# MVA and NYVAC as Vaccines against Emergent Infectious Diseases and Cancer

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**Abstract:** Recombinants based on poxviruses have been used extensively as gene delivery systems to study many biological functions of foreign genes and as vaccines against many pathogens, particularly in the veterinary field. Based on safety record, efficient expression and ability to trigger specific immune responses, two of the most promising poxvirus vectors for human use are the attenuated modified vaccinia virus Ankara (MVA) and the Copenhagen derived NYVAC strains. Because of the scientific and clinical interest in these two vectors, here we review their biological characteristics, with emphasis on virus-host cell interactions, viral immunomodulators, gene expression profiling, virus distribution in animals, and application as vaccines against different pathogens and tumors.

**Keywords:** Poxvirus vectors, mva and nyvac, vaccines, pathogens, tumours, preclinical, clinical.

## INTRODUCTION

Poxviruses, and in particular vaccinia virus (VACV), were among the first animal viruses to be investigated as gene transfer vectors. Recombinant gene expression by VACV was first demonstrated in 1982 [1, 2]. Since then, poxviruses have been successfully used for molecular biology studies, for *in vitro* production and functional characterization of proteins, as well as live vaccines and tools for vaccine research [3-5].

Several unique features make poxvirus recombinants excellent candidates as vaccine vectors: (i) The stability of freeze-dried vaccine [6], its low cost, ease of manufacture and administration. (ii) The cytoplasmic site of gene expression. (iii) The packing flexibility of the genome, which allows large amounts of the genome to be lost or deleted and foreign DNA to be integrated (at least 25 Kb) without loss of infectivity [5] and (iv) the ability to induce both antibody and cytotoxic T cell responses against foreign antigen with long lasting immunity after a single inoculation. Despite these advantages, complications observed in young children and immune compromised individuals during the Smallpox Eradication Program brought forth concerns regarding the safety of reintroduction of VACV as immunizing agent [7, 8]. Therefore, one of the approaches undertaken to enhance the safety of VACV has been the development of highly attenuated strains, like MVA or NYVAC.

The attenuated MVA virus was derived from chorioallantoid vaccinia Ankara (CVA), a Turkish smallpox vaccine strain that, after more than 570 passages in primary chicken embryo fibroblast cells (CEFs) became defective for replication in human cells and avirulent in test animals [9]. In the last decades of smallpox eradication campaign (1968–1980), MVA was inoculated into more than 120,000 individuals in Germany with no reported adverse side effects [10, 11] and it is now considered to be a suitable platform for the next generation of safer smallpox vaccines and recombinant poxvirus vectors [12, 13]. Genomic mapping and sequencing studies have revealed that MVA lost nearly 30 Kb of genomic information during its extended passage in CEF cells and has multiple deletions and mutations compared with the parental CVA strain [14]. Many of these genetic alterations are in genes implicated in the modulation of host response, and it is assumed that these deletions render MVA unable to complete its replication cycle in human cells [15, 16].

The attenuated NYVAC strain was derived from a plaque-cloned isolate of the Copenhagen vaccine strain (VACV-COP) by the precise deletion of 18 Open Reading Frames (ORFs) implicated in the pathogenicity and virulence of *Orthopoxviruses*, as well as host-range regulatory functions governing the replication competency of these viruses on cells derived from certain species. The resultant vector was proven to be highly attenuated since it failed to disseminate in immunodeficient mice, displayed a dramatically reduced ability to replicate on a variety of human tissue culture cells, and was unable to produce infectious virus in humans [17].

The main advantage of attenuated MVA and NYVAC strains is the safety record. Despite their limited replication in human and most mammalian cell types, both viruses provide a high level of gene expression and trigger strong immune responses when delivering foreign antigens in animals and humans [9, 18-20].

Due to the high interest in the clinical application of the poxvirus vectors, this review focus on the progress that has been made during the last few years in the biological characterization of MVA and NYVAC strains. The principal features of both vectors that might impact their ability to trigger

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specific immune responses are discussed. We also describe the use of MVA and NYVAC recombinants as candidate vaccines against viral, parasitic and bacterial diseases, as well as gene delivery systems in the prevention and treatment of cancer.

## THE VECTORS MVA AND NYVAC: SIMILARITIES AND DIFFERENCES

### Genomic Organization

The members of the poxvirus family have large double-stranded DNA genomes (167 to 224 Kb) encoding several hundred proteins. To date, there are more than 100 complete poxvirus genomes deposited in sequence databases (see: www.poxvirus.org), including the vaccinia virus Copenhagen strain (VACV-COP), used for the generation of NYVAC, encompassing 192 Kb [21] and MVA with 178 Kb [14].

Because of the cytoplasmic site of virus replication, VACV mRNAs are not spliced, and therefore vaccinia genes do not contain introns. Genes are closely spaced on the genome, and each gene appears to be controlled by its own transcriptional promoter. There are about 100 genes specifically conserved among the various poxviruses that are required for virus replication and morphogenesis. The remaining non-conserved genes, many of which are non-essential for replication in culture cells, dictate individual virus characteristics of host range and pathogenicity [22].

During the generation of the attenuated MVA and NYVAC strains, several non-essential genes were lost. A sequence comparison between MVA and NYVAC genomes revealed that there are multiple ORFs, (i) fragmented in MVA and intact in NYVAC, (ii) deleted in NYVAC and intact in MVA and, (iii) deleted in both diagram shown in Fig. (**1**). MVA and NYVAC share common deleted or non-functional ORFs including the 6 ORFs within the deletion d4817 (C5L-N1L), B13R (MVA181R) and B14R (MVA 182R) encoding the ICE inhibitor, the ATI remnant A26L and the K1L (MVA022L) host range gene. The MVA strain has a functional thymidine kinase gene (TK) (MVA086R), an intact C7L (MVA018L) host range gene and an intact C6L (MVA019L), A56R (MVA165R), N2L (MVA021L) and I4L (MVA065L) ORFs which are not present in NYVAC strain [14]. These differences in the genome are likely responsible for the distinct behaviour exhibited by MVA and NYVAC, both *in vitro* and *in vivo* systems [23, 24]. In fact, the C7L ORF was recently defined as a gene implicated in the control of viral protein synthesis and apoptosis [23]. Both host range genes C7L and K1L inhibit protein kinase (PKR) activation [23, 25-27] and have also been recently reported to inhibit antiviral activities induced by type I interferons [28].

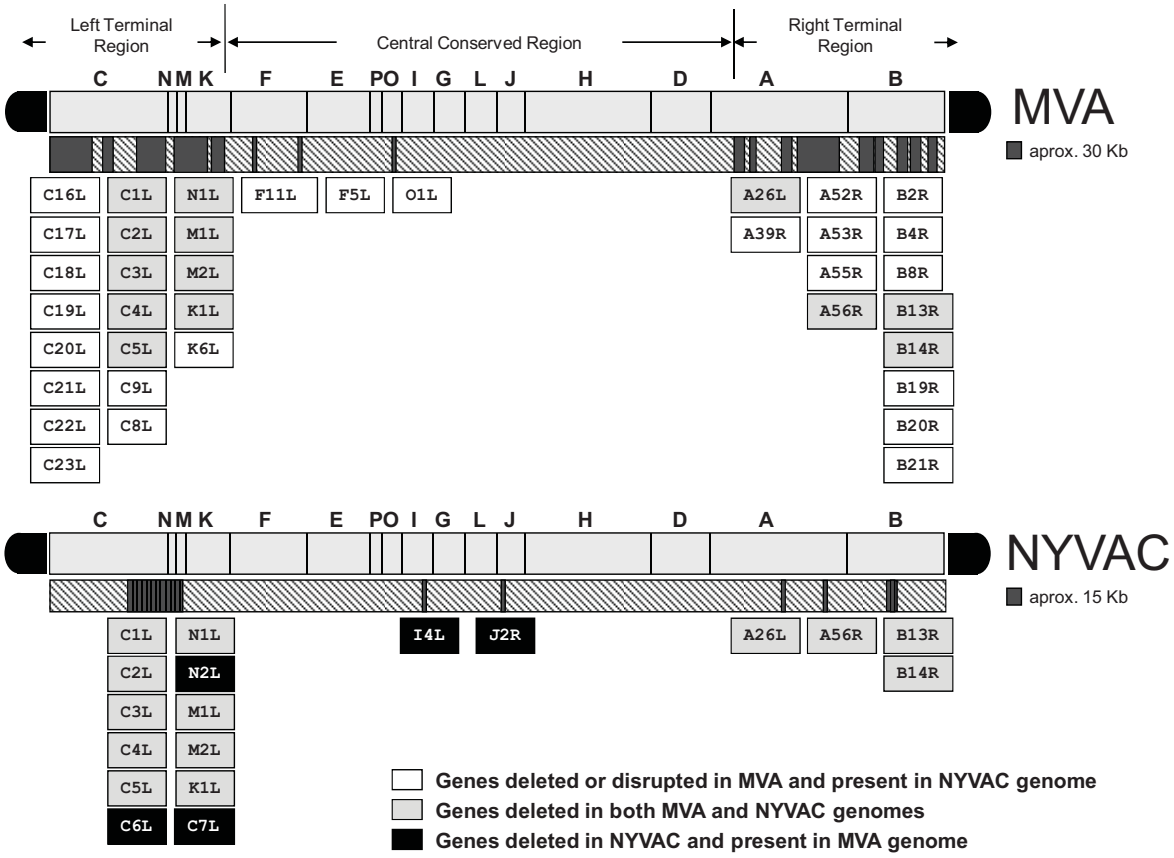


**Fig. (1).** Scheme of deleted genes in MVA and NYVAC genomes. Genome maps of MVA and NYVAC strains adapted from Antoine *et al.* [14] are represented. The deleted or fragmented genes in each genome are indicated.

## Virus Replication and Morphogenesis

Unlike most other DNA viruses, the poxvirus replicate in the cytoplasm of the target cell. Viral gene expression is stringently regulated by a cascade mechanism and virion assembly is a complex process that involves the production of multiple viral forms [29]. After virus entry, early genes are transcribed, leading to the expression of a number of molecules implicated in host-cell interactions as well as in viral DNA synthesis, which occurs in a juxtanuclear factory area, or replication centre, enclosed by rough endoplasmic reticulum [30]. Intermediate and late viral genes, including structural proteins, enzymes, and early transcription factors, are then transcribed. Viral DNA is incorporated into immature virions (IV) that mature into infectious intracellular mature virions (MV), which represent the first infectious particles produced during the viral life cycle. Some of MVs are wrapped by a double membrane derived from the *trans*-Golgi to form intracellular enveloped virions (WV). At the cell surface, WV fuse with the host cell membrane, lose their outer membrane, and form cell-associated enveloped virions (CEV). CEV become extracellular enveloped virions (EV) by detaching from the cell membrane directly or by inducing actin polymerization and detaching from the tips of actin-filled microvilli [31].

Although it has been described that MVA and NYVAC have lost the ability to replicate and to produce infectious particles in human cells and in a majority of mammalian cells [32-35], the knowledge about their cellular and biochemical properties is sparse. A head-to-head comparison of MVA and NYVAC infection under non-permissive conditions demonstrated that both viruses are unable to grow in cells from human origin, but there are clear differences among them in both the replication cycle and the morphogenetic program. As it has been previously reported, the MVA life cycle in HeLa cells is inhibited at late times post-infection, when only 4 % of MV are formed due to a block in virion assembly, but early and late viral proteins are produced like in permissive cells [15, 16, 36]. By contrast, in NYVAC infected human cells there is a translational block, with enhanced phosphorylation of the initiation factor eIF-2 alpha, that affects the synthesis of certain late viral proteins, some of them required in the maturation of virions. As a result, the block in morphogenesis occurs at or prior to the formation of IVs [23] Fig. (**2**). The inhibition in the synthesis of late viral proteins might also correlate with the potent apoptosis induced by NYVAC in infected HeLa cells, a phenomenon that is not observed after MVA infection [37]. As it has been recently proposed, the induction of apoptosis in cells, such as APC, that only express early viral genes may be related to the inability of the virus to express late viral proteins that interfere with host functions. It may be that to gain time to replicate, the virus expresses both early and late antiapoptotic factors, thereby circumventing a host-mediated proapoptotic response after viral infection [38]. A comparative analysis of expression profiles obtained by cDNA microarray screening of over 15,000 human genes, revealed in HeLa cells host genes differentially expressed in MVA versus NYVAC-infected cells [37, 39], that might also affect extend of virus maturation.

## Virus Distribution in Tissues

Poxviruses, and in particular VACV, may disseminate within the host by (*i*) direct cell to cell spread using actin tails, (*ii*) as free virus, (*iii*) infected leukocytes, and/or (*iv*) virus-induced cell motility. It is thought that the CEV and EV forms are particularly important for rapid cell to cell spread *in vivo*, whereas the MV form probably contributes to virus dissemination at distant sites only after late stage cell death and membrane rupture [40, 41]. In contrast to the replication competent VACV strain WR, the attenuated viruses MVA and NYVAC are unable to produce virus progeny in most mammalian cells. Hence, it is important to define how these viruses are distributed *in vivo* and the kinetics of expression of virus-encoded genes in different tissues. This concern was addressed for MVA [42, 43], but the comparative dissemination of MVA versus NYVAC *in vivo* was recently assayed by bioluminescence imaging using recombinants expressing the luciferase gene [24]. The study showed

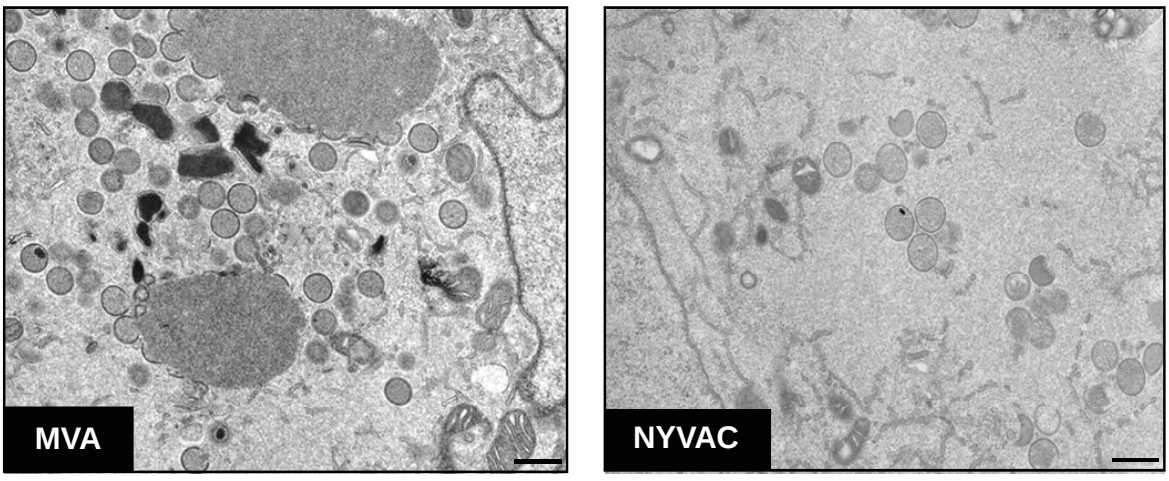


**Fig. (2).** Electron microscopy of MVA and NYVAC morphogenesis in HeLa cells. Electron micrographs of HeLa cells infected with 5 PFU/cell of either MVA or NYVAC strains at 16 h p.i. The magnification of each panel is indicated by bars in the lower right corner. All Bars = 500 nm. The EM images correspond to Fig. 5, panels A and B from reference [23], with permission.

that both attenuated viruses expressed transiently the reporter gene and were unable to produce infectious particles, demonstrating their restricted replication capacity. In NYVAC infected mice the heterologous antigen persisted longer than when expressed from MVA Fig. (**3**). Both viruses have the ability to reach and infect target tissues other than the site of inoculation; however, shortly after infection the efficiency of virus gene expression was higher for MVA than for NYVAC. In a recent study in macaques, aerosolized and radiolabeled MVA and NYVAC recombinants expressing HIV and HPV antigens gave safe and successful immunogenic responses to the foreign antigens that lasted for at least six months. In addition, *in vivo* scintigraphic imaging studies in the macaque model demonstrated that both viruses were absorbed primarily in the mucosal tissues of the lungs and respiratory tract, but not in the brain or eyes [44]. Thus, for vaccination purposes the systemic and mucosal routes are efficient ways to deliver MVA and NYVAC.

## Virus-Host Cell Interaction

The damage incurred by cells and tissues following viral infection stimulates a series of non-specific events that collectively make up the early inflammatory response. As it has been reported, mammalian cells are able to resolve virus infection by production of inflammatory cytokines and interferons (IFNs), a response that is mediated through virus-specific activation of Toll-like receptors (TLRs). These cytokines have been shown to be key elements of innate immune response and prevention of pathogenesis of virus-

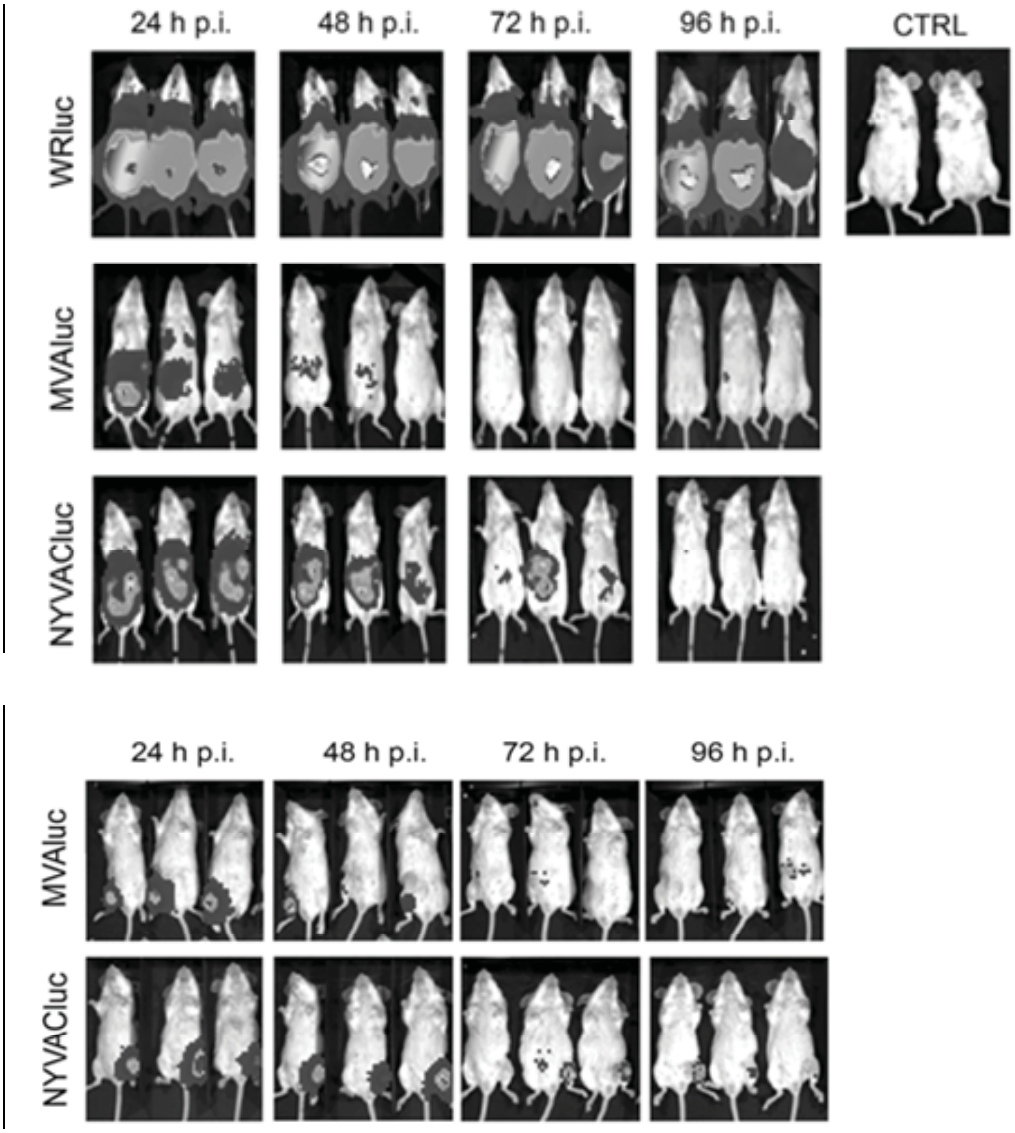


**Fig. (3).** Bioluminescence image distribution of WRluc, MVAluc and NYVACluc in mice inoculated by intraperitoneal (i.p) or intramuscular (i.m) route. In the right panel the mock-infected mice (CTRL) are shown. The images shown correspond to Fig. 1 panels A and C from reference [24], with permission.

induced diseases [45]. Immune sensing of MVA is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome, and MyD88 is critical in the production of IFNs [46]. VACV and other poxviruses express a variety of proteins which are non-essential for virus replication in culture cells but help the virus to evade the antiviral host response [47-50]. At present several genes involved in the immune evasion have been described for VACV strains including VACV-WR, MVA and VACV-COP (Table **1**). These viral immunomodulators mainly target the complement, cytokines, chemokines and TLR–signalling pathways, which are important host defence mechanisms activated in response to infection [50]. The viral genes involved in immune evasion mechanisms and specific site of action in the cell are shown in Fig. (**4**). The wide range of viral genes antagonizing selective pathways and host cell immune responses, highlight the multiple strategies that poxviruses used through evolution to escape immune surveillance and might help to explain why these viruses have caused animal and human diseases, some devastating like smallpox.

During the generation of the attenuated NYVAC and MVA strains some of those immunomodulatory genes were deleted or disrupted and this might explain why both viruses are highly immunogenic despite their restricted replication capacity. However, MVA has lost more immunomodulators than NYVAC. This fact correlates with the timing and strength of the inflammatory response induced by both viruses *in vivo*. MVA disseminates and induces faster than NYVAC the antiviral response, followed by rapid viral clearance (after 48 hours), whereas the NYVAC vector induces a delayed antiviral response and stays about 24 hours longer than MVA within tissues of the infected animal [24].

Another significant distinction between MVA and NYVAC is the marked difference in the ability to modulate host immune responses after infection of immature human monocyte-derived dendritic cells (mDCs) [124]. Compared to uninfected mDCs, these vectors have a profound impact on host gene expression profiles, upregulating 195 of the same genes, while differing in 359 genes that were specifically upregulated by MVA and 165 by NYVAC. At the mRNA level, although IL-12, IFN-β, and TNF-α were upregulated by both strains, they were increased to higher levels by MVA, whereas type I IFNs, IL-6 and Toll-like receptor pathways were selectively induced after MVA infection. The impact of such differences on the immune response generated by both vectors will be discussed in the following section.

## Immunogenic Differences between the Vectors in Preclinical Studies

Although the capacity of MVA and NYVAC vectors to produce similar levels of recombinant antigens as replication-competent viruses, and to elicit a potent specific immune response when administered by systemic and mucosal routes against multiple infectious disorders has been demonstrated [9, 13, 19, 32, 125, 126], only few preclinical studies have compared head-to-head the immune response induced by recombinants based on these strains.

This issue was originally addressed in Balb/c and transgenic HHD mice infected with MVA and NYVAC recombinants, both expressing four HIV-1 antigens (Env/Gag-Pol-Nef) from clades B and C [127, 128]. The MVA and NYVAC recombinants were able to induce potent cellular immune responses against peptide pools representing the HIV antigens included in the immunogen, but the magnitude and breath of such response differed between the poxvirus vectors depending on both the protocol and the animal model used.

A more detailed comparison between MVA and NYVAC vectors was recently performed in Rhesus macaques (*Macaca mulatta* of Indian origin) inoculated with recombinants expressing SIV/HIV-1 gene inserts and challenged with a pathogenic SHIV89.6P [129]. Although both vaccine candidates induced similar protective efficacy after virus challenge, the immune response was apparently exerted through different mechanisms. The MVA vector tended to induce specific CD8+ T cell responses in addition to CD4+, while the NYVAC vector boosted CD4+ T cells to a greater level than MVA. These findings highlight the differences in cellular responses triggered by MVA and NYVAC, which are likely to impact vaccine efficacy.

It is generally accepted that CD8+ T cells, in addition to antigen and costimulation, require a 'third signal' cytokines, that can be provided by either IL-12 and/or type I IFN (IFNα/β) produced by mature DC, which support strong clonal expansion, development of effector functions, or establishment of a long-lived, responsive memory population [130, 131]. The gene profiling data obtained from MVA-infected DCs revealed a severe upregulation at the mRNA level of IL-12, IFN-α and IFN-β, as well as interferon regulatory factor (IRF-7) and proteins implicated in the type I IFN production (MDA5, RIG) [124]. Consequently, interferon stimulated genes (ISGs) such as IFIT1 (ISG56), IFIT4 (ISG60) and SCYB10 were also upregulated. Furthermore, the differentiation programme initiated in common by IL-12 and IFNα/β regulate numerous genes involved in several functions. Among them, genes relevant for effector cell regulation of gene expression such as GADD45B [132] and the transcription factor NFAT5 [133], genes involved in signal transduction (MAP2K5) and cell cycle regulation (cyclin B1) [130], or members of the TNF family [134] were consistently upregulated shortly after MVA infection. Genes encoding for pro-inflammatory cytokines as TNF and IL-6, and for CC-chemokines as SCYA3 (MIP-1A), SCYA4 (MIP-1B) or SCYA5 (RANTES), which are involved in the modulation of the immune response, were differentially expressed between MVA and NYVAC. All these data support the preferential stimulation of CD8+ T cells by MVA.

It is noteworthy that in NYVAC infected mDCs all of the above CD8+ T cell stimulatory genes are markedly reduced. Additionally, the longer viral gene expression exhibited by NYVAC *in vivo* [24] may influence the preferential CD4+ T cell response induced by the vector, since it is described that to drive clonal expansion and differentiation of this T cell subset is required the persistence of the antigen throughout their expansion phase [135].

While it will be necessary to conduct more comparative studies between MVA and NYVAC expressing the same antigens, it is clear that both strains behave differently *in vitro* and *in vivo*. These observations have implications for

**Table 1. Immunomodulatory Genes Described for Vaccinia Virus Strains VACV-Cop, VACV-WR, MVA and NYVAC. Genes Deleted, Disrupted or Non-Functional are in Brackets. ORF Nomenclature from VACV-Cop Strain. V: VACV-WR; M: Modified Ankara Virus and N: NYVAC Virus. * Are Not Present in the Parental VACV-Cop Strain**

| ORF | V | M | N | Function | References |
|---|---|---|---|---|---|
| C23L/ B29R | C23L/ B29R | (001L/ 193R) | C23L/ B29R | Secreted type II CBP; binds CC-chemokines with high affinity, anti-inflammatory properties | [47] |
| C21L | C21L | (-) | C21L | Secreted; binds C3b and C4b, inhibits classical and alternative complement pathways | [51] |
| | C12L | 008L | (C12L*) | Secreted; binds IL-18 and inhibits IL-18 induced IFN-γproduction and NK response | [52, 53] |
| C10L | C10L | 006L | C10L | Secreted; IL-1 receptor antagonist (IL-1Ra) homologue. Blocks IL-1 receptor | [54] |
| C7L | C7L | 018L | (-) | Inhibits eIF2-α phosphorylation and apoptosis. Prevents PKR activation and inhibits antiviral activities induced by type I interferons | [23, 25, 28] |
| C3L | C3L | (-) | (-) | Secreted; functional complement 4b binding protein | [17] |
| C2L | C2L | (-) | (-) | Kelch-like protein, anti-inflammatory properties | [55] |
| N1L | N1L | (020L) | (-) | Inhibits TRAF6-induced NF-κB activation at the level of TRAF6 or downstream proteins preceding the IKK complex, inhibiting signalling to NF-κB by the TNF superfamily or by TLR. Recently described as a Bcl2-like anti-apoptotic protein | [56, 57] |
| M2L | M2L | (-) | (-) | Prevents phosphorylation of the ERK2 protein and subsequent NF-κB activation | [58, 59] |
| K1L | K1L | (022L) | (-) | Inhibits host NF−κB activation by preventing IκB degradation. Prevents PKR activation | [26, 27, 60] |
| K3L | K3L | 024L | K3L | Mimics the host factor eIF2-α. Blocks eIF2-α phosphorylation and PKR autophosphorylation, inhibiting translational arrest | [61-64] |
| K7R | K7R | 028R | K7R | Inhibits PRR-mediated induction of IFNβ by preventing TBK1/Ikki-mediated IRF activation owing to its ability to target human DEAD box protein 3 (DDX3) | [65] |
| F1L | F1L | 029L | F1L | Regulates mitochondria-mediated apoptosis: functions both as a suppressor of proapoptotic Bcl-2 family proteins (inhibits Bak and Bax activation via Bak-independent mechanism interacting with BH3-only protein BimL) and as an inhibitor of caspase-9 | [66-71] |
| F3L | F3L | 031L | F3L | Kelch-like protein, affects the innate immune response | [72] |
| E3L | E3L | 050L | E3L | Binds dsRNA and prevents PKR activation; inhibits PKR activity by direct binding; reduces the adenosine-to-inosine editing activity of IFN-induced ADAR: inhibits IRF3/7 activation; binds to and disables ISG15 function; binds dsRNA and prevent activation of 2´5´OAS/RnaseL; modulates expression of host cellular genes at the transcriptional level and inhibits apoptosis of host cell through Z-DNA binding | [64, 73-85] |
| H1L | H1L | 091L | H1L | Viral phosphatase that reverses STAT1 activation | [86] |
| H5R | H5R | 095R | H5R | Multifunctional protein involved in viral DNA replication, postreplicative gene transcription and virion morphogenesis; Transcription factor phosphorylated by B1R kinase; mediates the inhibition of CD1d1-mediated antigen presentation | [87, 88] |
| A39R | (A39R) | (150R/ 151R) | A39R | Secreted; semaphorin, binds Plexin C1and induces actin cytoskeleton rearrangement and inhibits integrin-mediated adhesion and chemokine-induced migration. Inhibits phagocytosis by dendritic cells and neutrophils imparing the cross-priming | [89-92] |
| A40R | A40R | 152R | A40R | Type II integral membrane protein related to C-type lectins, including NK cell receptors. Ligand unknown | [93, 94] |
| A41L | A41L | 153L | A41L | Secreted; vCKBP2 homologue. Ligands: CCL21, CCL25, CCL26 and CCL28, anti-inflammatory properties | [95, 96] |
| A44L | A44L | 157L | A44L | 3β-hidroxysteroid dehydrogenase that synthesized steroids. Anti-inflammatory properties | [97] |

**(Table 1) contd…..**

| ORF | V | M | N | Function | References |
|---|---|---|---|---|---|
| A46R | A46R | 159R | A46R | Targets TLR adaptors inhibiting both MyD88 and TRIF dependent pathways | [48, 98] |
| A52R | A52R | (-) | A52R | Targets IRAK-2 and TRAF-6 to block the NF-κB activation pathway by various TLRs. Enhances TLR-induced IL-10 production by binding TRAF6 | [98-100] |
| A53R | (A53R) | (-) | (A53R) | Secreted; soluble virus TNF receptor homologue | [101] |
| A55R | A55R | (-) | A55R | Kelch-like protein, anti-inflammatory properties, affects the outcome of infection in a murine intradermal model | [102] |
| B1R | B1R | 167R | B1R | Virus encoded kinase. Inhibits the CD1d1-mediated antigen presentation. Modulates the c-Jun-dependent signalling | [88, 103] |
| B7R | B7R | 175R | B7R | Resident in ER, might have a role in intracellular trafficking of proteins. Contains a CC-binding domain | [104, 105] |
| B8R | B8R | (176R) | B8R | Secreted protein that binds IFN-γ from various species but not mouse inhibiting binding of IFN-γ to its receptor | [106-111] |
| B13R/ B14R | B13R | (181R/ 182R) | (-) | Serine protease inhibitor SPI-2. Inhibits the proteolitic activity of IL-1β converting enzyme (ICE) that cleaves pro-IL-1β and pro-IL-18 precursors to produce IL-1β and IL-18. Inhibits apoptosis triggered by TNF-α and FasL | [49, 112, 113] |
| B15R | B14R | 183R | B15R | Inhibits the phosphorylation and subsequent degradation of IκBα, the inhibitor of NF-B, by its interaction with IKKβ;affects the infiltration and inflammatory response | [114, 115] |
| | B15R | 184R | (B16R*) | Secreted, binds IL-1β and blocks febrile response | [116, 117] |
| B19R | B18R | (187R) | B19R | Secreted and cell surface protein that binds type I IFN from various species (the affinity for mouse type I IFN is considerably lower than for the other species tested) inhibiting binding of type I IFNs to its receptor; virulence factor in VACV | [118-122] |
| | (B22R) | (-) | (B22R*) | Serine protease inhibitor SPI-1. Inhibits apoptosis through the serine protease Granzyme B and reduces killing by CTLs | [123] |

vaccination purposes, as it may be possible to combine the two pox vectors in a prime/boost protocol to elicit broader cellular immune responses, like it has been described for HIV antigens in vaccinated mice [127, 128].

## MVA AND NYVAC AS VACCINE CANDIDATES AGAINST VIRAL, PARASITIC AND BACTERIAL DISEASES

During the last years the emergence of new pathogens such as HIV, the severe acute respiratory syndrome associated coronavirus (SARS-CoV), henipaviruses (Hendra and Nipah) and most recently avian influenza viruses, has profoundly impact on the public health worldwide. In addition, historically established infectious diseases, such as West Nile fever, human monkeypox, dengue, tuberculosis, and malaria have emerged or resurged. Strains of common microbes such as *Staphilococcus aureus* and *Mycobacterium tuberculosis* have continued to develop resistance to the drugs that once were effective against them [136, 137]. All these evidences highlight the need for a robust pipeline of new antimicrobial agents based on innovative therapeutic strategies, new vaccines, and other preventive measures.

Poxviruses are useful tool for research and vaccine development. They have attractive properties for the generation of highly stable recombinants that may be able to respond promptly to these emerging diseases [3, 138, 139]. These live viral vector vaccines mimic viral infections hereby eliciting the appropriate innate "danger signals" to the adaptive immune system [9]. Additionally, the replication-defective viruses provide unique form of viral vaccines that combine the safety of a killed virus vaccine and the immunogenicity of a live virus vaccine by expressing gene products within cells so the antigens can be presented efficiently by both MHC class I and class II pathways [140]. Hence, and because of their safety profile, the poxvirus MVA and NYVAC strains are prime candidates for generation of recombinant virusvaccines against infectious diseases and cancer [13, 32].

The potential of MVA and NYVAC vectors as vaccine carriers against infectious diseases have been extensively studied in several pre-clinical and clinical trials using different administration approaches that are summarized in Table **2**. Several protocols have been applied to induce effective immune responses. The prime/boost strategy with heterologous vectors, but using poxvirus recombinants as the boosting immunogen, has proved to be an excellent regimen to elicit antigen-specific cellular immune responses with protection in animal model systems [141]. Different immunization protocols have demonstrated the efficacy of combining recombinants based on MVA or NYVAC with themselves, with DNA or with other recombinant viruses based on SFV or influenza, showing their capacity to modulate the quality of the immune response [127, 128, 142-144].

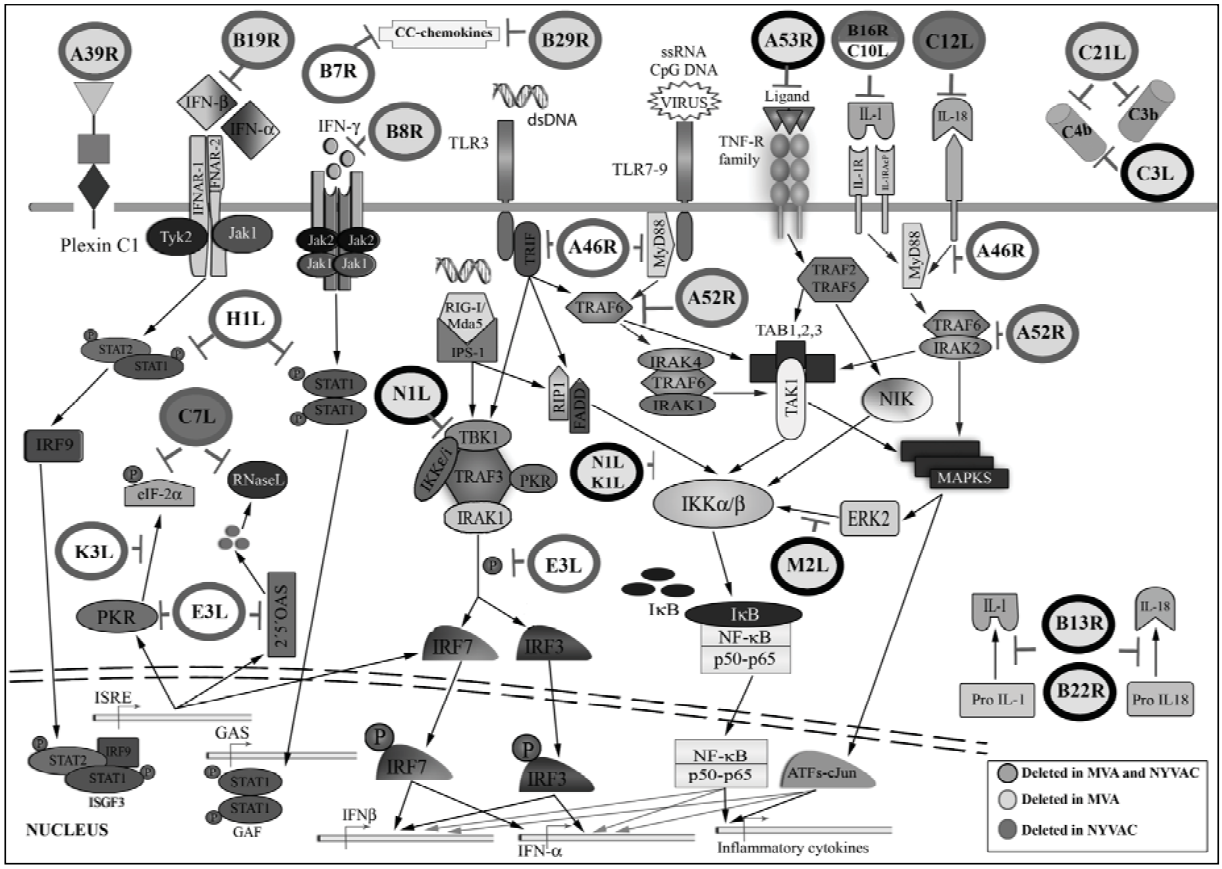


**Fig. (4).** Diagram showing immunomodulatory genes of MVA and NYVAC. Circles represent VACV genes that have been characterized as specific inhibitors of distinct pathways and are either present or absent in MVA or NYVAC, or are deleted in both viruses. The distinct behaviour of MVA versus NYVAC is likely to be determined by the absence of function in some of the indicated genes.

MVA and NYVAC expressing antigens derived from viruses, bacteria and parasites have being widely assayed in different animal models. Some preclinical studies that have being carried out are indicated in Table **2A**. These immunogens are able to induce humoral and/or cell-mediated immunity, which in some cases correlate with protection. Based on these preclinical findings, a wide range of recombinants based on MVA and NYVAC have being tested in clinical trials (Table **2B**).

In the last two years, attenuated MVA and NYVAC vectors have being used as vaccine candidates against disorders such as HIV/AIDS, influenza or tuberculosis. In the HIV-1 vaccine field the recent observation of about 31% protection against HIV-1 infection in a phase III Thai trial (RV144) using a combination of the recombinant poxvirus vector ALVAC and the protein gp120 [145], highlighted that improved poxvirus recombinants should be considered as components of an effective HIV/AIDS vaccine [146]. In fact, to date several clinical trials including MVA and NYVAC have been conducted using different combination approaches with encouraging results [147-151], and plans are to advance these vectors to phase II/III trials in the next few years.

Another emergent disease that severely affects the global public health is caused by influenza virus. The first pandemic of influenza virus this century has experienced, for which a vaccine was not available, was caused by a new influenza of swine-origin H1N1 strain, first detected in humans in April 2009. The global spread of the novel H1N1 influenza subtype has accelerated the prompt development of vaccines as a worldwide priority. Several strategies have being implemented to produce pandemic vaccines. One vaccine tested in humans includes the use of recombinant MVA vector expressing influenza viral antigens [152].

Despite of being an ancient disease, tuberculosis (TB) remains one of the most devastating causes of morbidity and mortality worldwide. In recent years, control of TB has been exacerbated by the deadly intersection of the HIV and TB epidemics and the emergence of multiple-drug-resistant tuberculosis. The failure of current TB immunization procedures to adequately protect against *M. tuberculosis* infections is largely responsible for the unsuccessful global control of this disease. The inadequacy of BCG (Bacillus Calmette-Guérin) immunization to control the TB epidemic has stimulated a worldwide effort to develop more-effective TB vaccination strategies. Some of these immunization approaches include the use of MVA expressing TB genes [153-155].

Finally, malaria parasite infections by Plasmodium are responsible for the loss of more young lives than any other

**Table 2. Summary of Preclinical (A) and Clinical (B) Studies Using Attenuated MVA and NYVAC Vectors as Vaccine Candidates Against Viral, Bacterial and Parasitic Diseases**

| **A: Preclinical Studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Target Disease** | **Animal Model** | **Vaccine** | **Antigen** | **Immune Response** | **Efficacy** | **References** |
| **Viral Infections** | | | | | | |
| Rabies | Mice | MVA-RG | RG (rabies glycoprotein) | Neutralizing Ab | Protection | [171] |
| | Mice | NYVAC-RG | RG | ND | Protection | [32] |
| HCV | HLA-I transgenic mice | MVA-HCV TG4040 | NS3, NS4, NS5B | Potent and long-lasting CD4+ and CD8+ T cell responses | Cross-protection | [172] |
| | Chimpanzees | P: DNA-CE1E2 + DNA-NS3 B: MVA-CE1E2 + MVA-NS3 | C (core), E1, E2, NS3 | Robust HCV-specific immune responses | Protection during the acute period but not in the chronic phase | [173] |
| | HLA.A2.1 mice | MVA-E1E2 | Native and modified E1 and E2 | Th1 response | ND | [174] |
| SARS | Mice and rabbits | P: MVA-S B: Ad-S | S (spike glycoprotein) | Neutralizing Ab | ND | [175] |
| | Rhesus monkeys | MVA-S | S | Neutralizing Ab | Protection | [176] |
| | Ferrets | MVA-S or MVA-N | S or N (nucleocapsid protein) | Neutralizing Ab | No protection | [177] |
| | Mice | MVA-S | S | Neutralizing Ab | Protection | [178] |
| Measles | Mice and rat | MVA-H | H (hemagglutinin glycoprotein) | Th1 and neutralizing Ab | Protection | [179] |
| | Newborn macaques | MVA-FH | F (fusion protein) and H | CTL and neutralizing Ab | Protection | [180] |
| | Macaques | MVA-FH | F and H | CTL and neutralizing Ab | Protection | [181] |
| Dengue | Rhesus monkeys | MVA-DEN2 or MVA-DEN4 | C-terminally truncated envelope protein from dengue type 2 (DEN2) or type 4 (DEN4) virus | Neutralizing Ab (MVA-DEN2) | Protection (MVA-DEN2) | [182] |
| EIV | Horses | MVA-HA or NP | HA (hemagglutinin), NP (nucleoprotein) | Ab and cellular responses | Protection | [183] |
| | Horses | MVA-HA or NP | HA, NP | Ab and cellular responses | ND | [184] |
| CMV | HLA.A2.1 mice | pp65-IE1-MVA | pp65 (tegument protein) and IE1 (immediate early gene product) | Robust primary CMI | ND | [185] |
| | Mice Tx recipient | MVA-CMV | UL55 (surface glycoprotein), UL83 (tegument protein) and UL123/e4 (nuclear protein) | Humoral and cellular responses | ND | [186] |
| | Mice | MVA-gB680 | gB (soluble glycoprotein B) | Neutralizing Ab and CTLs | ND | [187] |

**(Table 2) contd….**

| **A: Preclinical Studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Target Disease** | **Animal Model** | **Vaccine** | **Antigen** | **Immune Response** | **Efficacy** | **References** |
| **Viral Infections** | | | | | | |
| CDV | Ferrets | NYVAC-CDV | F and HA | Neutralizing Ab | Protection | [188] |
| EHV | Horses | NYVAC-64 | gene 64 | CMI | No protection | [189] |
| | Hamsters | MVA-gC | Complement receptor glycoprotein C | Humoral and cellular responses | ND | [190] |
| | Ponies | MVA-IE | Immediate early (IE) gene | CMI | Protection | [191] |
| RSV | Mice | MVA-F and MVA-G | F (fusion protein) and G (attachment protein) | Humoral and Th1 responses | No protection | [192] |
| | Infant macaques | MVA-F and MVA-G | F and G | Low Ab levels | No protection | [193] |
| | Mice | MVA-RSV | F and G | Humoral response | Protection | [194] |
| | Calves | MVA-bRSV | F and G | Humoral and cellular responses | Protection | [195] |
| JEV | Mice | MVA-JEV + (DNA-B7.1 or DNA-B7.2) | prM (precursor of the membrane) and E (envelope protein) | Specific JEV-immune responses | ND | [196] |
| | Swine | MVA-JEV | prM and E | Neutralizing Ab | Protection | [197] |
| | Mice | MVA-JEV | prM and E | Neutralizing Ab | Protection | [198] |
| | Rhesus monkeys | NYVAC-JEV | prM, E, NS1 | Neutralizing Ab | Protection | [199] |
| | Swine | NYVAC-JEV | prM, E, NS1 | Neutralizing Ab | Protection | [200] |
| Influenza | Mice | MVA-HA | HA (H5N1) | Ab and CTL responses | Protection | [201] |
| | Mice | MVA-HA-NP | HA and NP (H1N1) | Ab and CTL responses | Protection | [202] |
| | Mice | MVA-HA, NP | HA, NP | Ab and CTL responses | Protection | [203] |
| | Cynomolgus macaque | MVA-HA-VN/04 | HA (H5N1) | Cross-reactive Ab response | Protection | [204, 205] |
| | Pigs | NYVAC-HA | HA (H5N1) | Ab response | Partial protection (19/21) | [206] |
| | Mice | MVA-H1-Ca MVA-N1-Ca | HA and NA (H1N1) | Ab and CTL re-sponses | Protection | [207] |
| | Ferrets | MVA-HA | HA (H1N1) | Robust Ab response | Protection | [208] |
| **Bacterial Diseases** | | | | | | |
| Tuberculosis | Guinea pigs | P: BCG B: MVA-85A | Mycobacterial myco-lyltransferase antigen 85A | Cellular response | Protection | [209] |
| | Cattle | P: BCG B: MVA-85A | 85A | Cellular response | ND | [210] |
| | Mice | P: BCG B: MVA-85A | 85A | Specific CD4+ and CD8+ T cell responses | Protection | [211] |

**(Table 2) contd….**

| **A: Preclinical Studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Target Disease** | **Animal Model** | **Vaccine** | **Antigen** | **Immune Response** | **Efficacy** | **References** |
| **Bacterial Diseases** | | | | | | |
| Tuberculosis | Mice | MVA-64 | Early secreted protein MPT64 | Ab and CTL responses | ND | [212] |
| | Rhesus macaques | P: BCG<br>B: MVA-85A | 85A | Cellular response | Protection | [213] |
| | Cattle | P: BCG<br>B: MVA-85A | 85A | Long lasting cellular response | Protection | [214] |
| | Mice | P : ESAT6-85B fusion protein<br>B : MVA/IL15/5Mtb | 85A, 85B, ESAT6, HSP60 and Mtb39 | Long lasting cellular immune response | Protection | [215] |
| **Parasitic Diseases** | | | | | | |
| Toxoplasmosis | Mice | MVA-ROP2 | ROP2 (transmembrane protein of PVM) | Ab response | ND | [216] |
| | Mice | P: DNA-GRA4<br>B: MVA-GRA4 | GRA4 (dense granule antigen 4) | Ab and cellular responses | Protection | [217] |
| Leishmaniosis | Mice | P: DNA-TRYP<br>B: MVA-TRYP | TRYP (tryparedoxin peroxidase) | Cellular response | Protection | [218] |
| | Mice | P: DNA-LACK<br>B: MVA-LACK | LACK (p36) | Cellular response | Protection against *Leishmania major* | [219] |
| | Mice | P: DNA-LACK<br>B: MVA-LACK | LACK (p36) | Cellular response | Protection against *Leishmania infantum* | [220] |
| | Dogs | P: DNA-LACK<br>B: MVA-LACK | LACK (p36) | Cellular response | Protection against *Leishmania infantum* | [221] |
| | Dogs | P: DNA-TRYP<br>B: MVA-TRYP | TRYP (tryparedoxin peroxidase) | Th1 and memory cellular immune responses | Protection | [222] |
| **B: Clinical Studies** | | | | | | |
| **Target Disease** | **Clinical Trials** | **Vaccine** | **Antigen** | **Immune Response** | **Efficacy** | **References** |
| HIV-1 | Phase I | MVA-HIVA +/- DNA-HIVA prime | HIV-1 clade A gag p24 and p17 fused to 25 overlapping CD8+ T cell epitopes | Safe and well tolerated. Infrequent immune response | - | [223, 224] |
| | Phase I | P: DNA-HIVA<br>B: MVA-HIVA | HIV-1 clade A gag p24 and p17 fused to 25 overlapping CD8+ T cell epitopes | Multifunctional cellular responses | - | [225, 226] |
| | Phase I | NYVAC-C | Env, Gag-Pol-Nef (clade C) | Anti-HIV T cell response (5/12) | - | [227] |
| | Phase I | P: DNA-C<br>B: NYVAC-C | Env, Gag-Pol-Nef (clade C) | Specific HIV-immune responses (19/20) | - | [149, 150] |
| | Phase I/II | P: DNA HIVIS<br>B: MVA-CMDR | P:Env, Gag, Rev, RT (clade A, B) B: Env, Gag, Pol (clade C, A, E) | Specific HIV immune response (37/38) | - | [148, 228] |

**(Table 2) contd….**

| **B: Clinical Studies** | | | | | | |
|---|---|---|---|---|---|---|
| HIV-1 | Phase I | MVA-CMRD | Env, Gag and Pol (CRF01-AE) | Durable cell mediated and humoral immune responses | - | [229] |
| | Phase I | ADMVA | Env, Gag, Pol, Nef andTat (clade B′/C) | Neutalizing Ab and specific HIV immune response depending on the dose: LD (3/12), MD (6/12) and HD (8/13) | - | [147] |
| | Phase I | MVA (TBC-M4) | Env, Gag, Tat-Rev, Nef-RT (Clade C) | HIV immune response depending on the dose: LD (9/11) and HD (12/12). Ab response | - | [230] |
| Influenza | Phase I | MVA-NP+M1 | NP (Nucleoprotein) and M1 (Matrix protein 1) | High specific T cell responses | - | [152] |
| HCV | Phase I | MVA-HCV (TG4040) | NS3, NS4, NS5B | Safe and well tolerated | - | www.clinicaltrials.org |
| Smallpox | Phase I/IIb | MVA-BN | - | Cellular and humoral responses | Protection | [231-233] |
| JEV | Phase I | NYVAC-JEV | prM, E, NS1 | Humoral and CTL responses | - | [234, 235] |
| Malaria | Phase IIb | P: FP9<br>B: MVA-ME-TRAP | ME.TRAP | Cellular response | No protection | [236] |
| | Phase II | P: FP9 B: MVA-CS | CS | Low cellular response | No protection | [237] |
| | Phase II | P: RTS,S/AS02A<br>B: MVA-CS | CS | High Ab levels but low cellular response | Partial efficacy (33%) | [238] |
| | Phase II | P: DNA B: MVA | ME.TRAP<br>CS | Cellular and humoral responses | Protection | [239] |
| | Phase II | P: FP9 B: MVA | ME.TRAP | Cellular response | Protection | [144] |
| | Phase II | P: DNA<br>B: MVA + FP9 | ME.TRAP | Cellular response | No protection | [144] |
| | Phase II | P: DNA B: MVA | ME.TRAP | Cellular response | No protection | [240] |
| | Phase II | P: DNA B: MVA | ME.TRAP | Cellular response | No protection | [241] |
| | Phase I/IIa | NYVAC-Pf7 | 7 Ag from P. falciparum (CS, SSP2, LSA1, MSP1, SERA, AMA1, Pfs25) | Poor Ab levels and cellular response | No protection | [242] |
| Tuberculosis | Phase I | P: BCG<br>B: MVA-85A | Mycobacterial mycolyltransferase antigen 85A | Excellent safety profile and highly immunogenic | ND | [243, 244] |
| | Phase I | MVA-85A | Mycobacterial mycolyltransferase antigen 85A | Potent and durable T cell responses | ND | [153-155] |

infection. Ways to enhance cellular immunity to malaria have been developed and clinical trials are underway, specially a phase III trial in Africa with the CS-hepatitis B fused antigen in combination with an adjuvant [156]. Recombinant MVA has been used in heterologous immunization regimens with promising results [157].

As yet none of the MVA and NYVAC vectors have reached phase III, but as clinical studies progress it is feasible to foresee that these vectors will provide a clinical human benefit. Indeed, these vectors are used as effective vaccines against veterinary diseases, and parental MVA is currently being pursuit as an alternative human vaccine against smallpox, should a bioterrorism attack occur.

## RECOMBINANTS BASED ON MVA AND NYVAC AS PROPHYLACTIC AND THERAPEUTIC VACCINES AGAINST CANCER

Cancers arise through a multistage process involving sequential accumulation of molecular alterations such as the loss of tumor suppressor genes and gain of dominant oncogenes. These abnormalities drive neoplastic processes through different mechanisms including stimulation of cell proliferation, inhibition of cell death, cell-cycle arrest or enhanced angiogenesis in tumor environment as well as inactivation of DNA repair genes which increase mutation rate in other genes [158-160]. Additionally, cancer cells successfully evade the usual immunosurveillance mechanisms by altering the expression of MHC molecules [161, 162], defects in antigen processing and presentation machinery [163, 164], secretion of immunosuppressive soluble factors [165] and induction of T cell dysfunction [166] leading to an inability of the immune system to recognize and eliminate them. The failure of immune system surveillance is an integral component of tumor development.

The increasing knowledge of the molecular mechanisms underlying carcinogenesis has contributed to advance in the development of various gene therapy approaches that attempt to remedy these alterations. Current gene therapy strategies include corrective gene therapy (tumor suppressor gene and anti-oncogene gene approaches), cytoreductive gene therapy (suicide, pro-apoptotic, and anti-angiogenic gene therapy) and immunomodulation gene therapy.

Immunotherapy is considered one of the most promising approaches for cancer treatment. Recent insights on tumor evasion and tolerance mechanisms suggest that with proper stimulation of the immune cells and the local cytokine milieu it should be possible to induce an immune response to tumor cells. The use of vectors expressing genes to stimulate the host immune system or genes that attempt to bypass some of the defects that help cancer cells to evade its surveillance is an attractive anti-cancer strategy.

The efficient delivery of therapeutic genes and their appropriate expression are crucial issues for clinically relevant gene therapy. Viruses have been selected as gene delivery vehicles because their natural cell tropism, high efficiency at gene transfer and expression as well as their biological characteristics that can significantly enhance the immunogenicity of antigens carry within them. Numerous viral vectors systems have been developed, however the poxviruses are one of the most studied vectors for gene delivery and stimulation of immunity.

A variety of recombinant poxvirus vectors have been extensively used to express a multitude of foreign genes in preclinical studies and in clinical trials of cancer immunotherapy. Among them, attenuated strains of vaccinia virus MVA and NYVAC have been demonstrated as excellent vaccine candidates because of the high levels of recombinant protein expression, strong immunogenicity and their safety profile. Moreover, administration of VACV vectors provides a "danger signal" to the host that helps to prime T cell responses effectively [167, 168] and break immune tolerance to tumor associated antigens [169, 170].

The MVA and NYVAC vectors currently used in preclinical and clinical settings for cancer immunotherapy have been designed to trigger and/or enhance immune response to tumor cells by the delivery of tumor associated antigens (TAA). The data regarding efficacy and immune responses ind-uced by these attenuated strains are summarized in Table **3**.

Among TAA, viral antigens in virus-associated cancers are attractive targets for immunotherapy, since the cells capable of responding to these antigens should not be removed from the repertoire by central tolerance-inducing mechanisms. The MVA vectors expressing E2 or E6 and/or E7 viral antigens have shown therapeutic benefit in preclinical studies and clinical trials against HPV associated malignancies (Table **3A** and **3B**). Moreover, MVA recombinants expressing melanoma-specific differentiation antigens such as tyrosinase and melan-A as well as cancer/testis specific antigens MAGE1, MAGE3 and NY-ESO-1 have been used in HLA-A2 transgenic mice [286] and melanoma patients [267]. Additionally, MVA vector has been used to deliver oncofetal antigens such as MUC1, 5T4 and CEA and oncogene products like Her-2/neu and p53 to host immune system in transplantable tumor models and in clinical trials for immunotherapy of various types of cancer showing encouraging results (Table **3**). Since tumor-associated antigens are by definition weakly immunogenic, several strategies using MVA recombinants have been employed to improve immune response to TAAs including diversified prime/boost vaccination regimens [248, 249, 253, 267, 287] and expression of T cell costimulatory molecules or cytokines alongside with antigen [249, 288-290].

The poxviruses, including MVA and NYVAC vectors have been also used to transduce *in vitro* dendritic and tumor cells with genes for relevant tumor antigens or immunomodulatory products for whole cell vaccination providing feasible strategy for augmenting immune responses to tumor cells [256, 264, 266, 291].

In addition to the use of poxvirus vectors to deliver TAA and immunomodulatory molecules, members of this virus family have been explored as a potential oncolytic agents [292, 293]. Recently, an MVA vector has been used successfully for the transfer of suicide genes to cancer cells in preclinical tumor model showing therapeutic efficacy [261]. Overall, the use of MVA and NYVAC vectors as tumor vaccines represents a promising strategy to reduce tumor burden through activation of specific T cell immune responses. Considering that these vectors trigger a low immune response against themselves, several immunizations with the same vector could be applied. In addition, other attenuated poxvirus vectors that do not cross-react between them could be used in diversified prime/boost vaccination protocols. Furthermore, combination of tumor vaccines based on MVA or NYVAC vectors in multi-nodal strategies with other imm-

**Table 3. Gene Therapy Strategies Using MVA and NYVAC Vectors for Prevention and Treatment of Cancer in Preclinical (A) and Clinical (B) Settings**

| **A: Preclinical studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Cancer Type** | **Animal Model** | **Vaccine** | **Antigen** | **Reported Immune Responses** | **Result** | **References** |
| Sarcoma | Balb/c mice | MVA-p53 | Murine tumor protein p53 | p53-specific CTL response | Tumor rejection (12/16) | [245] |
| | | MVA-p53 + CTLA-4 mAb | | Vaccine effect was $CD8^+$ dependent and partially $CD4^+$ dependent | Tumor rejection (11/14) | |
| Tumors overexpressing mutated p53 | Balb/c and C57BL/6 mice | MVA-p53 + CpG ODN | Murine tumor protein p53 | Vaccine effect $CD8^+$ dependent and partially NK dependent | Tumor rejection and prolonged survival in the majority of treated animal | [246] |
| | | MVA-p53 + CTLA-4 mAb | | Vaccine effect $CD8^+$ dependent and partially $CD4^+$ dependent | | |
| | | MVA-p53 + CpG ODN + CTLA-4 mAb | | Enhanced p53-specific CTL response | | |
| Mammary carcinoma (hp53) | Hupki (Balb/cJ hp53 knock-in) | MVA-p53 + CpG ODN | Human tumor protein p53 | p53-specific $CD8^+$ and $CD4^+$ T cell responses | Tumor rejection in 50 % of animals | [247] |
| Mammary carcinoma (hup53) | Hupki (Balb/cJ hup53 knock in) | Prime: MVA-p53 Boost: *Listeria monocytogenes*-p53 (LmddA-LLO-p53) +/- Poly (I:C), CpG-ODN | Human tumor protein p53 | p53-specific $CD8^+$ and $CD4^+$ T cell responses<br>TLR agonists did not augment T cell responses | TLR agonists enhance antitumor effect of vaccine. Potent antitumor effect and improved survival. 50% complete regression of established tumors | [248] |
| Colon carcinoma (hCEA) | hCEA-Tg C57BL/6 mice | P: MVA-CEA/TRICOM B: F-CEA/TRICOM + GM-CSF + IL-2 | Human carcinoembryonic antigen | CEA-specific $CD8^+$ and $CD4^+$ T cells responses | Increased survival (5/7) | [249] |
| Melanoma (h5T4) | C57BL/6 mice | MVA-5T4 | Human trophoblast glycoprotein | No h5T4-specific CTL activity. Presence of anti-5T4 IgG2a, IgG2b and IgG1 | Delayed tumor development and increased survival | [250] |
| Colorectal carcinoma (h5T4) | Balb/c mice | | | | Reduction in the number of tumor nodules in the lungs and increased survival | |
| Melanoma (m5T4) | C57BL/6 mice | MVA-5T4 | Murine trophoblast glycoprotein | No m5T4-specific CTL activity. Presence of anti-5T4 IgG2a, IgG2b and IgG1 | Delayed tumor development and increased survival | [250] |

**(Table 3) contd….**

| **A: Preclinical studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Cancer Type** | **Animal Model** | **Vaccine** | **Antigen** | **Reported Immune Responses** | **Result** | **References** |
| Colorectal carcinoma (h5T4) | Balb/c mice | MVA-5T4 (TroVax) | Human trophoblast glycoprotein | Vaccine effect Ab mediated and $CD4^+$ T cells dependent | Protection against tumor challenge and >90% reduction in tumor burden | [251] |
| Pancreatic cancer | C57BL/6 mice | MVA-survivin +/- gemcitabine | Murine survivin | Survivin-specific CD4+ and $CD8^+$ T cell responses. Enhanced $CD8^+$ response in vaccine + gemcitabine group | Vaccine + gemcitabine: significant tumor regression and prolonged survival | [252] |
| Prostate Cancer | TRAMP mice | P: DNA-mPSCA + DNA-mSTEAP1 B: MVA-mPSCA + MVA-mSTEAP1 | Murine prostate stem cell antigen, Murine six transmembrane epithelial antigen of prostate | ND | Decreased primary tumor burden and suppression of the disease progression | [253] |
| | C57BL/6 mice | P: DNA-mPSCA or DNA-mSTEAP1 or combination B: MVA-mPSCA or MVA-mSTEAP1 or combination | | | Delayed TRAMP-C1 tumor growth | |
| Cervical carcinoma | Nude mice | MVA-E2 | E2 protein of BPV-1 | - | Prolonged life expectancy and reduced tumor growth | [254] |
| Transplantable papilloma carcinoma | Rabbits | MVA-E2 | E2 protein of BPV-1 | No CTL activity against tumor cells. Macrophage Ab-dependent cytotoxicity | Tumor regression in 80% of animals | [255] |
| Tumor cells expressing E6/E7 proteins of HPV 16 | C57BL/6 mice | DC/MVA-SigE7LAMP1 | E7 protein of HPV16 fused to the signal and transmembrane sequences of LAMP-1 (SigE7LAMP1) | Induction of immunity against tumor | Significant inhibition of tumor growth | [256] |
| | | P: CyaA/E7 B: MVA-SigE7LAMP1 | SigE7LAMP1 | E7-specific $CD8^+$ T cell response | Protection in 100% of animals | [257] |
| | | DNA-E7GGG MVA-GM-CSF | E7 protein of HPV16 | Increased infiltration of $CD3^+$ T cell in tumor | Significant inhibition of tumor growth | [258] |
| Mastocytoma Melanoma Renal carcinoma | B6D2 mice | MVA-CD3ε mAb | None (transgenes encode heavy and light chain of anti-mouse CD3ε IgG2a and anti-mouse TCRα/β IgG) | Tumor infiltration with $CD11c^+$ DC, $CD8^+$ and $CD4^+$ lymphocytes | Renal tumor rejection in 60 % of animals. No effect in melanoma and mastocytoma model | [259] |

**(Table 3) contd….**

**A: Preclinical studies**

| Cancer Type | Animal Model | Vaccine | Antigen | Reported Immune Responses | Result | References |
|---|---|---|---|---|---|---|
| Mastocytoma<br>Melanoma<br>Renal carcinoma | B6D2 mice | MVA-TCRα/β mAb | None (transgenes encode heavy and light chain of anti-mouse CD3ε IgG2a and anti-mouse TCRα/β IgG) | Tumor infiltration with $CD11c^+$ DC, $CD8^+$ and $CD4^+$ lymphocytes | Renal and melanoma tumor rejection in 33% and 30% of animals, respectively. No effect in mastocytoma model | [259] |
| Renal carcinoma | B6D2 mice | MVA-CD3ε mAb + Ad-IL-2 + Ad-IL-12 + Ad-IL-2/Ad-MIP1β + Ad-IL-12/Ad-MIP1β | None (transgene encodes heavy and light chain of anti-mouse CD3ε IgG2a) | NA | 60%-100% tumor rejection rate and prolonged survival | [260] |
| Colon cancer | Nude mice | MVA-FCU1 | None (transgene encodes FCU1) | - | Potent suppression of tumor growth and prolonged therapeutic levels of 5-FU in tumors | [261] |
| Sarcoma<br>Mammary carcinoma | Balb/c mice | P: ALVAC-p53<br>B: NYVAC-p53 | Murine tumor protein p53 | No p53-CTL specific response | No effect | [262] |
| Fibrosarcoma | Domestic cats | NYVAC-IL-2 | None (transgene encodes human IL-2) | ND | Tumor recurrence in 39% of animals | [263] |
| Colon adenocarcinoma | Balb/c mice | TC/NYVAC-B7.1<br>TC/NYVAC-IL-2 | None (transgenes encode murine IL-2 and murine B7.1) | NA | Protection and tumor burden reduction | [264] |

**B: Clinical studies**

| Cancer Type | Phase | Vaccine | Antigen | Reported Immune Responses | Reported Safely/ Clinical Observations | Reference |
|---|---|---|---|---|---|---|
| Melanoma<br>Stage II | I | MVA-Tyr | Tyrosinase | No tyrosinase-specific T cell or Ab responses | No side effects above grade 2 | [265] |
| Melanoma<br>Stage IV | I | DC/MVA-Tyr | Tyrosinase | Tyrosinase-specific T cell response (4/5) | Well tolerated. PR (1/6) | [266] |
| Melanoma<br>Stage II/III/IV | I | P: DNA-Mel3<br>B: MVA-Mel3 (Hi-8® MEL) | HLA-A*0201 and HLA-A*01 restricted CTL epitopes from melan-A, NY-ESO-1, MAGE-1, MAGE-3, tyrosinase | CTL response specific for epitope melan-$A_{26-35}$ (2/6) | Well tolerated. Only grade 1 side effects | [267] |
| | | P: MVA-Mel3<br>B: MVA-Mel3 | | CTL response specific for epitope melan-$A_{26-35}$ (4/7) | | |
| Melanoma<br>Stage III/IV | II | P: DNA-Mel3<br>B: MVA-Mel3 (Hi-8® MEL) | HLA-A*0201 and HLA-A*01-restricted CTL epitopes from melan-A, NY-ESO-1, MAGE-1, MAGE-3, tyrosinase | Melana-A/A2 tetramer responses (24/36). ELISPOT responses to at least 1 epitope (11/36) | Well tolerated. PR>24 months (1/39), SD>5 months (5/39), MxR (2/39). Increased TTP and survival in Melan-A responders | [268] |

**(Table 3) contd….**

| **B: Clinical studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Cancer Type** | **Phase** | **Vaccine** | **Antigen** | **Reported Immune Responses** | **Reported Safely/Clinical Observations** | **References** |
| MUC1-positive solid tumors | I | MVA-MUC1-IL-2 (TG4010) | Mucin 1 | MUC1-specific T cell activity (5/12). No MUC1-specific Ab response | Well tolerated. SD 6-9 months (4/12) | [269] |
| NSCLC Stage IIIb/IV | II | MVA-MUC1-IL-2 (TG4010) +/- Cisplatin Vinorelbine | Mucin 1 | MUC1-specific cellular immune responses in all responding patients | Well tolerated. Vaccine + chemo-therapy PR (13/37), SD (12/37). Favorable OS | [270] |
| Renal cell carcinoma | II | MVA-MUC1-IL-2 (TG4010) At progression combi-nation therapy with IFNα and IL-2 | Mucin 1 | MUC1-specific $CD4^+$ T cell response (6/28). MUC1-specific $CD8^+$ T cell response (4/23) correlated with better OS | No objective clini-cal responses. SD>6 months (5/27) TG4010 alone, SD>6 months (6/20) TG4010 + cytoki-nes. Overall TTF 9.3 months. OS 19.3 months for all patients | [271] |
| Prostate cancer | II | MVA-MUC1-IL2 (TG4010) | Mucin 1 | MUC1-specific ELISPOT responses (7/34) | Well tolerated. Primary study endpoint (50% decline in PSA) not achieved. Two fold improvement in PSA doubling time (13/40). Stabilized PSA >8 months (10/40) | [272] |
| Colorectal cancer Stage IV | I/II | MVA-5T4 (TroVax) | Trophoblast glycoprotein | 5T4-specificT cell esponse (16/17). 5T4-specific Ab response (14/17) | Well tolerated. SD 3-18 months (5/16) | [273] |
| Colorectal Cancer | II | MVA-5T4 (TroVax) | Trophoblast glycoprotein | 5T4-specific cellular and/or humoral immune responses (19/20) | Peripheral 5T4-specific responses and high $CD3^+$ infiltration at tumor site correlated with improved survival | [274] |
| Colorectal cancer Stage IV | II | MVA-5T4 (TroVax) + FOLFOX | Trophoblast glycoprotein | 5T4-specific Ab and T cell responses (10/11) | Well tolerated. CR or PR (6/11) | [275] |
| | II | MVA-5T4 (TroVax) + chemotherapy | Trophoblast glycoprotein | 5T4-specific cellular and/or humoral immune responses (12/12) | Safe and well tolerated. CR (1/19), PR (6/19), SD (5/19) | [276] |

**(Table 3) contd….**

| **B: Clinical studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Cancer Type** | **Phase** | **Vaccine** | **Antigen** | **Reported Immune Responses** | **Reported Safely/Clinical Observations** | **References** |
| Metastatic renal cell carcinoma | II | MVA-5T4 (TroVax) + IL-2 | Trophoblast glycoprotein | 5T4-specific Ab responses (23/23), 5T4-specific T cell reponses (13/23) | Well tolerated. No objective tumor responses. SD (12/23) | [277] |
| Renal cell carcinoma | I/II | MVA-5T4 (TroVax) + IFN-α | Trophoblast glycoprotein | 5T4-specific Ab responses (11/11), 5T4-specific cellular responses (5/11) | Well tolerated. No objective tumor responses. Increased median TTP as compared to IFNα alone arm. | [278] |
| | II | MVA-5T4 (TroVax) +/- IFN-α | Trophoblast glycoprotein | 5T4-specific Ab and /or cellular responses (22/23) similar in both arms. Magnitude of 5T4-specifc ELISPOT responses correlated with increased PFS; 5T4-specific humoral responses correlated with improved OS | Vaccine well tolerated in both arms. TroVax +IFNα: PR>7 months (1 patient); SD 1.7-9.6 months (14 patients, 7 TroVax and 7 TroVax +IFNα) | [279] |
| | II | MVA-5T4 (TroVax) + IL-2 | Trophoblast glycoprotein | 5T4-specific Ab responses (21/25). Correlation between magnitude of 5T4-specific Ab responses and PFS and OS | Safe and well tolerated. CR>24 months (2/25), PR>12 months (1/25), SD≥6 months (6/25) | [280] |
| Metastatic renal cell carcinoma | III | MVA-5T4 (TroVax) +SOC | Trophoblast glycoprotein | 5T4-specific Ab responses in 56% of patients<br>High 5T4-specific Ab response correlated with enhanced survival | Well tolerated.<br>No difference in OS | [281] |
| Prostate cancer | II | MVA-5T4 (TroVax) +/- GM-CSF | Trophoblast glycoprotein | 5T4-specific Ab responses (24/24), 5T4-specific ELISPOT responses (9/24). Similar immunological and clinical responses observed in both arms | Safe and well tolerated. No objective clinical responses. Increased median TTP in 5T4 ELISPOT responders | [282] |
| | I/II | MVA-BN®-PRO | PSA and PAP | PSA and PAP-specific immune responses<br>T-cell responses to antigens not included in vaccine | Well tolerated. | www.bavarian-nordic.com |

**(Table 3) contd….**

| **B: Clinical studies** | | | | | | |
|---|---|---|---|---|---|---|
| **Cancer Type** | **Phase** | **Vaccine** | **Antigen** | **Reported Immune Responses** | **Reported Safely/Clinical Observations** | **References** |
| CIN Grade I, II, III | I/II | MVA-E2 | E2 protein of BPV-1 | Ab and cytotoxic responses against HPV transformed cells | Well tolerated. Complete elimination of CIN (34/36). No evidence of HPV DNA in 50% of patients | [283] |
| CIN Grade II, III | II | MVA-E2 | E2 protein of BPV-1 | Ab and cytotoxic responses against HPV transformed cells | Elimination of high grade CIN (20/34), lesions reduction by 50% (11/34), lesions reduction to CIN II (2/34) and to CIN I (1/34). HPV DNA load was significantly decreased | [284] |
| | II | MVA-HPV-IL-2 (TG4001) | E6/E7 proteins of HPV 16 | NA | Well tolerated. No CIN II/III lesion (10/21). No E6/E7 mRNA detected | www.transgene.fr |
| Flat condyloma | I/II | MVA-E2 | E2 protein of BPV-1 | E2-specific Ab and HPV-transformed cells specific cytotoxic responses | No lesion or presence of HPV (28/30). No recurrent disease after 1 year of treatment | [285] |
| Breast cancer | I/II | MVA-BN®-HER2 (following chemotherapy and Herceptin® treatment or in combination with single-agent taxane chemotherapy) | Receptor tyrosine-protein kinase HER-2/neu | HER2-specific humoral and/or cellular responses (12/18) | Well tolerated. No disease progression after 6 months (15/30). Vaccine + chemotherapy<br>1 CR and 1 PR | www.bavarian-nordic.com |

notherapeutic agents, in particular those that aim at blocking immunosuppressive mechanisms, and/or with conventional therapeutic modalities may further improve their efficacy.

## IMMUNOGENIC IMPROVEMENTS OF MVA AND NYVAC VECTORS

The modest efficacy obtained with the RV144 Thai phase III trial against HIV infection highlights the need to develop improved attenuated poxvirus vectors as vaccines. Several strategies have been used to enhance the immunogenic capacity of MVA and NYVAC vectors. This has been accomplished in MVA after deleting viral genes that antagonize host specific immune responses [294-296], through co-expression of cytokines [297], as well as by combining the vector with adjuvants [298]. The immunogenicity of NYVAC has been improved by the incorporation of viral host range genes ([299]; Kibler, K. et al, submitted) or by deleting viral genes that antagonize the IFN system (Quakkelaar, ED et al, PLoS ONE, in press). Other viral genes with roles as immunomodulators and still present in the genomes of MVA and NYVAC are being analyzed for further improvements in immunogenicity of the two vectors. It will be important to establish in future studies whether anyone of the modified poxvirus vectors MVA and NYVAC could be used alone or in combination with other immunogens as more potent vaccines.

## CONCLUDING REMARKS

Undoubtedly, the poxvirus vectors MVA and NYVAC are promising candidates as delivery vehicles of foreign pro-

teins to tissues and as recombinant vaccines, as documented thus far by a number of preclinical and clinical studies. The information obtained highlights their safety record, efficient expression of the recombinant product, strong immunogenicity and, in many cases high protective efficacy against a pathogen. Since the two virus strains are derived from VACV the question emerges as if we should consider preferentially one vector over the other. Comparative studies of the two vectors in cultured cells and in animals showed distinct behaviour. In fact, while in HeLa cells MVA expresses all or nearly all of the early and late viral genes and morphogenesis is blocked after IV formation, NYVAC infection in the same cells has a restriction in the synthesis of some of the late viral proteins and morphogenesis is blocked at or before IV formation. In immature human mDCs, the replication of both viruses is largely restricted, but the two viruses differ in the magnitude and extend of induction of host genes. In fact, MVA triggered the expression of higher number of immunomodulatory genes than NYVAC, in particular the type I IFN and its signalling pathways. When inoculated by different routes in mice, the MVA vector expresses a heterologous antigen faster than NYVAC, but this vector stays longer (about 24 more hours) than MVA. Both vectors are cleared by 3 days postinfection. When the two vectors are administered to macaques by aerosol they are absorbed primarily in the mucosal tissues of the lungs and respiratory tract, but not in the brain or eyes, proven their safety. As documented, both systemic and mucosal routes are effective as MVA and NYVAC delivery systems of antigens, but the route to be applied will be selected as a function of the pathogen route of transmission and reservoir.

When the two vectors are compared head-to-head in preclinical studies with mice and monkeys, both vectors triggered specific immune responses to the foreign antigens and elicit protection in monkeys challenged with a pathogenic SHIV89.6p strain. Significantly, the type of cellular immune response triggered by MVA versus NYVAC differed clearly. While MVA triggers preferentially a CD8+ T cell response, NYVAC favours a CD4+ T cell response. These immunological differences must be taken into account when these vectors are considered as vaccines. Preclinical and clinical studies have been performed with these two vectors against diseases caused by viruses, bacteria, parasites and cancer. The conclusion of these studies is that both of these vectors are promising vehicles to deliver antigens and elicit specific immune responses that in some cases correlate with protection. Since the breath, magnitude and duration of the immune responses might be critical parameters in determining protection, further insights into the biology of MVA and NYVAC and their immune behaviour is needed. An important consideration is that these vectors still contain many other genes that counteract host immune responses, as illustrated in Table **1**. Understanding the contribution of these genes in virus-host cell interactions and in the immunogenicity of MVA and NYVAC recombinants is also needed. Some progress in this direction is emerging from studies by several groups. Overall, more efficient MVA vectors that enhance the magnitude, breath, polyfunctionality and durability of the immune response to antigens of pathogens are desirable. This is particularly relevant if instead of using combined immunogens a single product is target for mass vaccination purposes to simplify the immunization protocol and reduce manufacture cost. The hope is to know in the years ahead how viral genes antagonize the immune system and to make use of this knowledge in the generation of modified MVA and NYVAC gene delivery systems as newer vaccines and anti-tumor agents.

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## ABBREVIATIONS

| | | |
|---|---|---|
| 5-FU | = | 5-fluorouracil |
| Ab | = | Antibody |
| Ad | = | Adenovirus |
| Ag | = | Antigen |
| AIDS | = | Acquired immune deficiency syndrome |
| AMA1 | = | Apical membrane antigen 1 |
| APC | = | Antigen presenting cell |
| ATI | = | A-type inclusion body |
| B | = | Boost |
| B7.1 | = | Co-stimulatory protein B7-1 |
| BPV-1 | = | Bovine papillomavirus |
| CBP | = | Chemokine-binding protein |
| CDV | = | Canine distemper virus |
| CEA | = | Carcinoembrionic antigen |
| CIN | = | Cervical intraepithelial neoplasia |
| CMI | = | Cell mediated immune response |
| CMV | = | Cytomegalovirus |
| CpG | = | Cytosine-phosphate-guanine motifs |
| CR | = | Complete response |
| CS | = | Circumsporozoite protein |
| CTL | = | Cytotoxic T lymphocyte |
| CTLA-4 | = | CTL associated antigen 4 |
| CyaA | = | *Bordetella pertussis* adenylate cyclase toxoid |
| DCs | = | Dendritic cells |
| E7GGG | = | HPV16 E7 gene fused with *E. coli* β-glucuronidase gene |
| EHV | = | Equine herpes virus |
| EIV | = | Equine influenza virus |
| FCU1 | = | FCY1 and FUR1 fusion gene |
| FCY1 | = | S. cerevisiae cytosine deaminase gene |

FOLFOX = Combination chemotherapy (folinic acid, fluorouracil, oxaliplatin)
FP9 = Fowlpox virus 9
FPV = Fowlpox virus
FUR1 = S. cerevisiae uracil phosphoribosyltransferase gene
GM-CSF = Granulocyte-macrophage colony-stimulating factor
HCV = Hepatitis C virus
HIV = Human immunodeficiency virus
HPV 16 = Human papilloma virus 16
ICAM-1 = Intracellular adhesion molecule-1
ICE = Interleukin-1β-converting enzyme
IL-12 = Interleukin-12
IL-2 = Interleukin-2
IFNα = Interferon-alfa
JEV = Japanese encephalitis virus
LACK = Leishmania analogue of the receptors for activated C kinase
LAMP1 = Lysosomal-associated membrane protein 1
LFA-3 = Lymphocyte-function-associated antigen 3
LSA1 = Liver stage antigen 1
mAb = Monoclonal antibody
MAGE-1 = Melanoma associated antigen 1
MAGE-3 = Melanoma associated antigen 3
MAP2K5 = Mitogen-activated protein kinase kinase 5
MDA5 = Melanoma-differentiation-associated gene 5
ME.TRAP = Multiepitope thrombospondin related adhesion protein
MIP1β = Macrophage inflammatory protein-1-beta
MR = PR despite one new lesion
MSP1 = Merozoite surface protein 1
MVA = Modified vaccinia virus Ankara
MVA-BN = MVA Bavarian Nordic
MxR = Mixed responses
NA = Not available
ND = Not determined
NFAT5 = Nuclear factor of activated T cell 5
NK = Natural killer cell
NSCLC = Non-small cell lung cancer
NY-ESO-1 = Cancer/testis antigen 1B
NYVAC = New York vaccinia virus attenuated from Copenhagen
ODN = Oligodeoxynucleotide
OS = Overall survival
P = Prime
PAP = Prostate alkaline phosphatase
Pfs25 = 25 KDa sexual-stage antigen
PFS = Progression free survival
PFU = Plaque forming units
PR = Partial response
PSA = Prostate specific antigen
PVM = Parasitephorous vacuole membrane
RIG = Retinoid acid-inducible gene
RSV = Respiratory syncitial virus
SARS = Severe acute respiratory syndrome
SCYB10 = Chemokine (C-X-C motif) ligand 10
SD = Stable disease
SERA = Serine repeat antigen
SFV = Semliki forest virus
SHIV89.6P = Simian-human immunodeficiency virus 89.6P
SOC = Standard-of-care
SSP2 = Sporozoite surface protein 2
TC = Tumor cells
Tg = Transgenic
TTF = Time to treatment failure
TTP = Time to progression
TRICOM = B7.1, ICAM-1, LFA-3

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