor (IL-IR) family: delineation of a new class of IL-1R-related proteins based on signaling. J. Biol. Chem. 275, 29946.

Boukamp, P., Petrussevska, R.T., Breitkreutz, D., Hornung, J., Markham, A., Fusenig, N.E., 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell Biol. 106, 761.

Bratke, K.A., McLysaght, A., Rothenburg, S., 2013. A survey of host range genes in poxvirus genomes. Infect. Genet. Evol. 14, 406.

Breathnach, C.C., Clark, H.J., Clark, R.C., Olsen, C.W., Townsend, H.G.G., Lunn, D.P., 2006. Immunization with recombinant modified vaccinia Ankara (rMVA) constructs encoding the HA or NP gene protects ponies from equine influenza virus challenge. Vaccine 24, 1180.

Bundesamt für Vebraucherschutz und Lebensmittelsicherheit, 2002. Stellungnahme der ZKBS zur Risikobewertung des rekombinanten Vacciniavirus MVA. Az. 6790-10-74.

Calderara, S., Xiang, Y., Moss, B., 2001. Orthopoxvirus IL-18 binding proteins: affinities and antagonist activities. Virology 279, 22.

Carroll, M.W., Moss, B., 1995. E. coli beta-glucuronidase (GUS) as a marker for recombinant vaccinia viruses. Biotechniques 19, 352.
Carroll, M.W., Moss, B., 1997. Host range and cytopathogenicity of the highly attenuated MVA strain of vaccinia virus: propagation and generation of recombinant viruses in a nonhuman mammalian cell line. Virology 238, 198.
Chakrabarti, S., Brechling, K., Moss, B., 1985. Vaccinia virus expression vector: coexpression of beta-galactosidase provides visual screening of recombinant virus plaques. Mol. Cell. Biol. 5, 3403.
Chamcha, V., Kannanganat, S., Gangadhara, S., Nabi, R., Kozlowski, P.A., Montefiori, D.C., LaBranche, C.C., Wrammert, J., Keele, B.F., Balachandran, H., Sahu, S., Lifton, M., Santra, S., Basu, R., Moss, B., Robinson, H.L., Amara, R.R., 2016. Strong, but age-dependent, protection elicited by a deoxyribonucleic acid/modified vaccinia Ankara simian immunodeficiency virus vaccine. Open Forum Infect. Dis. 3, ofw034.
Chang, H.W., Watson, J.C., Jacobs, B.L., 1992. The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proc. Natl. Acad. Sci. U.S.A. 89, 4825.
Chapman, J.L., Nichols, D.K., Martinez, M.J., Raymond, J.W., 2010. Animal models of orthopoxvirus infection. Vet. Pathol. 47, 852.
Chen, Z., Zhang, L., Qin, C., Ba, L., Yi, C.E., Zhang, F., Wei, Q., He, T., Yu, W., Yu, J., Gao, H., Tu, X., Gettie, A., Farzan, M., Yuen, K.-y., Ho, D.D., 2005. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. J. Virol. 79, 2678.
Choi, J.H., Croyle, M.A., 2013. Emerging targets and novel approaches to Ebola virus prophylaxis and treatment. BioDrugs 27, 565.
Clark, R.H., Kenyon, J.C., Bartlett, N.W., Tscharke, D.C., Smith, G.L., 2006. Deletion of gene A41L enhances vaccinia virus immunogenicity and vaccine efficacy. J. Gen. Virol. 87, 29.
Cottingham, M.G., Andersen, R.F., Spencer, A.J., Saurya, S., Furze, J., Hill, A.V.S., Gilbert, S.C., 2008. Recombination-mediated genetic engineering of a bacterial artificial chromosome clone of modified vaccinia virus Ankara (MVA). PLoS One 3, e1638.
Coulibaly, S., Brühl, P., Mayrhofer, J., Schmid, K., Gerencer, M., Falkner, F.G., 2005. The nonreplicating smallpox candidate vaccines defective vaccinia Lister (dVV-L) and modified vaccinia Ankara (MVA) elicit robust long-term protection. Virology 341, 91.
Dai, P., Wang, W., Cao, H., Avogadri, F., Dai, L., Drexler, I., Joyce, J.A., Li, X.-D., Chen, Z., Merghoub, T., Shuman, S., Deng, L., 2014. Modified vaccinia virus Ankara triggers type I IFN production in murine conventional dendritic cells via a cGAS/STING-mediated cytosolic DNA-sensing pathway. PLoS Pathog. 10, e1003989.
Davies, M.V., Elroy-Stein, O., Jagus, R., Moss, B., Kaufman, R.J., 1992. The vaccinia virus K3L gene product potentiates translation by inhibiting double-stranded-RNA-activated protein kinase and phosphorylation of the alpha subunit of eukaryotic initiation factor 2. J. Virol. 66, 1943.
Davison, A.J., Moss, B., 1989. Structure of vaccinia virus late promoters. J. Mol. Biol. 210, 771.
de Jong, J.C., Claas, E.C.J., Osterhaus, A.D.M.E., Webster, R.G., Lim, W.L., 1997. A pandemic warning? Nature 389, 554.
de La Vega, M.-A., Stein, D., Kobinger, G.P., 2015. Ebolavirus evolution: past and present. PLoS Pathog. 11, e1005221.
De Vries, R.D., De Gruyter, H.L.M., Bestebroer, T.M., Pronk, M., Fouchier, R.A.M., Osterhaus, A.D.M.E., Sutter, G., Kreijtz, J.H.C.M., Rimmelzwaan, G.F., 2015.

Induction of influenza (H5N8) antibodies by modified vaccinia virus Ankara H5N1 vaccine. Emerg. Infect. Dis. 21, 1086.
Delaloye, J., Roger, T., Steiner-Tardivel, Q.-G., Le Roy, D., Knaup Reymond, M., Akira, S., Petrilli, V., Gomez, C.E., Perdiguero, B., Tschopp, J., Pantaleo, G., Esteban, M., Calandra, T., 2009. Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. PLoS Pathog. 5, e1000480.
Di Pilato, M., Mejías-Pérez, E., Gómez, C.E., Perdiguero, B., Sorzano, C.O.S., Esteban, M., 2013. New vaccinia virus promoter as a potential candidate for future vaccines. J. Gen. Virol. 94, 2771.
Di Pilato, M., Sánchez-Sampedro, L., Mejías-Pérez, E., Sorzano, C.O.S., Esteban, M., 2015. Modification of promoter spacer length in vaccinia virus as a strategy to control the antigen expression. J. Gen. Virol. 96, 2360.
Dobson, B.M., Tscharke, D.C., 2015. Redundancy complicates the definition of essential genes for vaccinia virus. J. Gen. Virol. 96, 3326.
Domi, A., Moss, B., 2002. Cloning the vaccinia virus genome as a bacterial artificial chromosome in Escherichia coli and recovery of infectious virus in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 99, 12415.
Domi, A., Moss, B., 2005. Engineering of a vaccinia virus bacterial artificial chromosome in Escherichia coli by bacteriophage [lambda]-based recombination. Nat. Methods 2, 95.
Douek, D.C., Kwong, P.D., Nabel, G.J., 2006. The rational design of an AIDS vaccine. Cell 124, 677.
Drexler, I., Heller, K., Wahren, B., Erfle, V., Sutter, G., 1998. Highly attenuated modified vaccinia virus Ankara replicates in baby hamster kidney cells, a potential host for virus propagation, but not in various human transformed and primary cells. J. Gen. Virol. 79, 347.
Drexler, I., Staib, C., Kastenmüller, W., Stevanović, S., Schmidt, B., Lemonnier, F.A., Rammensee, H.-G., Busch, D.H., Bernhard, H., Erfle, V., Sutter, G., 2003. Identification of vaccinia virus epitope-specific HLA-A*0201-restricted T cells and comparative analysis of smallpox vaccines. Proc. Natl. Acad. Sci. U.S.A. 100, 217.
Drillien, R., Spehner, D., Kirn, A., 1978. Host range restriction of vaccinia virus in Chinese hamster ovary cells: relationship to shutoff of protein synthesis. J. Virol. 28, 843.
Drillien, R., Koehren, F., Kirn, A., 1981. Host range deletion mutant of vaccinia virus defective in human cells. Virology 111, 488.
Earl, P.L., Wyatt, L.S., Montefiori, D.C., Bilska, M., Woodward, R., Markham, P.D., Malley, J.D., Vogel, T.U., Allen, T.M., Watkins, D.I., Miller, N., Moss, B., 2002. Comparison of vaccine strategies using recombinant env–gag–pol MVA with or without an oligomeric Env protein boost in the SHIV rhesus macaque model. Virology 294, 270.
Earl, P.L., Americo, J.L., Wyatt, L.S., Eller, L.A., Whitbeck, J.C., Cohen, G.H., Eisenberg, R.J., Hartmann, C.J., Jackson, D.L., Kulesh, D.A., Martinez, M.J., Miller, D.M., Mucker, E.M., Shamblin, J.D., Zwiers, S.H., Huggins, J.W., Jahrling, P.B., Moss, B., 2004. Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. Nature 428, 182.
Edghill-Smith, Y., Golding, H., Manischewitz, J., King, L.R., Scott, D., Bray, M., Nalca, A., Hooper, J.W., Whitehouse, C.A., Schmitz, J.E., Reimann, K.A., Franchini, G., 2005. Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. Nat. Med. 11, 740.
EMA, 2010. Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines. (EMA/CHMP/VWP/141697/2009).
Esposito, J., Brechling, K., Baer, G., Moss, B., 1987. Vaccinia virus recombinants expressing rabiesvirus glycoprotein protect against rabies. Virus Genes 1 (1), 7–21.

Esteban, D.J., Buller, R.M.L., 2005. Ectromelia virus: the causative agent of mousepox. J. Gen. Virol. 86, 2645.

European Medicines Agency, 2013. Imvanex: Modifiziertes Vacciniavirus Ankara Lebend. European Medicines Agency, London. EMA/490157/2013.

Ewer, K.J., O'Hara, G.A., Duncan, C.J.A., Collins, K.A., Sheehy, S.H., Reyes-Sandoval, A., Goodman, A.L., Edwards, N.J., Elias, S.C., Halstead, F.D., Longley, R.J., Rowland, R., Poulton, I.D., Draper, S.J., Blagborough, A.M., Berrie, E., Moyle, S., Williams, N., Siani, L., Folgori, A., Colloca, S., Sinden, R.E., Lawrie, A.M., Cortese, R., Gilbert, S.C., Nicosia, A., Hill, A.V.S., 2013. Protective CD8(+) T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. Nat. Commun. 4, 2836.

Ewer, K., Rampling, T., Venkatraman, N., Bowyer, G., Wright, D., Lambe, T., Imoukhuede, E.B., Payne, R., Fehling, S.K., Strecker, T., Biedenkopf, N., Krähling, V., Tully, C.M., Edwards, N.J., Bentley, E.M., Samuel, D., Labbé, G., Jin, J., Gibani, M., Minhinnick, A., Wilkie, M., Poulton, I., Lella, N., Roberts, R., Hartnell, F., Bliss, C., Sierra-Davidson, K., Powlson, J., Berrie, E., Tedder, R., Roman, F., De Ryck, I., Nicosia, A., Sullivan, N.J., Stanley, D.A., Mbaya, O.T., Ledgerwood, J.E., Schwartz, R.M., Siani, L., Colloca, S., Folgori, A., Di Marco, S., Cortese, R., Wright, E., Becker, S., Graham, B.S., Koup, R.A., Levine, M.M., Volkmann, A., Chaplin, P., Pollard, A.J., Draper, S.J., Ballou, W.R., Lawrie, A., Gilbert, S.C., Hill, A.V.S., 2016. A monovalent chimpanzee adenovirus Ebola vaccine boosted with MVA. N. Engl. J. Med. 374, 1635.

Excler, J.-L., Robb, M.L., Kim, J.H., 2014. HIV-1 vaccines. Hum. Vac. Immunother. 10, 1734.

Falivene, J., Del Médico Zajac, M.P., Pascutti, M.F., Rodríguez, A.M., Maeto, C., Perdiguero, B., Gómez, C.E., Esteban, M., Calamante, G., Gherardi, M.M., 2012. Improving the MVA vaccine potential by deleting the viral gene coding for the IL-18 binding protein. PLoS One 7, e32220.

Falkner, F.G., Moss, B., 1988. Escherichia coli gpt gene provides dominant selection for vaccinia virus open reading frame expression vectors. J. Virol. 62, 1849.

Ferguson, B.J., Benfield, C.T.O., Ren, H., Lee, V.H., Frazer, G.L., Strnadova, P., Sumner, R.P., Smith, G.L., 2013. Vaccinia virus protein N2 is a nuclear IRF3 inhibitor that promotes virulence. J. Gen. Virol. 94, 2070.

Ferrer, M.F., Del Médico Zajac, M.P., Zanetti, F.A., Valera, A.R., Zabal, O., Calamante, G., 2011. Recombinant MVA expressing secreted glycoprotein D of BoHV-1 induces systemic and mucosal immunity in animal models. Viral Immunol. 24, 331.

Fischer, S.F., Ludwig, H., Holzapfel, J., Kvansakul, M., Chen, L., Huang, D.C.S., Sutter, G., Knese, M., Hacker, G., 2005. Modified vaccinia virus Ankara protein F1L is a novel BH3-domain-binding protein and acts together with the early viral protein E3L to block virus-associated apoptosis. Cell Death Differ. 13, 109.

Fouchier, R.A.M., Garcia-Sastre, A., Kawaoka, Y., 2013. H5N1 virus: transmission studies resume for avian flu. Nature 493, 609.

Frey, S.E., Wald, A., Edupuganti, S., Jackson, L.A., Stapleton, J.T., Sahly, H.E., El-Kamary, S.S., Edwards, K., Keyserling, H., Winokur, P., Keitel, W., Hill, H., Goll, J.B., Anderson, E.L., Graham, I.L., Johnston, C., Mulligan, M., Rouphael, N., Atmar, R., Patel, S., Chen, W., Kotloff, K., Creech, C.B., Chaplin, P., Belshe, R.B., 2015. Comparison of lyophilized versus liquid modified vaccinia Ankara (MVA) formulations and subcutaneous versus intradermal routes of administration in healthy vaccinia-naïve subjects. Vaccine 33, 5225.

Garber, D.A., O'Mara, L.A., Zhao, J., Gangadhara, S., An, I., Feinberg, M.B., 2009. Expanding the repertoire of modified vaccinia Ankara-based vaccine vectors via genetic complementation strategies. PLoS One 4, e5445.

García-Arriaza, J., Nájera, J.L., Gómez, C.E., Tewabe, N., Sorzano, C.O.S., Calandra, T., Roger, T., Esteban, M., 2011. A candidate HIV/AIDS vaccine (MVA-B) lacking vaccinia virus gene C6L enhances memory HIV-1-specific T-cell responses. PLoS One 6, e24244.

García-Arriaza, J., Arnáez, P., Gómez, C.E., Sorzano, C.Ó.S., Esteban, M., 2013. Improving adaptive and memory immune responses of an HIV/AIDS vaccine candidate MVA-B by deletion of vaccinia virus genes (C6L and K7R) blocking interferon signaling pathways. PLoS One 8, e66894.

García-Arriaza, J., Gómez, C.E., Sorzano, C.Ó.S., Esteban, M., 2014. Deletion of the vaccinia virus N2L gene encoding an inhibitor of IRF3 improves the immunogenicity of modified vaccinia virus Ankara expressing HIV-1 antigens. J. Virol. 88, 3392.

Genzel, Y., 2015. Designing cell lines for viral vaccine production: where do we stand? Biotechnol. J. 10 (5), 728–740. http://dx.doi.org/10.1002/biot.201400388. Epub 2015 Apr 22.

Gilbert, S.C., Plebanski, M., Harris, S.J., Allsopp, C.E.M., Thomas, R., Layton, G.T., Hill, A.V.S., 1997. A protein particle vaccine containing multiple malaria epitopes. Nat. Biotechnol. 15, 1280.

Gilbert, S.C., Schneider, J., Hannan, C.M., Hu, J.T., Plebanski, M., Sinden, R., Hill, A.V.S., 2002. Enhanced CD8 T cell immunogenicity and protective efficacy in a mouse malaria model using a recombinant adenoviral vaccine in heterologous prime–boost immunisation regimes. Vaccine 20, 1039.

Gillard, S., Spehner, D., Drillien, R., Kirn, A., 1986. Localization and sequence of a vaccinia virus gene required for multiplication in human cells. Proc. Natl. Acad. Sci. U.S.A. 83, 5573.

Gire, S.K., Goba, A., Andersen, K.G., Sealfon, R.S.G., Park, D.J., Kanneh, L., Jalloh, S., Momoh, M., Fullah, M., Dudas, G., Wohl, S., Moses, L.M., Yozwiak, N.L., Winnicki, S., Matranga, C.B., Malboeuf, C.M., Qu, J., Gladden, A.D., Schaffner, S.F., Yang, X., Jiang, P.-P., Nekoui, M., Colubri, A., Coomber, M.R., Fonnie, M., Moigboi, A., Gbakie, M., Kamara, F.K., Tucker, V., Konuwa, E., Saffa, S., Sellu, J., Jalloh, A.A., Kovoma, A., Koninga, J., Mustapha, I., Kargbo, K., Foday, M., Yillah, M., Kanneh, F., Robert, W., Massally, J.L.B., Chapman, S.B., Bochicchio, J., Murphy, C., Nusbaum, C., Young, S., Birren, B.W., Grant, D.S., Scheiffelin, J.S., Lander, E.S., Happi, C., Gevao, S.M., Gnirke, A., Rambaut, A., Garry, R.F., Khan, S.H., Sabeti, P.C., 2014. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345, 1369.

Goebel, S., Johnson, G.P., Perkus, M.E., Davis, S.W., Winslow, J.P., Paoletti, E., 1990. The complete DNA sequence of vaccinia virus. Virology 179, 517.

Goepfert, P.A., Elizaga, M.L., Seaton, K., Tomaras, G.D., Montefiori, D.C., Sato, A., Hural, J., DeRosa, S.C., Kalams, S.A., McElrath, M.J., Keefer, M.C., Baden, L.R., Lama, J.R., Sanchez, J., Mulligan, M.J., Buchbinder, S.P., Hammer, S.M., Koblin, B.A., Pensiero, M., Butler, C., Moss, B., Robinson, H.L., the, H.S.G. the National Institutes of, A., Infectious Diseases, H.I.V.V.T.N., Donastorg, Y., Qin, L., Lawrence, D., Cardinali, M., Bae, J., Holt, R., Redinger, H., Johannessen, J., Broder, G., Moody-White, J., McKay, B., Calazans, G., Bentley, C., Kakinami, L., Skibinski, K., Estep, S., Tseng, J., Swenson, M., Madenwald, T., Overton, E.T., Edupuganti, S., Rouphael, N., Whitaker, J., Hay, C.M., Bunce, C.A., Gonzales, P., Hurtado, J.C., Dolin, R., Mayer, K., Walsh, S., Johnson, J., 2014. Specificity and 6-month durability of immune responses induced by DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 virus-like particles. J. Infect. Dis. 210, 99.

Goldblum, N., Sterk, V., Paderski, B., 1954. West Nile fever; the clinical features of the disease and the isolation of West Nile virus from the blood of nine human cases. Am. J. Hyg. 59, 89.

Gómez, C.E., Perdiguero, B., Jiménez, V., Filali-Mouhim, A., Ghneim, K., Haddad, E.K., Quakkerlaar, E.D., Delaloye, J., Harari, A., Roger, T., Dunhen, T., Sékaly, R.P., Melief, C.J.M., Calandra, T., Sallusto, F., Lanzavecchia, A., Wagner, R., Pantaleo, G., Esteban, M., 2012. Systems analysis of MVA-C induced immune response reveals its significance as a vaccine candidate against HIV/AIDS of clade C. PLoS One 7, e35485.

Goodpasture, E.W., Woodruff, A.M., Buddingh, G.J., 1931. The cultivation of vaccine and other viruses in the chorioallantoic membrane of chick embryos. Science 74, 371.

Goodpasture, E.W., Woodruff, A.M., Buddingh, G.J., 1932. Vaccinal infection of the chorio-allantoic membrane of the chick embryo. Am. J. Pathol. 8, 271.

Goonetilleke, N.P., McShane, H., Hannan, C.M., Anderson, R.J., Brookes, R.H., Hill, A.V.S., 2003. Enhanced immunogenicity and protective efficacy against mycobacterium tuberculosis of bacille calmette-guérin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. J. Immunol. 171, 1602.

Graham, K.A., Lalani, A.S., Macen, J.L., Ness, T.L., Barry, M., Liu, L.-Y., Lucas, A., Clark-Lewis, I., Moyer, R.W., McFadden, G., 1997. The T1/35 kDa family of poxvirus-secreted proteins bind chemokines and modulate leukocyte influx into virus-infected tissues. Virology 229, 12.

Graham, R.L., Donaldson, E.F., Baric, R.S., 2013. A decade after SARS: strategies for controlling emerging coronaviruses. Nat. Rev. Microbiol. 11, 836.

Greenberg, R.N., Hurley, Y., Dinh, D.V., Mraz, S., Vera, J.G., von Bredow, D., von Krempelhuber, A., Roesch, S., Virgin, G., Arndtz-Wiedemann, N., Meyer, T.P., Schmidt, D., Nichols, R., Young, P., Chaplin, P., 2015. A multicenter, open-label, controlled phase II study to evaluate safety and immunogenicity of MVA smallpox vaccine (IMVAMUNE) in 18–40 year old subjects with diagnosed atopic dermatitis. PLoS One 10, e0138348.

Haagmans, B.L., Al Dhahiry, S.H.S., Reusken, C.B.E.M., Raj, V.S., Galiano, M., Myers, R., Godeke, G.-J., Jonges, M., Farag, E., Diab, A., Ghobashy, H., Alhajri, F., Al-Thani, M., Al-Marri, S.A., Al Romaihi, H.E., Al Khal, A., Bermingham, A., Osterhaus, A.D.M.E., AlHajri, M.M., Koopmans, M.P.G., 2014. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect. Dis. 14, 140.

Haagmans, B.L., van den Brand, J.M.A., Raj, V.S., Volz, A., Wohlsein, P., Smits, S.L., Schipper, D., Bestebroer, T.M., Okba, N., Fux, R., Bensaid, A., Solanes Foz, D., Kuiken, T., Segalés, J., Sutter, G., Osterhaus, A.D.M.E., Osterhaus, A.D.M.E., 2016. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science 351 (6268), 77–81.

Haller, S.L., Peng, C., McFadden, G., Rothenburg, S., 2014. Poxviruses and the evolution of host range and virulence. Infect. Genet. Evol. 21, 15.

Hanke, T., Blanchard, T.J., Schneider, J., Ogg, G.S., Tan, R., Becker, M., Gilbert, S.C., Hill, A.V., Smith, G.L., McMichael, A., 1998. Immunogenicities of intravenous and intramuscular administrations of modified vaccinia virus Ankara-based multi-CTL epitope vaccine for human immunodeficiency virus type 1 in mice. J. Gen. Virol. 79, 83.

He, Y., Zhou, Y., Wu, H., Luo, B., Chen, J., Li, W., Jiang, S., 2004. Identification of immunodominant sites on the spike protein of severe acute respiratory syndrome (SARS) coronavirus: implication for developing SARS diagnostics and vaccines. J. Immunol. 173, 4050.

Herbert, M.H., Squire, C.J., Mercer, A.A., 2015. Poxviral ankyrin proteins. Viruses 7, 709.

Herfst, S., Schrauwen, E.J.A., Linster, M., Chutinimitkul, S., de Wit, E., Munster, V.J., Sorrell, E.M., Bestebroer, T.M., Burke, D.F., Smith, D.J., Rimmelzwaan, G.F., Osterhaus, A.D.M.E., Fouchier, R.A.M., 2012. Airborne transmission of influenza a/H5N1 virus between ferrets. Science (New York, N.Y.) 336, 1534.

Herrlich, A., Mayr, A., 1954. Comparative experimental works on cow pox virus vaccines. Arch. Hyg. Bakteriol. 138, 479.

Hessel, A., Schwendinger, M., Fritz, D., Coulibaly, S., Holzer, G.W., Sabarth, N., Kistner, O., Wodal, W., Kerschbaum, A., Savidis-Dacho, H., Crowe, B.A., Kreil, T.R., Barrett, P.N., Falkner, F.G., 2010. A pandemic influenza H1N1 live vaccine based on modified vaccinia Ankara is highly immunogenic and protects mice in active and passive immunizations. PLoS One 5, e12217.

Hessel, A., Schwendinger, M., Holzer, G.W., Orlinger, K.K., Coulibaly, S., Savidis-Dacho, H., Zips, M.-L., Crowe, B.A., Kreil, T.R., Ehrlich, H.J., Barrett, P.N., Falkner, F.G., 2011. Vectors based on modified vaccinia Ankara expressing influenza H5N1 hemagglutinin induce substantial cross-clade protective immunity. PLoS One 6, e16247.

Hinthong, O., Jin, X.L., Shisler, J.L., 2008. Characterization of wild-type and mutant vaccinia virus M2L proteins' abilities to localize to the endoplasmic reticulum and to inhibit NF-kappaB activation during infection. Virology 373 (2), 248–262. http://dx.doi.org/10.1016/j.virol.2007.11.034. Epub 2008 Jan 10.

Hirsch, V.M., Fuerst, T.R., Sutter, G., Carroll, M.W., Yang, L.C., Goldstein, S., Piatak, M., Elkins, W.R., Alvord, W.G., Montefiori, D.C., Moss, B., Lifson, J.D., 1996. Patterns of viral replication correlate with outcome in simian immunodeficiency virus (SIV)-infected macaques: effect of prior immunization with a trivalent SIV vaccine in modified vaccinia virus Ankara. J. Virol. 70, 3741.

Hoffman, S.L., Vekemans, J., Richie, T.L., Duffy, P.E., 2015. The march toward malaria vaccines. Am. J. Prev. Med. 49, S319.

Hornemann, S., Harlin, O., Staib, C., Kisling, S., Erfle, V., Kaspers, B., Häcker, G., Sutter, G., 2003. Replication of modified vaccinia virus Ankara in primary chicken embryo fibroblasts requires expression of the interferon resistance gene E3L. J. Virol. 77, 8394.

Isaacs, S.N., Kotwal, G.J., Moss, B., 1990. Reverse guanine phosphoribosyltransferase selection of recombinant vaccinia viruses. Virology 178, 626.

Ishii, K.J., Coban, C., Kato, H., Takahashi, K., Torii, Y., Takeshita, F., Ludwig, H., Sutter, G., Suzuki, K., Hemmi, H., Sato, S., Yamamoto, M., Uematsu, S., Kawai, T., Takeuchi, O., Akira, S., 2006. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nat. Immunol. 7, 40.

Iyer, S.S., Amara, R.R., 2014. DNA/MVA vaccines for HIV/AIDS. Vaccines 2, 160.

Iyer, S.S., Gangadhara, S., Victor, B., Gomez, R., Basu, R., Hong, J.J., Labranche, C., Montefiori, D.C., Villinger, F., Moss, B., Amara, R.R., 2015. Codelivery of envelope protein in alum with MVA vaccine induces CXCR3-biased CXCR5+ and CXCR5− CD4 T cell responses in rhesus macaques. J. Immunol. 195, 994.

Jahrling, P.B., Hensley, L.E., Martinez, M.J., LeDuc, J.W., Rubins, K.H., Relman, D.A., Huggins, J.W., 2004. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. Proc. Natl. Acad. Sci. U.S.A. 101, 15196.

Jeong-Sun, Y., SungHan, P., You-Jin, K., Hae Ji, K., Hak, K., Young Woo, H., Han Saem, L., Dae-Won, K., Kim, A.R., Deok Rim, H., Joo Ae, K., Su Jin, K., Jeong-Gu, N., Hee-Dong, J., Hyang-Min, C., Kisoon, K., Joo-Shil, L., Sung Soon, K., 2015. Middle east respiratory syndrome in 3 persons, South Korea, 2015. Emerg. Infect. Dis. 21, 2084.

Joachim, A., Nilsson, C., Aboud, S., Bakari, M., Lyamuya, E.F., Robb, M.L., Marovich, M.A., Earl, P., Moss, B., Ochsenbauer, C., Wahren, B., Mhalu, F., Sandström, E., Biberfeld, G., Ferrari, G., Polonis, V.R., 2015. Potent functional antibody responses elicited by HIV-I DNA priming and boosting with heterologous HIV-1 recombinant MVA in healthy Tanzanian adults. PLoS One 10, e0118486.

Jones, D.I., McGee, C.E., Sample, C.J., Sempowski, G.D., Pickup, D.J., Staats, H., 2016. MVA vaccination provides long-term protection against nasal rabbitpox virus challenge. Clin. Vaccine Immunol. 23 (7), 648–651.

Jordan, I., Northoff, S., Thiele, M., Hartmann, S., Horn, D., Höwing, K., Bernhardt, H., Oehmke, S., von Horsten, H., Rebeski, D., Hinrichsen, L., Zelnik, V., Mueller, W., Sandig, V., 2011. A chemically defined production process for highly attenuated poxviruses. Biologicals 39, 50.

Kamlangdee, A., Kingstad-Bakke, B., Anderson, T.K., Goldberg, T.L., Osorio, J.E., 2014. Broad protection against avian influenza virus by using a modified vaccinia Ankara virus expressing a mosaic hemagglutinin gene. J. Virol. 88, 13300.

Kapulu, M.C., Da, D.F., Miura, K., Li, Y., Blagborough, A.M., Churcher, T.S., Nikolaeva, D., Williams, A.R., Goodman, A.L., Sangare, I., Turner, A.V., Cottingham, M.G., Nicosia, A., Straschil, U., Tsuboi, T., Gilbert, S.C., Long, C.A., Sinden, R.E., Draper, S.J., Hill, A.V.S., Cohuet, A., Biswas, S., 2015. Comparative assessment of transmission-blocking vaccine candidates against plasmodium falciparum. Sci. Rep. 5, 11193.

Kieny, M.P., Lathe, R., Drillien, R., Spehner, D., Skory, S., Schmitt, D., Wiktor, T., Koprowski, H., Lecocq, J.P., 1984. Expression of rabies virus glycoprotein from a recombinant vaccinia virus. Nature 312 (5990), 163–166.

Kim, J.Y., Song, J.Y., Yoon, Y.K., Choi, S.-H., Song, Y.G., Kim, S.-R., Son, H.-J., Jeong, S.-Y., Choi, J.-H., Kim, K.M., Yoon, H.J., Choi, J.Y., Kim, T.H., Choi, Y.H., Kim, H.B., Yoon, J.H., Lee, J., Eom, J.S., Lee, S.-O., Oh, W.S., Choi, J.-H., Yoo, J.-H., Kim, W.J., Cheong, H.J., 2015. Middle east respiratory syndrome infection control and prevention guideline for healthcare facilities. Infect. Chemother. 47, 278.

Knitlova, J., Hajkova, V., Voska, L., Elsterova, J., Obrova, B., Melkova, Z., 2014. Development of eczema vaccinatum in atopic mouse models and efficacy of MVA vaccination against lethal poxviral infection. PLoS One 9, e114374.

Kreijtz, J.H.C.M., Suezer, Y., van Amerongen, G., de Mutsert, G., Schnierle, B.S., Wood, J.M., Kuiken, T., Fouchier, R.A.M., Löwer, R.A.M., Osterhaus, A.D.M.E., Sutter, G., Rimmelzwaan, G.F., 2007. Recombinant modified vaccinia virus Ankara-based vaccine induces protective immunity in mice against infection with influenza virus H5N1. J. Infect. Dis. 195, 1598.

Kreijtz, J.H.C.M., Suezer, Y., de Mutsert, G., van Amerongen, G., Schwantes, A., van den Brand, J.M.A., Fouchier, R.A.M., Löwer, J., Osterhaus, A.D.M.E., Sutter, G., Rimmelzwaan, G.F., 2009a. MVA-based H5N1 vaccine affords cross-clade protection in mice against influenza a/H5N1 viruses at low doses and after single immunization. PLoS One 4, e7790.

Kreijtz, J.H.C.M., Suezer, Y., de Mutsert, G., van den Brand, J.M.A., van Amerongen, G., Schnierle, B.S., Kuiken, T., Fouchier, R.A.M., Löwer, J., Osterhaus, A.D.M.E., Sutter, G., Rimmelzwaan, G.F., 2009b. Recombinant modified vaccinia virus Ankara expressing the hemagglutinin gene confers protection against homologous and heterologous H5N1 influenza virus infections in macaques. J. Infect. Dis. 199, 405.

Kreijtz, J.H.C.M., Süzer, Y., Bodewes, R., Schwantes, A., van Amerongen, G., Verburgh, R.J., de Mutsert, G., van den Brand, J., van Trierum, S.E., Kuiken, T., Fouchier, R.A.M., Osterhaus, A.D.M.E., Sutter, G., Rimmelzwaan, G.F., 2010. Evaluation of a modified vaccinia virus Ankara (MVA)-based candidate pandemic influenza A/H1N1 vaccine in the ferret model. J. Gen. Virol. 91, 2745.

Kreijtz, J.H.C.M., Goeijenbier, M., Moesker, F.M., van den Dries, L., Goeijenbier, S., De Gruyter, H.L.M., Lehmann, M.H., Mutsert, G.d., van de Vijver, D.A.M.C., Volz, A., Fouchier, R.A.M., van Gorp, E.C.M., Rimmelzwaan, G.F., Sutter, G., Osterhaus, A.D.M.E., 2014. Safety and immunogenicity of a modified-vaccinia-virus-

Ankara-based influenza a H5N1 vaccine: a randomised, double-blind phase 1/2a clinical trial. Lancet Infect. Dis. 14, 1196.
Kremer, M., Suezer, Y., Volz, A., Frenz, T., Majzoub, M., Hanschmann, K.-M., Lehmann, M.H., Kalinke, U., Sutter, G., 2012a. Critical role of perforin-dependent CD8+ T cell immunity for rapid protective vaccination in a murine model for human smallpox. PLoS Pathog. 8, e1002557.
Kremer, M., Volz, A., Kreijtz, J.H.C.M., Lehmann, M.H., Fux, R., Sutter, G., 2012b. Easy and efficient protocols for working with recombinant vaccinia virus MVA. Methods Mol. Biol. 890, 59–92.
Kroon, E.G., Mota, B.E.F., Abrahão, J.S., Fonseca, F.G.d., Trindade, G.d.S., 2011. Zoonotic Brazilian vaccinia virus: from field to therapy. Antivir. Res. 92, 150.
Kuiken, T., Fouchier, R., Rimmelzwaan, G., van den Brand, J., van Riel, D., Osterhaus, A., 2011. Pigs, poultry, and pandemic influenza: how zoonotic pathogens threaten human health. In: Curtis, N., Finn, A., Pollard, J.A. (Eds.), Hot Topics in Infection and Immunity in Children VIII. Springer New York, New York, p. 59.
Kwa, S., Sadagopal, S., Shen, X., Hong, J.J., Gangadhara, S., Basu, R., Victor, B., Iyer, S.S., LaBranche, C.C., Montefiori, D.C., Tomaras, G.D., Villinger, F., Moss, B., Kozlowski, P.A., Amara, R.R., 2015. CD40L-adjuvanted DNA/modified vaccinia virus Ankara simian immunodeficiency virus (SIV) vaccine enhances protection against neutralization-resistant mucosal SIV infection. J. Virol. 89, 4690.
Lambe, T., Carey, J.B., Li, Y., Spencer, A.J., van Laarhoven, A., Mullarkey, C.E., Vrdoljak, A., Moore, A.C., Gilbert, S.C., 2013. Immunity against heterosubtypic influenza virus induced by adenovirus and MVA expressing nucleoprotein and matrix protein-1. Sci. Rep. 3, 1443.
Lanciotti, R.S., Roehrig, J.T., Deubel, V., Smith, J., Parker, M., Steele, K., Crise, B., Volpe, K.E., Crabtree, M.B., Scherret, J.H., Hall, R.A., MacKenzie, J.S., Cropp, C.B., Panigrahy, B., Ostlund, E., Schmitt, B., Malkinson, M., Banet, C., Weissman, J., Komar, N., Savage, H.M., Stone, W., McNamara, T., Gubler, D.J., 1999. Origin of the west Nile virus responsible for an outbreak of encephalitis in the northeastern united states. Science 286, 2333.
Lehmann, M.H., Kastenmuller, W., Kandemir, J.D., Brandt, F., Suezer, Y., Sutter, G., 2009. Modified vaccinia virus Ankara triggers chemotaxis of monocytes and early respiratory immigration of leukocytes by induction of CCL2 expression. J. Virol. 83, 2540.
Lillie, P.J., Berthoud, T.K., Powell, T.J., Lambe, T., Mullarkey, C., Spencer, A.J., Hamill, M., Peng, Y., Blais, M.-E., Duncan, C.J.A., Sheehy, S.H., Havelock, T., Faust, S.N., Williams, R.L., Gilbert, A., Oxford, J., Dong, T., Hill, A.V.S., Gilbert, S.C., 2012. Preliminary assessment of the efficacy of a T-cell-based influenza vaccine, MVA-NP+M1, in humans. Clin. Infect. Dis. 55, 19.
Liu, J., McFadden, G., 2015. SAMD9 is an innate antiviral host factor with stress response properties that can be antagonized by poxviruses. J. Virol. 89, 1925.
Lohr, V., Rath, A., Genzel, Y., Jordan, I., Sandig, V., Reichl, U., 2009. New avian suspension cell lines provide production of influenza virus and MVA in serum-free media: studies on growth, metabolism and virus propagation. Vaccine 27, 4975.
Loubet, P., Samih-Lenzi, N., Galtier, F., Vanhems, P., Loulergue, P., Duval, X., Jouneau, S., Postil, D., Rogez, S., Valette, M., Merle, C., Régis, C., Costa, Y., Lesieur, Z., Tattevin, P., Lina, B., Carrat, F., Launay, O., 2016. Factors associated with poor outcomes among adults hospitalized for influenza in France: a three-year prospective multicenter study. J. Clin. Virol. 79, 68.
Ludwig, H., Mages, J., Staib, C., Lehmann, M.H., Lang, R., Sutter, G., 2005. Role of viral factor E3L in modified vaccinia virus Ankara infection of human HeLa cells: regulation of the virus life cycle and identification of differentially expressed host genes. J. Virol. 79, 2584.

Ludwig, H., Suezer, Y., Waibler, Z., Kalinke, U., Schnierle, B.S., Sutter, G., 2006. Double-stranded RNA-binding protein E3 controls translation of viral intermediate RNA, marking an essential step in the life cycle of modified vaccinia virus Ankara. J. Gen. Virol. 87, 1145.
Mackett, M., Smith, G.L., Moss, B., 1982. Vaccinia virus: a selectable eukaryotic cloning and expression vector. Proc. Natl. Acad. Sci. U.S.A. 79, 7415.
Mackett, M., Smith, G.L., Moss, B., 1984. General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. J. Virol. 49, 857.
Mahnel, H., Mayr, A., 1994. Experiences with immunization against orthopox viruses of humans and animals using vaccine strain MVA. Berl. Munch. Tierarztl. Wochenschr. 107, 253.
Mayr, A., Munz, E., 1964. Veränderung von Vaccinevirus durch Dauerpassagen in Hühnerembryofibroblastenkulturen. Zentralbl. Bakteriol. B 195, 24.
Mayr, A., Herrlich, A., Mahnel, H., 1955. Experimental research on the S-antigen in the viruses of animal smallpox. Arch. Hyg. Bakteriol. 139 (8), 580.
Mayr, A., Hochstein-Mintzel, V., Stickl, H., 1975. Abstammung, Eigenschaften und Verwendung des attenuierten vaccinia-Stammes MVA. Infection 3, 6.
McCollum, A.M., Damon, I.K., 2014. Human monkeypox. Clin. Infect. Dis. 58, 260.
McFadden, G., 2005. Poxvirus tropism. Nat. Rev. Microbiol. 3, 201.
McShane, H., Williams, A., 2014. A review of preclinical animal models utilised for TB vaccine evaluation in the context of recent human efficacy data. Tuberculosis 94, 105.
McShane, H., Behboudi, S., Goonetilleke, N., Brookes, R., Hill, A.V.S., 2002. Protective immunity against mycobacterium tuberculosis induced by dendritic cells pulsed with both CD8+- and CD4+-T-cell epitopes from antigen 85A. Infect. Immun. 70, 1623.
McShane, H., Pathan, A.A., Sander, C.R., Keating, S.M., Gilbert, S.C., Huygen, K., Fletcher, H.A., Hill, A.V.S., 2004. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat. Med. 10, 1240.
Memish, Z.A., Cotten, M., Meyer, B., Watson, S.J., Alsahafi, A.J., Al Rabeeah, A.A., Corman, V.M., Sieberg, A., Makhdoom, H.Q., Assiri, A., Al Masri, M., Aldabbagh, S., Bosch, B.-J., Beer, M., Müller, M.A., Kellam, P., Drosten, C., 2014. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. Emerg. Infect. Dis. 20, 1012.
Mercer, A.A., Fleming, S.B., Ueda, N., 2005. F-box-like domains are present in most poxvirus Ankyrin repeat proteins. Virus Genes 31, 127.
Meseda, C.A., Atukorale, V., Kuhn, J., Schmeisser, F., Weir, J.P., 2016. Percutaneous vaccination as an effective method of delivery of MVA and MVA-vectored vaccines. PLoS One 11, e0149364.
Meyer, H., Sutter, G., Mayr, A., 1991. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. J. Gen. Virol. 72, 1031.
Meyer, B., Müller, M.A., Corman, V.M., Reusken, C.B.E.M., Ritz, D., Godeke, G.-J., Lattwein, E., Kallies, S., Siemens, A., van Beek, J., Drexler, J.F., Muth, D., Bosch, B.-J., Wernery, U., Koopmans, M.P.G., Wernery, R., Drosten, C., 2014. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. Emerg. Infect. Dis. 20, 552.
Min, C.-K., Cheon, S., Ha, N.-Y., Sohn, K.M., Kim, Y., Aigerim, A., Shin, H.M., Choi, J.-Y., Inn, K.-S., Kim, J.-H., Moon, J.Y., Choi, M.-S., Cho, N.-H., Kim, Y.-S., 2016. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Sci. Rep. 6, 25359.

Moorthy, V.S., Pinder, M., Reece, W.H.H., Watkins, K., Atabani, S., Hannan, C., Bojang, K., McAdam, K.P.W.J., Schneider, J., Gilbert, S., Hill, A.V.S., 2003. Safety and immunogenicity of DNA/modified vaccinia virus Ankara malaria vaccination in African adults. J. Infect. Dis. 188, 1239.

Moorthy, V.S., Good, M.F., Hill, A.V.S., 2004. Malaria vaccine developments. Lancet 363, 150.

Mosavi, L.K., Cammett, T.J., Desrosiers, D.C., Peng, Z.-y., 2004. The Ankyrin repeat as molecular architecture for protein recognition. Protein Sci. 13, 1435.

Moss, B., 2011. Smallpox vaccines: targets of protective immunity. Immunol. Rev. 239, 8.

Mullarkey, C.E., Boyd, A., van Laarhoven, A., Lefevre, E.A., Veronica Carr, B., Baratelli, M., Molesti, E., Temperton, N.J., Butter, C., Charleston, B., Lambe, T., Gilbert, S.C., 2013. Improved adjuvanting of seasonal influenza vaccines: preclinical studies of MVA-NP+M1 coadministration with inactivated influenza vaccine. Eur. J. Immunol. 43, 1940.

Munseri, P.J., Kroidl, A., Nilsson, C., Joachim, A., Geldmacher, C., Mann, P., Moshiro, C., Aboud, S., Lyamuya, E., Maboko, L., Missanga, M., Kaluwa, B., Mfinanga, S., Podola, L., Bauer, A., Godoy-Ramirez, K., Marovich, M., Moss, B., Hoelscher, M., Gotch, F., Stöhr, W., Stout, R., McCormack, S., Wahren, B., Mhalu, F., Robb, M.L., Biberfeld, G., Sandström, E., Bakari, M., 2015. Priming with a simplified intradermal HIV-1 DNA vaccine regimen followed by boosting with recombinant HIV-1 MVA vaccine is safe and immunogenic: a phase IIa randomized clinical trial. PLoS One 10, e0119629.

Murray, K.O., Mertens, E., Desprès, P., 2010. West Nile virus and its emergence in the United States of America. Vet. Res. 41, 67.

Nakano, E., Panicali, D., Paoletti, E., 1982. Molecular genetics of vaccinia virus: demonstration of marker rescue. Proc. Natl. Acad. Sci. U.S.A. 79, 1593.

Nash, D., Mostashari, F., Fine, A., Miller, J., O'Leary, D., Murray, K., Huang, A., Rosenberg, A., Greenberg, A., Sherman, M., Wong, S., Campbell, G.L., Roehrig, J.T., Gubler, D.J., Shieh, W.-J., Zaki, S., Smith, P., Layton, M., 2001. The outbreak of West Nile virus infection in the New York City area in 1999. N. Engl. J. Med. 344, 1807.

Ng, T., Hathaway, D., Jennings, N., Champ, D., Chiang, Y.W., Chu, H.J., 2003. Equine vaccine for West Nile virus. Dev. Biol. (Basel) 114, 221–227.

Nilsson, C., Hejdeman, B., Godoy-Ramirez, K., Tecleab, T., Scarlatti, G., Bråve, A., Earl, P.L., Stout, R.R., Robb, M.L., Shattock, R.J., Biberfeld, G., Sandström, E., Wahren, B., 2015. HIV-DNA given with or without intradermal electroporation is safe and highly immunogenic in healthy swedish HIV-1 DNA/MVA vaccinees: a phase I randomized trial. PLoS One 10, e0131748.

Oie, K.L., Pickup, D.J., 2001. Cowpox virus and other members of the orthopoxvirus genus interfere with the regulation of NF-kB activation. Virology 288, 175.

Ondondo, B.O., 2014. The influence of delivery vectors on HIV vaccine efficacy. Front. Microbiol. 5, 439.

Ondondo, B., Murakoshi, H., Clutton, G., Abdul-Jawad, S., Wee, E.G.T., Gatanaga, H., Oka, S., McMichael, A.J., Takiguchi, M., Korber, B., Hanke, T., 2016. Novel conserved-region T-cell mosaic vaccine with high global HIV-1 coverage is recognized by protective responses in untreated infection. Mol. Ther. 24, 832.

Overton, E.T., Stapleton, J., Frank, I., Hassler, S., Goepfert, P.A., Barker, D., Wagner, E., von Krempelhuber, A., Virgin, G., Meyer, T.P., Müller, J., Bädeker, N., Grünert, R., Young, P., Rösch, S., Maclennan, J., Arndtz-Wiedemann, N., Chaplin, P., 2015. Safety and immunogenicity of modified vaccinia Ankara-Bavarian Nordic smallpox vaccine in vaccinia-naive and experienced human immunodeficiency virus-infected

individuals: an open-label, controlled clinical phase II trial. Open Forum Infect. Dis. 2, ofv040.
Panicali, D., Paoletti, E., 1982. Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. 79, 4927.
Paran, N., Suezer, Y., Lustig, S., Israely, T., Schwantes, A., Melamed, S., Katz, L., Preuß, T., Hanschmann, K.-M., Kalinke, U., Erez, N., Levin, R., Velan, B., Löwer, J., Shafferman, A., Sutter, G., 2009. Postexposure immunization with modified vaccinia virus Ankara or conventional lister vaccine provides solid protection in a murine model of human smallpox. J. Infect. Dis. 199, 39.
Parker, S., Siddiqui, A.M., Oberle, C., Hembrador, E., Lanier, R., Painter, G., Robertson, A., Buller, R.M., 2009. Mousepox in the C57BL/6 strain provides an improved model for evaluating anti-poxvirus therapies. Virology 385, 11.
Perkus, M.E., Goebel, S.J., Davis, S.W., Johnson, G.P., Limbach, K., Norton, E.K., Paoletti, E., 1990. Vaccinia virus host range genes. Virology 179, 276.
Postigo, A., Cross, J.R., Downward, J., Way, M., 2006. Interaction of F1L with the BH3 domain of Bak is responsible for inhibiting vaccinia-induced apoptosis. Cell Death Differ. 13, 1651.
Quaglio, G., Goerens, C., Putoto, G., Rübig, P., Lafaye, P., Karapiperis, T., Dario, C., Delaunois, P., Zachariah, R., 2016. Ebola: lessons learned and future challenges for Europe. Lancet Infect. Dis. 16, 259.
Raj, V.S., Mou, H., Smits, S.L., Dekkers, D.H.W., Muller, M.A., Dijkman, R., Muth, D., Demmers, J.A.A., Zaki, A., Fouchier, R.A.M., Thiel, V., Drosten, C., Rottier, P.J.M., Osterhaus, A.D.M.E., Bosch, B.J., Haagmans, B.L., 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495, 251.
Raj, V.S., Farag, E.A.B.A., Reusken, C.B.E.M., Lamers, M.M.L., Pas, S.D., Voermans, J., Smits, S.L., Osterhaus, A.D.M.E., Al-Mawlawi, N., Al-Romaihi, H.E., AlHajri, M.M., El-Sayed, A.M., Mohran, K.A., Ghobashy, H., Alhajri, F., Al-Thani, M., Al-Marri, S.A., El-Maghraby, M.M., Koopmans, M.P.G., Haagmans, B.L., 2014. Isolation of MERS coronavirus from a dromedary camel, Qatar, 2014. Emerg. Infect. Dis. 20, 1339.
Reardon, S., 2014. Forgotten' NIH smallpox virus languishes on death row. Nature 514, 544.
Reusken, C.B.E.M., Raj, V.S., Koopmans, M.P., Haagmans, B.L., 2016. Cross host transmission in the emergence of MERS coronavirus. Curr. Opin. Virol. 16, 55.
Ricci, P.S., Schäfer, B., Kreil, T.R., Falkner, F.G., Holzer, G.W., 2011. Selection of recombinant MVA by rescue of the essential D4R gene. Virol. J. 8, 529.
Rivers, T.M., Ward, W.t.T.A.o.S.M., 1931. Cultivation of vaccine virus for Jennerian prophylaxis in man. J. Exp. Med. 54, 453.
Rivers, T.M., Ward, S.M., 1933. Further observations on the cultivation of vaccine virus for Jennerian prophylaxis in man. J. Exp. Med. 58, 635.
Rodríguez, D., González-Aseguinolaza, G., Rodríguez, J.R., Vijayan, A., Gherardi, M., Rueda, P., Casal, J.I., Esteban, M., 2012. Vaccine efficacy against malaria by the combination of Porcine Parvovirus-Like particles and vaccinia virus vectors expressing cs of plasmodium. PLoS One 7, e34445.
Roederer, M., Keele, B.F., Schmidt, S.D., Mason, R.D., Welles, H.C., Fischer, W., Labranche, C., Foulds, K.E., Louder, M.K., Yang, Z.-Y., Todd, J.-P.M., Buzby, A.P., Mach, L.V., Shen, L., Seaton, K.E., Ward, B.M., Bailer, R.T., Gottardo, R., Gu, W., Ferrari, G., Alam, S.M., Denny, T.N., Montefiori, D.C., Tomaras, G.D., Korber, B.T., Nason, M.C., Seder, R.A., Koup, R.A., Letvin, N.L., Rao, S.S., Nabel, G.J., Mascola, J.R., 2014. Immunological and virological mechanisms of vaccine-mediated protection against SIV and HIV. Nature 505, 502.
Russell, C.A., Fonville, J.M., Brown, A.E.X., Burke, D.F., Smith, D.L., James, S.L., Herfst, S., van Boheemen, S., Linster, M., Schrauwen, E.J., Katzelnick, L.,

Mosterín, A., Kuiken, T., Maher, E., Neumann, G., Osterhaus, A.D.M.E., Kawaoka, Y., Fouchier, R.A.M., Smith, D.J., 2012. The potential for respiratory droplet-transmissible A/H5N1 influenza virus to evolve in a mammalian host. Science 336, 1541.

Sánchez-Puig, J.M., Blasco, R., 2005. Isolation of vaccinia MVA recombinants using the viral F13L gene as the selective marker. Biotechniques 39, 665.

Sasse, J., Gelderblom, R.H., 2015. Lessons learnt from the German smallpox outbreaks after World War II. Bundesgesundheitsbl. Gesundheitsforsch. Gesundheitsschutz 58, 730.

Schneider, J., Gilbert, S.C., Blanchard, T.J., Hanke, T., Robson, K.J., Hannan, C.M., Becker, M., Sinden, R., Smith, G.L., Hill, A.V., 1998. Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. Nat. Med. 4, 397.

Sebastian, S., Gilbert, S.C., 2016. Recombinant modified vaccinia virus Ankara-based malaria vaccines. Expert Rev. Vaccines 15, 91.

Sheehan, S., Harris, S.A., Satti, I., Hokey, D.A., Dheenadhayalan, V., Stockdale, L., Manjaly Thomas, Z.-R., Minhinnick, A., Wilkie, M., Vermaak, S., Meyer, J., O'Shea, M.K., Pau, M.G., Versteege, I., Douoguih, M., Hendriks, J., Sadoff, J., Landry, B., Moss, P., McShane, H., 2015. A Phase I, open-label trial, evaluating the safety and immunogenicity of candidate tuberculosis vaccines AERAS-402 and MVA85A, administered by prime-boost regime in BCG-vaccinated healthy adults. PLoS One 10, e0141687.

Shisler, J.L., Jin, X.L., 2004. The vaccinia virus K1L gene product inhibits host NF-kappaB activation by preventing IkappaBalpha degradation. J. Virol. 78 (7), 3553–3560.

Sigal, L.J., 2016. Chapter six—the pathogenesis and immunobiology of mousepox. In: Frederick, W.A. (Ed.), Advances in Immunology. Academic Press, New York, p. 251.

Sivan, G., Ormanoglu, P., Buehler, E.C., Martin, S.E., Moss, B., 2015. Identification of restriction factors by human genome-wide RNA interference screening of viral host range mutants exemplified by discovery of SAMd9 and WDR6 as inhibitors of the vaccinia virus K1L(−)C7L(−) mutant. mBio 6, e01122.

Smith, V.P., Bryant, N.A., Alcamí, A., 2000. Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. J. Gen. Virol. 81, 1223.

Smith, E.J., Marié, I., Prakash, A., García-Sastre, A., Levy, D.E., 2001. IRF3 and IRF7 phosphorylation in virus-infected cells does not require double-stranded RNA-dependent protein kinase R or Ikappa B kinase but is blocked by vaccinia virus E3L protein. J. Biol. Chem. 23, 8951.

Smith, G.L., Benfield, C.T.O., Maluquer de Motes, C., Mazzon, M., Ember, S.W.J., Ferguson, B.J., Sumner, R.P., 2013. Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. J. Gen. Virol. 94, 2367.

Song, F., Fux, R., Provacia, L.B., Volz, A., Eickmann, M., Becker, S., Osterhaus, A.D.M.E., Haagmans, B.L., Sutter, G., 2013. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. J. Virol. 87, 11950.

Sperling, K.M., Schwantes, A., Schnierle, B.S., Sutter, G., 2008. The highly conserved orthopoxvirus 68k ankyrin-like protein is part of a cellular SCF ubiquitin ligase complex. Virology 374, 234.

Sperling, K.M., Schwantes, A., Staib, C., Schnierle, B.S., Sutter, G., 2009. The orthopoxvirus 68-kiloDalton ankyrin-like protein is essential for DNA replication and complete gene expression of modified vaccinia virus Ankara in nonpermissive human and murine cells. J. Virol. 83, 6029.

Spriggs, M.K., Hruby, D.E., Maliszewski, C.R., Pickup, D.J., Sims, J.E., Buller, R.M.L., VanSlyke, J., 1992. Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. Cell 71, 145.

Staib, C., Drexler, I., Ohlmann, M., Wintersperger, S., Erfle, V., Sutter, G., 2000. Transient host range selection for genetic engineering of modified vaccinia virus Ankara. Biotechniques 28, 1137.
Staib, C., Drexler, I., Sutter, G., 2004. Construction and isolation of recombinant MVA vaccinia virus and poxvirology. Methods Mol. Biol. 269, 77.
Staib, C., Kisling, S., Erfle, V., Sutter, G., 2005. Inactivation of the viral interleukin 1β receptor improves CD8+ T-cell memory responses elicited upon immunization with modified vaccinia virus Ankara. J. Gen. Virol. 86, 1997.
Staib, C., Suezer, Y., Kisling, S., Kalinke, U., Sutter, G., 2006. Short-term, but not post-exposure, protection against lethal orthopoxvirus challenge after immunization with modified vaccinia virus Ankara. J. Gen. Virol. 87, 2917.
Stanley, D.A., Honko, A.N., Asiedu, C., Trefry, J.C., Lau-Kilby, A.W., Johnson, J.C., Hensley, L., Ammendola, V., Abbate, A., Grazioli, F., Foulds, K.E., Cheng, C., Wang, L., Donaldson, M.M., Colloca, S., Folgori, A., Roederer, M., Nabel, G.J., Mascola, J., Nicosia, A., Cortese, R., Koup, R.A., Sullivan, N.J., 2014. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against Ebola Virus challenge. Nat. Med. 20, 1126.
Steffen, D.L., Xu, K., Nikolov, D.B., Broder, C.C., 2012. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 4, 280.
Stickl, H., Hochstein-Mintzel, V., 1971. Intracutaneous smallpox vaccination with a weak pathogenic vaccinia virus ("MVA virus"). Munch Med. Wochenschr. 27, 1149.
Stickl, H., Hochstein-Mintzel, V., Mayr, A., 1974. MVA-Stufenimpfung gegen Pocken. Dtsch. Med. Wochenschr. 99, 2386.
Stittelaar, K.J., van Amerongen, G., Kondova, I., Kuiken, T., van Lavieren, R.F., Pistoor, F.H.M., Niesters, H.G.M., van Doornum, G., van der Zeijst, B.A.M., Mateo, L., Chaplin, P.J., Osterhaus, A.D.M.E., 2005. Modified vaccinia virus Ankara protects macaques against respiratory challenge with Monkeypox virus. J. Virol. 79, 7845.
Sui, J., Li, W., Murakami, A., Tamin, A., Matthews, L.J., Wong, S.K., Moore, M.J., Tallarico, A.S.C., Olurinde, M., Choe, H., Anderson, L.J., Bellini, W.J., Farzan, M., Marasco, W.A., 2004. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc. Natl. Acad. Sci. U.S.A. 101, 2536.
Suthar, M.S., Diamond, M.S., Gale Jr., M., 2013. West Nile virus infection and immunity. Nat. Rev. Microbiol. 11, 115.
Sutter, G., Moss, B., 1992. Nonreplicating vaccinia vector efficiently expresses recombinant genes. Proc. Natl. Acad. Sci. U.S.A. 89, 10847.
Sutter, G., Wyatt, L.S., Foley, P.L., Bennink, J.R., Moss, B., 1994. A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. Vaccine 12, 1032.
Sutter, G., Ohlmann, M., Erfle, V., 1995. Non-replicating vaccinia vector efficiently expresses bacteriophage T7 RNA polymerase. FEBS Lett. 371, 9.
Symons, J.A., Adams, E., Tscharke, D.C., Reading, P.C., Waldmann, H., Smith, G.L., 2002. The vaccinia virus C12L protein inhibits mouse IL-18 and promotes virus virulence in the murine intranasal model. J. Gen. Virol. 83, 2833.
Tameris, M.D., Hatherill, M., Landry, B.S., Scriba, T.J., Snowden, M.A., Lockhart, S., Shea, J.E., McClain, J.B., Hussey, G.D., Hanekom, W.A., Mahomed, H., McShane, H., 2013. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet 381, 1021.

Tapia, M.D., Sow, S.O., Lyke, K.E., Haidara, F.C., Diallo, F., Doumbia, M., Traore, A., Coulibaly, F., Kodio, M., Onwuchekwa, U., Sztein, M.B., Wahid, R., Campbell, J.D., Kieny, M.-P., Moorthy, V., Imoukhuede, E.B., Rampling, T., Roman, F., De Ryck, I., Bellamy, A.R., Dally, L., Mbaya, O.T., Ploquin, A., Zhou, Y., Stanley, D.A., Bailer, R., Koup, R.A., Roederer, M., Ledgerwood, J., Hill, A.V.S., Ballou, W.R., Sullivan, N., Graham, B., Levine, M.M., 2016. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. Lancet Infect. Dis. 16, 31.

Tartaglia, J., 1992. Highly attenuated poxvirus vectors. AIDS Res. Hum. Retroviruses 8, 16.

Taylor, J.M., Barry, M., 2006. Near death experiences: poxvirus regulation of apoptotic death. Virology 344, 139.

Tree, J.A., Hall, G., Rees, P., Vipond, J., Funnell, S.G.P., Roberts, A.D., 2016. Repeated high-dose (5 × 108TCID50) toxicity study, of a third generation smallpox vaccine (IMVAMUNE), in New Zealand white rabbits. Hum. Vac. Immunother. 12 (7), 1795–1801.

Troy, J.D., Hill, H.R., Ewell, M.G., Frey, S.E., 2015. Sex difference in immune response to vaccination: a participant-level meta-analysis of randomized trials of IMVAMUNE® smallpox vaccine. Vaccine 33, 5425.

Valentin, A., McKinnon, K., Li, J., Rosati, M., Kulkarni, V., Pilkington, G.R., Bear, J., Alicea, C., Vargas-Inchaustegui, D.A., Patterson, L.J., Pegu, P., Liyanage, N.P.M., Gordon, S.N., Vaccari, M., Wang, Y., Hogg, A.E., Frey, B., Sui, Y., Reed, S.G., Sardesai, N.Y., Berzofsky, J.A., Franchini, G., Robert-Guroff, M., Felber, B.K., Pavlakis, G.N., 2014. Comparative analysis of SIV-specific cellular immune responses induced by different vaccine platforms in rhesus macaques. Clin. Immunol. 155, 91.

Van Rompay, K., 2012. The use of nonhuman primate models of HIV infection for the evaluation of antiviral strategies. AIDS Res. Hum. Retroviruses 28, 16.

Veits, J., Römer-Oberdörfer, A., Helferich, D., Durban, M., Suezer, Y., Sutter, G., Mettenleiter, T.C., 2008. Protective efficacy of several vaccines against highly pathogenic H5N1 avian influenza virus under experimental conditions. Vaccine 26, 1688.

Volz, A., Langenmayer, M., Jany, S., Kalinke, U., Sutter, G., 2014. Rapid expansion of CD8+ T cells in wild-type and type I interferon receptor-deficient mice correlates with protection after low-dose emergency immunization with modified vaccinia virus Ankara. J. Virol. 88, 10946.

Volz, A., Kupke, A., Song, F., Jany, S., Fux, R., Shams-Eldin, H., Schmidt, J., Becker, C., Eickmann, M., Becker, S., Sutter, G., 2015. Protective efficacy of recombinant modified vaccinia virus Ankara delivering Middle East respiratory syndrome coronavirus spike glycoprotein. J. Virol. 89, 8651.

Volz, A., Lim, S., Kaserer, M., Lülf, A., Marr, L., Jany, S., Deeg, C.A., Pijlman, G.P., Koraka, P., Osterhaus, A.D.M.E., Martina, B.E., Sutter, G., 2016. Immunogenicity and protective efficacy of recombinant modified vaccinia virus Ankara candidate vaccines delivering West Nile virus envelope antigens. Vaccine 34, 1915.

von Sonnenburg, F., Perona, P., Darsow, U., Ring, J., von Krempelhuber, A., Vollmar, J., Roesch, S., Baedeker, N., Kollaritsch, H., Chaplin, P., 2014. Safety and immunogenicity of modified vaccinia Ankara as a smallpox vaccine in people with atopic dermatitis. Vaccine 32, 5696.

Vora, N.M., Li, Y., Geleishvili, M., Emerson, G.L., Khmaladze, E., Maghlakelidze, G., Navdarashvili, A., Zakhashvili, K., Kokhreidze, M., Endeladze, M., Mokverashvili, G., Satheshkumar, P.S., Gallardo-Romero, N., Goldsmith, C.S., Metcalfe, M.G., Damon, I., Maes, E.F., Reynolds, M.G., Morgan, J., Carroll, D.S.,

2015. Human infection with a zoonotic orthopoxvirus in the country of Georgia. N. Engl. J. Med. 372, 1223.
Vuola, J.M., Keating, S., Webster, D.P., Berthoud, T., Dunachie, S., Gilbert, S.C., Hill, A.V.S., 2005. Differential immunogenicity of various heterologous prime-boost vaccine regimens using dan and viral vectors in healthy volunteers. J. Immunol. 174, 449.
Wahl-Jensen, V., Cann, J.A., Rubins, K.H., Huggins, J.W., Fisher, R.W., Johnson, A.J., de Kok-Mercado, F., Larsen, T., Raymond, J.L., Hensley, L.E., Jahrling, P.B., 2011. Progression of pathogenic events in cynomolgus macaques infected with Variola virus. PLoS One 6, e24832.
Waibler, Z., Anzaghe, M., Ludwig, H., Akira, S., Weiss, S., Sutter, G., Kalinke, U., 2007. Modified vaccinia virus Ankara induces toll-like receptor-independent type I interferon responses. J. Virol. 81, 12102.
Waibler, Z., Anzaghe, M., Frenz, T., Schwantes, A., Pöhlmann, C., Ludwig, H., Palomo-Otero, M., Alcamí, A., Sutter, G., Kalinke, U., 2009. Vaccinia virus-mediated inhibition of type I interferon responses is a multifactorial process involving the soluble type I interferon receptor B18 and intracellular components. J. Virol. 83, 1563.
Wasilenko, S.T., Stewart, T.L., Meyers, A.F.A., Barry, M., 2003. Vaccinia virus encodes a previously uncharacterized mitochondrial-associated inhibitor of apoptosis. Proc. Natl. Acad. Sci. U.S.A. 100, 14345.
Wasilenko, S.T., Banadyga, L., Bond, D., Barry, M., 2005. The vaccinia virus F1L protein interacts with the proapoptotic protein Bak and inhibits Bak activation. J. Virol. 79, 14031.
Webster, D.P., Dunachie, S., Vuola, J.M., Berthoud, T., Keating, S., Laidlaw, S.M., McConkey, S.J., Poulton, I., Andrews, L., Andersen, R.F., Bejon, P., Butcher, G., Sinden, R., Skinner, M.A., Gilbert, S.C., Hill, A.V.S., 2005. Enhanced T cell-mediated protection against malaria in human challenges by using the recombinant poxviruses FP9 and modified vaccinia virus Ankara. Proc. Natl. Acad. Sci. U.S.A. 102, 4836.
Wennier, S.T., Brinkmann, K., Steinhäußer, C., Mayländer, N., Mnich, C., Wielert, U., Dirmeier, U., Hausmann, J., Chaplin, P., Steigerwald, R., 2013. A novel naturally occurring tandem promoter in modified vaccinia virus Ankara drives very early gene expression and potent immune responses. PLoS One 8, e73511.
Werden, S.J., Rahman, M.M., McFadden, G., 2008. Chapter 3 poxvirus host range genes. In: Advances in Virus Research. Academic Press, New York, p. 135.
Williams, A., Goonetilleke, N.P., McShane, H., Clark, S.O., Hatch, G., Gilbert, S.C., Hill, A.V.S., 2005a. Boosting with Poxviruses enhances Mycobacterium bovis BCG efficacy against tuberculosis in guinea pigs. Infect. Immun. 73, 3814.
Williams, A., James, B.W., Bacon, J., Hatch, K.A., Hatch, G.J., Hall, G.A., Marsh, P.D., 2005b. An assay to compare the infectivity of mycobacterium tuberculosis isolates based on aerosol infection of guinea pigs and assessment of bacteriology. Tuberculosis 85, 177.
Wittek, R., Moss, B., 1980. Tandem repeats within the inverted terminal repetition of vaccinia virus DNA. Cell 21, 277.
Wittek, R., Cooper, J.A., Barbosa, E., Moss, B., 1980. Expression of the vaccinia virus genome: analysis and mapping of mRNAs encoded within the inverted terminal repetition. Cell 21, 487.
Wyatt, L.S., Shors, S.T., Murphy, B.R., Moss, B., 1996. Development of a replication-deficient recombinant vaccinia virus vaccine effective against parainfluenza virus 3 infection in an animal model. Vaccine 14, 1451.
Wyatt, L.S., Earl, P.L., Eller, L.A., Moss, B., 2004. Highly attenuated smallpox vaccine protects mice with and without immune deficiencies against pathogenic vaccinia virus challenge. Proc. Natl. Acad. Sci. U.S.A. 101, 4590.
Wyatt, L.S., Earl, P.L., Xiao, W., Americo, J.L., Cotter, C.A., Vogt, J., Moss, B., 2009. Elucidating and minimizing the loss by recombinant vaccinia virus of human

immunodeficiency virus gene expression resulting from spontaneous mutations and positive selection. J. Virol. 83, 7176.
Xiang, Y., Condit, R.C., Vijaysri, S., Jacobs, B., Williams, B.R.G., Silverman, R.H., 2002. Blockade of interferon induction and action by the E3L double-stranded RNA binding proteins of vaccinia virus. J. Virol. 76, 5251.
Yang, Z.-F., Mok, C.K.P., Peiris, J.S.M., Zhong, N.-S., 2015. Human infection with a novel avian influenza A(H5N6) virus. N. Engl. J. Med. 373, 487.
Yuan, M., Zhang, W., Wang, J., Al Yaghchi, C., Ahmed, J., Chard, L., Lemoine, N.R., Wang, Y., 2015. Efficiently editing the vaccinia virus genome by using the CRISPR-Cas9 system. J. Virol. 89, 5176.
Zhao, J., Li, K., Wohlford-Lenane, C., Agnihothram, S.S., Fett, C., Zhao, J., Gale, M.J., Baric, R.S., Enjuanes, L., Gallagher, T., McCray, P.B., Perlman, S., 2014. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc. Natl. Acad. Sci. U.S.A. 111, 4970.
Zimmerling, S., Waibler, Z., Resch, T., Sutter, G., Schwantes, A., 2013. Interleukin-1β receptor expressed by modified vaccinia virus Ankara interferes with interleukin-1β activity produced in various virus-infected antigen-presenting cells. Virol. J. 10, 34.
Zitzmann-Roth, E.-M., von Sonnenburg, F., de la Motte, S., Arndtz-Wiedemann, N., von Krempelhuber, A., Uebler, N., Vollmar, J., Virgin, G., Chaplin, P., 2015. Cardiac safety of modified vaccinia Ankara for vaccination against smallpox in a young, healthy study population. PLoS One 10, e0122653.