Investigator's brochure

A multi-center, randomized controlled phase Ib/IIa trial to assess the safety, tolerability and immunogenicity of the candidate vaccine MVA-SARS-2-ST in adults	
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Confidentiality statement

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Abbreviations	
2019-nCoV	2019 novel coronavirus, now SARS-CoV-2
ACE2	Angiotensin-converting enzyme 2
ADE	Antibody-dependent enhancement
AE	Adverse event
APC	Antigen presenting cell
AVV	Adeno-associated virus
BSL	Biosafety level
CVA	Chorioallantois Vaccinia virus Ankara
CEF	Chicken embryonic fibroblasts
CEPI	Coalition of Epidemic Preparedness Innovations
CFR	Case fatality rate
CoA	Certificate of Analysis
CoC	Certificate of Compliance
COVID-19	Coronavirus disease 2019
CTL	Cytotoxic T lymphocyte
CTM	Clinical trial material
CTU	Clinical trial unit
DC	Dendritic cell
DLT	Dose-limiting toxicity
dpc	Day post challenge
DPP4	Dipeptidyl peptidase 4
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immune absorbent spot
EMA	European Medicines Agency
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FHD	Full human dose
GLP	Good laboratory practice
GMO	Genetically modified organism
GMP	Good manufacturing practice
HA-tag	Human influenza hemagglutinin tag
HD	High dose
HIV	Human immunodeficiency virus
ICS	Intracellular cytokine staining
i.d.	Intradermal
IFN-γ	Interferon gamma
IFR	Infection fatality ratio
IGS	Incoming Goods Specification
i.m.	Intramuscular
i.n.	Intranasal
i.t.	Intratracheal
IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
IU	Infectious Units
LD	Low dose
LMU	Ludwig-Maximilians-Universität München
MERS	Middle East respiratory syndrome
MERS-CoV	MERS-related coronavirus
MERS-S	MERS glycoprotein or spike (S) protein

MOI	Multiplicity of infection
(r)MVA	(Recombinant) Modified Vaccinia virus Ankara
MVS	Master virus seed
MVA-BN®	Modified Vaccinia virus Ankara Bavarian Nordic®
N/A	Not available
NOAEL	No observed adverse effect level
NSAID	Non-steroidal anti-inflammatory drug
p.b.	Prime-boost
PBS	Phosphate buffered saline
NZWR	New Zealand white rabbit
PFU	Plaque forming units
PRNT80	Plaque reduction neutralization test
RSV	Research seed virus
SAE	Severe adverse event
SARS	Severe acute respiratory syndrome
SARS-CoV(-2)	Severe acute respiratory syndrome coronavirus (2)
s.c.	Subcutaneous
TCID50	Median tissue culture infective dose
SFC	Spot-forming cells
TNF-α	Tumor necrosis factor-α
VNT	Virus neutralization titer
WHO	World Health Organization
Wk	Week

Summary of Changes

Summary of changes since last version of protocol (IB version 2.0 to version 3.0)		
Amendment Number	**Date of Amendment**	**Section Affected by Change**
02	30APR2021	Whole document
Brief description of change: Harmonization of titer and unit notations.		
02	30APR2021	Cover page
Brief description of change: Update of study information.		
02	30APR2021	Summary
Brief description of change: Update of available information about SARS-2, COVID-19 and the new vaccine candidate MVA-SARS-2-ST.		
02	30APR2021	Section 2.2 and Section 2.3
Brief description of change: Update of COVID-19 case numbers and newly available treatments and vaccines.		
02	30APR2021	Section 2.4
Brief description of change: Addition of information regarding the new vaccine candidate MVA-SARS-2-ST. Integration of available information on clinical trial with MVA-SARS-2-S.		
02	30APR2021	Section 3
Brief description of change: Addition of details regarding MVA-SARS-2-ST characterization and production.		
02	30APR2021	Section 4.1
Brief description of change: Addition of information on MVA-SARS-2-ST and MVA-SARS-2-S preclinical data.		
02	30APR2021	Section 4.3
Brief description of change: Addition of information on MVA-SARS-2-S clinical data and thereof resulting plans on MVA-SARS-2-ST toxicology studies.		
02	30APR2021	Section 5.3
Brief description of change: Addition of clinical data on safety, tolerability and immunogenicity of MVA-SARS-2-S.		
02	30APR2021	Section 6

Brief description of change:
Correction and update of risk analysis.

1 Summary

We herein propose a Phase Ib/IIa clinical trial to determine the safety, tolerability and immunogenicity of two ascending doses MVA-SARS-2-ST in human volunteers in stable health condition. MVA-SARS-2-ST is an investigational vaccine aimed at preventing COVID-19 in risk groups. The causative agent SARS-CoV-2 first emerged in December 2019 in Wuhan, China and quickly spread over the whole world with currently more than 114 million confirmed cases of primary infection leading to more than 2.5 million deaths in at least 223 countries (https://www.who.int/emergencies/diseases/novel-coronavirus-2019; accessed on 2021-03-02). The Word Health Organization (WHO) declared the outbreak a "Public Health Emergency of International Concern" on 30st of January 2020 and only 41 days later, on 11th of March, a pandemic.

COVID-19 presents as a respiratory disease, ranging from asymptomatic to severe pneumonia with acute respiratory distress syndrome, multi-organ failure and septic shock potentially leading to death. SARS-CoV-2 is easily transmitted by human-to-human contact particularly via exposure to infectious droplets. The virus is thought to be of zoonotic origin due to its high genetic similarity to bat coronaviruses.

MVA-SARS-2-ST represents a replication-deficient vector vaccine built on the recombinant Modified Vaccinia virus Ankara (MVA) platform. MVA-SARS-2-ST expresses a prefusion stabilized version of the full-length spike (S) glycoprotein of SARS-CoV-2, an antigen functionally implicated in virus cell entry and the target of naturally occurring neutralizing antibodies to coronaviruses. Multiple lines of evidence support the choice of the S glycoprotein as vaccine target as described in detail below. The MVA vector platform, due to its replication-deficiency, is considered a safe, well tolerated and immunogenic vaccine platform capable of inducing a multimodal humoral and cell-based immunological antigen response. MVA in its dozens of different clinical stage explorations in thousands of patients has never been associated with vaccine-related serious adverse events. The licensed IMVAMUNE®/IMVANEX® smallpox vaccine by Bavarian Nordic is also an MVA-based product line.

The MVA-SARS-2-ST study medication is obtained from GMP-compliant manufacturing of drug substance and drug product by IDT Biologika GmbH in Dessau (Germany), a renowned manufacturer and quality leader of human vaccines. The study medication was subjected to extensive quality analyses and stability testing at the level of final vial and met stringent quality specifications as described in great detail in the corresponding IMPD.

Preclinical testing was done in mice using up-to full-human-dose equivalents (1x 10^8 PFU) in a prime-boost vaccination scheme. MVA-SARS-2-ST was well tolerated and led to the production of S antigen-specific CD8+ T cells and neutralizing antibodies. Preclinical and clinical testing of the predecessor vaccine candidate MVA-SARS-2-S harboring the native SARS-CoV-2 S protein demonstrated its safety and tolerability.

Owing to its replication-deficiency, MVA-SARS-2-ST, even as a recombinant virus, can be handled under BSL1 laboratory conditions. With minor precautions regarding the intramuscular administration of MVA-SARS-2-ST to study participants and the dressing of the administration site, a safe vaccine profile can be expected to emerge from this work. Mild to moderate reactogenicity, both local and systemic, is expected to be reported by a number of study participants.

In the light of the current global COVID-19 pandemic caused by SARS-CoV-2 and the lack of sufficient treatment or vaccination possibilities, the present trial can be justified on grounds of expected clinical benefit exceeding the risk of exposure.

2 Introduction

2.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

The coronavirus family consists of large positive-stranded RNA viruses that generally cause mild cold-like symptoms in humans. Human coronaviruses were first identified in the mid-1960s and belong to the alpha and beta coronavirus genera. Four coronaviruses are known to induce a mild disease: 229E, NL63, OC43 and HKU1.[1] Humans around the world frequently get infected with these common human coronaviruses. But the emergence of zoonotic viruses like the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2002, the Middle East Respiratory Coronavirus (MERS-CoV) in 2012 and the novel SARS-CoV-2 (formerly 2019-nCoV) in 2019 demonstrates the pathogenic potential of the *Betacoronavirus* genus to humans.

SARS-CoV-2 has been identified in January 2020 following a series of severe viral pneumonia cases of unknown origin reported in Wuhan, China. The first observed case of a SARS-CoV-2 infection was reported on 31st December 2019 presenting symptoms of an atypical pneumonia. The virus is believed to be of zoonotic origin, although the original animal source causing the spill over into human population has not been identified yet. Due to its high infectivity even in asymptotic patients, SARS-CoV-2 quickly spread worldwide, mostly by traveling.

Phylogenetic analysis revealed a close relationship between SARS-CoV and the novel coronavirus which was therefore named SARS-CoV-2.[2] Both viruses use the same cell entry receptor, the angiotensin-converting enzyme 2 (ACE2).[3] The spike (s) surface glycoprotein mediates viral cell entry by binding to ACE2 exposed on the cell surface.[4] Further whole genome analysis showed a close similarity to bat coronaviruses.[5]

2.2 Coronavirus Disease 2019 (COVID-19)

SARS-CoV-2 is the cause of the coronavirus disease 2019 (COVID-19), which is an infectious respiratory disease. The most common symptoms are fever, dry cough and fatigue. Other, less common symptoms include loss of taste and/or smell, nasal congestion, conjunctivitis, sore throat, headache, muscle or joint pain, different types of skin rash, nausea or vomiting, diarrhea, chills or dizziness. Symptoms of severe COVID-19 disease are shortness of breath, loss of appetite, confusion, persistent pain or pressure in the chest and high temperature. While most cases are either asymptomatic or come along with only mild symptoms, a severe form of pneumonia including acute respiratory distress syndrome, multi-organ failure, septic shock and blood clots can occur that can lead to death.[6] Due to the high number of asymptotic or mild COVID-19 cases, it is expected that many cases are never clinically verified, especially in countries with low surveillance rate. It is therefore currently not possible to reliably measure or calculate the case fatality rate (CFR) or infection fatality ratio (IFR) of COVID-19. Early claims which stated that COVID-19 has a significantly lower CFR compared to SARS and MERS need to be reconsidered as it is highly likely that the percentage of unidentified SARS and MERS cases is similar to what we can currently observe during the ongoing pandemic. However, precisely these observations support a rather low fatality rate. In general, it has been proven that the CFR increases significantly at higher ages and in combination with certain underlying medical conditions such as high blood pressure, heart and lung problems, diabetes, obesity or cancer.[7]

The incubation period lasts for around five days, although ranges from two to fourteen days have been reported.[8] SARS-CoV-2 is easily transmitted between humans and infections mainly occur during close contact through the exposure to infective respiratory droplets produced by coughing, sneezing and talking or, less frequently, through contact with contaminated surfaces. Although people are most infectious when showing symptoms, they may already be infectious two days before the first symptoms appear and can stay infectious for two weeks or longer in severe cases.[a] Even asymptotically infected people are able to shed infectious virus particles, which greatly hampers efforts to stop the spreading of COVID-19.[9]

The World Health Organization (WHO) declared the COVID-19 outbreak a "Public Health Emergency of International Concern" on 30st of January 2020 and, since 11th of March 2020, classifies the situation as pandemic. As of March 2nd 2021, 114,140,104 confirmed cases and 2,535,520 deaths have been reported, affecting at least 223 countries, areas or territories.[b] Hesitant travel and social restrictions, limited testing capacities and the lack of treatments or vaccines against the newly emerged virus promoted the initial inexorable spreading of SARS-CoV-2 around the globe (see Figure 1).

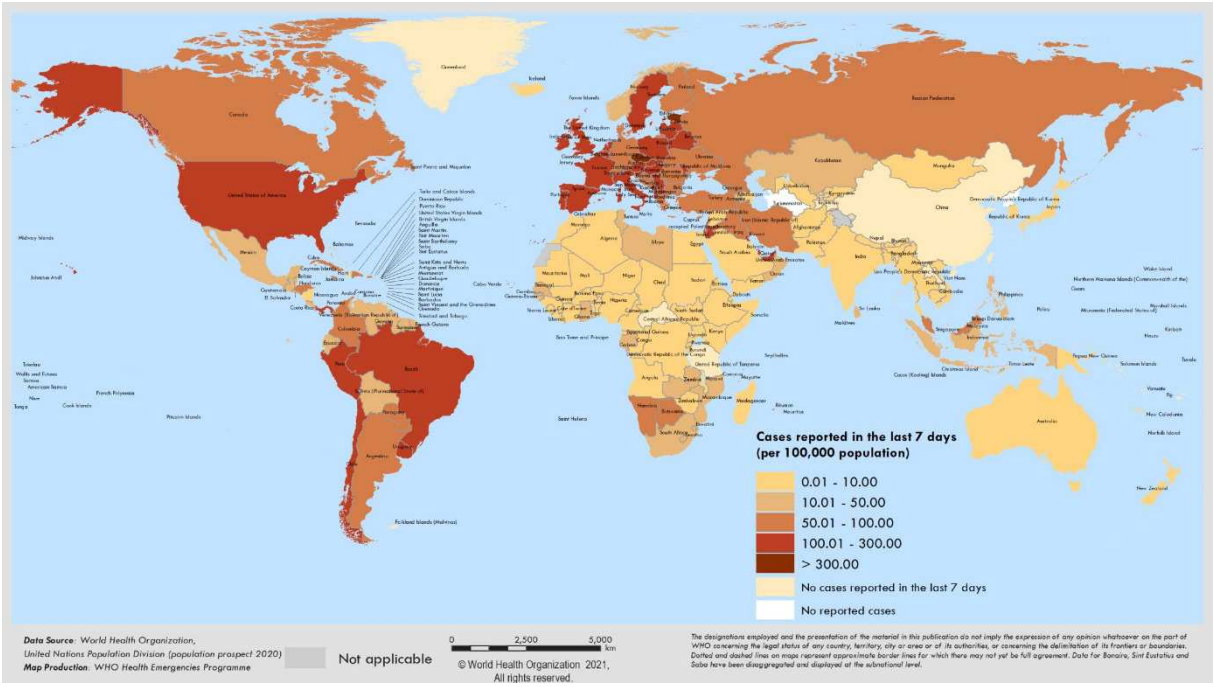

Figure 1: COVID-19 cases per 100 000 population reported in the last seven days by countries, territories and areas, 15 February through 21 February 2021. (https://www.who.int/publications/m/item/weekly-epidemiological-update---23-february-2021).

Consequently, the pandemic led to a global collapse of economy and already caused the largest global recession since the Great Depression in the 1930s. In large parts of the world, social life was or still is strictly regulated, which additionally affects the health of millions of people. As new and recurring outbreaks continue to be reported worldwide (see Figure 2), the current hunt for COVID-19 treatments and vaccines is of uttermost importance.

[a] https://www.ecdc.europa.eu/en/covid-19/questions-answers accessed on 2020-06-22
[b] https://www.who.int/emergencies/diseases/novel-coronavirus-2019 accessed on 2021-03-02

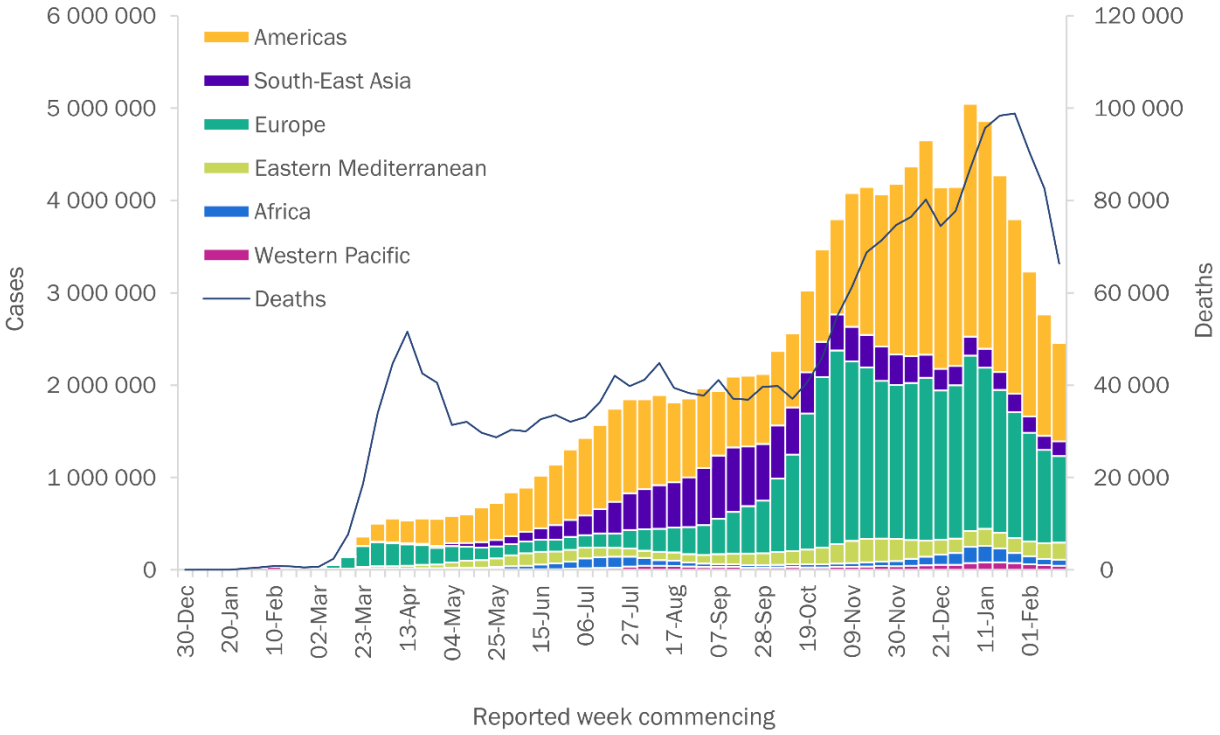

Figure 2: COVID-19 cases reported weekly by WHO Region, and global deaths, as of 21 February 2021. (https://www.who.int/publications/m/item/weekly-epidemiological-update---23-february-2021).

2.3 Treatment Strategies and Vaccine Candidates

Giving the extremely heavy impact the SARS-CoV-2 outbreak continuous to have on the whole world, a so far unprecedented research effort has started, uniting private and public research facilities and biotechnology and pharmaceutical companies. Due to the short time frame from the first description of the newly emerging disease until today, only a hand full of repurposed therapeutics have been approved to treat COVID-19: dexamethasone in the UK and Japan; Avigan (favilavir) in China, Italy and

Russia; and Veklury (remdesivir) in the US, UK and Japan. Emergency use authorizations have been issued to therapeutics using monoclonal antibodies and covalescent plasma. However, until now the mainstay interventions remain supportive therapy and contact isolation.

The WHO recommends the following preventive measures for the general public to minimize the risk of infection:

- Regular hand cleaning with alcohol-based hand rubs or washing with soap and water
- Maintaining at least 1 meter distance between yourself and others
- Wear a face mask when being around other people
- Avoiding crowded or indoor setting
- Meet other people outside whenever possible
- Avoid touching eyes, nose and mouth
- Covering mouth and nose when coughing or sneezing
- Clean and disinfect surfaces frequently especially those which are regularly touched
- Self-isolation in case of minor symptoms like cough, headache and mild fever

Enormous financial and scientific efforts have been initiated on international scale to find exploratory treatments and vaccine candidates. Organizations like the WHO, the Coalition of Epidemic Preparedness Innovations (CEPI) and the Bill & Melinda Gates Foundation as well as numerous government-funded programs have allocated huge funds to support various investigations. Here we want to highlight a few of these but in addition refer to eight review articles that provide further details of the current work.[10-17] Besides searching for so far unknown compounds that can be developed into therapeutic drugs to alleviate the severity of a COVID-19 infection, medicines already licensed in a different context (so-called repurposing) are tested for beneficial activity against SARS-CoV-2. If successful, this approach has the potential to make treatment options available to a large number of patients relatively quickly as challenges regarding regulatory processes and (large-scale) production are considerably smaller. By June 2020, over 300 potential COVID-19 therapies were in the state of preclinical or clinical testing.[12]

By March 2021, the WHO counted 258 COVID-19 candidate vaccines, with 76 candidates in clinical trials, including the work at DZIF with the Modified Vaccinia Virus Ankara (MVA) platform of the TTU Emerging Infections.[c] The ”Verband der forschenden Arzneimittelhersteller” (vfa) lists a dozen additional attempts at developing a prophylactic vaccine against SARS-CoV-2. Table 1 lists all SARS-CoV-2 vaccine candidates approved by the European Medicines Agency (EMA) until end of February 2021.

Table 1: SARS-CoV-2 vaccines approved by EMA until end of February 2021

Vaccine	Platform	Institution
Comirnaty (BNT162b2)	modified RNA and self-amplifying RNA in lipid nanoparticle	BioNTech, Pfizer, Fosun Pharma, Germany
COVID-19 Vaccine Moderna (mRNA-1273)	mRNA encoding SARS-CoV-2 spike protein in lipid nanoparticle	Moderna Inc, NIAD, USA

[c] https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

Vaccine	Platform	Institution
COVID-19 Vaccine AstraZeneca (ChAdOx-1-S)	Chimpanzee adenovirus viral vector expressing SARS-CoV-2 spike protein	University of Oxford, AstraZeneca, UK

2.4 MVA-SARS-2-ST: A Viral Vector Vaccine Candidate against COVID-19

Attenuated vaccinia viruses like the Modified Vaccinia virus Ankara (MVA) have been extensively used in the last two decades as platforms to generate recombinant vaccine candidates. MVA is a replication-deficient viral vector that holds great promise as a safe and efficacious vaccine platform. It can be engineered to encode one or more foreign antigens and thus function as a multivalent vaccine, eliciting cell-based and humoral immune responses.[18] The vector can be used at biosafety level 1 and has intrinsic adjuvant properties.

MVA was originally derived from Chorioallantois Vaccinia virus Ankara (CVA) through serial passaging in chicken embryo fibroblasts (CEF).[19,20] Between 1968–1985, the Bavarian State Vaccine Institute produced MVA as a human smallpox vaccine. The application of this MVA vaccine successfully increased the safety of the conventional smallpox vaccination as documented by the absence of any SAE in large field trials involving more than 120,000 individuals in Germany.[20,21]

In 2013, the Journal *Vaccine* used one of their Editorials to focus on poxvirus vectors and comprehensively summarized the experiences by using MVA as a vaccine platform during the last two decades.[22] They described that more than 35 publications reported results of clinical trials using MVA vectors for viruses, parasites or malaria. MVA produces the heterologous antigen intracellularly and has strong immunostimulatory capacities especially in targeting the innate immune system, and thereby possesses two functions: it produces an authentically processed (foreign) antigen within the infected cell and functions as an adjuvant supporting efficient antigen presentation to the adaptive immune system. In consequence, recombinant MVA vaccines elicit both antigen-specific humoral (e.g. circulating antigen-binding and virus-neutralizing antibodies) and cellular immune responses (CD8+ T cells, CD4+ T cells, Th1 type-like immunity). In the past, no single serious adverse event (SAE) has been reported when MVA was used as a vaccine candidate. All studies that tested MVA as a vaccine candidate demonstrated a favorable safety and immunogenicity profile and even immunization in immunocompromised individuals did not show any severe or serious adverse events.[23]

We herein have taken the approach to deliver the SARS-CoV-2 S glycoprotein gene by recombinant MVA as a way of active immunization against SARS-2. The construction of MVA-SARS-2-ST was done at the Ludwig-Maximilians-Universität (LMU) in Munich, Germany by the team of Prof. Dr. Gerd Sutter.

The investigational medicinal product (IMP) is obtained from IDT Biologika GmbH in Dessau, Germany. IDT is a renowned manufacturer and quality leader for the GMP-compliant manufacturing of human vaccines. The study medication is derived from the spontaneously immortalized avian cell line DF-1. It was subjected to extensive quality analyses and stability testing at the level of the final vial and met stringent quality specifications as described in detail in the corresponding IMPD.

In summary, the MVA-SARS-2-ST candidate vaccine demonstrated robust synthesis of a prefusion stabilized version of the S antigen in cells of mammalian origin, appropriate growth characteristics in cell cultures, and genetic stability upon serial amplification in DF-1 cell substrate. The vaccine was tested in mice using intramuscular applications of MVA-SARS-2-S up-to full-human-dose equivalents (1x 10^8 PFU). The prime-boost vaccinations were tolerated without any signs of disease (body weight monitoring, clinical score). At the end of the study after macroscopic and histopathological examination, there were no findings supporting any systemic effect related to MVA-SARS-2-ST administration in prime or prime-boost vaccinated mice. Lesions at the administration sites and draining lymph nodes were interpreted as treatment-related. All vaccinated mice produced S antigen-specific CD8+ T cells, serum antibodies binding to S glycoprotein and neutralizing SARS-CoV-2 in tissue culture infections.

A phase I clinical trial assessing the safety, tolerability and immunogenicity of two ascending doses of the predecessor candidate vaccine MVA-SARS-2-S did demonstrate the safety and tolerability of the vaccine. However, immunogenicity results were disappointing, as the majority of individuals in both dose cohorts did not seroconvert after vaccination.

The development of MVA-SARS-2-ST is firmly based on MVA-SARS-2-S which was inspired by another promising MVA based coronavirus vaccine candidate. MVA-MERS-S is carrying the S glycoprotein gene of the Middle East Respiratory Syndrome coronavirus MERS-CoV. The vaccine was developed and published in 2013 by a group of German and Dutch researchers and is also produced by IDT Biologika GmbH.[24] Non-clinical studies in rodents and dromedary camels demonstrated safety, immunogenicity and protection against challenge infections.[24-26] The 1st generation vaccine MVA-MERS-S produced on CEF cells was tested in a phase Ia study between 2017-2019. The trial was an open, single-center phase I trial to assess the safety, tolerability and immunogenicity of two ascending doses (PI: Prof. Marylyn M. Addo, EudraCT No. 2014-003195-23, ClinicalTrials.gov: NCT03615911). MVA-MERS-S was administered in a prime-boost regimen in 23 healthy volunteers using a 28 day interval. The data revealed a beneficial safety and immunogenicity profile. Vaccinees experienced no single serious adverse event.[27] Total antibodies induced and virus neutralizing capacity were correlated after boost vaccination. The RBD of MERS-CoV S turned out to be a particularly rich source of T cell epitopes. A phase Ib study with the 2nd generation vaccine produced on DF-1 cells is planned to start end of December 2020 including 145 study participants in Germany and the Netherlands (PIs: Prof. Marylyn M. Addo (DE) and Prof. Dr. Eric van Gorp (NL), EudraCT No. 2019-000715-83, ClinicalTrials.gov: NCT04119440). The auspicious results obtained for MVA-MERS-S so far and its close homology to MVA-SARS-2-S(T) are strongly encouraging us to expand our work onto MVA-SARS-2-ST in search for a safe and effective SARS-CoV-2 vaccine. Given the fact that by now, the majority of the clinically successful vaccine candidates is based on a stabilized version of the S protein to elicit an optimal immunogenicity and taking the preclinical data for MVA-SARS-2-S and MVA-SARS-2-ST into account, we are now highly optimistic upon exploring the potential of our optimized COVID-19 vaccine candidate.

3 Physical, chemical, pharmaceutical properties and formulation

The single active drug substance of MVA-SARS-2-ST was developed in 2020/2021 by a group of German researchers at the Ludwig-Maximilians-University (LMU) in Munich, Germany. The drug substance is engineered on the recombinant Modified Vaccinia virus Ankara (rMVA) platform, details of which we briefly review as follows.

Modified Vaccinia virus Ankara (MVA) was originally derived from Chorioallantois Vaccinia virus Ankara (CVA) through serial passaging in chicken embryo fibroblasts (CEF).[19,20] In the period 1968–1985, the Bavarian State Vaccine Institute produced MVA as a human smallpox vaccine. The application of this MVA vaccine successfully increased the safety of the conventional smallpox vaccination as documented by the absence of any serious adverse event in large field trials involving more than 120,000 individuals in Germany.[20,21]

The serial passage of MVA in CEF cultures resulted in major deletions in the viral genome and many mutations that affect known vaccinia virus virulence and immune evasion factors.[28,29] Consequently, MVA replication is highly restricted to avian cells and the virus is unable to produce infectious progeny in most cells of mammalian origin.[18,30,31]

Analysis of the host cell restriction phenotype of MVA demonstrated a late block in the assembly of viral particles in non-permissive cells. This implies an unimpaired synthesis of viral early, intermediate and abundant late gene products, which supported its development as safe and particularly effective viral vector.[18] Moreover, the biological safety and replication deficiency of MVA has been confirmed in various *in vivo* models, including avian species and animals with severe immunodeficiencies.[32-35] For this reason, rMVA viruses as genetically modified organisms (GMO) have been categorized as biosafety level 1 (BSL1) in most countries, provided that innocuous heterologous gene sequences are expressed.

We therefore summarize the specific advantages afforded by the rMVA vaccine platform as follows:

- unimpaired synthesis of MVA gene products
- capable of foreign antigen expression
- produces native conformation antigens
- BSL1 GMO derivatives
- elicits multimodal immune responses
- inherent immunostimulating (adjuvans) properties
- excellent safety profile due to replication deficiency

The vaccine is engineered on the above platform to comprise the following features:

- The cDNA SARS-2-S containing the entire gene sequence for SARS-CoV-2 S glycoprotein (based on GenBank ID MN908947.1 CDS 21579…25400) was obtained by DNA synthesis, including removal of termination signals for vaccinia virus early transcription by silent gene mutation and the exchange of five amino acids by point mutation (R682G, R683S, R685S, K986P, V987P) to stabilize the protein in the prefusion conformation
- SARS-2-ST was cloned under transcriptional control of the vaccinia virus early/late promoter PmH5 and introduced by homologous recombination into the existing deletion site III of the MVA genome
- Clonal isolate MVA F6 has been used as stock virus as before[37]

- MVA-SARS-2-ST was found to be genetically stable and to replicate efficiently in DF-1 cells but not in human HeLa, HaCat or A549 cells
- the resulting MVA-SARS-2-ST can be handled under BSL1

The scheme in Figure 3 depicts the generation of MVA-SARS-2-ST and the identity testing by PCR analysis of genomic viral RNA and cell replication. For a detailed insight into the general construction procedure please refer to Song F. et al., 2013 describing MVA-MERS-S, a homologous vaccine candidate for MERS.

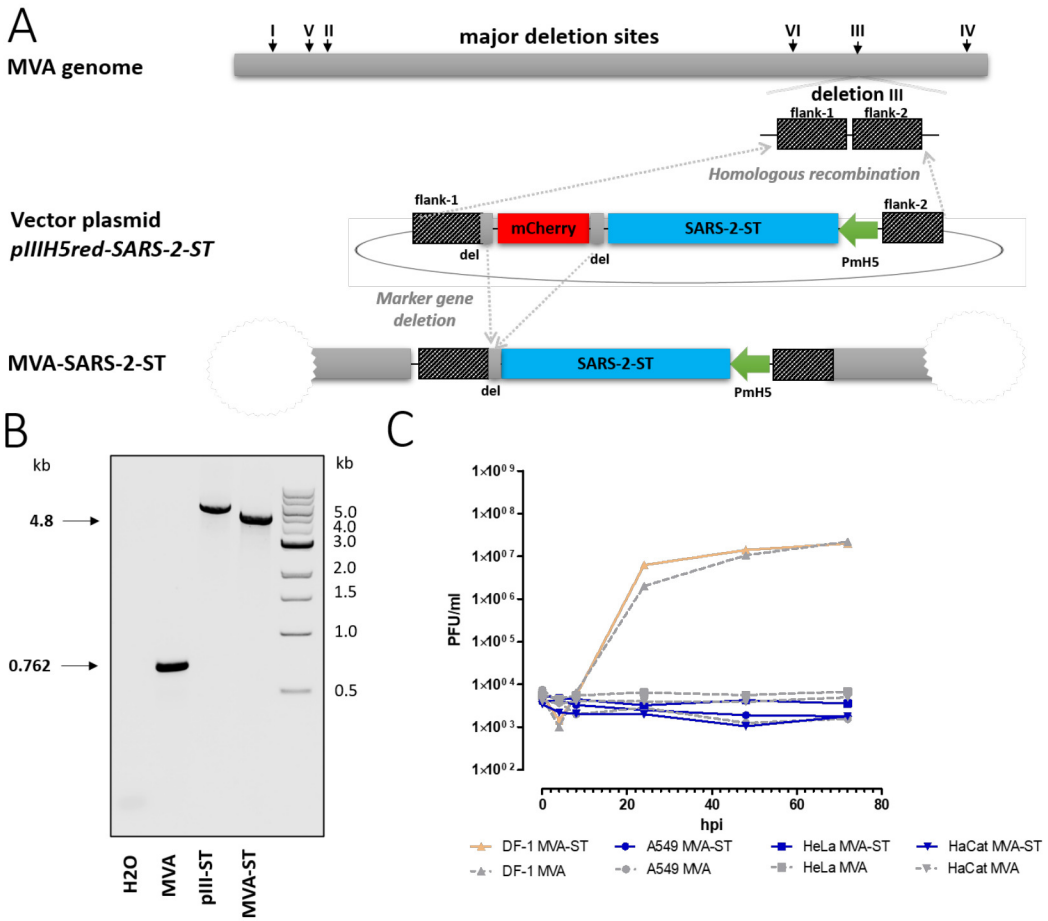

Figure 3: Schematic drawing of the construction process, identity testing and cell replication of MVA-SARS-2-ST. A, vector design and construction process. B, Genetic integrity of MVA-SARS-2-ST (MVA-ST) verified by PCR analysis. The precise intragenomic deletion of the marker gene mCherry during plaque purification is revealed by amplification of an about 1 kb smaller PCR product compared *pIIIH5red-SARS-2-ST* (pIII-S). C, Multiple-step growth analysis: MVA and MVA-SARS-2-ST (MVA-ST) can efficiently be amplified on DF-1 but failed to productively grow in cells of human origin (cell lines HaCat, HeLa and A549). Cells were infected at a MOI of 0.05 with MVA or MVA-ST and collected at the indicated time points.

Figure 4 provides an overview of the stages of the vaccine manufacturing process of MVA-SARS-2-ST in comparison to MVA-MERS-S.

[redacted]

For replacement of MVA-SARS-2-S by MVA-SARS-2-ST the same process is implemented. That was confirmed by the regulatory agency in the informal Meeting on 05.02.2021 for MVA-SARS-ST.

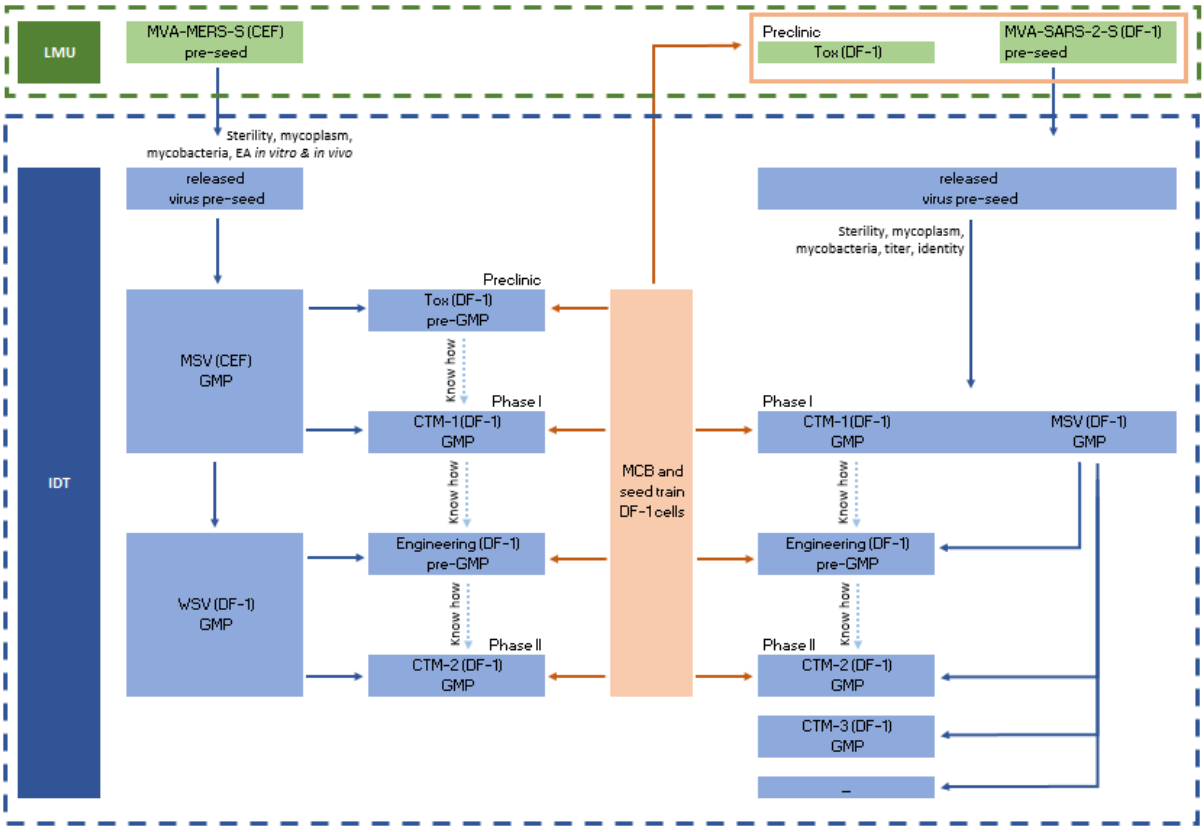

Figure 4: Manufacturing process overview of MVA-MERS-S from virus seed to clinical trial material (left) and of MVA-SARS-2-S (right).

A detailed listing of the QC testing of MVA-SARS-2-S clinical trial material and the placebo material across all manufacturing stages and including the final vial drug and placebo product can be found in the IMPD.

A summary of physical, chemical and pharmaceutical properties of the MVA-SARS-2-ST drug product is listed in Table 2.

Table 2: MVA-SARS-2-ST drug product summary

Product Name	**MVA-SARS-2-ST**
Dosage form	Injectable
Unit dose / injection volume	0.5 mL each of Low dose: 1x 10^7 IU/dose (Target confidence interval P=0.95) Middle dose: 5x 10^7 IU/dose (Target confidence interval P=0.95) High dose: 1x10^8 IU/dose (Target confidence interval P=0.95) The Injection is repeated as a booster after 28 days.
Vial	Sterile 2.0 mL vial with 0.5 mL extractable volume per dose

Vial label low dose	Zur klinischen Prüfung bestimmt Prüfcode: UKE-SARS-COV-2-ST EudraCT-Nr.: 2021-000548-23 Wirkstoff: MVA-SARS-2-ST_LD (1 x 10^7 IU/dose) Inhalt: 0.5 mL (nominal) Injektionslösung zur i.m. Anwendung gemäß Prüfplan **Lagerung bei ≤-65 °C, Vernichtung gemäß Anweisung des Sponsors/Prüfplans** **Sponsor:** Universitätsklinikum Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg; **CRO:** CTC North GmbH & Co. KG am Universitätsklinikum Hamburg-Eppendorf, Martinistr. 64, 20251 Hamburg Probanden-Nr.: _____ Ch.-B.: verw. bis: 3042292102
Vial label middle dose	Zur klinischen Prüfung bestimmt Prüfcode: UKE-SARS-COV-2-ST EudraCT-Nr.: 2021-000548-23 Wirkstoff: MVA-SARS-2-ST_MD (5 x 10^7 IU/dose) Inhalt: 0.5 mL (nominal) Injektionslösung zur i.m. Anwendung gemäß Prüfplan **Lagerung bei ≤-65 °C, Vernichtung gemäß Anweisung des Sponsors/Prüfplans** **Sponsor:** Universitätsklinikum Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg; **CRO:** CTC North GmbH & Co. KG am Universitätsklinikum Hamburg-Eppendorf, Martinistr. 64, 20251 Hamburg Probanden-Nr.: _____ Ch.-B.: verw. bis: 3042302102
Vial label high dose	Zur klinischen Prüfung bestimmt Prüfcode: UKE-SARS-COV-2-ST EudraCT-Nr.: 2021-000548-23 Wirkstoff: MVA-SARS-2-ST_HD (1 x 10^8 IU/dose) Inhalt: 0.5 mL (nominal) Injektionslösung zur i.m. Anwendung gemäß Prüfplan **Lagerung bei ≤-65 °C, Vernichtung gemäß Anweisung des Sponsors/Prüfplans** **Sponsor:** Universitätsklinikum Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg; **CRO:** CTC North GmbH & Co. KG am Universitätsklinikum Hamburg-Eppendorf, Martinistr. 64, 20251 Hamburg Probanden-Nr.: _____ Ch.-B.: verw. bis: 3042312102
Vial label placebo	Zur klinischen Prüfung bestimmt Prüfcode: UKE-SARS-COV-2-ST EudraCT-Nr.: 2021-000548-23 Wirkstoff: Placebo Inhalt: 0.5 mL (nominal) Injektionslösung zur i.m. Anwendung gemäß Prüfplan **Lagerung bei ≤-65 °C, Vernichtung gemäß Anweisung des Sponsors/Prüfplans** **Sponsor:** Universitätsklinikum Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg; **CRO:** CTC North GmbH & Co. KG am Universitätsklinikum Hamburg-Eppendorf, Martinistr. 64, 20251 Hamburg Probanden-Nr.: _____ Ch.-B.: verw. bis: 3042322102
Secondary packaging label low dose	**MVA-SARS-2-ST LD** **0,5 ml (1x 10^7 IU/dose)**

	Anzahl Vials/Box: 49 Vials (0,5ml) Ch.-B.: LDXXYYZZ Verwendbar bis: MM/JJJJ Injektionslösung zur i.m. Anwendung Arzneimittel zur klinischen Prüfung. Anwendung nach Prüfplan Lagerung: ≤-65 °C EudraCT-Nr.: 2021-000548-23 Prüfcode: UKE-SARS-COV-2-ST Sponsor: Universitätsklinikum Hamburg-Eppendorf (UKE), Tel. 040 7410-0 Martinistr. 52, 20246 Hamburg; CRO: CTC North GmbH & Co. KG, Tel. 040 524719-0 Martinistraße 64, 20251 Hamburg
Secondary packaging label middle dose	**MVA-SARS-2-ST MD** **0,5 ml (5x 10^7 IU/dose)** Anzahl Vials/Box: 49 Vials (0,5ml) Ch.-B.: MDXXYYZZ Verwendbar bis: MM/JJJJ Injektionslösung zur i.m. Anwendung Arzneimittel zur klinischen Prüfung. Anwendung nach Prüfplan Lagerung: ≤-65 °C EudraCT-Nr.: 2021-000548-23 Prüfcode: UKE-SARS-COV-2-ST Sponsor: Universitätsklinikum Hamburg-Eppendorf (UKE), Tel. 040 7410-0 Martinistr. 52, 20246 Hamburg; CRO: CTC North GmbH & Co. KG, Tel. 040 524719-0 Martinistraße 64, 20251 Hamburg
Secondary packaging label high dose	**MVA-SARS-2-ST HD** **0,5 ml (1x 10^8 IU/dose)** Anzahl Vials/Box: 49 Vials (0,5ml) Ch.-B.: HDXXYYZZVerwendbar bis: MM/JJJJ Injektionslösung zur i.m. Anwendung Arzneimittel zur klinischen Prüfung. Anwendung nach Prüfplan Lagerung: ≤-65 °C EudraCT-Nr.: 2021-000548-23 Prüfcode: UKE-SARS-COV-2-ST Sponsor: Universitätsklinikum Hamburg-Eppendorf (UKE), Tel. 040 7410-0 Martinistr. 52, 20246 Hamburg; CRO: CTC North GmbH & Co. KG, Tel. 040 524719-0 Martinistraße 64, 20251 Hamburg
Secondary packaging label placebo	**Placebo** Anzahl Vials/Box: 49 Vials (0,5ml) Ch.-B.: PLXXYYZZ Verwendbar bis: MM/JJJJ Injektionslösung zur i.m. Anwendung Arzneimittel zur klinischen Prüfung. Anwendung nach Prüfplan Lagerung: ≤-65 °C

	EudraCT-Nr.: 2021-000548-23 Prüfcode: UKE-SARS-COV-2-ST Sponsor: Universitätsklinikum Hamburg-Eppendorf (UKE), Tel. 040 7410-0 Martinistr. 52, 20246 Hamburg; CRO: CTC North GmbH & Co. KG, Tel. 040 524719-0 Martinistraße 64, 20251 Hamburg
Formulation	30 mM Tris, 6 % sucrose, 0.05 % PS-80
Route of administration	i.m. injection in deltoid muscle
Physical description	Clear to turbid liquid solution
Manufacturer	IDT Biologika GmbH Am Pharmapark D-06861 Dessau-Rosslau
Drug Product batch number	Low dose batch:LD010321 (SAP No 2103220080) Middle dose batch: MD010321 (SAP No 2103220084) High dose batch: HD010321 (SAP No 2103220088) Placebo batch: PL011020 (SAP No 2010140057)
IDT Product specification	Release specification for Bulk Drug Substance – RS - 000366 Release specification for Final Bulk – RS - 000367 Release specification for Drug Product visual inspected – RS – 000368 Release specification for Drug Product visual inspected – RS – 000369 Release specification for Placebo – RS - 000304

The IMP will be delivered to the participating clinical trial sites or responsible pharmacy directly by IDT Biologika GmbH and will be stored there at ≤-65 °C in rooms with restricted access only for members of the unblinded team. Temperature will be continually monitored and recorded. For use at the clinical trial site, the IMP will be shipped on dry ice.

For usage of the IMP, the vial will be removed from the freezer by a member of the unblinded team. The IMP will be thawed for 20-30 min at room temperature. Once it is thawed, the liquid will be clear to opaque-white liquid. The vaccine is stable at room temperature for up to 4 hours. Details of IMP handling can be accessed in the Pharmacy Manual.

For a comprehensive summary of the batch characteristics of MVA-SARS-2-ST at drug product level, the reader is referred to the IMPD and final CoA.

4 Nonclinical studies

4.1 Nonclinical pharmacology

Given the short time since the first description of the new respiratory disease in December 2019 and the identification of the responsible virus SARS-CoV-2 in January 2020, the development of suitable animal models for the investigation of COVID-19 and possible cures and vaccines is still an ongoing process. Here we want to give a brief summary of the currently most promising animal models but in addition refer to the review of Mundoz-Fontela et al. which provides a deep discussion of the advantages and disadvantages of the different models.[38]

Wild-type mice are not susceptible to SARS-CoV-2 infections but can be made susceptible either as human transgenic ACE2 receptor mice or by transduction of murine lung tissue with human ACE2 through an adenovirus or adenovirus-associate virus expressing hACE2. Mice constantly expressing hACE2 under the control of a promotor are susceptible to SARS-CoV-2 infection and show mild to lethal disease progression depending on receptor expression rates.[39-41] Substitution of murine ACE2 with human ACE2 leads to SARS-CoV-2 replication in respiratory and brain tissues without severe disease manifestation.[42] Adenovirus mediated transduction of the respiratory tract of mice leads to transient expression of hACE2 in the respective tissue and clinical disease development after SARS-CoV-2 challenge.[43-45] This system can be quickly adapted to different mouse strains and allows to study lung infections and histopathological changes consistent with viral pneumonia.

Ferrets are naturally susceptible to SARS-CoV-2 and only show weak to no clinical disease manifestation.[46] However, virus shedding and virus transmission to uninfected ferrets can be detected in experimental settings.[47-49] Syrian hamsters can be infected with SARS-CoV-2 and show mild-to-moderate disease including signs of respiratory distress.[50] Two weeks post infection, hamsters have typically recovered without intervention. Interestingly, infections of hamsters with SARS-CoV-2 reflect differences both in age and sex as observed for COVID-19 in humans, leading to more serious disease in aged and male animals. Furthermore, transmission to control animals has also been observed.[51,52]

Primary pharmacology data of MVA-SARS-2-S immunization has been studied in BALB/c mice and is currently studied in a Syrian hamster challenge model. Additional data of the predecessor construct MVA-SARS-2-S was by now generated in the following models and studies:

- immunogenicity in BALB/c and C57/BL6 mice
- protective efficacy from SARS-CoV-2 challenge in BALB/c mice transiently transduced with hACE2
- protective efficacy from SARS-CoV-2 challenge and infectiousness in ferrets and Syrian hamsters.

Table 3 summarizes the information already available on those studies.

Table 3: Nonclinical pharmacology studies for MVA-SARS-2-ST and MVA-SARS-2-S

Model	IMP/dose/ route/n=x	Setup	Challenge	Read-out	Findings
MVA-SARS-2-ST					
BALB/c mice, female	MVA-SARS-2-ST/ PBS prime-boost (21 day interval), 1x 10^6 or 1x 10^8 PFU, i.m., n=6-12	Safety, tolerability, immunogenicity	no	Body weight, clinical observation, humoral and cellular immunity, histopathology	No adverse effects, no abnormal macroscopic lesions, no systemic effects, administration site and draining lymph nodes show signs of expected physiological immune reaction against vaccination, significant humoral and cellular immune responses compared to PBS-vaccinated animals
Syrian hamster, male and female	MVA-SARS-2-ST/ MVA wt, prime/ prime-boost (21 day interval), 1x 10^8 PFU, i.m. n=24	Protection, immunity, disease enhancement	1x 10^4 TCID50 SARS-CoV-2, 28 days after last immunization	Body weight, clinical observation, humoral immunity, viral excretion after challenge (pPCR & TCID50), pathology & histopathology	*Results expected for mid of 2021*
MVA-SARS-2-S					
BALB/c mice, female	MVA-SARS-2 S/ PBS, prime/ prime-boost (21 day interval), 1x 10^7 or 1x 10^8 PFU, i.m., n=7-18	Safety, tolerability, immunogenicity	no	Body weight, clinical observation, humoral and cellular immunity, histopathology only for 1x 10^8 PFU and PBS	No adverse effects, no abnormal macroscopic lesions, no systemic effects, administration site and draining lymph nodes show signs of expected physiological immune reaction against vaccination, significant humoral and cellular immune responses compared to PBS-vaccinated animals (prime-boost >> prime only), vague dose-relation for humoral immunity
BALB/c mice, female, transduced with 5x 10^8 PFU Ad-hACE2-mCh after second immunization	MVA-SARS-2-S/ PBS, prime-boost (21 day interval), 1x 10^7 or 1x 10^8 PFU, i.m., n=4-6	Protection, disease enhancement	1.5x 10^4 TCID50 SARS-CoV-2, 3 days after transduction	Body weight, clinical observation, humoral immunity, viral excretion after challenge (qPCR & TCID50), histopathology	No adverse effects, no enhanced disease after challenge, significant reduction of detectable viral RNA compared to control animals, no infectious virus isolation possible, significant humoral immune response compared to control animals, *histopathology: interstitial pneumonia (moderate to severe, mainly lymphohistiocytic, multifocal) in control mice, no significant reduction of severity in vaccinated mice, mild BALT (bronchus-associated lymphatic tissue) hyperplasia and small aggregates of lymphocytes around blood vessels in vaccinated mice; in situ hybridization: strong reduction of viral RNA in vaccinated mice.*

Model	IMP/dose/ route/n=x	Setup	Challenge	Read-out	Findings
BALB/c mice, female, transduced with 5x 10^8 PFU Ad-hACE2-mCh after second immunization	MVA-SARS-2-S/ PBS, prime-boost (56 day interval), 1x 10^8 PFU, i.m., n=4-6	Protection, disease enhancement	1.5x 10^4 TCID50 SARS-CoV-2, 3 days after transduction	Body weight, clinical observation, humoral immunity, viral excretion after challenge (qPCR & TCID50), histopathology	No adverse effects, no enhanced disease after challenge, significant reduction of detectable viral RNA compared to control animals, no infectious virus isolation possible, significant humoral immune response compared to control animals, *histopathology: interstitial pneumonia (moderate to severe, mainly lymphohistiocytic, multifocal) in control mice, no significant reduction of severity in vaccinated mice, mild BALT (bronchus-associated lymphatic tissue) hyperplasia and small aggregates of lymphocytes around blood vessels in vaccinated mice; in situ hybridization: strong reduction of viral RNA in vaccinated mice.*
C57BL/6 mice, female	MVA-SARS-2-S/ no treatment, i.n. prime/ i.m. prime-i.n. boost (24 day interval), 1x 10^7 PFU, n=6-9	Safety, tolerability, immunogenicity	no	Body weight, clinical observation, humoral and cellular immunity	No adverse effects, polyfunctional CD8+ T cell and a CD4+ T helper type 1 (TH1)-biased immune response
Ferrets	MVA-SARS-2-S/ no treatment, prime-boost (21 day interval), 1-2x 10^8 PFU, i.n./i.m., n=4-8	Protection, transmission, immunity, disease enhancement	1x $10^{5.6}$ TCID50 SARS-CoV-2, 14 days after second immunization	Body weight, clinical observation, humoral immunity, viral excretion after challenge (qPCR & TCID50), transmission on unvaccinated contact animals	No adverse effects, no enhanced disease after challenge, no clinical disease manifestation after challenge, no sterile immunity, no transmission of SARS-CoV from i.n. vaccinated animals on contact animals
Syrian hamster, male and female	MVA-SARS-2-S/ MVA wt, prime/ prime-boost, (21 day interval), 1x 10^8 PFU, i.m. n=24	Protection, immunity, disease enhancement	1x 10^4 TCID50 SARS-CoV-2, 28 days after last immunization	Body weight, clinical observation, humoral immunity, viral excretion after challenge (pPCR & TCID50), pathology & histopathology	No adverse effects no overt clinical disease in vaccinated animals (incl. preliminary gross pathology); preliminary data show significant reduction of SARS-CoV-2 loads in lungs of vaccinated animals suggesting solid protection from challenge, histopathology ongoing

Preclinical immunogenicity of MVA-SARS-2-ST was evaluated in BALB/c mice and induction of significant S-antigen specific humoral and cellular immune responses could be demonstrated. Using 1x 10^8 PFU MVA-SARS-2-ST, IgG antibody titers directed against the full-length S protein and the S1 subunit were assessed by different ELISAs and clearly showed the supremacy of a prime-boost vaccination scheme (see Figure 5).

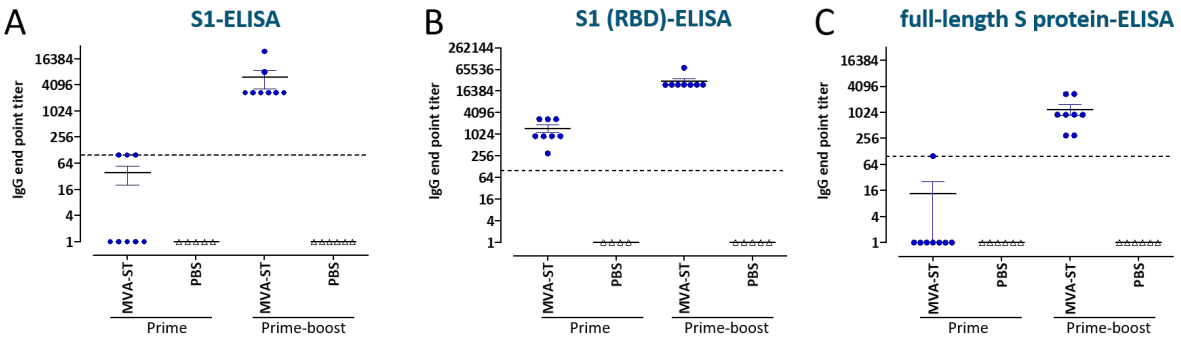

Figure 5: Antigen-specific IgG responses induced by treatment with recombinant MVA-SARS-2-ST (MVA-ST). Groups of BALB/c mice (n=6-8) were vaccinated in a prime-boost regime (21-day interval) with 1x 10^8 PFU of MVA-ST via the i.m. route. Mice inoculated with PBS served as controls. Sera were collected 18 days after the first immunization (prime) and 14 days after the second immunization (prime-boost) and analyzed for SARS-2-S specific IgG titers by ELISA. A) S1 subunit antigen; B) S protein RBD antigen; C) full-length S protein antigen.

Virus neutralization was determined by VNT100 assay using sera of mice vaccinated in a prime-boost schedule with 1x 10^6 or 1x 10^8 PFU MVA-SARS-2-ST. 100 % of all sera revealed neutralizing activity as shown in Figure 6.

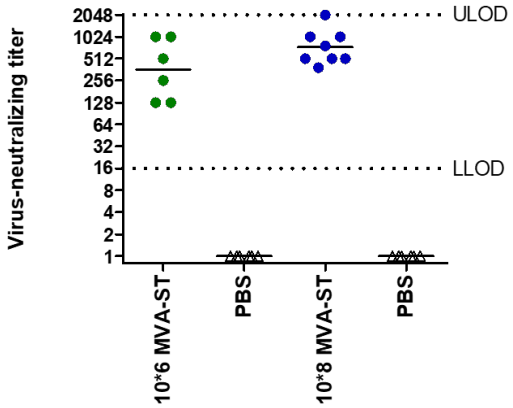

Figure 6: Antigen-specific virus neutralization capacity induced by treatment with recombinant MVA-SARS-2-ST (MVA-ST). Groups of BALB/c mice (n=7-12) were vaccinated in a prime-boost regime (21-day interval) with 1x 10^6 (10*6) or 1x 10^8 (10*8) PFU of MVA-ST via the i.m. route. Mice inoculated with PBS served as controls. Sera were collected 14 days after the second immunization (prime-boost) and analyzed for SARS-2-ST specific IgG titers by virus neutralization (VNT100).

Activation of a SARS-CoV-2 specific cellular immunity was monitored through S-specific CD8+ T cells elicited in BALB/c mice vaccinated with 1x 10^8 PFU MVA-SARS-2-ST in a prime-boost scheme. Identification and quantification of virus-specific T cells was done by IFN-γ ELISPOT ICS plus SFACS analysis using isolated splenocytes 8 days after the second immunization. Stimulation with an S1-specific peptide was done *in vitro*. All MVA-SARS-2-ST vaccinated animals showed significant levels of activated SARS-CoV-2-S specific CD8+ T cells (see Figure 7).

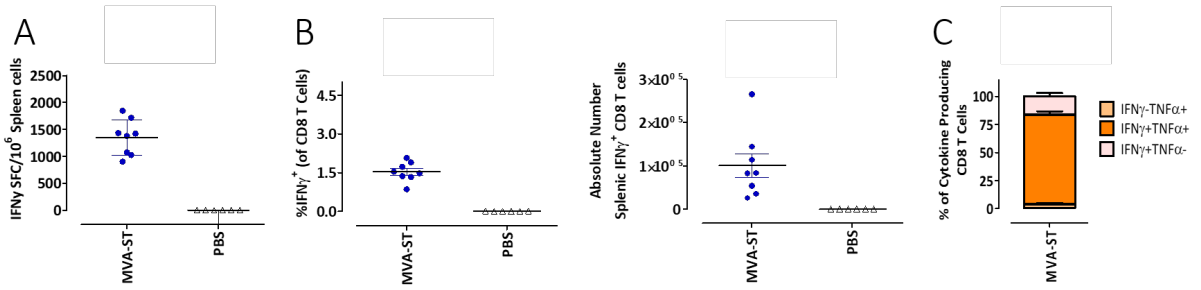

Figure 7: Activation of SARS-2-ST specific CD8+ T cells after prime-boost immunization with MVA-SARS-2-ST. Groups of BALB/c mice (n=4-6) were immunized twice with 1x 10^8 PFU MVA-SARS-2-ST (MVA-ST) over a 21-day interval via the i.m. route. Mock immunized mice (PBS) served as controls. Splenocytes were collected and prepared on day 14 after prime-boost immunization. Total splenocytes were stimulated with the H2d restricted peptide of the SARS-2-S protein S268-276 (S1; GYLQPRTFL) and were measured by IFN-γ and TNF-α ICS plus FACS analysis. IFN-γ production by CD8+ T cells was measured by FACS analysis. Graphs show A) IFN-γ SFC for stimulated splenocytes measured by ELISPOT assay. B) IFN-γ production by CD8+ T cells measured by FACS analysis. Graphs show the frequency and absolute number of IFN-γ+ CD8+ T cells. C) Cytokine profile of S1-specific CD8+ T cells. Graphs show the mean frequency of IFN-γ-TNF-α+, IFN-γ+TNF-α+ and IFN-γ+TNF-α- cells within the cytokine positive CD8+ T cell compartment.

We additionally want to highlight the most important results of the conducted preclinical studies with the predecessor vaccine candidate MVA-SARS-2-S. Vaccination of BALB/c mice led to the induction of S-antigen-specific humoral and cellular immunity. Humoral immunity was assessed by ELISA and (surrogate) virus neutralization test (VNT100, sVNT) as shown in Figure 8. At day 35 after the prime-boost vaccination, detection of S-antigen binding IgG antibodies in sera from all vaccinated animals was possible. The booster immunization on day 21 increased the serum IgG levels about 10-fold for the 1x 10^7 PFU vaccination group and about 5-fold for the 10^8 PFU vaccination group. The sera of mice inoculated with 1x 10^8 PFU MVA-SARS-2-S revealed neutralizing activities in 88 % of all sera for the VNT assay and in 100 % of all sera for the sVNT assay. The reported data on humoral immunity supports a clear dose response effect with the higher dose of 1x 10^8 PFU MVA-SARS-2-S inducing higher levels of SARS-CoV-2 specific antibodies.

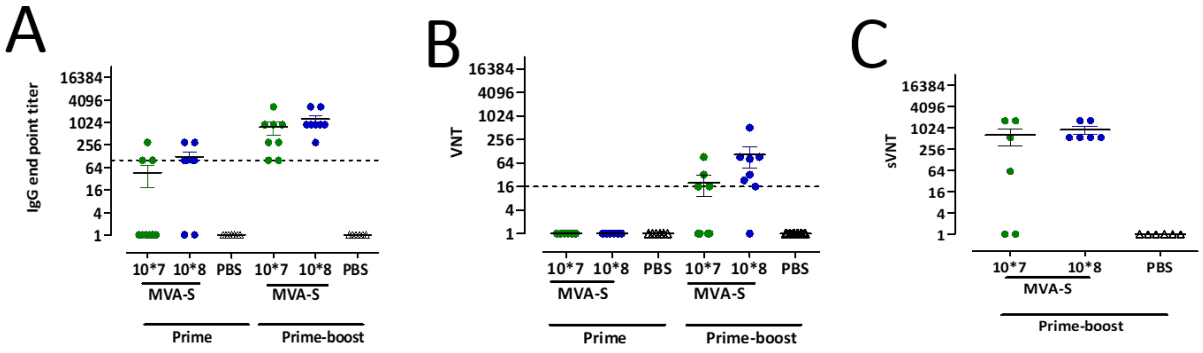

Figure 8: Antigen-specific humoral immunity induced by treatment with recombinant MVA-SARS-2-S (MVA-S). Groups of BALB/c mice (n=7-12) were vaccinated in a prime-boost regime (21-day interval) with 1x 10^7 (10*7) or 1x 10^8 (10*8) PFU of MVA-SARS-2-S via the intramuscular (i.m.) route. Mice inoculated with saline (PBS) served as controls. Sera were collected 18 days after the first immunization (prime) and 14 days after the second immunization (prime-boost) and analyzed for SARS-2-S specific IgG titers by ELISA (A) and SARS-CoV-2 neutralizing antibodies by virus neutralization (VNT100; B) or surrogate virus neutralization test (sVNT; C).

To assess the activation of SARS-CoV-2-specific cellular immunity, we monitored for S-specific CD8+ T cells in BALB/c mice vaccinated with 1x 10^7 PFU or 1x 10^8 PFU MVA-SARS-2-S in prime-boost immunization schedules using a 3-week interval. To identify and enumerate virus-specific T cells by IFN-γ ELISPOT or ICS (intracellular cytokine staining) FACS analysis we isolated splenocytes at day 8 after the last MVA-SARS-2-S immunization and used S1-specific peptide stimulation for activation upon *in vitro* culture. All MVA-SARS-2-S immunizations elicited significant levels of activated SARS-CoV-2-specific CD8+ T cells irrespective of the dosage (see Figure 9).

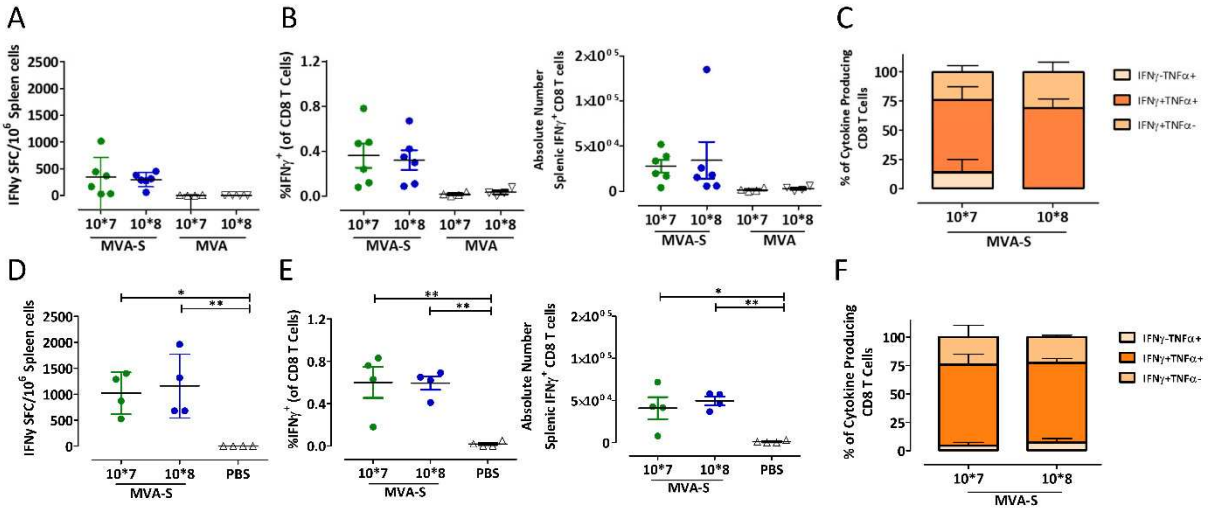

Figure 9: Activation of SARS-2-S specific CD8+ T cells after prime-boost immunization (21-day interval) with MVA-SARS-2-S. Groups of BALB/c mice (n=4-6) were immunized twice with 1x 10^7 (10*7) or 1x 10^8 (10*8) PFU of MVA-SARS-2-S (MVA-S) over a 21-day interval via the i.m. route. Mock immunized mice (PBS) were used as negative controls. Splenocytes were collected and prepared on day 8 after prime (A,B,C) or prime-boost immunization (D,E,F). Total splenocytes were stimulated with the H2-d restricted peptide of the SARS-2-S protein S268-276 (S1; GYLQPRTFL) and were measured by IFN-γ ELISPOT assay and IFN-γ and TNF-α ICS plus FACS analysis. A,D) IFN-γ SFC for stimulated splenocytes measured by ELISPOT assay. B,E) IFN-γ production by CD8+ T cells measured by FACS analysis. Graphs show the frequency and absolute number of IFN-γ+ CD8+ T cells. (C,F) Cytokine profile of S1-specific CD8+ T cells. Graphs show the mean frequency of IFN-γ-TNF-α+, IFN-γ+TNF-α+ and IFN-γ+TNF-α- cells within the cytokine positive CD8+ T cell compartment. Differences between groups were analyzed by one-way ANOVA and Tukey post-hoc test. Asterisks represent statistically significant differences between two groups. * p <0.05, ** p <0.01.

Further data on cellular immunity was generated in C57BL/6 mice vaccinated with 1x 10^7 PFU MVA-SARS-2-S. One group received an i.n. prime-only vaccination and a second group was treated first with an i.m. prime and, 24 days later, with an i.n. boost vaccination. Untreated age matched animals served as controls. A polyfunctional CD8+ T cell and a CD4+ T helper type 1 (TH1)-biased immune response was induced by MVA-SARS-2-S vaccination, similar to T cell responses known to be associated with protective immunity to viruses.[57]

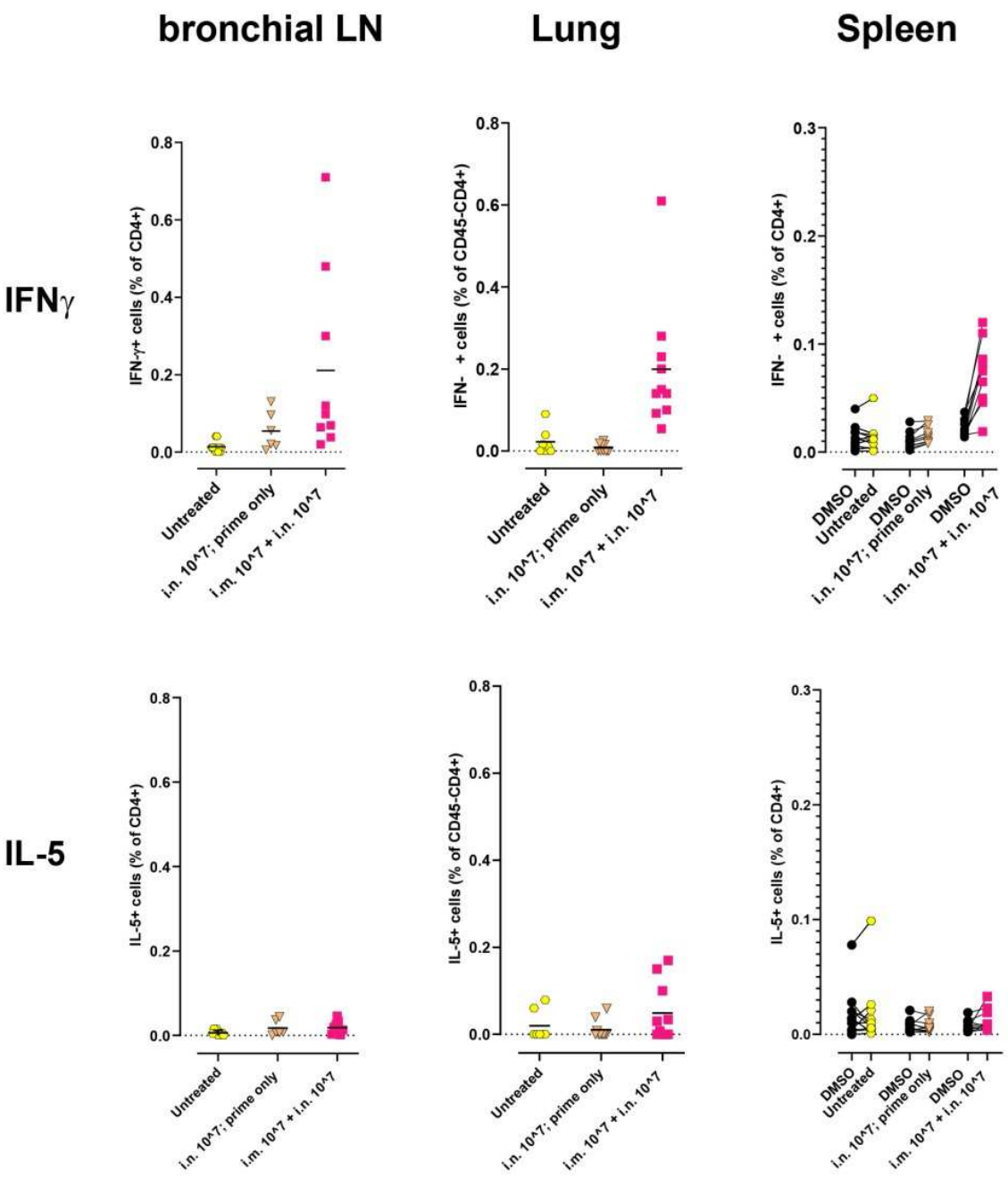

Figure 10: Application of MVA-SARS-CoV-2-S induces TH1 immune responses. Female C57BL/6 mice were intranasally primed with 1x 10^7 (10^7) PFU MVA-SARS-CoV-2-S (beige triangles, n=6) or intramuscularly primed with 1x 10^7 (10^7) PFU MVA-SARS-CoV-2-S followed by an intranasal boost with the same dose of the vaccine 24 days later (magenta quadrants, n=9). Mice were sacrificed 40 days after priming. Untreated age matched controls (yellow hexagons, n=6) served as control. Before scarification, mice intravenously received 5 µg anti-CD45-FITC antibodies to label cells within the lungs' blood vasculature. Five minutes later the organs indicated were harvested. Cells were isolated, counted, and cultured in medium containing brefeldin A, and an DMSO-dissolved pool of 128 overlapping, 15-amino-acid-long peptides covering the first 640 amino acids of SARS-CoV-2-S protein as well as 31 predicted 8-15 amino-acid- long immunodominant peptides. The final concentration of each peptide was 2 µg/ml and the final concentration of DMSO was <1 %. An aliquot of spleen cells was cultured in medium containing the same concentration of brefeldin A and DMSO but not peptides. After 6 h of incubation, cells were stained for extracellular markers (CD3, CD4, CD44), washed, fixed, permeabilized, and stained with anti-cytokine antibodies. The percentage of IFN-γ+ and IL-5+ of tissue CD3+CD4+CD44+ T cells has been determined.

To analyze if the MVA-SARS-2-S-mediated immunity protects from a SARS-CoV-2 infection, BALB/c mice were vaccinated with a prime-boost (21 days) regiment with 1x 10^8 or 1x 10^7 PFU MVA-SARS-2-S or saline (PBS) as control. Subsequently, mice were intranasal transduced with 5x 10^8 PFU of Ad-ACE2-mCh to ensure the expression of the human receptor ACE2 (hACE2) in the lungs. Three days post transduction the mice were i.n. infected with 1.5x 10^4 TCID50 SARS-CoV-2. Four days post infection, serum samples were collected before mice were sacrificed. Afterwards, the lungs were isolated and analyzed.

MVA-SARS-2-S-vaccinated (1x 10^7 or 1x 10^8 PFU) BALB/c mice challenged with SARS-CoV-2 indicated significantly reduced viral RNA loads in lungs compared to control animals (see Figure 11 A). In line with these findings, no infectious SARS-CoV-2 was present in the lungs of MVA-SARS-2-S-vaccinated BALB/c mice compared to control animals (see Figure 11 B). Quantification of the SARS-CoV-2-neutralizing antibodies by virus neutralization test (VNT100) showed that all mice vaccinated with the higher MVA-SARS-2-S dose (Fig. 8 C, 1x 10^8 PFU, blue) and four of five mice vaccinated with the low dose (see Figure 11 C, 1x 10^7, green) had neutralizing antibodies as opposed to control animals which showed no neutralization activity.

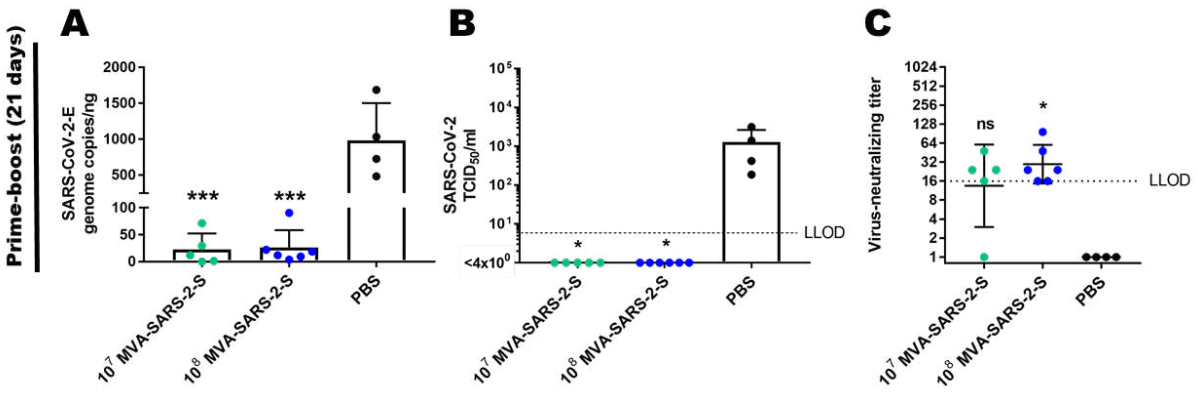

Figure 11: Application of MVA-SARS-CoV-2-S reduces viral load in mice. Groups of BALB/c mice (n=4-6) were vaccinated in a prime-boost regime (21-day interval) with 1x 10^7 or 1x 10^8 PFU of MVA-SARS-2-S via the i.m. route. Mice inoculated with saline (PBS) served as controls. After the second vaccination, mice were intranasally transduced with 5x 10^8 PFU of Ad-ACE2-mCh to ensure expression of the SARS-CoV-2 receptor ACE2. Three days post transduction, mice were i.n. infected with 1.5x 10^4 TCID50 SARS-CoV-2. Four days post infection, serum samples were collected and SARS-CoV-2 neutralizing antibodies were quantified by virus neutralization test (VNT100; C). Mice were sacrificed before lungs were isolated and homogenized. RNA was isolated out of homogenized lungs and SARS-CoV-2-E-specific qRT-PCR was performed (A). TCID50 analysis were performed from lung homogenates (B).

The data obtained so far establishes the need for a prime-boost vaccination scheme. The knowledge gained from the experiments found a benefit promise in respect to protection from COVID-19.

Ferrets were used as an additional preclinical model since they are naturally susceptible to a SARS-CoV-2 infection. The animals were vaccinated in a prime-boost scheme (21-day interval) either i.n. or i.m. with 1-2x 10^8 PFU MVA-SARS-2-S. Control animals did not receive any vaccination or placebo and were kept separated from the vaccinated animals. Additional to the control animals, further untreated contact animals were kept together with the treated ferrets. Clinical disease manifestation after challenge with SARS-CoV-2 could not be observed neither in vaccinated nor in control animals. Treatment with MVA-SARS-2-S did not lead to any obvious effect on body weight in any group both before and after challenge with SARS-CoV-2 (see Figure 12).

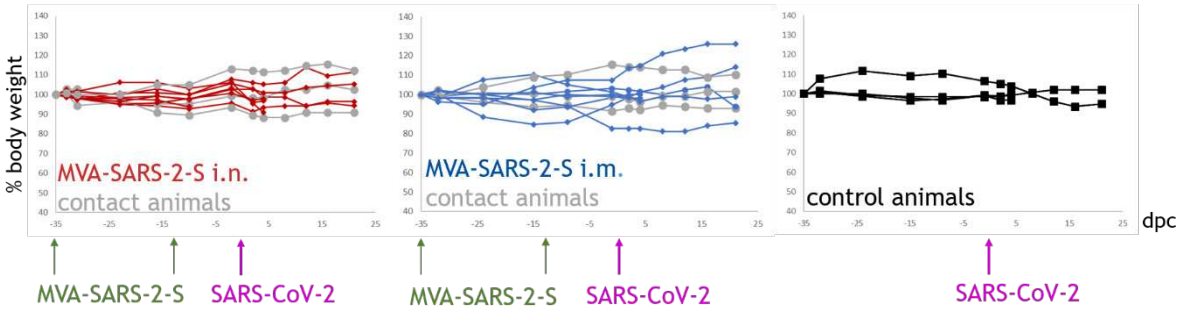

Figure 12: Body weight changes of ferrets after vaccination and challenge. Ferrets (n=7-8) were vaccinated in a prime-boost regime (21-day interval) with 1-2x 10^8 PFU of MVA-SARS-2-S via the intranasal (red) or intramuscular (blue) route. Untreated contact animals (grey, n=3) were kept together with the vaccinated animals but did not receive any treatment. Control animals (black, n=4) did not receive any vaccination or placebo but were challenged with SARS-CoV-2. Body weight was measured regularly.

Evaluation of macroscopic lesions did not reveal a negative impact of the vaccination with MVA-SARS-2-S in comparison to control animals. Since the left lung lobes were in general more consistently affected by macroscopic lesion, they were examined in greater histopathological detail (see Figure 13). The results clearly show that the vaccination did not have a negative impact on the grade of pulmonary inflammation. The predominant lesions were characterized by perivascular infiltrates.

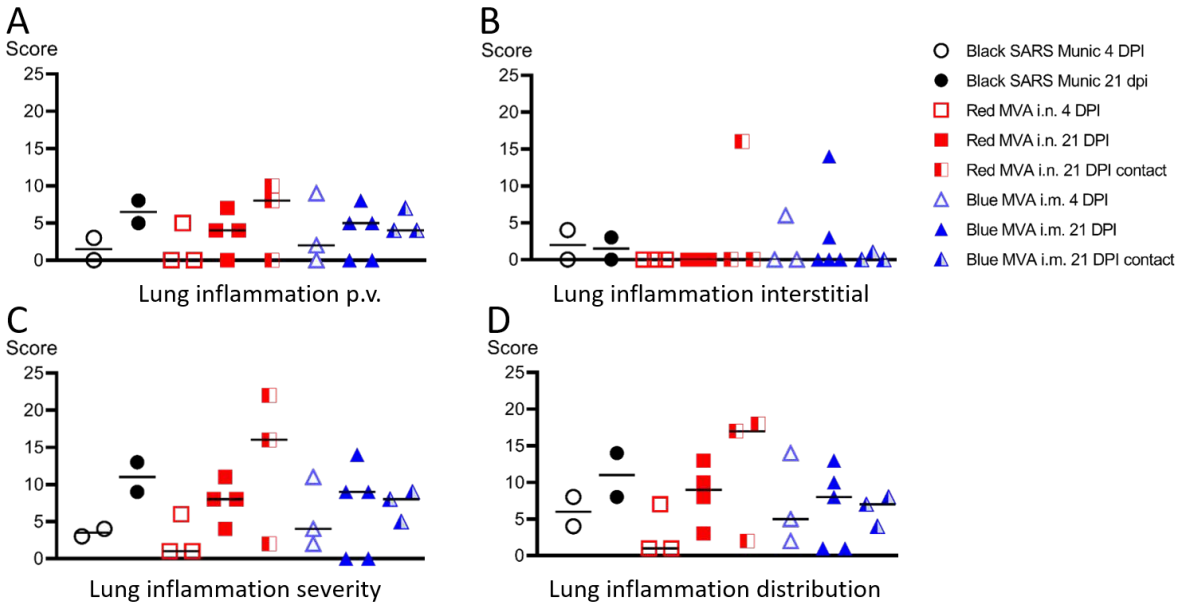

Figure 13: Histopathology of pulmonary inflammation in vaccinated and challenged ferrets. (A) Total score for severity and distribution of perivascular) inflammation in both left lung lobes. (B) Total score for severity and distribution of interstitial inflammation in both left lung lobes. (C) Total score for severity of any inflammation in both left lung lobes. (D) Total score for distribution of any inflammation in both left lung lobes.

In summary, the data obtained from the ferret MVA-vaccination SARS-CoV-2-challenge model did not hint towards notably adverse events caused by the treatment with MVA-SARS-2-S both before and after challenge with SARS-CoV-2. The experiments did not show any evidence for an enhanced disease triggered by vaccination and subsequent SARS-CoV-2 challenge.

4.2 Pharmacokinetics and product metabolism in animals

Biodistribution and clearance of MVA-SARS-2-ST was not studied. Instead, we want to highlight the results of the investigation of MVA-MERS-S obtained after a single i.m. vaccine application in C57BL/6 mice.[58] In summary, Langenmayer et al. did not observe tissue reactions in organs/tissues other than the injection site and the draining lymph nodes. The histological findings at the injection site and draining lymph nodes reflect the immune competence of the animals to develop an adequate immunological response to an intracellular organism. PCR monitoring of the intramuscular injection of MVA-MERS-S led to MVA-DNA detection mainly at the inoculation site with very few other PCR-positive organ samples. Following inoculation, the number of PCR positive sample sites steadily decreased, indicating a continuous clearance of the recombinant MVA from the host organism.

Overall, the described findings are in clear accordance with other studies performed to assess the distribution of parental virus MVA in immune-suppressed macaques and mice[33,59] and immune-competent mice[60,61]. These studies included exhaustive organ/tissue sampling for pathological examination complemented by PCR analysis in a subset of tissues. In these studies, inflammatory tissue reactions to MVA application were restricted to the injection sites. Viral DNA detected by PCR analysis was found mainly at the inoculation sites with very few positive samples from organ/tissues peripheral to the inoculation site.

We expect MVA-SARS-2-ST to show an identical biodistribution and clearance pattern as described for MVA-MERS-S. The glycoproteins of MERS-CoV and SARS-CoV-2 are closely related and the resulting recombinant MVA constructs can be expected to exhibit comparable biochemical properties.

4.3 Toxicology

The MVA platform has been used in laboratory animal species and uniformly found well tolerated and safe. This includes studies in chicken,[62,63] dog,[64] cotton rat[65,66] and mouse[65,67].

MVA-SARS-2-S was studied in a combined, non-GLP preclinical proof-of-concept and safety study in female BALB/c mice, which were kept under SPF conditions and obtained from Charles River Laboratories. Due to practical aspects, male BALB/c mice were not part of the study. Toxicology endpoints have been included in the study design. MVA-SARS-2-S was administered at a full human dose (1x 10^8 PFU/dose) in a prime-only (7-day recovery period) or prime-boost vaccination scheme (21-day interval, 14 days recovery period until study day 35) via the intramuscular route (Mm. semimembranosus/ semitendinosus left thigh). PBS was used as vehicle in the control group.

Table 4 describes the experimental groups and doses as follows:

Table 4: Preclinical study of MVA-SARS-2-S: experimental groups and doses

Group	Treatment	Nominal dose# [PFU/animal]	Treatment volume [mL]	No. of animals
1	Vehicle	-	0.25	18*
2	MVA-SARS-2-S prime	1x 10^8		12
3	MVA-SARS-2-S prime boost	1x 10^8		18

#The same dose volume was used for all vaccination time points. There was no correction for actual body weight.
*****Six animals were mock-vaccinated on day 7 and 12 animals on day 21 followed by a 14 day recovery period.

The relevant clinical and gross pathology findings reported for this study are summarized in Table 5.

Table 5: Findings in preclinical study of MVA-SARS-2-S in vaccinated BALB/c mice

Findings	Number of Animals	Comment
Mortality	0/30	No mortality
Daily clinical observation	0/30	No clinical findings
Body weight development	0/30	Regular body weight development within range of controls
Gross pathology	0/30	No adverse findings

A detailed progression of the body weight development shown in Figure 14.

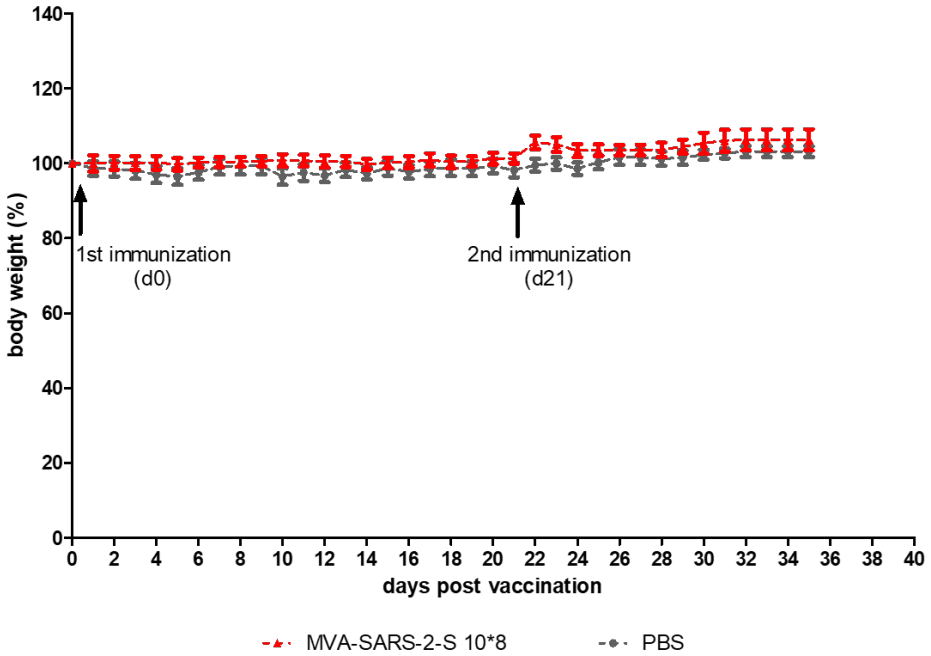

Figure 14: Body weight changes of mice after vaccination. BALB/c mice (n=12) were vaccinated in a prime-boost regime (21 day interval) with 1x 10^8 (10*8) PFU of MVA-SARS-2-S via the intramuscular route. Vaccination with saline (PBS; n=12) was used as a control. Body weight was measured daily.

Necropsy was performed after euthanasia by cervical dislocation seven days after prime-only vaccination or, after boost vaccination on day 21, at the end of the recovery period (study day 35). After external examination, the thoracic and abdominal cavity was opened and a macroscopic examination was performed. Table 6 summarizes macroscopically examined organs and preserved tissues. Since the spleen is necessary for the analysis of cellular immunity, this organ was not available for histopathology from every animal.

Table 6: Organs examined macroscopically and tissue asservated during post-mortem examination

Organs examined macroscopically	Tissue preserved for histopathology
adrenal glands, caecum, colon, duodenum, eye, heart, ileum, jejunum, kidney, liver, lymph nodes (Ln. mandibularis, Ln. subiliacus, Ln. ischiadicus, Ln. popliteus, Ln. iliacus medialis left side), larynx, lungs, mammary gland, mesenteric lymph nodes, pancreas, oesophagus, ovary, oviduct, parathyroid gland, peripheral nerve, salivary gland (sublingual, submandibular), skeletal muscle (esp. thigh of both sides including application side), skin, stomach, spleen, thyroid gland, thymus, ureter, urinary bladder, uterus, tongue, trachea, vagina	adrenal gland (left side), heart, kidney (left side), liver, lung, lymph nodes (Ln. subiliacus, Ln. ischiadicus, Ln. popliteus, Ln. iliacus med. of left side), skeletal muscle (application side), thymus, particularly spleen

The organs were preserved in paraformaldehyde for histopathological examination. Tissues were routinely trimmed, paraformaldehyde-fixed, paraffin-embedded and cut. Sections were stained with hematoxylin and eosin; additional staining was not prepared.

The findings of the histopathological analysis are summarized in Table 11 and listed in detail in Tables 12 to 16 in the appendix of this document.

At the site of intramuscular administration, a predominantly minimal to mild, mixed cellular infiltrate was often observed in the interstitium of muscle fibers and adjacent adipose tissue associated with separation of muscle fibers through edema and signs of myodegeneration in MVA-SARS-2-S vaccinated animals. Control animals showed minimal to mild marks of myodegeneration. Necrosis of individual myofibers was seen rarely in prime-boost treated animals. Additionally, muscular regeneration characterized by internalized nuclei was observable in mice vaccinated twice and necropsied on day 35. The draining lymph nodes often showed a minimal to mild increased number of paracortical lymphocytic cellularity, which was interpreted as lymphoid hyperplasia, due to the lesions in the skeletal muscle. Further alterations like marked inflammation were not observed in the lymph nodes.

There was absence of lesions attributable to MVA-SARS-S-2 inoculation in any examined tissue other than the administration site and draining lymph nodes. The nature of all other changes was consistent with commonly seen unspecific lesions normally found in BALB/c mice.

The most relevant findings of the above study are summarized and discussed as follows:

- **No mortality and no clinical observation** was made in MVA-SARS-2-S treated mice in comparison to PBS vaccinated control animals.
- Body weight development and food consumption of all animals was within normal limits and comparable to control animals.
- The stomach of all vaccinated animals was filled with ingesta and the consistency of the faeces in the rectum was normal, underlining a good general condition of the mice.
- No abnormal macroscopic lesions were detected at necropsy.
- Macroscopically, skeletal muscles around the injection side were mildly edematous in comparison to the opposite thigh muscle of the same animal or of PBS-treated animals, which is considered a normal local irritation due to vaccine injection and thus procedure-related. Draining lymph nodes on the injection side were mildly enlarged, which demonstrates the immune competence of the animals to develop an adequate immunological response against the administered vaccine and as such is not considered an adverse finding.
- **Histopathologically, there were no lesions attributable to MVA-SARS-S-2 inoculation in any tissue other than the administration site as well as the draining lymph nodes.** Systemic effects related to vaccination were not seen. Signs of myodegeneration in the administration site were observed in treated and control mice, but only to a minimal to mild degree. Local inflammation of the myofiber interstitium and the adjacent adipose tissue can be interpreted as part of the physiologic immune reaction to the vaccine virus, respectively as a consequence to treatment procedure. The degree and extent of inflammation, myodegeneration and -necrosis is in accordance with the ratio of inoculum volume in relation to the administration site, the murine thigh muscle, which is considerably smaller than a human thigh. The lymphoid hyperplasia observed in draining lymph nodes is interpreted as a sign of immune competence of the animals and is characteristic for any early response to inflammation at a draining site.[68]

In conclusion, in this proof-of-concept study we observed no evidence for a potential toxicity of the full human dose of MVA-SARS-2-S in BALB/c mice. The repeated vaccination was well tolerated and

caused no adverse events and no relevant macroscopic or histopathological changes. The observed reactions were comparable to previous experiments using non-recombinant MVA or other recombinant MVA vaccine constructs and are considered to be part of the pharmacodynamic principle of a MVA-based vaccination.[58] **This establishes a No Observed Adverse Event Level (NOAEL) for MVA-SARS-2-S of 1x 10^8 PFU for repeated i.m. administration.** Furthermore, MVA-SARS-2-S was shown to elicit humoral and cellular immune responses against SARS-CoV-2 as highlighted in chapter 4.1.

The related MVA-MERS-S vaccine candidate was tested in two GLP-compliant toxicity studies. The 1st generation of the vaccine produced in CEF cells was tested in a compressed n+1 dosing schedule and at Full Human Dose (FHD) in Wistar rats. In summary, the experimental vaccine was well tolerated and had a NOAEL of >1.5x 10^8 PFU for the repeat dosing (study report attached). The 2nd generation of the vaccine, MVA-MERS-S_DF-1 produced in DF-1 cells was tested in New Zealand White Rabbits (NZWR) in an n+1 dosing schedule using FHD. Again, the vaccine was well tolerated and a NOAEL of 2.4x 10^8 PFU for repeated dosing was reported (study report attached).

The choice of the SARS-S glycoprotein for vaccination of laboratory animals during the SARS outbreak in 2003/2004 has been met with skepticism. *In vitro*, in cell cultures, experimental conditions were developed under which SARS immune sera could enhance cell entry of the SARS virus independently from the ACE2 receptor via the FcyR mechanism, so called antibody dependent enhancement (ADE).[69,70] While the conditions under which ADE could be demonstrated *in vitro* were narrow, also *in vivo* experiments in ferrets pointed to a potential ADE mechanism in this species.[71,72] Liu and coworkers demonstrated an enhanced lung pathology in rhesus macaques receiving anti-S-IgG in addition to SARS-CoV virus challenge. The authors speculate this being caused by a skewing of an inflammation-resolving macrophage response and reported similar observations in patients deceased of SARS.[73] On the other hand, primary human macrophages infected *in vitro* with SARS-CoV through FcyR mediated ADE were incapable of supporting a productive replication of the virus.[74]

A recent study demonstrated a dose dependent enhancement of the FcyR mediated viral cell entry mechanism of both SARS-CoV and MERS-CoV.[75] The same study also showed, that neutralizing antibodies binding and stabilizing the MERS-CoV S protein receptor binding domain can activate the same conformational changes and proteolytic processing of the S protein *in vitro* which are normally triggered by binding to the human DPP4 receptor and thus enhance viral uptake.[75] Besides SARS-CoV and MERS-CoV also feline coronavirus (Feline CoV) S glycoprotein has also been associated with ADE.[76]

Whether any of these concerns hold for SARS-2-S antigen is under debate. It has been proposed that ADE could account for the severity of COVID-19 cases in patients previously infected with other coronaviruses.[77] In this context it is also mentioned that elderly people, who for unknown reasons have a significantly higher risk for severe courses of COVID-19, concurrently have a higher likelihood of previous contact with other, less harmful coronaviruses and thus might experience SARS-CoV-2 ADE.[78] Cross-reactivity and cross-neutralization of antibodies against the S proteins of SARS-CoV-2 with SARS-CoV and other coronaviruses are under close investigation but so far no final conclusions can be drawn. Encouraging results from severely diseased COVID-19 patients treated with plasma from convalescent patients and showing no severe adverse events on the other hand oppose the fears of ADE effects in the context of SARS-CoV-2 infections.[79,80] Furthermore, data from SARS-CoV-2 vaccine studies in macaques showed the development of high titers of spike-specific antibodies and protection against challenge infection without increasing lung pathology or ADE.[81,82] By now, at least 44 candidate

vaccines have entered different phases of clinical evaluation[d] and the ClinicalTrials.gov platform alone lists almost 200 vaccine trials in post-recruiting state (accessed on 2020-10-27). Considering the ongoing spread of SARS-CoV-2 all over the world and the therefore considerably high risk of infection for participants of clinical trials, the absence of reports describing ADE effects can definitely be interpreted as an encouraging signal.

The Coalition of Epidemic Preparedness Innovation (CEPI), the Food and Drug Administration (FDA; U.S. Department of Health and Human Services) and others have developed guidance to product developers to not delay phase I clinical testing of vaccines but to study Enhanced Respiratory Disease (ERD) prior to the start of phase II trials.[e] We have studied potential disease enhancement in two different preclinical challenge models: hACE2-transduced BALB/c mice and naturally susceptible ferrets (see chapter 4.1). In both setups, the dose of MVA-SARS-2-S used to vaccinate the animals corresponded to the full human dose. **Neither in mice nor in ferrets could we detect any evidence for disease enhancement after challenge with SARS-CoV-2 compared to unvaccinated control animals.** Instead, vaccinated BALB/c mice showed a significant immune response and reduced viral loads compared to control animals and i.n. immunized ferrets could no longer transmit the disease to contact animals. Furthermore, **the recombinant MVA vaccine platform has in general been shown to elicit both antigen-specific humoral and cellular immune responses[22] and therefore is not suspected to trigger an enhanced disease following vaccination**.

ERD might be relevant to vaccinees upon natural infection with SARS-CoV-2.[83] However, careful evaluation of all available information regarding ADE for various viral diseases in general as well as specific information obtained in the context of SARS-CoV, MERS-CoV and SARS-CoV-2 infectious *in vitro* and *in vivo* models clearly support the investigation of experimental COVID-19 vaccine candidates in human trials without undue delay.[84]

[d] https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines accessed on 2020-07-27

[e] https://www.fda.gov/media/139638/download accessed on 2020-07-13

5 Effects in human

5.1 Experiences with MVA Vaccination in Humans

As alluded to before, recombinant replication deficient MVA has been used clinically for decades by now. Back in 1978, Mayr and coworkers reported the successful vaccination of >120,000 individuals with the replication deficient smallpox vaccination strain MVA.[20] Bavarian Nordic, a private sector firm, developed MVA-BN® as a smallpox vaccine and registered the product in the EU (IMVANEX®) and Canada (IMVAMUNE®). Based on company information[f], MVA-BN was tested extensively in more than a dozen clinical trials involving more than 7,600 individuals including at least 1,000 individuals with a compromised immune system. A significant body of literature from various applications of the MVA platform across indications and patient groups backs the efficacy and safety claims. **Use of replication deficient MVA has never been associated with vaccine-related severe adverse events and is generally considered safe**.[85,86] This includes clinical experience from vaccinating risk groups including HIV patients[87,88], cancer patients[89], the vulnerable patient group of pre-school children[90] and further includes scenarios where MVA-based therapeutic vaccination was co-administered with chemotherapy[91]. A query for MVA in relevant databases resulted in the following number of hits (Table 7).

Table 7: Body of literature and clinical trial records for MVA based vaccines

Database	Query	Number of hits
Pubmed (accessed 2020-10-23)	Modified vaccinia virus Ankara	1,101
	Modified vaccinia virus Ankara, article type 'Clinical Trial'	142
Clinicaltrials.gov (accessed 2020-10-23)	Modified vaccinia virus Ankara	61
	MVA	310
	MVA 'with results'	43

We herein summarize and tabulate the literature references retrieved from the Pubmed database (see Table 17 in the appendix). We also refer to three articles published on the MVA vaccine platform and summarize the key features as follows.[85,86,92]

- **no single vaccine-related SAE** has been reported therein
- adverse events are being reported and include **local and systemic reactogenicity**, which is frequent, mild-to-moderate in nature and clearly dose-dependent; of the tested routes the **intramuscular administration** seems to strike a good balance of immunogenicity and reduced incidence of local events
- **local events** are indurations, pain and redness at the injection site
- **systemic events** include headache, myalgia, flu-like symptoms, fever and fatigue
- all AEs reported are transient and resolve spontaneously
- typical **dose ranges** for MVA vaccines are 1x 10^6 to 1x 10^9 PFU (alternatively $TCID_{50}$), more frequently doses of 1x 10^7 to 2.5x 10^8 PFU are chosen; the trend towards higher

[f] http://www.bavarian-nordic.com/what-we-do/technology/mva-bn.aspx

immunogenicity of doses of 1x 10^8 PFU and higher is accompanied by an increased incidence of reactogenicity
- **Immune responses** to MVA vaccines are generally **multimodal**, including humoral and cell-based immunity, addressing the CD4+ and CD8+ cell compartments; however, immunogenicity is antigen and context dependent and certain indications like HIV, tuberculosis and malaria are notorious for the weak immunogenicity of their antigens or transient immune responses
- a heterologous **prime and boost scheme** is frequently applied where a strong cell-based immunity is targeted; here, DNA vector vaccines are frequently used as prime with MVA used for boosting immunity after approximately 28 days of priming; more recently, ChAd-63 based priming vaccines have been tested with good outcomes; previously also fowl pox virus vectored vaccines have been used in combination with MVA vaccines
- a typical human **immunization schedule** with MVA based vaccines for prime and boost involved two or three injections at intervals of typically four weeks

With local injection site findings being poorly represented by the preclinical rodent model we extracted representative information from recent literature and summarize the findings as follows:

- for ACAM3000 MVA and doses of 1x 10^6 to 1x 10^8 $TCID_{50}$, a direct comparison of administration routes revealed severe local reactogenicity (discomfort, erythema, induration at injection site) for 8/20 study participants in the i.d. route, for 5/20 participants in the s.c. route and for 0/20 participants in the i.m. route; no serious AE; symptoms resolve within four to seven days[93]
- for MVA-NP+M1 and an equal dose of 5x 10^7 PFU, a head-to-head comparison of i.d. and i.m. administration routes revealed frequent mild-to-moderate local adverse events, with local symptoms (erythema, swelling, warmth) being more frequent for the i.d. route than the i.m. route except pain, which is reported invariantly by 90 % of study participants; no serious AEs reported by the authors; all symptoms resolve spontaneously within two to three days after immunization[93]
- for MVA-H5-sfMR and doses of 1x 10^7 or 1x 10^8 PFU with one or two administrations by the i.m. route, between 20 % and 90 % of study participants reported mild to moderate local reactions in the form of itch, redness or swelling; between 10 % and 45 % reported severe local pain; no serious AE; local reactions resolve within six days after immunization[37]

The vaccine-related information presented herein was used to inform the study protocol of our phase II study.

5.2 Safety and Immunogenicity of the Vaccine MVA-MERS-S

The design of MVA-SARS-2-S is based on MVA-MERS-S, an experimental vaccine developed in 2013 to prevent Middle East Respiratory Syndrome (MERS).[24] The first generation of MVA-MERS-S was produced on CEF cells by IDT Biologika GmbH in Dessau, Germany. In 2017/2018 the vaccine was submitted to a single-center open-label phase I clinical trial to assess safety, tolerability and immunogenicity of a homologous 28-day prime-boost regimen with promising results (ClinicalTrial.gov: NCT03615911).[27]

In total 26 healthy adults aged 18-55 years were enrolled and 23 completed the study. A previous MVA vaccination was a key exclusion criterion. The primary objectives were safety and tolerability of the two dosage levels 1x 10^7 PFU and 1x 10^8 PFU and reactogenicity after administration. In the monitoring

of acute reactogenicity following immunizations, no serious adverse events (SAE) were reported. A total of 178 vaccine-related AE were reported in 20/26 (77 %) of the study participants. The majority was solicited and mild, with a few moderate and no severe AE. Most AE appeared early after administration of the vaccine. The HD showed more AE after prime and boost immunizations compared to the LD cohort. All AE resolved quickly and generally required no treatment. Local reactions (pain, swelling and induration) were the most common AE and were observed in 65 % (17/26), 38 % (10/26) and 38 % (10/26) of participants, respectively. Headaches (62 %, 16/26) and fatigue/malaise (65 %, 17/26) were the most common systemic AE.

An ELISA was used to monitor binding antibody responses against MERS-CoV-S1 throughout the study. The value 0.5 OD was defined as seropositivity threshold. Seroconversion was detected in 87 % (20/23) of all vaccinees; with 75 % (9/12) and 100% (11/11) in the LD and HD cohorts at any time point throughout the study, respectively.

Titers of neutralizing antibodies were assessed using the VNT assay and the $PRNT_{80}$ assay. For both assays, live MERS-CoV (EMC/2012 isolate) was used. At any time point throughout the study, sera from 58 % (12/23) of all vaccinees neutralized MERS-CoV using VNT. A single injection of MVA-MERS-S showed no induction of neutralizing antibodies. Only after boosting, the VNT test revealed detectable neutralizing capacity, but mainly in vaccinees from the LD cohort, with a peak response on day 42, when seven participants (58 %, 7/12) of the LD cohort reacted positively, compared to two vaccinees from the HD cohort (18 %, 2/11). In the more sensitive PRNT80 assay, antibody responses mirrored a similar pattern as observed using the ELISA. A single vaccine administration induced neutralizing antibodies only in one vaccinee of the LD cohort. After boost immunization, the LD cohort showed responses in 75 % vaccinees (9/12) compared to 82 % (9/11) in the HD cohort. Boosting elicited a significant increase of neutralizing antibody responses at day 42 compared to baseline in both cohorts. A strong correlation between binding and neutralizing antibodies was observed at day 35 post prime immunization.

MERS-CoV-S-specific T-cell responses were evaluated by IFN-γ-ELISpot, using five overlapping peptide pools. T-cell responses against MERS-CoV-S already emerged after a single shot of MVA-MERS-S in some of the vaccinees and were enhanced after boost immunization. Overall, IFN-γ secretion was detected in 87 % (20/23) of all participants at one or more time points throughout the study. Taking into account the number of assay responders over time, both dose cohorts showed a similar pattern: the earliest assay responders were already identified at day 28. At day 180, the LD cohort still showed positive T-cell responses in 55 % (6/11), while the HD showed a response in only 9 % (1/11).

5.3 Safety Data for the Vaccine MVA-SARS-2-S

A single-center open-label phase I clinical trial is currently running to evaluate safety, tolerability and immunogenicity of two intramuscular dose administrations and two ascending dose levels of MVA-SARS-2-S in healthy adults aged 18 to 55. Key exclusion criteria included evidence of an active COVID-19 infection and previous rMVA immunization. Inclusion and exclusion criteria are listed on ClinicalTrial.gov NCT04569383. The study design was reviewed and approved by the competent national authority (Paul-Ehrlich-Institute) and the Ethics Commission of the Hamburg Medical Association. The vaccine was manufactured by IDT Biologika GmbH in Dessau, Germany.

The primary objectives are safety, tolerability and reactogenicity of the two dosage levels after administration. SARS-CoV-2-S-specific antibody response is assessed as a secondary endpoint.

Participants receive two doses of either 1x 10^7 + 0.5 log IU (low dose cohort, n=15) or 1x 10^8 + 0.5 log IU (high dose cohort, n=15) of MVA-SARS-2-S.Prior to prime and boost immunization, each individual underwent physical examination, drug and pregnancy testing and received an electrocardiogram at screening. Heart rate, blood pressure and body temperature were recorded. Participants were monitored for four hours after immunization. In addition, blood was drawn to perform clinical, chemical and haematological safety as well as immunogenicity analyses. A Local Safety Board (LSB) reviewed the safety data (adverse events vital signs and laboratory results) collected up to day 7 after the 1st vaccination. Based on the data set, the LSB decided on dose escalation to the higher planned dosed and on the administration of the second dose in both dose cohorts, planned to be administered on day 28 after prime vaccination.

At the time of IB generation, the study has completed recruitment. All participants who were enrolled in the study (n=30) completed both vaccinations and are currently followed-up for safety and immunogenicity analyses until day 168, which marks the end of study visit. After all participants from both dose cohorts had received both vaccinations, an interim analysis of the safety data was performed (January 28, 2021). The IMP was well-tolerated in both dose cohorts and no severe or serious adverse events were observed. In total, 24/30 participants reported a total of 162 adverse events, of which 89 were considered related to the IMP. 83/89 were of mild, 6/89 of moderate severity. Related adverse events were almost exclusively solicited and consisted of local reactions, headaches, fatigue/malaise, arthralgia, myalgia, gastrointestinal symptoms, fever and chills. Dry mouth was reported by one vaccinee after both vaccinations and, therefore, marked an unsolicited adverse event, which was considered to be possibly related to the IMP and which was of mild nature. In the high dose cohort, one participant developed COVID-19 after the second vaccination with MVA-SARS-2-S, which was classified as mild and was treated in an outpatient setting. Vital signs, temperature, as well as laboratory results, including hematology and clinical chemistry values (complete blood count, electrolytes, liver and renal function values, C-reactive protein, and others) were assessed during all study visits and did not reveal any clinically significant findings.

6 Summary of data and guidance for the investigator

In order to arrive at a balanced risk/benefit assessment of the clinical investigation of MVA-SARS-2-ST as a prophylactic vaccine, the various risk dimensions should be dissected and risk mitigation strategies developed. In general alignment with our study protocol, we plan to enroll volunteers in stable health conditions including a subset of people with a previous SARS-CoV-2 infection. The phase Ib/IIa study aims to evaluate safety, tolerability and immunogenicity of the IMP. These Individuals might have a health benefit of the trial as SARS-CoV-2 is still causing an ongoing pandemic including an increasing number of active cases in Germany. Effective risk mitigation is of paramount importance. Tables 8 to 10 summarize identified risk factors of the use of MVA-SARS-2-ST and the suggested mitigation strategies by categories individual risks, virus contact risks, and environmental risks.

Table 8: MVA-SARS-2-ST risk analysis and mitigation, individual risks

Risk	Risk evaluation	Identified risk mitigation strategy	Suggested implementation
Uncontrolled MVA-SARS-2-ST virus replication	No risk; profound replication deficiency of MVA-F6 parental virus and study medication well documented	None	N/A
Reversion to pathogenic MVA	No risk; major genomic deletions lead to replication deficiency	None	N/A
SARS-2-S glycoprotein associated enhanced pathology	Very low risk; enhanced disease considered a theoretical risk; effects only seen under narrow experimental conditions	Careful selection of study subjects in stable health conditions (in- and exclusion criteria)	Monitoring, emergency phone numbers, follow-up
Local and systemic reactogenicity	Likely and frequent, mild-to-moderate and transient	NSAIDs	Monitoring, emergency phone numbers
Type 1 hypersensitivity reactions	Standard	Advanced life support drugs and resuscitation equipment	Immunization in fully equipped CTU, staggered dosing on both dose groups
AEs & SAEs (solicited and unsolicited)	IMP-related mild and moderate AE expected, low risk for severe AE and SAE	Open-label run-in study design, safety holding rules, monitoring, DSMB	Dose escalation after safety call, safety holding rules, monitoring, DSMB

Table 9: MVA-SARS-2-ST risk analysis and mitigation, virus contact risk

Risk	Risk evaluation	Identified risk mitigation strategy	Suggested implementation
Vaccine virus spread by unintended contact with vaccine	Very low risk; MVA is replication deficient	Cover injection site to absorb leaking virus	Dressing of injection site
		Inactivation by detergent in case of surface contamination	Implementation of hygiene plan
Vaccine virus spread by viral shedding	No risk; MVA is replication deficient	None	N/A

Table 10: MVA-SARS-2-ST risk analysis and mitigation, environmental risk

Risk	Risk evaluation	Identified risk mitigation strategy	Suggested implementation
Viral spread by shedding of MVA-SARS-2-ST	No risk; no shedding expected due to replication deficiency in study subjects; few (avian) cells permissible in terms of virus propagation (as such not available in environment)	None	N/A
Viral spread by blood-feeding insects	No risk	None	N/A

In conclusion, the relevant risk dimensions, in particular to study subjects can be effectively addressed at the level of the study protocol. **Given the pandemic spreading of SARS-CoV-2, the lack of effective treatment or prevention of COVID-19 and the severity of the disease, the present experimental vaccine MVA-SARS-2-ST deserves to be tested for safety, tolerability and immunogenicity in humans.**

7 Publications and Reports

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8 Appendix

Table 11: Histopathological findings, summary data

Main groups											Sex: female		
Group		1						2			3		
Treatment		Vehicle						MVA-SARS-2-S prime			MVA-SARS-2-S prime boost		
Dose								10E8 pfu/animal			10E8 pfu/animal		
Day of necropsy		7			35			7			35		
Grading		.	1	2	.	1	2	.	1	2	.	1	2
Organs:	Microscopic findings:												
Adrenal gland	No finding(s)	6/6	0/6	0/6	12/12	0/12	0/12	12/12	0/12	0/12	18/18	0/18	0/18
Kidney, L	No finding(s)	6/6	0/6	0/6	12/12	0/12	0/12	12/12	0/12	0/12	18/18	0/18	0/18
Liver	Inflammatory cells, multifocal	5/6	1/6	0/6	11/12	1/12	0/12	12/12	0/12	0/12	17/18	1/18	0/18
Lungs	Prominent BALT	0/6	0/6	0/6	12/12	0/12	0/12	12/12	0/12	0/12	17/18	1/18	0/18
Ln. subiliacus, L	Lymphoid hyperplasia	0/6	0/6	0/6	12/12	0/12	0/12	2/12	10/12	0/12	6/18	12/18	0/18
Ln. ischiadicus, L	Lymphoid hyperplasia	0/6	0/6	0/6	12/12	0/12	0/12	5/12	7/12	0/12	10/18	8/18	0/18
Ln. popliteus, L	Lymphoid hyperplasia	0/6	0/6	0/6	12/12	0/12	0/12	0/12	12/12	0/12	2/18	16/18	0/18
Ln. iliacus medialis, L	Lymphoid hyperplasia	0/6	0/6	0/6	12/12	0/12	0/12	1/12	11/12	0/12	1/18	17/18	0/18
Thymus	No finding(s)	0/6	0/6	0/6	12/12	0/12	0/12	12/12	0/12	0/12	18/18	0/18	0/18
Spleen	No finding(s)	0/6	0/6	0/6	12/12	0/12	0/12	12/12	0/12	0/12	18/18	0/18	0/18
Site of administration, L	Mixed cell infiltration, interstitial, multifocal	0/6	0/6	0/6	12/12	0/12	0/12	9/12	3/12	0/12	3/18	13/18	2/18
Site of administration, L	Necrosis, myofibres	0/6	0/6	0/6	12/12	0/12	0/12	12/12	0/12	0/12	16/18	2/18	0/18
Site of administration, L	Degeneration, myofibres	5/6	1/6	0/6	7/12	5/12	0/12	0/12	12/12	0/12	0/18	18/18	0/18

No. of animals affected / total No. of animals. Abbreviations: ., 1, 2 = normal, minimal to mild, moderate alteration; L = left site; Ln. = Lymphonodus.

Table 12: Histopathological findings, individual data, vehicle group, day 7 (prime only)

Main groups		Day of necropsy: 7					Sex: female
Group		1					
Treatment		Vehicle, prime					
Dose							
Organs:	Microscopic findings:	Animal ID					
		121	122	123	124	125	126
Adrenal gland	No finding(s)	.	.	.	.	.	.
Kidney, L	No finding(s)	.	.	.	.	.	.
Liver	Inflammatory cells, multifocal	.	1	.	.	.	.
Lungs	Prominent BALT	.	.	.	.	.	.
Ln. subiliacus, L	Lymphoid hyperplasia	.	.	.	M	.	.
Ln. ischiadicus, L	Lymphoid hyperplasia	.	M	.	.	.	.
Ln. popliteus, L	Lymphoid hyperplasia	.	.	.	.	.	.
Ln. iliacus medialis, L	Lymphoid hyperplasia	.	.	.	.	.	.
Thymus	No finding(s)	.	.	.	.	.	.
Spleen	No finding(s)	.	.	.	.	.	.
Site of administration, L	Mixed cell infiltration, interstitial, multifocal	.	.	.	.	.	.
Site of administration, L	Necrosis, myofibres	.	.	.	.	.	.
Site of administration, L	Degeneration, myofibres	.	.	.	.	1	.

Abbreviations: ., 1, 2, 3, 4 = normal, minimal to mild, moderate, marked or severe alteration; M = missing organ; L = left site; Ln. = Lymphonodus.

Table 13: Histopathological findings, individual data, vehicle group, day 35 (prime-boost)

Main groups		Day of necropsy: 35											Sex: female
Group		1											
Treatment		Vehicle, prime boost											
Dose													
Organs:	Microscopic findings:	Animal ID											
		127	128	129	130	131	132	133	134	135	136	137	138
Adrenal gland	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Kidney, L	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Liver	Inflammatory cells, multifocal	.	.	.	.	.	1	.	.	.	.	.	.
Lungs	Prominent BALT	.	.	.	.	.	.	.	.	.	.	.	.
Ln. subiliacus, L	Lymphoid hyperplasia	.	.	.	.	.	.	M	M	M	M	.	.
Ln. ischiadicus, L	Lymphoid hyperplasia	.	M	.	M	.	.	.	M	.	.	.	.
Ln. popliteus, L	Lymphoid hyperplasia	M	.	.	.	.	.	.	.	.	.	.	.
Ln. iliacus medialis, L	Lymphoid hyperplasia	.	.	.	.	.	.	.	.	.	M	.	.
Thymus	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Spleen	No finding(s)	M	M	M	M	.	.	M	M	M	M	M	M
Site of administration, L	Mixed cell infiltration, interstitial, multifocal	.	.	.	.	.	.	.	.	.	.	.	.
Site of administration, L	Necrosis, myofibres	.	.	.	.	.	.	.	.	.	.	.	.
Site of administration, L	Degeneration, myofibres	1	1	.	1	1	.	.	.	1	.	.	.

Abbreviations: ., 1, 2, 3, 4 = normal, minimal to mild, moderate, marked or severe alteration; M = missing organ; L = left site; Ln. = Lymphonodus.

Table 14: Histopathological findings, individual data, MVA-SARS-2-S, day 7 (prime only)

Main groups		Day of necropsy: 7											Sex: female
Group		2											
Treatment		MVA-SARS-2-S prime											
Dose		10E8 pfu/animal											
Organs:	Microscopic findings:	Animal ID											
		221	222	223	224	225	226	227	228	229	230	231	232
Adrenal gland	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Kidney, L	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Liver	Inflammatory cells, multifocal	.	.	.	.	.	.	.	.	.	.	.	.
Lungs	Prominent BALT	.	.	.	.	.	.	.	.	.	.	.	.
Ln. subiliacus, L	Lymphoid hyperplasia	1	1	1	1	1	1	M	M	1	1	1	1
Ln. ischiadicus, L	Lymphoid hyperplasia	M	.	.	1	.	.	1	1	1	1	1	1
Ln. popliteus, L	Lymphoid hyperplasia	1	1	1	1	1	1	1	1	1	1	1	1
Ln. iliacus medialis, L	Lymphoid hyperplasia	1	1	1	1	1	M	1	1	1	1	1	1
Thymus	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Spleen	No finding(s)												
Site of administration, L	Mixed cell infiltration, interstitial, multifocal	.	.	.	1	.	.	.	.	.	1	1	.
Site of administration, L	Necrosis, myofibres	.	.	.	.	.	.	.	.	.	.	.	.
Site of administration, L	Degeneration, myofibres	1	1	1	1	1	1	1	1	1	1	1	1

Abbreviations: ., 1, 2, 3, 4 = normal, minimal to mild, moderate, marked or severe alteration; M = missing organ; L = left site; Ln. = Lymphonodus.

Table 15: Histopathological findings, individual data, MVA-SARS-2-S, day 35 (prime-boost), animals 321-332

Main groups		Day of necropsy: 35											Sex: female
Group		3											
Treatment		MVA-SARS-2-S prime boost											
Dose		10E8 pfu/animal											
Organs:	Microscopic findings:	Animal ID											
		321	322	323	324	325	326	327	328	329	330	331	332
Adrenal gland	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Kidney, L	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Liver	Inflammatory cells, multifocal	.	.	.		1	.	.	.	.	.	.	.
Lungs	Prominent BALT	.	.	.	.	.	.	.	.	.	1	.	.
Ln. subiliacus, L	Lymphoid hyperplasia	1	.	M	1	1	1	M	1	1	1	1	1
Ln. ischiadicus, L	Lymphoid hyperplasia	M	.	.	.	.	.	.	1	1	.	1	1
Ln. popliteus, L	Lymphoid hyperplasia	.	1	1	1	1	1	1	1	1	1	1	1
Ln. iliacus medialis, L	Lymphoid hyperplasia	1	.	1	1	1	1	1	1	1	1	1	1
Thymus	No finding(s)	.	.	.	.	.	.	.	.	.	.	.	.
Spleen	No finding(s)	M	M	M	M	.	.	.	.	.	.	.	.
Site of administration, L	Mixed cell infiltration, interstitial, multifocal	.	.	.	1	1	1	1	1	1	1	1	2
Site of administration, L	Necrosis, myofibres	.	.	.	.	.	.	.	.	.	1	.	1
Site of administration, L	Degeneration, myofibres	1	1	1	1	1	1	1	1	1	1	1	1

Abbreviations: ., 1, 2, 3, 4 = normal, minimal to mild, moderate, marked or severe alteration; M = missing organ; L = left site; Ln. = Lymphonodus.

Table 16: Histopathological findings, individual data, MVA-SARS-2-S, day 35 (prime-boost), animals 333-338

Main groups		Day of necropsy: 35					Sex: female
Group		2					
Treatment		MVA-SARS-2-S prime boost					
Dose		10E8 pfu/animal					
Organs:	Microscopic findings:	Animal ID					
		333	334	335	336	337	338
Adrenal gland	No finding(s)	.	.	.	.	.	.
Kidney, L	No finding(s)	.	.	.	.	.	.
Liver	Inflammatory cells, multifocal	.	.	.	.	.	.
Lungs	Prominent BALT	.	.	.	.	.	.
Ln. subiliacus, L	Lymphoid hyperplasia	1	M	1	M	1	M
Ln. ischiadicus, L	Lymphoid hyperplasia	1	M	1	1	1	.
Ln. popliteus, L	Lymphoid hyperplasia	1	1	.	1	1	1
Ln. iliacus medialis, L	Lymphoid hyperplasia	1	1	1	1	1	1
Thymus	No finding(s)	.	.	.	.	.	.
Spleen	No finding(s)	M	M	M	M	M	M
Site of administration, L	Mixed cell infiltration, interstitial, multifocal	1	2	1	1	1	1
Site of administration, L	Necrosis, myofibres	.	.	.	.	.	.
Site of administration, L	Degeneration, myofibres	1	1	1	1	1	1

Abbreviations: ., 1, 2, 3, 4 = normal, minimal to mild, moderate, marked or severe alteration; M = missing organ; L = left site; Ln. = Lymphonodus.

Table 17: Tabulated summary of Pubmed hits for MVA, article type 'Clinical Trial'

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
MVA-MERS-S	MERS	I	$1x10^7$ PFU $1x10^8$ PFU i.m.	Humoral and cell-mediated immune responses	No safety concerns	NCT03615911/ 32235883
Ad26.Mos.HIV MVA-Mosaic	HIV	I/IIa, n=27	2x $5x10^{10}$ vp Ad26.Mos.HIV i.m. at weeks 0 and 12 and 2x 10^8 PFU MVA-Mosaic at weeks 24 and 48	delayed time to viral rebound compared to that in placebo recipients by only several days	No safety concerns	NCT02919306/ 32235883
MVA-BN/ ACAM2000	Smallpox	III, n=440	2x MVA-BN ($1x10^8$ $TCID_{50}$) s.c. at weeks 0 and 4 and 1x ACAM2000 ($2.5\text{-}12.5x10^5$ PFU) scarification at week 8 or 1x ACAM2000 ($2.5\text{-}12.5x10^5$ PFU) scarification at week 0	MVA-BN immunogenic, MVA-BN protects against ACAM2000 challenge	No safety concerns	NCT01913353/ 31722150
MVA85A	Tuberculosis	I, n=37	$5x10^7$ PFU at day 0 and 28; prime by aerosol (group 1) or i.d. (groups 2 & 3) and boost by aerosol (group 2) or i.d. (group 1 & 3)	Aerosol vaccination induced potent cellular Ag85A-specific mucosal and systemic immune responses	No serious adverse events, AE profile induced by aerosolised MVA85A as a boost vaccination 1 month after intradermal prime does not support the further development of this specific vaccination regimen.	NCT01954563/ 31039172
PanAd3-RSV MVA-RSV	Respiratory syncytial virus	I,.n=30 aged 60–75 years	10^8 PFU MVA-RSV prime only, i.m.; 10^8 PFU PanAd3-RSV at week 0 and 4, i.m.; 10^8 PFU PanAd3-RSV i.m. at week 0 and 10^8 PFU MVA-RSV i.m. at week 8,; 10^8 PFU PanAd3-RSV i.n. at week 0 10^8 PFU MVA-RSV i.m. at week 8	Induction of RSV-specific humoral and cellular immunity	Favorable safety and tolerability	-/30742894
AdCh3NSmut1 MVA-NSmut ChAdV63.HIVco MVA.HIVconsv	HCV HIV	I, n=33	$2.5x10^{10}$ vp AdCh3NSmut1 at week 0 and $2x10^8$ PFU MVA-NSmut at week 8 or $5x10^{10}$ vp ChAdV63.HIVconsv at week 0 and $2x10^8$ PFU MVA.HIVconsv at week 8 or $2.5x10^{10}$ vp AdCh3NSmut1 and $5x10^{10}$ vp	High magnitude and broad T cell responses: polyfunctional CD4+ and CD8+ T cells	No vaccine-related serious adverse events	NCT02362217/ 30713538

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
			ChAdV63.HIVconsv at week 0 and $1x10^8$ PFU MVA-NSmut and $1x10^8$ PFU MVA.HIVconsv at week 8 , i.m.			
MVA E2	Recurrent respiratory papillomatosis	I/II, n=29 RRP patients	10^7 vp injected into papilloma lesions at weeks 0, 2, 4 and 6	Lesions completely eliminated in 44.8%; lesions recurred in 55.2% after prime, no recurrences after second treatment	No adverse events	-/ 30605254
ChAd63 MVA ME-TRAP	Malaria	IIb, n=700 children aged 5-17 months		Moderate T cell responses, no significant protective efficacy	Safe	-/ 30540808
DNA-MVA-rgp140/GLA	HIV	II, n= 191	HIV-DNA i.d. priming at weeks 0, 4 and 12; boosting with10^8 PFU HIV-MVA twice, alone or combined with CN54rgp140/GLA-AF, i.m.		No safety concerns	-/ 30496299
p53MVA + pembrolizumab	Advanced solid cancers	n=11	up to 3 triweekly vaccines in combination with pembrolizumab	Clinical responses in 3/11 patients	One patient had a grade 5 fatal myocarditis related to pembrolizumab and possibly related to the vaccine. After the study was amended for enhanced cardiac monitoring, no additional cardiac toxicities were noted.	-/ 30094792
Ad26/Ad26 + gp140 HIV-1	HIV	I/IIa, n= 393 + parallel study in rhesus monkeys, n= 72	Priming at weeks 0 and 12 with Ad26.Mos.HI-V; boosting at weeks 24 and 48 with Ad26.Mos.HIV or MVA i.m.	Comparable and robust immune responses in humans and rhesus monkeys	Favorable safety and tolerability.	NCT02315703/ 30047376
rMVA-H5	Influenza A(H5N1)	I/IIa, n=79	1 or 2 immunizations 10^7 or 10^8 PFU, i.m.	Broadly reactive antibody responses		-/ 29912453
MVA.mos1 and MVA.mos2	HIV	I, n=25	10^8 PFU, i.m.	Multiclade HIV-1-specific immune responses were elicited	Safe and generally well tolerated, no vaccine-related SAEs	-/ 29669026
ChAd63-MVA RH5	Malaria	Ia, n=24	i.m.	RH5-specific responses have been induced	No SAEs or unexpected reactions and no safety concerns	-/ 29093263
late MVA-B boost	HIV	n=13	10^8 PFU	Moderate increases in HIV-specific T cell responses in 38% of volunteers	Safe	-/ 29065142

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
HIV-DNA priming + HIV-MVA boosts	HIV	n=24	600 µg or 1,200 µg of HIV-DNA, boosts of 10^8 PFU HIV-MVA		Safe and well tolerated	-/ 28969431
MVA-brachyury-TRICOM vaccine	Advanced cancer	I, n = 38		Activates brachyury-specific T cells *in vitro* and in patients	Can be administered safely	NCT02179515 / 28855356
Clade B HIV-1 vaccine combination	HIV	I, n=48		Durable, functional humoral responses	Well tolerated and safe	NCT01571960 / 28727817
MVA85A	Tuberculosis			No long-term effectiveness		-/ 28633702
MVA.HIVconsv	HIV	N=19	5.5×10^7 PFU, or 2.2×10^8 PFU,	Modest immunogenicity	Safe	NCT01024842 / 28537062
MVA-BN-Filo & Ad26-ZEBOV	Ebola virus	I	Prime Ad26-ZEBOV Boost MVA-BN-Filo: $1x10^8$ PFU. Or Prime MVA-BN-Filo and Boost: Ad26-ZEBOV	Immunogenicity	Immunogenic profile	28291882
MVA vaccine	Smallpox	II, n= 435		Does not support alternative strategies of administering MVA vaccine by syringe and needle on compressed schedules or administration by jet injector on the standard schedule		NCT01827371 / 28256358
TG4023 (MVA-FCU1)	Liver tumors	I, n=16	10^7 PFU, 10^8 PFU or $4x10^8$	Feasible	Well tolerated	NCT00978107 / 28177438
ChAd63 ME-TRAP, MVA ME-TRAP	Malaria	IIb, n=120	priming $5x10^5$ viral particles, booster $2x10^8$ PFU	Efficient and cost-effective clinical trial design	No SAEs related to the vaccination	-/ 27978537
MVA-BN	Atopic eczema and allergic rhinitis	I, n=60	s.c.		Good safety profile	-/ 27357167
Triplex vaccine	CMV	I, n=24	3 doses i.m.	Expansion of durable CMV-specific T cells with potential for viremia control	Safe	-/ 27760761
GOVX-B11	HIV	I, n=9		Major limitations for therapeutic vaccination	Safe and well tolerated	NCT01378156 / 27711228
SAAVI DNA-C2, SAAVI MVA-C and Novartis gp140 with MF59 adjuvant in various combinations	HIV	I, n=184		Induced neutralizing and binding antibodies and cellular immune responses	Safe	NCT01418235 / 27583368
MVA-BN®	Smallpox	II, n=120	s.c.	Immunogenic	Safe	NCT00857493 / 27327616
RTS,S/AS01$_B$, ChAd63 ME-TRAP, MVA ME-TRAP	Malaria	IIa, N=37	i.m.	Significantly reduced risk of infection over controls	No SAEs related to vaccination	NCT01883609 / 27307573

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
Boosting with CN54rgp140/GLA-AF	HIV	I, n=40	2 doses, i.m.	Enhances Immune Responses	Safe	-/ 27192151
ChAd63 and MVA ME-TRAP	Malaria	I, n=138		Strong T-cell responses	No vaccine related SAEs	-/ 27109630
Boosting with gp140/MF59 (in addition to SAAVI DNA-C2 and SAAVI MVA-C)	HIV	N=27	2 doses, i.m.	Boosting enhanced levels of binding and neutralizing antibodies as well as CD4(+) T-cell responses to HIV-1 envelope	Safe and well tolerated	NCT00574600 / 27098021
Ad26.ZEBOV, MVA-BN-Filo	Ebola virus	I, n=87		At least 86% of vaccine recipients showed Ebola-specific T-cell responses	No SAEs	-/ 27092831
ChAd3-EBO-Z	Ebola virus	I, n=20; Ib, n=91	I: $1x10^{10}$ or $1x10^{11}$ pu Ib: $2,5x10^{10}$or $5x10^{10}$ pu	$1x10^{11}$ pu elicited strong antiglycoprotein antibody responses in all participants	No safety concerns	NCT02231866 and NCT02267109 / 26546548
PanAd3-RSV and (MVA)-RSV	Respiratory syncytial virus	I, n=40	Heterologous and homologous p.b.			NCT01805921 / 26510727
IMVAMUNE	Smallpox	II, n=350	2 doses, s.c.	Ability to elicit vaccinia-specific immune responses	Favorable safety profile	NCT00316602 / 26439129
PanAd3-RSV, MVA-RSV	Respiratory syncytial virus	calf model		Induction of neutralizing antibodies and cellular immunity in calves		-/ 26268314
PanAd3-RSV, MVA-RSV	Respiratory syncytial virus	I, n=42	4 combinations of p.b.	Immunogenic	Safe and well tolerated	NCT01805921 / 26268313
ChAd63, MVA ME-TRAP	Malaria	IIb, n= 121	5×10^{10} viral particles i.m. + 2×10^{8} PFU i.m.	Efficacy was partial	No SAEs	NCT01666925 / 25947165
HIVIS03	HIV	using sera from previously conducted HIVIS03 trial		Elicited potent antibody-dependent cellular cytotoxicity responses		-/ 25874723
MVA85A	TB in HIV-1 infected adults	II, n=650	2 doses i.d.	Immunogenic	Well tolerated	NCT01151189 / 25726088
MVA-B	HIV	n=30	3 or 4 doses	Minor but significant increase in the T cell responses	Safe and well tolerated	NCT01571466 / 25724985
ChAd3, MVA booster	Ebola	I, n=60	1 dose, $1x10^{10}$, $2,5x10^{10}$ or $5x10^{10}$	Elicited B-cell and T-cell immune responses	No safety concerns were identified	NCT02240875 / 25629663
ChAd63 CS alone and with MVA CS	Malaria	Ia. n=24	Heterologous p.b.	Immunogenic	Safe	NCT01450280 / 25522180
ChAd3-NSmut and MVA-NSmut	HCV	I	Heterologous p.b.	Highly immunogenic	Well tolerated	NCT01296451 / 25378645
ChAd63 and MVA ME-TRAP	Malaria	flow cytometry and additional	Heterologous p.b.			-/ 24930599

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
		interferon (IFN)-γ ELISpot data characterizing				
MVA-H5-sfMR	Influenza	I/IIa, n=80	10^7, 10^8 PFU, p.b., i.m.	Immunogenic (10^8>10^7 PFU)	0 SAE, 11/80 AE, local and systemic reactions (10^8>10^7 PFU)	NTR3401/ 25455987
ChAd63+MVA (CS); Cad63+MVA (ME-TRAP)	Malaria	CHMI	Heterologous p.b.	Low level protection only		NCT01623557/ 25336730
PedVacc002 (MVA.HIVA)	HIV	I/II infants, n=73	Single dose i.m.	Low	AE rare	-/ 25173483
MVA85A	TB	I, n=24	i.d. vs aerosol	Immunogenic; i.d. > aerosol	Well tolerated	NCT01497769/ 25151225
MVA-BN (Imvamune)	Atopic dermatitis	I, n=60	P.b., s.c.	100% seroconversion after boost	0 SAE, mild, moderate and transient AEs	-/ 25149431
MVA-EL (EBV)	EBV+ cancer	I, n=16	$5x10^7$, $5x10^8$ PFU, 3 doses, i.d.	8/14 functional T cell responses	Well tolerated and safe	-/ 25124688
DNA+Chad63+ MVA ($MVA.HIV_{consv}$)	HIV	I, n=32		Highly immunogenic	0 SAE, 27/32 grade 1 local and systemic events	NCT01151329/ 25007091
MVA85A	TB	IIb infants		Does not boost BCG effect		-/ 24828094
TG4040 MVA (NS3, 4, 5B)	Chronic HCV	II, n=153	6-7 doses	Up to 64% complete virological response	Tolerated in combination with PEG-INF/RBV	NVT01055821/ 24657484
JS7+MVA/HIV62B	HIV naïve	IIa, n=299	Doses 0,2,4,6, months	Durable humoral and T cell response		-/ 24403557
ChAd+MVA (ME-TRAP)	Malaria tropica	n=14	p.b.	Sterile protection in heterologous challenge in 3/14		-/ 24284865
MVA 2nd boost	HIV	N=24	10^8 PFU	23/24 show T cell and humoral responses	Well tolerated	-/ 24090081
TroVax MVA-5T4	Prostate cancer	II, n=25		6/10 humoral responses	Well tolerated	-/ 23877659
MVA-BN (Imvamune)	Smallpox	N=208	$5x10^7$ $TCID_{50}$, d0,7 vs d0,28; s.c.	T cell and humoral response d0,28>d0,7	30% of patients report moderate to severe reactogenicity	-/ 23664987
ChAd63ME-TRAP+MVA-ME-TRAP	Malaria tropica	Ib, n=46 malaria exposed		Highly immunogenic	Safe	NCT01373879/ 23526949
MVA-BN (Imvamune)	HSCT recipients	N=24	10^7, 10^8 $TCID_{50}$, p.b. d0,28; s.c.	Neutralizing antibodies 10^8>10^7 $TCID_{50}$	Well tolerated at both doses, no SAEs; transient local reactogenicity	NCT00565929 23482644
MVA-85A	TB	IIb infants, n=2797	One dose, i.d.	Modest immunogenicity, no protection afforded	Local adverse events verum > placebo; no single SAE related to MVA85A	NCT00953927/ 23391465
MVA-EL (EBV)	NPC	I, n=18	3 doses, $5x10^7$ and $5x10^8$ PFU; i.d.	15/18 patients with T cell response	No DLTs, safe	-/ 23348421
Advax+MVA.TBC-M4	HIV	N=32, HIV naive		9/9 T cell responses	Safe and well tolerated	23345881
rMVA+rFPV (HIV)	HIV	I, n=150		Anti MVA Nabs with increasing dose		-/ 23142302
MVA-85A	TB	I adults	10^7 or 10^8 PFU	T cell response 10^8 >10^7, broader epitope coverage	0 SAEs, systemic AEs more frequent at higher dose, resolved	-/ 22789508
MVA-VP2	Poultry IBDV					-/22705743

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
DNA+MVA-mBN32	HIV	N=36 Uninfected	Het. p.b. schedule	3/25 HIV epitope Responders after p.b.		-/ 22398243
ChAd-63 (AMA1 3D7)+ MVA (AMA-1 FVO)	Malaria tropica blood-stage antigen	Ia, n=16	Het. p.b. schedule	Highly immunogenic, T cell and humoral responses	Safe	NCT01095055/ 22363582
ChAd-63 (ME-TRAP)+MVA(ME-TRAP) booster	Malaria	I, n=54	Het. p.b. schedule	CD8+ and CD4+ T cell responses	Excellent safety profile	NCT00890019/ 22275401
TG4010 (MVA MUC1 Il-2)	NSCLC in combination chemo-therapy	IIb, n=148	10^8 PFU	TG4010 enhances effect of chemotherapy	Local and systemic AEs in 17/73 patients receiving TG4010	NCT00415818/ 22019520
MVA-B	HIV naïve	I, n=30	3 doses wk0,4,16; 10^8 PFU; i.m.	75% ELISpot responders, strong and durable	164 AEs grade 1-2, 5 AEs grade 3 (not related to vaccination); safe	NCT00679497/ 21907749
ChAd-63 (MSP-1) + MVA (MSP-1)	Malaria	Ia adults	Het. p.b. schedule	Strong T cell responses, CD4+ and CD8+	Safe and generally well tolerated	-/ 21862998
pSG2.HBs+MVA.HBs	Chronic HBV	N=32	Het. p.b. schedule, 5x10^7 or 1.5x10^8 PFU at 3-week intervals, i.d.	No control of HBV infection	Mild local and systemic AEs, no changes in AST or ALT	ISRCTN67270384 / 21347224
GeoVax pGA2/JS7 DNA+MVA.HIV 62	HIV	I	Het. p.b. schedule, 2-3 doses, i.m.	DDMM>DMM>MMM (CD4+ and CD8+ response)	Local and systemic symptoms were mild or moderate	-/ 21282192
MVA-HIV & FPV-HIV	HIV-negative and MVA naive	I, n=150	Up to 10^9 PFU MVA; Het p.b., mo 0,1,3,5,7; n=150	~50% induction of HIV CD8+ and CD4+ T cell responses		-/ 21216311
MVA-NP+M1	Influenza	I,		Highly immunogenic (CD8+ T cells) in high-dose i.m. group	Generally safe and well tolerated; local side effects i.m. <i.d.; systemic side effects increase with dose	-/ 21148512
MVA-CMDR (HIV env, gag, pol)	HIV	I, n=48	10^7 and 10^8 PFU i.m. or 10^6 and 10^7 PFU i.d., mo 0,1,3	Durable cell-mediated and humoral immune responses	Safe and well tolerated, no SAEs	NCT00376090/ 21085591
MVA-CS	Malaria					-/ 20838432
MVA.HIVA	HIV			52% response rate in HIV seronegative and 93% in seropositive patients		-/ 20816902
HIV-1 DNA vaccine + HIV-MVA	HIV	N=38	Het p.b. with MVA at 9 mo	35/38 reactive lymphoproliferation; 32/38 CD4+; 7/38 CD8+ reactive		-/ 20463104
MVA (ACAM3000)	Smallpox	N=72	Two-dose p.b. schedule, d0,28; 10^7 and 10^8 $TCID_{50}$; various routes	NAbs 10^8>10^7 $TCID_{50;}$ Cell-mediated immunity no clear relationship to dose or route	Well tolerated at all doses and by all routes; local reactogenicity i.d. and s.c > i.m.; i.d. may be dose sparing	-/ 20350191
MVA (ACAM3000)+Dryvax	Smallpox	N=36	10^7 $TCID_{50}$ i.d. or i.m.	MVA protects against Dryvax challenge		-/ 20350190
ADMVA	HIV	I, n=50	p.b. schedule mo 0,1,6; 10^7,	Durable cellular and humoral immune response	Study follow-up 18 mo; well tolerated, no SAEs or cardiac events; local	NCT00252148/ 20111599

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
			$5x10^7$, $2.5x10^8$ PFU; i.m.		and systemic reactogenicity in 77/78%, of mild intensity	
DNA+MVA expressing Mel antigens (Mel3)	Melanoma	N=41	One/two doses DNA.Mel3, escalating doses of MVA.Mel3	22/31 CD8+ T cell responses	High dose vaccine well tolerated; flu-like and injection site symptoms	-/ 20043222
MVA-85A	TB	BCG vaccinees, n=36		Potent and durable T cell response	Well tolerated, no vaccine-related SAEs	-/ 20017188
IMVAMUNE	Smallpox	II, n=164	P.b. schedule	94% antibody response at 10^8 TCID50, 100% after boost	Favorable safety profile with local reactions as most frequent observation	-/ 19944151
MVA-TBC-M4	HIV	I, n=32	p.b. schedule mo 0,1,6; $5x10^7$ (LD), $2.5x10^8$ (HD) PFU; i.m.	Dose-dep cellular and humoral immune response directed against Env and Gag	0 SAEs; local and systemic reactogenicity HD>LD in approx. 80% of patients	-/ 19943789
TroVax (MVA-5T4)	Metastatic renal cell cancer	II, n=28		22/23 ITT humoral or cellular immune responses; no clinical benefit	Well tolerated, 0 SAEs	-/ 19561532
HIV-1 DNA-prime MVA-boost			3xprime, 1x boost MVA.HIV-1	Pre-existing immunity to MVA does not prevent immune response		-/ 19450644
MVA.TRYP	Visceral leishmania in dogs					-/ 19386420
TroVax (MVA-5T4)	Metastatic renal cell cancer	I/II, n=11	MVA co-administered with IFNalpha	11/11 patients mount 5T4 antibodies, 5/11 cellular response	Well tolerated, no SAEs	-/ 19342962
TroVax (MVA-5T4)	Metastatic renal cell cancer	II, n=25	MVA co-administered with high dose IL-2, 3 doses wk0,3,6	Stable disease; 25/25 develop 5T4 antibodies, 13/25 develop T cell response	No vaccine related SAEs	ISRCTN83977250 / 19128501
DNA/MVA.TRYP/LACK	Visceral leishmania in dogs	N=22	DNA prime 0.1/1.0 mg d0, $1x10^8$ PFU MVA d28		Safe, no clinical side effects	-/ 19095029
TroVax (MVA-5T4)	Metastatic renal cell cancer	II, n=25	MVA co-administered with IL-2	21/25 mount antibody response, good clinical response rate	No vaccine related SAEs	-/ 19010868
MVA.HIV-1 + FPV.HIV-1	HIV infected on HAART			Increase in HIV-1 specific CD4+ and CD8+ cells	Safe and well tolerated	-/ 18940219
DNA.HIV-1 (Gp-160)+MVA.HIV-1 (Env, Gag, Pol) boost	HIV naïve	N=40		34/37 vaccinees have HIV-specific IFNgamma response (CD4+ and CD8+)	Vaccine-related adverse events were mild and tolerable	ISRCTN32604572 / 18808335
MVA85A	TB	BCG primed subjects		Reduced expression of TGFbeta in PBMCs		-/ 18682270
TG4010 (MVA-Muc1-IL-2)	NSCLC	II, n=65	MVA+cisplatin or MVA +(MVA+cisplatin)	13/37 PR arm 1 (MVA+cisplatin)	TG4010 was well tolerated, mild to moderate injection site and systemic reactions	-/ 18594319
MVA85A	TB (uninfected)	BCG primed subjects, n=24		Induction of potent and	Well tolerated	NCT00460590/ 18582195

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
				durable T cell responses		
TroVax (MVA-5T4)	Prostate cancer	II, n=27	MVA+GM-CSF	24/24 mount antibody response, no objective clinical response	Well tolerated in all patients, no SAEs attributed to vaccine	-/ 18528296
pTHr DNA+MVA.HIV-1		4xphase I		Cell-mediated immune response ~ placebo	Safe and well-tolerated	-/ 18440674
MVA-BN (IMVAMUNE)+ Dryvax challenge	Smallpox	N=90	MVA-BN d0,28 s.c. or i.m.; Dryvax challenge d112	2 doses MVA-BN (p.b.) ~ 1 dose Dryvax; MVA-BN protects from Dryvax cutaneous lesions	Vaccination with MVA-BN was safe and well tolerated	-/ 18036708
MVA85A	TB	I, BCG naïve,	BCG d0, MVA85A d28	Highly immunogenic; MVA boosts BCG primed response	Safe	NCT00427453/ 17957238
FP9(ME-TRAP)+MVA (ME-TRAP)	Malaria tropica	N=405 children age 1-6y	Heterologous p.b.	No clinical efficacy for vaccination program		ISRCTN88335123 / 17710125
TroVax (MVA-5T4)	Colorectal cancer	II, n=17	Administered with chemotherapy	10/11 humoral and cellular 5T4 responses, 6 complete or partial responses	TroVax safe and well tolerated, no SAEs	-/ 17671134
MVA.HIV-1	HIV under HAART	N=10		MVA induced HIV humoral and cellular responses in the majority of individuals		-/ 17604541
MVA E2	HPV infection, condyloma	I/II, n=30	4 weekly doses of 10^6 virus particles injected into the urethra	28/30 elimination of condyloma, no recurrence	No local or systemic adverse events	-/ 17263589
DNA (HIVA) + MVA (HIVA)	HIV naïve	N=192	Heterologous p.b.; $5x10^6$ to $2.5x10^8$ PFU i.d., i.m., s.c.	Infrequent immune response	No SAEs, AEs were mild to moderate; some severe local reactions	-/ 17250931
MVA+Dryvax	Smallpox		2-3 doses of MVA or placebo prime + Dryvax challenge at mo 3	MVA priming reduces extent of Dryvax lesion formation	MVA found safe	-/ 17126963
DNA (ME-TRAP)+MVA(ME-TRAP) vs CS antigen	Malaria tropica	CHMI, n=16	Heterologous p.b., 2 doses DNA +1dose MVA	DDM (ME-TRAP) > DDM (CS); delay to parasitemia (partial protection)	Vaccines well tolerated	-/ 16988273
FP9 (ME-TRAP)+MVA(ME-TRAP) vs CS antigen	Malaria tropica	N=54	Heterologous p.b., alternating immunization	Durable effector functions		-/ 16856208
pGA2/JS2 DNA+MVA	HIV	I, n =30	0.3 or 3 mg JS2, i.m., mo 0,2			-/ 16831092
TroVax (MVA-5T4)	Colorectal cancer	I/II, n=22	p.b. schedule wk 0,4,8, $5x10^7$ to $5x10^8$ PFU	16/17 with cellular and 14/17 with humoral immune response	Well tolerated in all patients, no SAEs	
DNA+MVA (HIVA)	HIV negative	I, n=24	Various heterologous p.b. schedules	Polyfunctional HIV-1 specific T cells in majority of vaccinees		-/ 16641265
FP9 + MVA (ME-TRAP)	Malaria tropica	I, n=22 children	Half or full dose of FP9 priming vector	Strong T cell responses		-/ 16621181

Drug (antigen)	Indication	Phase, n=x	Dose, schedule and route	Efficacy	Toxicology findings and assessment	NCT / PMID
FP9 + MVA (ME-TRAP)	Malaria tropica					-/ 16488059 [Review]
MVA	Melanoma			Melan-A26-35 epitope characterization		-/ 16339586
IMVAMUNE	Smallpox	I, n=86	Different doses and routes, dose up to 10^8 $TCID_{50}$	All 38 subjects in HD group seroconverted	All vaccinations well tolerated with mild to moderate pain at the injection site	-/ 16337719
DNA + MVA (HIVA)	HIV uninfected	I, n=18	p.b. schedule; i.m.		Both vaccines were safe and well tolerated, no AEs, redness and induration at injection site	-/ 16176847
MVA (HIV Nef)	HIV patients on HAART	I, n=14	Wk 0,4,16	Immunogenic, potential of therapeutic vaccination	Well tolerated except for local reactions, mild systemic side effects	-/ 15865223
FP9 + MVA	Malaria tropica		Heterologous p.b. schedule	Sterile protection for up to 20 months		-/ 15781866
MVA-hTyr	Melanoma	I, n=20	Wk 0,4,8; $5x10^8$ IU	No hTyr directed immune response	Safe and no side effects beyond grade 2	-/ 15627214
DNA/MVA-ME-TRAP	Malaria tropica	N=372	Heterologous p.b. immunization scheme; 3 doses	Highly immunogenic for effector T cell induction	MVA-ME-TRAP was safe and well tolerated	-/ 15526058
MVA-85A	TB, BCG primed					-/ 15502839 [Review]
DNA+MVA (7 Melanoma antigens)	Melanoma	I, n=13	p.b. DNA/MVA or MVA/MVA	Poor immunogenicity		-/ 15386406
MVA-hTyr transduced dendritic cells	Melanoma	I, n=6	Wk 0,2,4,6 $1x10^8$ dendritic cells i.v., 3xs.c.	Tyrosinase-specific T cells	Well tolerated, low grade fever	-/ 15328176
DNA/FP9/MVA-ME-TRAP	Malaria tropica	I, n=29	Heterologous p.b., dose $1.5x10^8$ PFU for MVA, i.m.	DNA/MVA and FP9/MVA most potent inducers of effector T cells	No SAEs	-/ 15181568
DNA/MVA-HIVA	HIV	I, n=18	DNA/MVA p.b. scheme	HIV-specific effector T cell response (14/18)	Safe	-/ 15039533
MVA-HIV-1 (Nef)	HIV infected	I, n=10		CD4 T cell response	No adverse effects	-/ 14604567
DNA/MVA-ME-TRAP	Malaria tropica		Heterologous p.b.	Immunogenic for effector T cell function	Safe	-/ 14551895
DNA/MVA-ME-TRAP			Heterologous p.b.; i.d. for MVA	Immunogenic		-/ 12766765
DNA/MVA-SIV	Rhesus macaques			Virus specific CD8+ and CD4+ responses, no NAbs		-/ 12072518