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Modified vaccinia virus Ankara as antigen delivery system: how can we best use its potential?

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Safety-tested modified vaccinia virus Ankara (MVA) has been established as a potent vector system for the development of candidate recombinant vaccines. The versatility of the vector system was recently demonstrated by the rapid production of experimental MVA vaccines for immunization against severe acute respiratory syndrome associated coronavirus. Promising results were also obtained in the delivery of Epstein-Barr virus or human cytomegalovirus antigens and from the clinical testing of MVA vectors for vaccination against immunodeficiency virus, papilloma virus, *Plasmodium falciparum* or melanoma. Moreover, MVA is considered to be a prime candidate vaccine for safer protection against orthopoxvirus infections. Thus, vector development to challenge dilemmas in vaccinology or immunization against poxvirus biothreat seems possible, yet the right choice should be made for a most beneficial use.

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Abbreviations

CEF	chicken embryo fibroblast
CMV	cytomegalovirus
HIV	human immunodeficiency virus
HPV	human papillomavirus
MVA	modified vaccinia virus Ankara
SARS	severe acute respiratory syndrome
SIV	simian immunodeficiency virus
VV	vaccinia virus

Introduction

Poxviruses engineered to express foreign genes are established tools for target protein synthesis and vaccine development in biomedical research. A large packaging capacity for recombinant DNA, precise virus-specific control of target gene expression, lack of persistence or genomic integration in the host, high immunogenicity as vaccine, and ease of vector and vaccine production were

important features enabling this success story. Concerns about the safety of poxviruses, including vaccinia virus (VV) as the former smallpox vaccine, have been addressed by the use of viruses that are replication-defective in human cells. Among these, modified vaccinia virus Ankara (MVA) can be considered as one of the virus strains of choice for preclinical and clinical research [1].

Historically, MVA was developed through attenuation by serial passage in primary chicken embryo cells to serve as a safer vaccine against smallpox (for a review see [2]). After more than 570 passages in tissue culture MVA had lost the broad cellular host range of VV, being unable to productively grow in many cells of mammalian origin. The concurrent avirulence of MVA for laboratory animals and its entirely unproblematic use for primary smallpox vaccination in more than 100 000 humans founded a high safety profile for recombinant MVA that, depending on the nature of the inserted target gene, can be used under conditions of biosafety level 1. The capacity to produce similar levels of viral and recombinant antigen when compared with replication-competent viruses is a relevant feature of MVA vaccines. In recent years, significant progress has been made in the development of MVA vaccine technologies.

This review informs about the newest developments in the generation of recombinant MVA and illustrates the principal features that have an impact on MVA immunogenicity. We also describe advances made in the pre-clinical and clinical evaluation of MVA as a second-generation poxvirus vaccine or for the delivery of heterologous antigens targeting infectious diseases and cancer. Finally, we consider the compatibility of different MVA exploitations and raise the question as to what future use priority should be given.

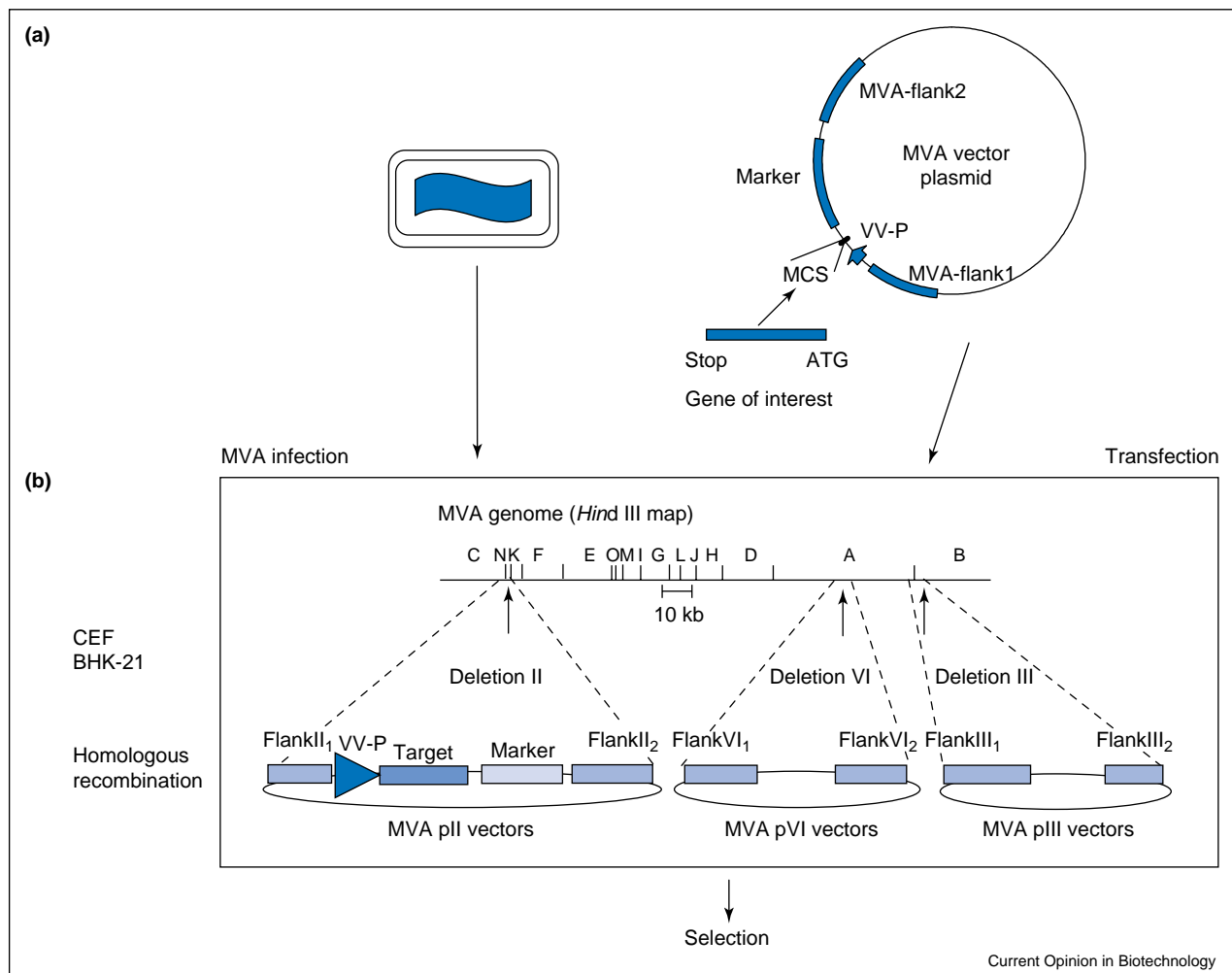
Genetic engineering of recombinant MVA

Recombinant MVA are among the most promising live viral vector systems, because of their well-established safety and their versatility for the production of heterologous proteins. The recent engineering of recombinant MVA to synthesize all components necessary for the assembly and delivery of alphavirus replicon particles serves as an elegant example of their application [3]. For most purposes, however, the generation of MVA vectors is straightforward requiring a single genomic insertion mediated by homologous recombination between the virus genome and DNA from a plasmid that carries one or two recombinant genes being placed under

control of a VV-specific promoter (Figure 1). The sites of naturally occurring deletions within the MVA genome or the classical gene loci encoding for the VV proteins thymidine kinase or hemagglutinin serve as sites for the insertion of recombinant gene sequences. There are several well-established techniques for clonal isolation of recombinant MVA involving the coexpression of chromogenic agents (e.g. *Escherichia coli* β -galactosidase and β -glucuronidase) or providing resistance against antimicrobials (e.g. *E. coli* xanthine-guanine-phosphoribosyl-transferase) (for an overview see [2,4]). Recently, methods relying on growth selection of recombinant MVA have been developed. These protocols take advantage

of selective propagation in cell cultures that are non-permissive for non-recombinant MVA. In one protocol, the VV host range gene K1L is transiently introduced into the MVA genome and recombinant MVA can subsequently be isolated in rabbit kidney RK-13 cells [4,5]. After clonal isolation of the vector virus, the K1L marker gene is again removed from the viral genome bearing the advantage that the same marker can be re-used subsequently to generate MVA recombinant viruses harboring multiple gene insertions. Another recently developed host range selection technique relies on growth rescue of mutant MVA in chicken embryo fibroblast (CEF) cells [6]. MVA- Δ E3L lacks the interferon resistance gene E3L

Figure 1



The generation of recombinant MVA. (a) Schematic representation of an MVA particle on the left and the MVA transfer plasmid on the right. MVA DNA sequences adjacent to the deletion site (MVA-flank1, MVA-flank2) were cloned into the plasmid and target genes are inserted between these sequences and placed under transcriptional control of vaccinia virus-specific promoters (VV-P). Recombinant MVA are generated by infection of chicken embryo fibroblast (CEF) or baby hamster kidney (BHK-21) cells with MVA and concurrent transfection with transfer vectors, resulting in recombination between homologous DNA sequences of vector and virus sequences. (b) An MVA-infected, transfected cell. Schematic map of the MVA genome and plasmids designed for the insertion of foreign DNA. Sites of the restriction endonuclease *Hind* III within the genome of MVA are indicated at the top. The positions of the naturally occurring deletions II, III, and VI are marked by arrows. MVA DNA sequences adjacent to the deletion sites (flank1, flank2) were cloned into plasmids to generate the pII, pIII and pVI transfer vectors.

and is unable to grow in CEF. Stable re-insertion of the E3L gene together with a target gene sequence allows for quick isolation of MVA recombinant viruses on CEF.

Basics of MVA immunogenicity and smallpox vaccination

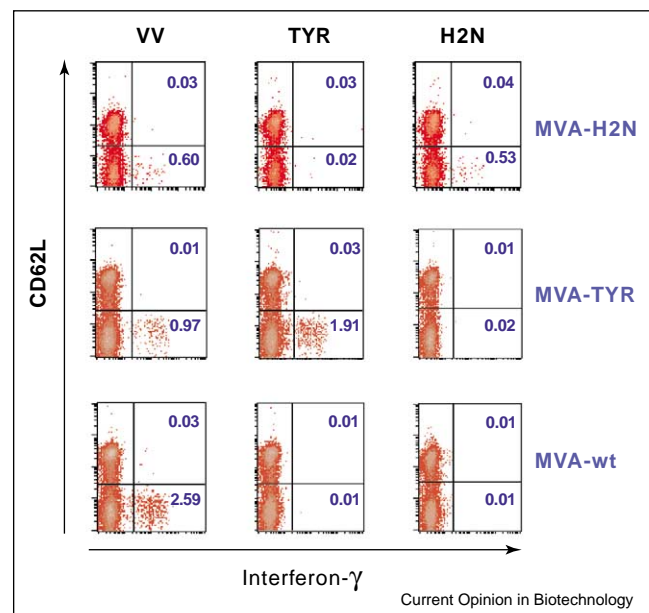
In the 1970s, highly attenuated MVA was primarily considered to provide a means for safer vaccination against smallpox. Although there was knowledge about its avirulence and immunostimulating capacity from infection experiments in multiple animal models, more systematic efforts to characterize the molecular basis of MVA vaccine immunogenicity date from the more recent past. Important achievements include the characterization of the MVA molecular life cycle upon infection of non-permissive mammalian cells, the elucidation of the MVA genome, and our understanding that MVA has lost relevant poxvirus immune evasion genes that target innate host responses based on cytokine and chemokine functions. Consequently, defects in inhibitory virus genes are likely to be responsible for the MVA-specific induction of host cytokine synthesis proposed decades ago (for a review see [2]). Interestingly, inactivation of the VV interferon resistance gene E3L in the MVA genome resulted in enhanced production of type I interferon in CEFs, suggesting that the capacity of MVA to stimulate innate responses may be further enhanced by rational mutagenesis [6]. It can be assumed that such immunostimulation contributes to the particular immunogenicity of MVA and might compensate for the advantage of live replication-competent VV in sustained antigen production. In contrast to other viruses (e.g. members of the herpesvirus family), MVA or replication-competent VV do not appear to specifically interfere with host cell antigen processing or presentation, allowing for an apparently unimpaired induction of adaptive immune responses. This assumption is in agreement with the finding that VV-specific humoral and cellular immunity can be detected decades after primary vaccination [7]. When recently compared with conventional VV vaccines, MVA vaccines were found to elicit similar patterns of VV-specific immune responses that provided protection against experimental orthopoxvirus infections but required inoculation of higher dosage [8,9,10,11]. Remarkably, Earl and coworkers [11] demonstrated substantial protective capacity of MVA vaccines in non-human primates against challenge with monkeypox virus, thus making MVA a valuable candidate as second-generation smallpox vaccine.

Antivector immunity and impact on MVA vaccination

With more recombinant MVA used for antigen delivery in clinical research [12,13,14,15], there is an increasing need to evaluate MVA-specific immune responses following immunization. Although there are established means to monitor vaccine-induced antibodies, cell-mediated

immunity has scarcely been assessed [11,16–18]. Several approaches allow VV-induced T-cell immunity to be quantified without knowledge of defined VV-specific target antigens [19–21]. In addition, the use of recently identified human leukocyte antigen HLA-A*0201-restricted VV-specific CD8⁺ T-cell epitopes [8,22] now makes it possible to compare epitope-specific responses elicited against vector or recombinant antigens (Figure 2; I Drexler and G Sutter, unpublished). Further knowledge as to how these responses might influence each other is highly relevant for developing optimal modes of MVA vector immunization. Although replication-competent VV vaccines and other viral vectors are more likely to be hampered by antivector immunity than MVA-based vectors [23,24], vaccinia-specific immune responses can be assumed to affect immunogenicity of the target antigen delivered by the MVA vector vaccine. Yet, pre-existing MVA or VV immunity did not interfere with subsequent immunizations of recombinant MVA expressing a cytomegalovirus (CMV) glycoprotein gB antigen [25]. In addition, a variety of methods has been

Figure 2



Comparative monitoring of epitope-specific CD8⁺ T-cell responses directed against vaccinia virus and recombinant antigens. Quantitation of epitope-specific T cells was performed following one immunization of humanized HLA-A*0201-transgenic mice with 10⁸ IU of either wild-type MVA (MVA-wt), recombinant MVA-TYR or MVA-H2N expressing the human tumor antigens tyrosinase and Her-2/neu, respectively. Ten days post vaccination, peptide-specific intracellular cytokine release of splenocytes was determined after stimulation with vaccinia epitope VP35#1 (VV), tyrosinase (TYR) or Her-2/neu peptides (H2N). Cells were analyzed by flow cytometry for the presence of peptide-specific, activated (CD62L^{low}) CD8⁺ T cells. The magnitude of the specifically induced T-cell response is depicted as the cells shifted to the lower right and indicated as a percentage (numbers in blue) of interferon- γ -secreting CD8⁺ T cells within the CD8⁺ cell population.

derived to possibly circumvent the influence of vector-specific responses, including the application of DNA prime MVA boost immunization [26]. One particularly promising approach to enhance target antigen specific immune responses is based on combining different viral vector vaccines (e.g. influenza [24], avipox [27,28], Semliki Forest [29] or vesicular stomatitis virus [30]), non-viral vector vaccines (e.g. DNA- [31,32] or *Salmonella*-based vaccines [33]) or protein vaccines [34] with MVA vaccines producing a common antigen. These so-called prime-boost regimens can vary in dosage, or in numbers and intervals of application [13[•]], and can combine different application routes with various vector systems [35]. Depending on the kind of disease, the type of immune response that needs to be induced by the test vaccine can also influence the formulation. In the case of cancer, human immunodeficiency virus (HIV) or malaria, an immune response biased towards T helper 1 (Th1) cell immunity will be promoted using a combined DNA and MVA vaccination [36].

Recombinant MVA as a candidate vaccine against viral diseases

Much previous research has been dedicated to the development of MVA candidate recombinant vaccines against multiple virus infections of humans, including those causing AIDS, influenza, early childhood respiratory diseases, measles, Japanese encephalitis or dengue fever (for a review see [2]). As an effective vaccine against AIDS is urgently needed, recombinant MVA producing immunodeficiency virus antigens are among the first vector viruses to be evaluated as candidate vaccines in humans [14[•],37]. Substantial data from studies in different simian immunodeficiency virus (SIV) or simian human immunodeficiency virus (SHIV, a chimera of SIV and HIV) infection models strongly support thorough clinical evaluation of MVA-based vaccines. The use of heterologous prime-boost strategies including MVA vaccines and delivering multiple SIV or HIV antigens proved successful to elicit antibody and high-level cytotoxic T-cell responses in macaques. The immune response effectively controlled a mucosal challenge with SHIV 89.6P and SIVmac251-derived viruses or significantly reduced viral loads after challenge infection with neutralization-resistant and highly replication-competent SIVmac239 [30,38–42]. Importantly, recent data suggest that SIV/HIV-specific mucosal immunity can be boosted by peripheral MVA immunization after oral priming with a *Salmonella* vector vaccine [33] and intranasal inoculation of recombinant MVA can stimulate immunodeficiency virus-specific immunity in the genital or rectal tract [35,43]. Elicitation of potent mucosal immunity and induction of broadly virus-neutralizing antibody responses are important milestones still to be reached for the derivation of a successful HIV vaccine. The principal capacity of MVA to elicit highly effective virus-neutralizing antibody responses has been highlighted by the characterization of vector vac-

cines against severe acute respiratory syndrome (SARS) coronavirus and human CMV [25,44[•]]. The successful engineering of MVA expressing SARS coronavirus spike protein also demonstrated the suitability of the vector system to readily respond to the potential threat of rapidly emerging infectious diseases. A recently developed recombinant MVA delivering multiple CMV antigens represents a promising candidate for the clinical testing of MVA-based T-cell vaccines [25,45[•],46]. This study also mirrors a general interest in the development of MVA vector vaccines against human herpesvirus infections [47,48].

Recombinant MVA as candidate vaccines against cancer, parasitic and bacterial diseases

The potential to activate robust cellular major histocompatibility complex (MHC) class I- and II-restricted CD8⁺ and CD4⁺ T-cell responses against recombinant antigens has made MVA vaccines attractive for immunotherapeutic approaches against cancer and selected intracellular parasitic or bacterial infectious diseases. For experimental cancer therapy, virus-associated malignancies seem to be predestined targets for MVA vaccines. Taylor and co-workers [48] demonstrated the immunogenicity of an Epstein-Barr virus-associated nasopharyngeal carcinoma vaccine by reactivating Epstein-Barr virus-specific CD8⁺ and CD4⁺ memory T cells *in vitro*. Recently, Corona Gutierrez and colleagues [49^{••}] showed evidence for the therapeutic efficacy of an MVA vaccine delivering human papillomavirus (HPV) E2 antigen against cervical cancer associated with HPV infection in a phase I/II clinical trial. Vaccines based on recombinant MVA expressing different tumor-associated antigens specific for a variety of cancers are currently being tested in mice [27,50,51] and humans [52]. Often these are combined with cytokines such as interleukin-2 [52], costimulatory molecules such as B7-1 [27], measures to circumvent immune inhibitory signalling (by CTLA-4 blockade) [51] or cellular adjuvants like dendritic cells [15] to enhance immune responses against antigens that are likely to be tolerogenic self-proteins.

Major efforts are underway to develop vaccines against malaria caused by *Plasmodium* spp. parasites. Preclinically, heterologous prime-boost immunization regimens have elicited strong CD8⁺ T-cell immunity and have shown substantial protection in mouse malaria challenge models against *Plasmodium berghei* [28] or *Plasmodium yoelii* [53]. In addition, safety and immunogenicity have been established in clinical trials with human volunteers, experimentally [13[•]] or naturally exposed to *Plasmodium falciparum* [54]. Recently, the first prime-boost vaccinations against tuberculosis — combining DNA [55] or the classical bacille Calmette-Guérin vaccine [56] with recombinant MVA expressing *Mycobacterium tuberculosis* antigen 85A — proved to be protective in mice.

Table 1

First clinical evaluation of recombinant MVA vaccines.

Target disease	Antigen	Clinical trial	Reference
AIDS	HIV-1 Nef	Phase I/II, immunotherapy	[14*]
AIDS	HIVA multiantigen	Phase I, prophylaxis	[37]
Cervical cancer	HPV E2	Phase I/II, immunotherapy	[49**]
Breast cancer	MUC1	Phase I, immunotherapy	[52]
Melanoma	Tyrosinase	Phase I/II	[15,57*]
Malaria	<i>P. falciparum</i> ME-Trap	Phase I, prophylaxis	[54]
Malaria	<i>P. falciparum</i> ME-Trap	Phase I, prophylaxis	[13*]

Conclusions

Recombinant MVA is a prime candidate poxvirus vector for a generation of new vaccines against infections and tumors. The portrait of the virus combines desirable elements such as high-level biological safety, the ability to activate appropriate innate immune mediators upon vaccination, and the capacity to deliver substantial amounts of heterologous antigens. The adoption of up-to-date methodology for convenient vector generation, vector quality control, and vector vaccine immune monitoring has increased the pace of development, bringing recombinant MVA vaccines into clinical trials. Initial studies testing MVA vaccines for prophylaxis or immunotherapy of AIDS, malaria, human papilloma virus-associated cancer or melanoma have already been completed (Table 1). First results are, overall, very encouraging and confirm clinical safety. Importantly, however, they also demonstrate clinical efficacy, despite the intrinsic difficulties associated with these target diseases. Renewed interest in the development of MVA as candidate vaccine against an orthopoxvirus-related biothreat is likely to provide the basis for feasible large-scale manufacturing of MVA vaccines. Indeed, first studies suggest that MVA would provide a suitable orthopox vaccine, if necessary. The use of recombinant MVA to simultaneously provide immunity against smallpox has been proposed. Nevertheless, one should keep in mind that hasty population-wide smallpox vaccinations might not be desirable, as high level antivector immunity could limit the future potential of poxvirus-based vector vaccines more urgently needed for prophylaxis or therapy of uncontrolled infectious diseases, cancer or emerging infections.

Update

Recent data from a phase I clinical trial for treatment of metastatic melanoma indicated that vaccination with MVA-transduced dendritic cells can *in vivo* activate T-cell responses directed against the recombinant antigen tyrosinase and against the recently identified epitope within vaccinia virus envelope antigen H3 [57*,8*].

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