

REVIEW

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Biosafety considerations for attenuated measles virus vectors used in virotherapy and vaccination

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ABSTRACT

Attenuated measles virus (MV) is one of the most effective and safe vaccines available, making it attractive candidate vector to prevent infectious diseases. Attenuated MV have acquired the ability to use the complement regulator CD46 as a major receptor to mediate virus entry and intercellular fusion. Therefore, attenuated MV strains preferentially infect and destroy a wide variety of cancer cells making them also attractive oncolytic vectors. The use of recombinant MV vector has to comply with various regulatory requirements, particularly relating to the assessment of potential risks for human health and the environment. The present article highlights the main characteristics of MV and recombinant MV vectors used for vaccination and virotherapy and discusses these features from a biosafety point of view.

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Introduction

Attenuated measles virus (MV) represents an attractive vector candidate for virotherapy and vaccination. This is due to several features including: (i) impressive track record of safety and efficacy in human population, (ii) lack of genomic integration in the host cells due to their cytoplasmic replication, (iii) high immunogenicity as vaccine, (iv) compared to other RNA viruses MV maintain high levels of genetic stability,¹ (v) large foreign gene(s) insertion capacity (>6 kb)² and (vi) selectivity to tumor cells in case of virotherapy applications.

Many of the attenuated strains in use are derived from the MV Edmonston strain (MV-Edm) isolated in 1954 from the throat washings and blood of a child with measles, in a primary culture of human kidney cells.³ This isolate was subsequently adapted to various types of cultured cells, giving rise to attenuated Edmonston A and B seeds.⁴ Further passages of Edmonston A and B seeds on chicken embryo fibroblasts (CEF) produced the more attenuated Schwarz and Moraten viruses.⁵ Table 1 summarizes the characteristics of the different measles vaccine strains derived from the Edmonston strain. Administration of these vaccines has dramatically reduced the incidence of measles. These vaccines are among the most effective and safe human vaccines in use providing long-lasting protection.¹⁷ These characteristics make measles vaccine attractive as a viral vector backbone for the development of recombinant vaccines against other viral infections such as human immunodeficiency virus (HIV), SARS coronavirus (SARS-CoV) and flavivirus infections.^{18–20} Nucleotide differences between the different attenuated vaccine strains used as vectors and MV-Edm are indicated in Table 1. Attenuation is not determined by the absolute number of substitutions, because the Zagreb strain which

is more attenuated than Edmonston B strain has fewer substitutions, as compared with MV-Edm. Schwarz and Moraten strains have identical nucleotide sequences, despite their divergent passage histories. Because both strains have been passaged in CEF at reduced temperatures, it is possible that similar cell culture conditions may have resulted in similar nucleotide substitutions. Despite the diverse geographic origins of the progenitors and the variations in cell culture systems, incubation temperatures, and passage numbers, the genome of vaccines demonstrates sequence similarity.⁵ The choice of a strain for the design of the recombinant vector is based on the attenuation of the strain and its safe use as vaccine rather than nucleotide sequence analysis.

Other measles vaccines were derived from wild-type progenitors isolated independently in Russia (Leningrad-4), Japan (CAM-70), and China (Shangai-191).⁵

The wild-type (wt) MV enters cells predominantly via the signaling lymphocyte activation molecule (SLAM also known as CD150), mainly expressed on subsets of lymphocytes, thymocytes, macrophages and mature dendritic cells (DCs). In contrast all laboratory adapted attenuated MV-Edm strains have acquired the ability to use, besides CD150, the complement regulator CD46 (also known as membrane cofactor protein; MCP) as receptor to mediate virus entry and intercellular fusion. This receptor is overexpressed on the surface of malignant cells protecting them against complement mediated cell lysis.²¹ Another cellular receptor, Nectin-4 (also known as poliovirus receptor-like protein 4; PVRL4), expressed on primary airway epithelial cells and also overexpressed in many tumor types, has also been identified as a receptor for MV viral entry.^{22,23} Therefore, attenuated MV strains preferentially

Table 1. Main characteristics of the wild-type MV strain and the live-attenuated measles vaccines derived from the Edmonston virus.

Strains	Main characteristics		Receptor used to enter cells	Who develops the strain
MV	Wild-type strain		CD150 - Nectin 4	
MV Edmonston isolate/MV Edmonston (wild-type) strain	MV strain isolated from a child with measles in primary human kidney cells. ³ This strain has undergone 13 passages in cell culture.		CD150 – Nectin 4	
Edmonston seed A and B ⁴	passages of the Edmonston strain in: primary human kidney cells primary human amnion cells adapting to chick embryos and further passaging in chick embryo cells (CEF) ⁴		CD150 – Nectin 4	
Live-attenuated vaccines derived from the Edmonston virus				
		Nucleotide changes compared to MV Edmonston isolate		
Edmonston B strain	Additional passages on CEF	36	CD46 – CD150 – Nectin 4. ⁶ Unidentified receptor other than CD150 and CD46. ⁷	1963 licensed for use in the US Rubeovax [®] . Adverse reactions: fever, rash, upper respiratory symptoms, occasionally convulsions. It was abandoned in 1975. ⁸
Schwarz strain*	Additional passages on CE cells	42 (16 nucleotides differences compared to Edmonston B strain)	CD46 – CD150 – Nectin 4	1965 (GSK Priorix [®] , vaccine for immunization against measles, mumps and rubella, "heat-stable" vaccine suitable for use in tropical countries, developing countries) Rouvax [®] , vaccine produced by manufacturers in Europe and Brazil, Sanofi-Pasteur, Institute Pasteur, France
Moraten strain*	Additional passages on CEF	42* Schwarz and Moraten strains have identical nucleotide sequences	CD46 – CD150 – Nectin 4	1968 Use as vaccine in the USA, Attenuvax [®] , M-M-R [®] II (measles, mumps, and rubella vaccine), Merck and Co
Zagreb strain [also referred to as Edmonston-Zagreb strain (EZ)] ⁹	Additional passaging and plaque purification in human diploid cells WI-38. ¹⁰ Produced either on CEF and on human diploid cells MRC-5. ^{11,12}	33	CD46 – CD150 – Nectin 4. ⁶	The most commonly used measles vaccine in the Expanded Program on Immunization (EPI) of the WHO. Developed by the Institute of Immunology in Zagreb
AIK-C strain ¹³	Passage in: human amnion cells sheep kidney cells CEF adapted to MRC-5 cells. ^{13,14}	42	CD46 – CD150 – Nectin 4. ⁶	Strain used as measles vaccine in Japan. ¹⁵ One of the candidate vaccine strain for EPI.
MVbv strain ¹⁶	Produced in MRC-5 cells.	36	CD46 – CD150 – Nectin 4	Moraten [®] Berna vaccine, University of Zurich, Berna Biotech, Switzerland, can be applied parenterally or as aerosol vaccine

infect and destroy a wide variety of cancer cells making them attractive oncolytic vectors.²⁴

Recombinant, attenuated MV strains are currently being tested in several phase I clinical trials as vaccine against HIV²⁵ or chikungunya virus²⁶ and as oncolytic vector in ovarian

cancer, glioblastoma multiforme, multiple myeloma, head and neck cancer, and mesothelioma.^{24,27-29}

The use of recombinant, genetically modified (GM) viral vectors for pre-clinical and clinical trials must comply with several European Union legislations including the legal

provisions on biosafety aiming at protecting public health and the environment against potentially adverse effects of genetically modified organisms. Activities involving manipulation of GM viral vectors in contained conditions (e.g. laboratories, animal husbandries, production facilities, hospital rooms) may comply with Directive 2009/41/EC.³⁰ Activities involving their deliberate release into the environment require that a case-by-case environmental risk assessment (ERA) should be carried out before release according to the principles defined in annex II of Directive 2001/18/EC.³¹ The ERA is also part of the procedure for marketing authorization.³² The general steps underlying an ERA of viral vectors have been discussed in Baldo et al.³³

This article focuses on biosafety issues in the European Union when performing clinical trials with recombinant attenuated MV vectors. The risk related to research and development activities and large scale production of these vectors are not developed in the present review.

Hazard related to the molecular and biological characteristics of measles viruses

Wild-type measles virus

Measles virus is an enveloped negative-strand RNA virus of the genus *Morbillivirus* within the *Paramyxoviridae* family and is the causative agent of the acute, exanthemous, infectious measles disease.³⁴ The MV genome encodes 8 proteins: 2 non-structural proteins (V and C), a phosphoprotein (P), the large polymerase protein (L) and the nucleoprotein (N) forming the viral nucleocapsid which contains the viral RNA genome, the matrix protein (M), and 2 envelop glycoproteins, the hemagglutinin protein (H) and the fusion protein (F) responsible for receptor binding and membrane fusion respectively.³⁵ Upon infection of susceptible cells, MV causes cell-cell fusion producing multinucleated giant cells, the typical cytopathic effect of MV infection.³⁶

Infection through the natural route is initiated in the respiratory tract via infection of CD150⁺ alveolar macrophages and/or DCs in the airway lumen or just below the epithelial cell layer by receptor-mediated recognition of MV.³⁶ Measles virus is captured by the DCs, which are widely distributed throughout the respiratory epithelium. Instead of being degraded, MV is protected and transported into the regional lymph nodes where it is efficiently transmitted to CD150⁺ lymphocytes which amplify the virus establishing primary viremia.^{36,37} After amplification of the virus in lymph nodes, lymphocytes and monocytes carry the virus to various organs throughout the body including the skin, kidneys, gastrointestinal tract and liver, and throughout the respiratory tract.²³ MV-infected lymphocytes migrate into the lung tissue from the blood vessels where they may induce degradation of the basement membrane. They travel between adjacent epithelial cells and transmit the virus to epithelial cells via the receptor Nectin-4 expressed in adherens junctions.^{22,23} This causes extensive epithelial damage and apical shedding of MV resulting in transmission to the next host.

Humans are the only known natural host for MV.²³ Non-human primates can also be infected and are commonly used

as model of infection. To date there is no known animal reservoir and no asymptomatic carrier state has been documented.

Measles (Rubeola) is one of the most contagious viral diseases known. Measles virus is efficiently transmitted by aerosols entering the respiratory tract or by direct contact with respiratory secretions.^{36,38} Its capacity of spreading is high, one person infected with MV can infect 15 to 20 others ($R_0 = 11-18$),³⁹ meaning that the interruption of endemic transmission in population requires that more than 95% of the population is immune.⁴⁰ The infectious dose for wt MV is 0.2 units by intranasal spray.⁴¹

Measles virus has a cytoplasmic replication cycle eliminating the possibility of integration into the host cell DNA and then a possible insertional mutagenesis.

Measles virus is an enveloped virus unstable in the environment surviving less than 2 hours on surfaces or objects.⁴² Respiratory droplets can remain infective for at least one hour in confined spaces.³⁸ MV is susceptible to a variety of disinfectants (e.g., povidone iodine, 1% sodium hypochlorite, peracetic acid, hydrogen peroxide, 70% Ethanol).⁴¹

The World Health Organization (WHO) has defined criteria for the classification of microorganisms into 4 Risks Groups, taking into account the severity of the disease that pathogens may cause in humans or animals, their ability to spread among the population, and the availability of prophylaxis or efficient treatment.⁴³ In Belgium, classification lists for human, animal, or plant pathogens provide a tool for identifying biological hazards associated with the contained use^a of wild-type pathogenic organisms.⁴⁴

MV is classified as a biological agent of class of risk 2 for humans: it can cause human disease and might be a hazard for directly exposed persons; it is however unlikely to spread to the community because an effective prophylactic vaccine against measles is available.⁴³⁻⁴⁵

Attenuated measles virus generation

Attenuation of MV strains is the result of adaptation of the virus to growth conditions in non-permissive cell culture, especially avian cell lines.⁵ MV isolation and passages in CD150 negative cells such as human kidney cells and Vero cells may have selected the viruses capable of using CD46 as a receptor for viral entry into host cells.^{7,37,46} The use of CD46 by some MV strains may be considered as an *in vitro* adaptation rather than *in vivo* property of those strains.⁷ Likewise, the vaccine strains must have adapted to chicken embryo fibroblasts by using an unknown receptor present on them.⁷ CD46 is a complement regulatory protein that plays an important role in protecting autologous cells from complement attack. CD46 is ubiquitously expressed at low density by all normal human cell types except erythrocytes. CD46 level on tumor cells can be up to 7-10 fold higher compared with normal cells and protects tumor cells from complement mediated lysis.⁴⁷ High CD46 receptor density on tumor cells is a key determinant of oncolytic specificity of attenuated MV strains. Whereas virus entry increases progressively with CD46 density, there is a threshold number of CD46 receptors required for cell-to-cell fusion, which leads to death of all the cells incorporated into the syncytia. The

differential expression of CD46 in tumor cells versus normal cells significantly increases the susceptibility of tumor cells to the oncolytic activity of MV attenuated strains. The *in vivo* tropism of attenuated MV and wt MV was compared using an animal model: in cynomolgus macaques experimentally infected via intratracheal or aerosol route, only the wt MV caused significant viremia and viral dissemination to the skin and the submucosa of respiratory epithelia.⁴⁸ MV strains that have acquired the ability to use CD46 as a receptor might gain a growth advantage *in vitro* in human and monkey cells because distribution of CD46 is ubiquitous, unlike that of CD150. However, *in vivo*, MV attenuated strains down-regulate CD46 in infected cells, which are then subject to complement-mediated cell lysis that may limit the spread of MV infection.⁴⁹

In vitro, attenuated MV has a much wider tropism than wt MV as it can use both CD46 and CD150 as cellular receptors to enter host cells. Nectin-4, the third MV receptor is expressed abundantly in placental trophoblasts, glandular cells of the stomach, and adenocarcinomas of the lung, breast and ovary. Moderate amounts are expressed in the epithelium of tonsils, oral mucosa, esophagus, and the epithelial cells of the nasopharynx and the trachea. Nectin-4 is expressed in the adherens junctions, on the basolateral side of epithelial cells in close contact to infected MV lymphocytes and dendritic cells and it is necessary for infection of human airway epithelia.⁵⁰ Smaller amounts are expressed in the lung macrophages and neuronal cells of the cerebral cortex. In cancer cells, Nectin-4 is highly up-regulated, and expressed on both apical and basolateral surfaces.⁵⁰ Reverse genetics technology using a helper-cell-based rescue system^{2,51} allowed the rescue of replicating measles viruses from cloned DNA able to stably express heterologous antigens. This technology enables the rescue of clinically approved and genetically relevant measles vaccine strains.^{12,16,20,52} Edmonston B strain has been used for the construction of essentially all genetically modified oncolytic MV-Edm derivatives described until now and for all those which entered the clinical trial phase (Table 2). Oncolytic activities of MV have been demonstrated for at least 12 different cancer types.⁶⁰ Furthermore attenuated MV has been used worldwide to vaccinate children with an excellent safety profile and with

no reversion to the wt MV making it attractive as oncolytic vector compared to other oncolytic viruses that are not used in vaccination.⁶⁰

Attenuated oncolytic MV vectors retain some characteristics enabling them to replicate in the human host. Compared to replication defective viral vectors, the likelihood of exposure of the environment around the patient is increased.⁶¹ However, dissemination of the viral vector from the patient into the environment is not an adverse event *per se*. Its impact will mostly depend on the characteristics of the recombinant vector itself, such as its pathogenicity, its infectious dose, its transmission mode, the availability of effective prophylaxis or treatment, its susceptibility to disinfection.³³

The vectors used to develop attenuated recombinant MV for vaccination against infectious diseases and which entered the clinical trial phase have been engineered by reverse genetics from the cDNA of different measles vaccine strains as described by Radecke et al.⁵¹ Additional transcription units (ATU) were introduced in the viral genome in order to construct the vector expressing the sequences corresponding to foreign antigens. Table 3 summarizes the clinical and pre-clinical studies performed using recombinant MV vector as prophylaxis vaccine candidates against infectious diseases.

History of safe use of attenuated measles virus

Although the wt MV can result in potentially serious infectious disease, the attenuated MV strains have a significant safety record, with millions of vaccine doses having been safely administered in more than 40 y of use.⁷⁰ However, fatal infections have been documented in immunodeficient vaccinated children⁷¹⁻⁷⁴ and in a vaccinated adult with AIDS.⁷⁵

Administration of a high-titer (HT) Edmonston-Zagreb vaccine in areas with a high incidence of measles in children younger than 9 months shown an increased incidence of female mortality.^{76,77} Investigators suggested that the HT measles vaccine had caused immune suppression similar to that of measles infection.⁷⁸⁻⁸⁰ A review summarizing HT studies suggests that the HT vaccine itself is unlikely to be the cause of immunosuppression, indeed the effect was not found in all studies. Moreover, the HT studies with excess mortality rates showed

Table 2. Clinical trials using oncolytic measles virus derived vectors. Legend: CEA: carcinoembryonic antigen, NIS: sodium iodide symporter, TCID₅₀: medium tissue culture infective dose. The MV vectors have been produced as reported by Radecke et al.⁵¹

Target disease	Transgenic expressed proteins	Administration route	Measles dosage level	Clinical trials	Parental strain	Reference(s)
Recurrent ovarian cancer	CEA; NIS; NIS (MV-NIS transduced mesenchymal stem cells)	Intraperitoneal	MV-CEA: 10 ³ to 10 ⁹ TCID ₅₀ MV-NIS: 10 ⁸ and 10 ⁹ TCID ₅₀ MV-NIS transduced MSC: 10 ⁹ TCID ₅₀ of virus inoculum	Phase I (finished); Phase I/II	Edm B vector	27, 28,53, 54
Glioblastoma multiforme	CEA	Intracranial	10 ⁵ to 2 × 10 ⁷ TCID ₅₀	Phase I	Edm B vector	55
Multiple myeloma	NIS	Intravenous (with or without cyclophosphamide)	10 ⁶ to 10 ¹¹ TCID ₅₀	Phase I; Phase II	Edm B vector	29, 56, 57
Mesothelioma	NIS	Intrapleural	3 × 10 ⁹ TCID ₅₀	Phase I	Edm B vector	58
Squamous cell carcinoma of head and neck	NIS	Intratumoral	3 × 10 ⁹ TCID ₅₀	Phase I	Edm B vector	59

Table 3. Clinical and preclinical studies using recombinant attenuated measles virus as prophylaxis vaccine candidates against infectious diseases. Legend: intramuscular, i.m.; intraperitoneal, i.p.; subcutaneous, s.c.; TCID₅₀: medium tissue culture infective dose; pfu, plaque forming units.

Target disease	Transgenic expressed proteins	Administration route	Measles dosage level	Clinical trial or preclinical assays	Parental strain	Reference(s)
HIV	F4: a fusion protein comprising the viral Ag p17, p24, RT, Nef	i.m.	2.9 log CCID50 or 4 log CCID50	Human (Phase I trial)	Schwarz vector	25
HIV	HIV proteins	ip	10 ⁴ – 10 ⁵ pfu	Mice	MVb vector (derived from the MVbv vaccine strain)	16, 62
Chikungunya fever virus (CHIKV)	Virus-like particles (VLP)	i.m.	1.5 × 10 ⁴ – 3 × 10 ⁵ TCID ₅₀	Human (Phase I trial)	Schwarz vector	26
West Nile Virus (WNV)	Secreted form of WNV envelope glycoprotein	i.p. (mice); i.m. (Squirrel monkeys)	10 ⁶ TCID ₅₀	mice, squirrel monkeys	Schwarz vector	63, 64
Nipah virus (NIV)	NIV glycoprotein	i.p. (Hamster); s.c. (Monkeys)	2 × 10 ⁴ TCID ₅₀ ; 10 ⁵ TCID ₅₀	Hamster, monkeys	Eclm B vector	65
Dengue virus (DV)	Tetravalent DV antigens incorporating the domain III of the envelope E glycoprotein (EDIII) of DV 1–4 in combination with the ectodomain of the membrane M protein (ectoM)	i.p.	10 ⁵ TCID ₅₀	Mice	Schwarz vector	66
SARS Coronavirus (SARS-CoV)	Membrane-anchored SARS-CoV spike (S) protein or its secreted soluble ectodomain (Ssol)	i.p.	10 ⁵ pfu	Mice	Schwarz vector	19
Simian immunodeficiency virus (SIV)	Gag protein	Aerosol immunization or i.m and i.p	5 × 10 ⁴ – 10 ⁶ pfu	Macaques	Edmonston Zagreb vector	12
Hepatitis B (HBV)	HB surface antigens	intranasal (macaques)	5 × 10 ⁴ TCID ₅₀	macaques	Schwarz vector	67
Respiratory syncytial virus (RSV)	RSV M2–1 or Nucleoprotein (NP)	i.m.	10 ⁵ TCID ₅₀	Cotton rats	AIK-C vector	68
Human papilloma virus (HPV)	Major viral capsid protein L1	i.p.	10 ⁵ pfu	Mice	MVb vector	69

increased female mortality rates that could be due to environmental or contextual conditions.⁸¹

According to their high attenuation profiles and their history of safe use, MV attenuated strains currently used as measles vaccines could be classified as agents of class of risk 1 because these strains are non-pathogenic for human and not harmful for the environment and present a negligible risk during contained use.⁴³ MV-Edm B strain should also be classified in class of risk 1. MV-Edm strain is reactogenic and can cause fever and rash in measles-naïve children.⁸² However, this strain is not able to cause a disease and is not transmissible to other persons.⁷⁰

Reconversion to wild-type

In addition and as already mentioned, the MV genome is very stable and reversion of measles vaccine strains to pathogenicity and subsequent transmission to other individuals have to date not been reported.⁷⁰

Hazards related to the recombinant MV vectors

In addition to its use as vaccine against measles, attenuated MV is used as a backbone for the development of prophylactic vaccine and as recombinant oncolytic vector. MV vector has been shown to stably express large, heterologous antigen-coding sequences up to 6 kb long.² Unlike primary strains, attenuated vaccine strains cause no immunosuppression.¹⁸ In addition, MV infects cells of the immune system, including macrophages and DCs, thus providing an opportunity to deliver antigens directly to the most effective antigen presenting cells, a major advantage for a vaccine vector. Several studies provide good indications of the clinical safety and efficacy of these vectors. Preclinical studies have tested recombinant MV vaccine for prophylaxis against HIV-1,²⁰ SARS-CoV,¹⁹ Chikungunya virus,⁸³ Dengue virus,⁶⁶ West Nile virus⁶⁴ and other infectious disease or cancer (Table 3). MV recombinant vector elicits strong and long-term HIV-specific neutralizing antibodies and cellular immune responses, even in the presence of preexisting immunity to MV^{20,84-86} arboviruses such as West Nile virus,¹⁸ Dengue virus⁶⁶ or SARS-CoV¹⁹ in animal models. The strong capacity to raise T-cell responses that persist on long term in lymphoid organs is a hallmark of live attenuated vaccines. MV is particularly efficient at generating live long memory CD4 and CD8 T cells that help to maintain neutralizing antibodies and to prevent from reinfection.⁸⁷ The long-term protective immunity in humans against expressed foreign transgenes should still be evaluated.

A phase I clinical study with recombinant MV (MV1-F4) used to immunize healthy people against HIV-1 is ongoing²⁵ and the results of a phase I clinical trial with the vaccine candidate (MV-CHIK) used to immunize people against chikungunya virus are now available.²⁶

Hazard related to the transgene

When recombinant MV vector is used, risk assessment should also take into account the potential risk associated with the transgene.

The vaccine candidate MV1-F4 has been developed by reverse genetics from the cDNA of the Schwarz strain and has been genetically modified to express the F4 HIV antigen.²⁰ F4 is a single fusion protein comprising 4 HIV-1 clade antigens, p17 and p24 encoded by *gag*, reverse transcriptase encoded by *pol* and the regulatory protein Nef.⁸⁸ P24 is a structural protein present on the capsid. Its association with the viral protein p17 results in a matrix protein between the envelope and the capsid, ensuring the integrity of the virion. Nef acts both to increase the infectivity of viral particles and to reduce the expression of the CD4 receptor molecule on the cell surface.⁸⁹ And finally, reverse transcriptase is the enzyme used to generate cDNA from viral RNA. The F4 fusion protein alone has been administered as vaccine candidate by intramuscular route to healthy HIV seronegative adults and no toxic or allergic effects have been observed in a phase I clinical trial.^{88,90}

The vaccine candidate MV-CHIK has also been developed with the Schwarz strain as vector backbone and modified to express structural genes from chikungunya virus.²⁶ The structural proteins capsid (C), the envelope E1 and E2 glycoproteins and 2 small peptides, E3 and 6K allows the formation of self-assembling virus particles (VLP) that mimic the alphavirus external structure.⁹¹ These structural proteins have been administered as vaccine candidate in healthy adults in a phase I trial and has been shown to be safe and well tolerated.⁹² Furthermore, the results of a phase I trial showed that MV-CHIK is safe and had an overall acceptable tolerability profile when administered in healthy humans.^{26,92}

The MV vector pTM-MV-Schw that was used to construct MV1-F4 and MV-CHIK contains an infectious cDNA corresponding to the antigenome of the Schwarz vaccine strain.⁹³ Additional transcription units (ATU) were introduced in the viral genome in order to construct the vector expressing the sequence corresponding to the F4 fusion protein for MV-F4 or to the structural protein from CHIK for MV-CHIK. The ATU was introduced into the plasmid backbone by site-directed mutagenesis between the MV P and M genes. Rescue of recombinant virus from the plasmid was performed using helper-cell-based rescue system cells.⁵¹

MV Edm B strain has been used in virotherapy and modified to express reporter proteins. MV-CEA expresses the soluble N-terminal domain of human carcinoembryonic antigen (CEA). CEA is a biological inert tumor marker and has only minimal immunogenicity. In a phase I clinical trial, MV-CEA has been administered by intraperitoneal route in 21 patients and no dose-limiting toxicity or treatment-induced immunosuppression was observed.²⁷ CEA expression allows non-invasive monitoring of viral gene expression but does not provide any information about the anatomic localization of virally infected cells and does not enhance the oncolytic effect of the viral vector. Therefore, MV has been modified to express another reporter protein, the sodium iodide symporter (NIS). The NIS protein is a membrane ion channel that is normally expressed in the thyroid, mammary glands, stomach and salivary tissue. Expression of NIS allows cells to actively transport ions into the cell. It may be used as therapeutic transgene capable of further increasing the oncolytic potency of MV-NIS by facilitating the intracellular entry of radioisotopes, which can cause direct radiation damage to tumor cells, thereby

Table 4. Preclinical studies using retargeted attenuated measles virus.

Target disease	Transgenic expressed proteins	Targeted receptor and cells	Administration route	Preclinical assay	Parental strain	Receptors used for viral entry	References
Multiple Myeloma	A disintegrin ML-28L echistatin (MV-ERV measles virus echistatin vector)	Integrin alpha(v)beta3 expressed on activated but not quiescent endothelial cells (targeting tumors and their associated blood vessels)	Intratumoral	mice	MV Edmonston B	CD150, CD46, integrin $\alpha v \beta 3$	96
Breast cancer	Amino-terminal fragment of the urokinase plasminogen activator (uPA) (of human or mice) targeting uPAR (MV-uPA).	Urokinase receptor (uPAR) on activated endothelial cells (tumor vascular targeting)	IV	mice	Edmonston vaccine lineage NSe strain ¹	uPAR, CD46, CD150	98
Non-Hodgkin's Lymphoma (NHL)	CD-20 targeted MV vector expressing the prodrug convertase purine nucleoside phosphorylase (PNP)	CD20 (B-cell specific antigen CD20)	Intracerebral administration	mice	Edmonston vaccine lineage NSe strain	CD20, (ablation of CD46 and CD150 interactions)	99
Glioblastoma multiforme (GBM)	Human IL-13, GFP	Interleukin-13-receptor $\alpha 2$ (IL-13R $\alpha 2$)	Intracerebral administration	mice	Edmonston vaccine lineage NSe strain	IL-13R $\alpha 2$, (ablation of CD46 and CD150 interactions)	100
Glioblastoma multiforme	a single chain antibody recognizing EGFR, GFP	Epidermal growth factor receptor (EGFR) or EGFRvIII (the most common EGFR mutant) (EGFR is a glioma-specific receptor)	Intracerebral administration	mice	Edmonston vaccine lineage NSe strain	EGFR or EGFRvIII, (ablation of CD46 and CD150 interactions)	101
Myeloma	Oncolytic MV displaying $\alpha v \beta 3$ integrin-binding peptides, cyclic arginine-glycine-aspartate (RGD) or echistatin (integrins) ⁴ recombinant MV displaying cyclic RGD or echistatin and GFP	Both viruses had expanded tropisms, and efficiently entered target cells via binding to integrinsNeovessel endothelial cells <i>in vivo</i>	iv	mice	cDNAs of the virulent IC-B strain isolated in a lymphoid cell line, B95a ²	CD46-CD150-EGFR	103
Ovarian cancer	MV- α FV: a single-chain antibody (scFv) specific for α -folate receptor	Folate receptor	Sc, ip	Mice	Edmonston vaccine lineage NSe strain	Folate receptor (ablation of CD46 and CD150 interactions)	104

¹The virus is derived from the Edmonston vaccine lineage NSe strain; it was rescued using the pseudoreceptor STAR (6 His tagging and retargeting) system propagated on Vero-HIS cells. The viral H protein contains the CD46-ablating mutation Y481A and the SLAM-ablating mutation R533A; it also contains the GFP which facilitates viral rescue and allows visualization of infection *in vitro* and *in vivo*.⁹⁷

²The rescue system uses Chinese hamster ovary cells expressing CD150 (CHO/hSLAM) and the recombinant vaccinia virus LO-T7-1 (the lister strain had been used as a vaccine in the WHO smallpox eradication programme).¹⁰²

enhancing the therapeutic efficacy (radiovirotherapy). In a phase I clinical trial, MV-NIS has been administered by intraperitoneal route in 16 patients and no dose-limiting toxicity was observed.²⁸ MV-CEA and MV-NIS have been achieved via the introduction of the transgene as additional transcription units upstream of the viral N gene for MV-CEA and downstream of the viral H gene between H and L gene for MV-NIS.²⁴

In preclinical studies, MV-NIS was administered intravenously in measles-naïve squirrel monkeys and measles-susceptible transgenic mice and no toxicity was observed.⁹⁴ There were no premature or unscheduled deaths during the study. No adverse clinical signs or meaningful effects on body weight or temperature were seen. No MV-NIS treatment-related effects were observed for any hematology, clinical chemistry, thyroid hormone, or cytokine parameter, but reticulocyte counts were transiently suppressed in the cyclophosphamide-treated animals. No treatment-related lesions were seen in any of the animals at the time of necropsy, and no histopathological changes were observed in any of the organs tested.⁹⁴ A phase I clinical trial was therefore initiated to determine the maximum tolerated dose of intravenously administered MV-NIS in patients with advanced refractory multiple myeloma.⁵⁶ Preliminary data on 2 patients from this study has been reported.²⁹ These patients who were seronegative for prior measles exposure received the highest feasible dose level (10^{11} TCID₅₀, 50% tissue culture infectious dose). They had secondary effects, they became febrile, tachycardic and hypotensive with severe nausea and vomiting. Toxicity resolved within the first week after therapy and was probably due to the high dose of recombinant virus administered combined with the absence of pre-existing immunity.

These inserts (F4, VLP, CEA and NIS) are currently being used in phase I/II clinical trials. Other inserts used in combination with oncolytic MV are tested in pre-clinical studies. For a review see Msaouel et al.²⁴ Their safety profile has still to be characterized, taking into account that some of the expressed gene product may have intrinsic hazardous properties such as toxic or allergenic properties. Gene products that may be considered as potentially hazardous in the particular context of gene therapy using viral vectors have been reviewed by Bergmans et al.⁹⁵ and Van den Akker et al.⁶¹ have also discussed inserts with unknown or novel characteristics produced by synthetic biology that could be considered as potentially hazardous. Finally, the hazard related to the transgene also depends on the conditions of use (dose, administration route, expression level of the transgene).

Some foreign genes could potentially lead to retargeting oncolytic MV vectors to other cells to increase specificity against tumor cells. MV vector can be retargeted to specific tumor cells by linking a single-chain antibody (single chain fragment variable, scFv) or naturally occurring ligand to the virus attachment H glycoprotein displayed on the virus surface. The ablation of receptor CD46 and CD150 binding sites of some vectors allows entry only into cells expressing the receptor for the scFv or ligand linked to H. A variety of scFv's have been displayed on H against different receptor (Table 4). Broadening viral tropism to molecules expressed on the luminal endothelial surface of tumor neovascularization may increase targeted viral delivery to tumor sites following

systemic administration.^{97,98,103} Retargeted attenuated MV Edm vector lack neurotoxic activity even when administered directly to the CNS of measles-naïve IFN type I receptor deficient (IFNAR^{KO}) CD46 Ge transgenic mice, a very sensitive model of viral neurotoxicity.⁹⁹⁻¹⁰¹ However, toxicity of the retargeted vector should be evaluated in non-human primates before initiation of a clinical study because transgenic mice do not express human receptors.¹⁰⁴

Finally, the recombinant MV shows an excellent stability of transgene expression. Various genes or combinations of genes were expressed stably in MV vectors for more than 12 passages.^{18,93}

Possibility of recombination with other viruses and genetic stability of MV strains

There has been no conclusive evidence to date of any genetic recombination events between MV vaccine and wt strains in people co-infected with both viruses.²⁴ Recombination, which is an efficient means for rapid genetic change for many viruses, does not occur in paramyxoviruses. No recombinant viruses have been isolated from natural infections. Unlike other RNA viruses such as influenza and HIV, MV vaccine strains demonstrate high genetic stability even after prolonged replication in human host.¹⁰⁵ Consequently, the probability of generating mutant strains is very low.^{18,93}

Influence of pre-existing immunity

Like all viral vectors, the MV vector efficacy in inducing a protecting immune answer could be affected by the pre-existing immunity among the human population.¹⁰⁶ However, numerous studies have shown that revaccinating already immunized individuals results in a boost of anti-MV antibodies, indicating that the live vaccine replicates despite pre-existing immunity.^{107,108} Likewise, the presence of maternal anti-MV antibodies has been shown to limit the induction of humoral but not CMI responses in immunized infants.¹⁰⁹ Moreover, vaccination of pre-immunized mice and primates with recombinant MV expressing HIV antigens has shown induction of anti-HIV env antibodies in the presence of anti-MV antibodies.²⁰ In another study, mice immunized with a recombinant MV-SARS vector developed SARS-specific immune responses in the presence of anti-MV immunity.¹¹⁰

The route of administration of the vector could also influence the immune response. The vaccine strain MVb and the MV vector developed from this vaccine strain could be administered by aerosol and it could therefore circumvent systemic MV pre-immunity more efficiently than parenteral administration.¹⁰⁶ Studies evaluating the safety of aerosolized measles vaccine did not identify severe side effects. However, aerosolized measles vaccine could trigger or exacerbate asthma in young children¹¹¹ as it was observed for intranasal administration of attenuated influenza vaccine.¹¹² Anti-measles immunity enhances the safety of oncolytic MV and has been a prerequisite in the majority of the MV virotherapy trials.²⁴ However, neutralizing antibodies may limit infection of tumor cells by MV. Indeed, cell-associated viruses are protected from antibody neutralization and have been tested to overcome antiviral

pre-existing immunity.¹¹³ Mesenchymal stem cells (MSC) transduced with MV-NIS were injected into ovarian tumor xenografts. The MV-NIS transduced MSC significantly extended the survival of measles immune tumor bearing mice in contrast to MV-NIS injected alone.¹¹³ Mesenchymal stem cells transduced with MV-NIS are currently being tested in a phase I/II clinical trial.⁵⁴

Risk classification of the recombinant vector

The risk classification of the recombinant MV vectors depends on the nature of the vector but has also to take into account any potential risk associated with the inserted gene(s). We recommend classifying MV1-F4 and MV-CHIK in class of risk 1. Indeed, the MV strain backbone derived from the attenuated measles vaccine strains, and the F4 protein and VLP have no known toxic or allergic effects when administered to humans.^{92,93}

The recombinant vectors MV-CEA and MV-NIS should also be classified in class of risk 1, based on the class of risk of the Edm B strain used as vector backbone. There is no reason to think that the presence of CEA and NIS could change the safety profile of the recombinant virus.

Hazard related to exposure pathways

An important step in the risk assessment of viral vectors is the evaluation of exposure pathways whereby personnel, non-patients and/or the environment may be exposed to recombinant MV administered to patients.

Biodistribution and shedding

Biodistribution is defined as the dispersion of the vector within the patient's body from the site of administration. Indeed, the presence of viral vectors in organs might be indicative of potential shedding which corresponds to the dissemination of viral vector in any form into the environment via excreta (urine, faeces, sweat, saliva, nasopharyngeal fluids), blood and semen from the treated patient.¹¹⁴ Biodistribution and shedding of MV vector depends on the dose, the route of administration and the MV strain used.

Wild-type MV strains use primarily the CD150 receptor expressed on lymphocytes, thymocytes, macrophages and mature DCs cells and Nectin-4 receptor expressed on epithelial cells for viral entry to host cells. Infection with wt MV has been thought to begin by infection of DCs in the respiratory tract followed by lymphoid organs.¹¹⁵ This phase of localized replication is followed by primary and secondary viremia, with viral spread to multiple epithelial tissues including the skin, kidneys, gastrointestinal tract, liver and the respiratory tract. Shedding of the virus occurs from the nasopharynx.³⁶ In natural infection, MV can also be isolated from urine up to 10 d after rash onset.¹¹⁶ MV RNA persists at multiple sites for many months after resolution of the rash and apparent recovery in a proportion of children. Experimental infection of rhesus macaques with MV showed that persistence of viral RNA in blood, respiratory tract, or lymph nodes is characteristic of primary MV infection.¹¹⁷

In addition to the receptors used by wt MV, attenuated MV strains can use the CD46 receptor for viral entry into host cells and were therefore expected to have a wider tropism than the wt MV strains. The biodistribution of attenuated MV (Edm B strain) was compared to that of wt MV in cynomolgus macaques experimentally infected via intratracheal or aerosol route.⁴⁸ Both viruses predominantly infected alveolar macrophages and DCs in the lungs. However, strikingly, only the wt MV caused viremia and was disseminated to lymphoid tissues, the respiratory submucosa, and the skin. Some mechanism must operate *in vivo* to suppress the growth of the virus capable of using CD46 as receptor.⁷ It is also important to take into account that the standard route of administration of live-attenuated MV vaccine, the subcutaneous route has not been evaluated in this study. Intramuscular vaccination of macaques with a MV Edmonston-Zagreb strain expressing GFP demonstrated that muscle cells were not infected and dendritic cells and macrophages were the predominantly the target cells.¹¹⁸

Published data on shedding of MV vaccine strains are limited. After Measles vaccination in humans, MV RNA could be detected in urine as late as 14 d post immunization¹¹⁹ and was rarely detected in throat or nasopharyngeal secretions. Morfin et al.¹²⁰ reported a case of Measles Schwarz virus isolation in throat swabs of a child, 12 d after vaccination and in a study realized until 2002 and 2006, 9 children presented a symptomatic post vaccinal excretion of MV in nasopharynx¹²¹ showing that subcutaneous injection of the attenuated MV Schwarz strain can result in respiratory excretion of this virus. However, person to person transmission of measles vaccine has never been reported.¹⁷

The biodistribution of the HIV-1 candidate vaccine MV1-F4 was similar to that of the parental MV Schwarz strain in monkeys immunized by intramuscular route, with both vaccines replicating preferentially in secondary lymphoid organs and epithelium-rich tissues.²⁰

To assess the potential shedding of the parental Schwarz vaccine strain and MV1-F4 vaccine candidate, excretions and body fluids of monkeys immunized with these vaccine strains were tested for the presence of MV viral sequences by RT-qPCR assay. Of the 8 time points at which shedding analysis was performed, MV viral RNA was only detected at day 11, the expected peak of viremia, in some biological fluids samples from few immunized monkeys. However, none of these samples contained infectious virus, indicating that no shedding of infectious virus particles was observed for either of the 2 vaccines.²⁰ The introduction of HIV F4 fusion protein did not alter the tropism of the parental strain or its shedding capacity.

No data are available regarding the biodistribution and shedding of MV-CHIK. However, no alteration in tropism is expected for the same vector containing the genes coding for structural proteins from chikungunya virus.

With regards to the 2 recombinant oncolytic MV vectors described before, the following data are available:

MV-CEA and MV-NIS: In order to maximize viral infection of the tumor cells, MV-CEA and MV-NIS were administered by intraperitoneal route to patients with recurrent ovarian cancer.^{21,27,28} In pre-clinical studies performed in measles naïve IFNAR^{KO} CD46 Ge mice, there was no evidence of viral replication outside the peritoneal cavity (i.e., the brain, heart and

skeletal muscle).¹²² In phase I clinical studies, 21 women were administered and low levels of MV-CEA genomes were found by RT-qPCR in peripheral blood mononuclear cells (PBMCs) of 4 asymptomatic patients.²⁷ Sixteen women received MV-NIS and no detection of viral genome in peripheral blood was observed.²⁸

Shedding of oncolytic MV has also been evaluated in pre-clinical and clinical studies. In phase I clinical studies, no viral RNA in urine or saliva was observed after intraperitoneal administration of MV-CEA or MV-NIS.^{27,28}

In one of the phase I clinical trial⁵⁶ listed in Table 2, MV-NIS is administered by intravenous route with or without pretreatment with cyclophosphamide. In addition to its chemotherapeutic activity, cyclophosphamide is an immunosuppressive agent. Therefore, it can potentially prolong and enhance viral dissemination and replication in the tumor.¹²³ To evaluate the biodistribution of intravenous MV-NIS administration, IFNAR^{KO} CD46 Ge mice pre-treated with cyclophosphamide demonstrated alterations in the biodistribution of MV-NIS-infected cells and the kinetics of MV-NIS elimination.⁹⁴ Virus was sporadically detected by RT-qPCR in the brains of cyclophosphamide-pretreated mice whereas those not treated with cyclophosphamide remained negative.

In two patients with advanced refractory multiple myeloma treated with intravenous infusion of MV-NIS, radioiodine SPECT-CT scan provided clear evidence of tumor-targeted MV-NIS infection and propagation of the MV-NIS infection in tumor cells. There was no evidence for spread of MV-NIS from the tumor to adjacent normal tissues.²⁹

Shedding of MV-NIS administered by intravenous route alone or with pretreatment with cyclophosphamide has been evaluated by RT-qPCR.¹²³ Viral RNA was detected in buccal swabs of monkeys after intravenous administration of MV-NIS.⁹⁴ A higher level of virus RNA was detected in the buccal swabs in the cyclophosphamide treated monkey and viral RNA remained detectable for a longer period of time indicating that this drug enhance the propagation of the vector in animals.⁹⁴

Measles virus transcripts were still detectable in the circulating cells of one patient at 6 weeks after intravenous infusion of MV-NIS.²⁹

During the course of a clinical trial, the possible consequences of leakage of the vector outside the patient's body may include, for example, adverse effects associated with the infection of personnel or people in general coming into contact with the vaccinated individuals. However, it is important to take into account that most individuals in industrialized countries are immune to the WT measles virus as a result of natural infection or vaccination. Moreover, as mentioned before, measles virus is very rapidly degraded in the environment. The consequences of dissemination will also depend on the characteristics of the recombinant vector itself, in particular the safety profile of the transgene which have been shown to be safe.

Direct exposure

Some manipulations might lead to exposure to the recombinant MV vector, e.g. the preparation of the viral vector, its administration to the patient and waste disposal. Direct exposure may

also result from accidental inoculation of personnel during the treatment of the patient via droplets or aerosols contacting mucous membrane, non-intact skin or eyes. The risk of piercing by needle or injury due to sharps, cutting with broken vials is the greatest for the personnel handling vials and syringes. Medical and paramedical staff and the people visiting the treated patient could also be exposed to contaminated material, waste material or spoiled surfaces. These exposure pathways can be reduced or eliminated by application of appropriate risk management strategies.

Considerations for risk management (containment, workers protection measures, waste treatment)

When attenuated MV recombinant vectors are used in the clinical setting (under contained conditions), appropriate containment and other measures to protect human health and the environment should be implemented as a result of a risk assessment taking into account the characteristics of the biological agent manipulated, the nature of the transgene and the nature of the activity. Four containment levels (CL-1 to CL-4, CL-4 being the most stringent) are defined in the EU legislation and consist in a combination of technical characteristics of the facility, safety equipment, laboratory practices and operational procedures such as waste management procedures.³⁰

The attenuation in MV1-F4 and MV-CHIK vaccine candidates and the history of safe use of MV Schwarz strain allows handling this recombinant virus under CL-1 in the clinical setting. As mentioned before the addition of the transgenes does not change its safety profile.

A CL-1 should also be recommended for handling MV-CEA and MV-NIS oncolytic vectors.

Transduction of MSC cells by recombinant MV-NIS should be carried out in CL-2. When manipulating mesenchymal stem cells which are primary cell cultures obtained directly from organs or tissues,¹¹³ the presence of adventitious contaminating agents constitutes the main hazard. As they are characterized by a finite life span, the time available for characterization and detection of contaminating agents remains limited. Therefore, primary cell cultures derived from human must be transduced in a CL-2 and manipulated in a class II biosafety cabinet guaranteeing the product sterility and personnel should wear gloves.

To prevent or manage risks associated with dissemination of recombinant MV vectors into the environment during viral vector administration, application of specific work practices should be applied and personnel should wear personal protective equipment to prevent or manage risks:

Operations producing aerosols such as the puncture of a vial should be limited or strictly contained during preparation and administration of the recombinant MV vector. Consequently, the preparation of the GM vector should preferably be performed in a class II biosafety cabinet. Personnel administering the vector should wear adequate protective clothing such as lab coats, gloves, goggles and masks. Work with needles and other sharp objects should be strictly limited and workers should never recap nor remove needles from syringes.

Spills should be inactivated by an appropriate disinfectant (e.g., 1% sodium hypochlorite extemporaneously prepared), allowing sufficient contact time before disposal.

Contaminated waste and personal protective equipment should be inactivated using an appropriate method (autoclave or incineration) before disposal and potentially contaminated non-disposable materials (e.g., the material used for the transport, preparation or administration of the GM vector, instruments, surfaces) need to be properly decontaminated.

If an incident occurs that could lead to infection (e.g., breakage of a vial containing the vector, piercing with a needle), applicable first aid should be performed (i.e., flushing eyes for ocular exposure, placing an absorbent tissue on the affected area in order to absorb viral particles and apply disinfectant directly to the tissue and after removing this tissue washing the skin thoroughly), followed by reporting to the supervisor.

Personnel manipulating the recombinant vector and persons in close contact with the treated patient should be vaccinated against measles.

After injection of a recombinant MV, the treated patient should avoid contacts with immunosuppressed individuals or individuals lacking protective immunity for a minimum of 8 d or until saliva, urine and blood PBMC test negative for the virus (whichever is longer). As the number of patients treated with different MV strains for specific clinical indications increases, and clinical information regarding the likelihood and duration of shedding accumulates, biosafety guidelines are expected to evolve.

As MV is a human virus with no known animal reservoir, there is no risk for animals and plants.

Conclusion

Attenuated MV is one of the most effective and safe human vaccine in use providing long-lasting protection. Attenuated MV is now widely used as recombinant vector for vaccination against various infectious diseases or cancers and for virotherapy. Measles virus has a cytoplasmic replication cycle eliminating the possibility of integration into the host cells DNA avoiding a possible insertional mutagenesis. Furthermore, the MV genome is very stable and MV vectors have been shown to stably express large sizes of foreign inserted genetic sequences. We have presented and discussed in this paper several biosafety issues which need to be considered carefully when performing clinical trials with recombinant attenuated MV vectors. After having performed a comprehensive risk assessment of the MV vectors currently used in clinical trials as prophylactic vaccine or oncolytic vector we can conclude that these MV vectors belong to class of risk 1 for humans. In order to maximize risk mitigation and avoid dissemination into the environment, these MV vectors should be manipulated under containment level 1.

Note

- a. Any activity in which micro-organisms are genetically modified or in which GMM pathogenic or not are used and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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