European Medical Agency*

Non-interventional registry-based Study – Study Report

Prospective Cohort Study and Emulated Target Trial to Estimate the Safety and Effectiveness of MVA-BN vaccination against MPXV infection in at-risk individuals in Germany (SEMVAc/TEMVAc)

Protocol: EUPAS50093

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DATE STUDY INITIATED: Start of data collection - 07 July 2022

DATE STUDY COMPLETED: End of data collection - 31 December 2023

PASS Information

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Country(-ies) of study	Germany
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List of abbreviations

AESI	Adverse event of special interest
aHR	Adjusted Hazard ratio
AR	Adverse Reaction
ART	Antiretroviral therapy
ASD	Absolute standardised mean difference
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CED	Cohort Entry Date
CI	Confidence interval
DAGNAE	German Association of Outpatient Physicians for Infectious Diseases and HIV Medicine
eCRF	Electronic case report form
EMA	European Medicines Agency
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
GEP	Good Epidemiologic Practise
GPP	Good pharmacoepidemiology/pharmacovigilance practise
HIV	Human immunodeficiency virus
HR	Hazard ratio
HSMV	History of previous smallpox vaccination
ICD-10-CM	ICD-10-CM International Classification of Diseases, Tenth Revision, Clinical Modification
ICF	Informed consent form
ICU	Intensive Care Unit
ID	Index date
IDe	Index date of effectiveness
IDs	Index date of safety
IQR	Interquartile range
IR	Incidence rate
ISPE	International Society of Pharmacoepidemiology
MedDRA	Medical Dictionary for regulatory activities
Mpox	Mpox disease
MPXV	Mpox virus
MSM	Men who have sex with men
MVA-BN	Modified Vaccinia Ankara - Bavarian Nordic
NA	Not available
OR	Odds ratio
PASS	Post-Authorisation Safety Study
PCR	Polymerase chain reaction

PEP	Post-exposure prophylaxis
PLWHIV	Persons living with HIV
PRAC	Pharmacovigilance Risk Assessment Committee
PrEP	Pre-exposure prophylaxis
PS	Propensity score
PT	Preferred term
RMP	Risk Management Plan
ROA	Route of Administration
RR	Risk ratio
SAP	Statistical Analysis Plan
SAR	Severe adverse reaction
SEMVAC	Safety and Effectiveness of MVA-BN vaccination against MPXV infection in at-risk individuals in Germany
SMR	Standardised mortality ratio
SOC	System organ class
STI	Sexually transmitted infection
STIKO	German Standing Committee on Vaccination
TEMVAC	Emulated Target trial for Effectiveness of MVA-BN Vaccination against MPX infection in at-risk individuals
USMVAC	Effectiveness and safety of Modified Vaccinia Ankara - Bavarian Nordic (MVA-BN) vaccination against monkeypox (mpox) in at-risk individuals in the United States
VE	Vaccine effectiveness
VIGIV	Intravenous vaccinia immune globulin

1. Abstract

Title: Effectiveness and safety of MVA-BN vaccination against mpox in at-risk individuals in Germany, a multicentric prospective cohort study (SEMVAc).

Keywords: mpox, vaccine, effectiveness, safety

Background and Rationale: Mpox is an infectious disease caused by the human mpox virus (hMPXV) belonging to the same genus (*Orthopox*) as the variola virus. An outbreak in April 2022 revealed epidemiological patterns in historically non-endemic countries in Europe and North America, associated with human-to-human viral transmission rather than contact with animal reservoirs. Notably, men who have sex with men (MSM) and transgender persons between the ages of 18 and 50, were the population at highest risk of mpox disease in these non-endemic countries.

In July 2022, the European Medicines Agency (EMA)'s Committee for Medicinal Products for Human Use (CHMP) recommended extending the indication of the third-generation smallpox vaccine MVA-BN to include protecting adults from mpox disease, primarily based on non-clinical data and limited clinical experience. EMA, in collaboration with the European Centre for Disease Prevention and Control (ECDC), coordinates and supports the conduct of post-authorisation studies on vaccine effectiveness (VE) and safety as part of the EU Vaccine Monitoring Platform. As part of this endeavour, this study aimed to generate evidence to support regulatory-decision making on the benefit/risk profile of the MVA-BN vaccine.

Research Question and Objectives: To assess effectiveness of the MVA-BN vaccine and to describe the incidence of safety events (SAR, AR, AESIs) and reactogenicity (tolerability), and the influence of sexual behaviour, HIV status, PrEP use, and history of smallpox vaccination (HSMV) on the safety and effectiveness of mpox vaccination.

Study Design: SEMVAc is a prospective, non-interventional, multicentric cohort study of the safety and effectiveness of the MVA-BN vaccine in a population of MSM and transgender persons. The additional analysis, TEMVAc, uses a retrospective, target trial emulation approach only for the primary objective of VE.

Setting: The data for the SEMVAc study was prospectively collected by 31 participating HIV and infectious disease healthcare clinics in Germany, predominantly in Berlin. TEMVAc data was collected retrospectively in seven of the 31 centres participating in the SEMVAc study.

Subjects and Study Size: Recruitment was carried out in and around specialised infectious diseases centres or HIV clinics. Potential participants were informed via flyers and websites or by study centre physicians (written and oral). Individuals who met the inclusion criteria and who did not meet any exclusion criteria were invited to participate in the study. 6459 participants were initially recruited prior to exclusions or dropouts. Of the 6265 participants enrolled in the study from 7 July 2022 to 31st of December 2023, there were 5077 vaccinated, 1188 unvaccinated and 542 in the crossover group (i.e., unvaccinated participants at enrolment with subsequent vaccination). In addition to this prospective enrolment, a total of 9350 subjects between 1 July 2022 and 31 Oct 2022 were identified retrospectively, based on manual medical record review, of which 6054 were included in the final TEMVAc VE analysis.

Variables: *Baseline characteristics* included sociodemographic characteristics, comorbidities (including chronic and sexually transmitted infections), medical history (i.e., immunocompromising conditions), HIV treatment and prevention medications (i.e., ART, PreP), and baseline sexual history collected through questionnaires. MVA-BN *exposure* was defined as the documented receipt of the vaccine either at the study centre or reported by the participant as indicated on the Vaccination and Infection intake form for inclusion in

the study. The *primary outcome* of vaccine effectiveness was mpox, defined as the confirmation of a positive Polymerase chain reaction (PCR) laboratory test result indicating mpox virus (MPXV) infection and reported by the study centre physician on the electronic case report form (eCRF). The safety outcomes included any event that classifies as adverse reaction, severe adverse reaction, or specified AESI's myo- and pericarditis and encephalitis. The assessment of causality is defined by the national pharmacovigilance reporting system and is reported by each study centre. Participants completed *reactogenicity* questionnaires regarding symptoms experienced within 7 days of receiving the vaccination. In addition to *sexual behaviour* collected on a monthly basis for the entire cohort, participants who were vaccinated during the study period completed questionnaires after each vaccination in reference to the sexual behaviour four weeks prior and post vaccination.

Data Source: Study participants were asked to complete questionnaires during their participation in the study including the following items: baseline characteristics (including age, sex, height, body weight, previous smallpox vaccinations, comorbidities including previous sexually transmitted infections (STIs), intake of selected medications (i.e. antiretroviral therapy, immunosuppression, HIV pre-exposure prophylaxis), exposure risk to MPXV infection (i.e. sexual behaviour), tolerability (reactogenicity) of the vaccination, and symptoms that may indicate mpox disease. The data collected from the study participants is documented electronically in an eCRF. Personal data were pseudonymised before being transmitted. The data is stored within the Charité server system of the study department and is only accessible through the study team. In the emulated target trial component (TEMVAc), data from electronic medical records (EMRs) of subjects meeting the adapted inclusion and exclusion criteria were collected by study centre physicians via manual chart review.

Results: 6265 participants were enrolled between 7th of July 2022 and 31st December 2023. 14 MPXV infections were documented in the study cohort in the course of the study, all occurred in the vaccinated group. For the primary objective of VE, no cases of MPXV infection were reported in the unvaccinated group, thus VE could not be calculated. Eleven and two cases of MPXV infection were reported in those with one and two doses of MVA-BN, respectively. Cumulative incidence for those vaccinated with one dose 0.0034 [95%CI 0.0014-0.0054] was higher than those with two doses (0.0016 [95% CI 0.00-0.0041]). Similar patterns between doses were observed in persons living with HIV (PLWHIV) and PrEP user subgroups, and in the HSMV subgroup after the first dose was 0.0022 (95% CI 0.00-0.0053). The IR per 1000 person-years in those vaccinated with 1 dose was 8.88 (95% CI 4.61-15.21), while in those who received the second dose was 0.91 (95% CI 0.15-2.8). Similar patterns were seen in the PLWHIV (16.51 [95% CI 7.09-31.92], 1.27 [95% CI 0.07-5.59]), PrEP users, (7.52 [95% CI 2.33-17.46], 0.98 (95% CI 0.06-4.33]) and HSMV subgroups (4.68 [95% CI 0.78-14.45] with 1 dose). No cases were reported in the HSMV subgroup in those with a second dose.

In the TEMVAc analysis, 9328 subjects were eligible for the target trial emulation after chart review, and 6054 subjects were matched and included in the final VE analysis. During the observation period from 1 July 2022 to 31 October 2022, a total of 48 mpox cases were reported, 32 occurred in the unvaccinated and 16 after the first vaccination. Cumulative incidence after one dose was 0.661% (95% CI 0.403-1.082) with a VE of 54.15 (95% CI 21.09 to 73.36). PrEP users had an estimated VE of 63.64 (95% CI 14.92 to 84.46), which was higher than in HSMV (60.71 [95% CI -33.37 to 88.42]) and PLWHIV (41.25 [95% CI -20.88 to 71.45]). Generally, due to a limited number of mpox cases in the PLWHIV and HSMV subgroups, VE estimates had wider confidence intervals and were less conclusive. No mpox cases were observed in those vaccinated with a second dose and therefore, VE could not be calculated in this group. Symptoms were less severe in those who experienced breakthrough infections post-vaccination compared to those who were unvaccinated.

For the secondary objective of safety, a very low number of adverse reactions, and no serious adverse reactions or AESI were reported throughout the study period, indicating that the MVA-BN vaccine was well tolerated and showing a favourable safety profile. Pain at the injection site was the most common reaction reported (65.3% after the first dose, 51.8% after the second dose), with mostly mild or moderate pain (46.7% and 40.6% mild, 17.7% and 10.8% moderate after first and second vaccination, respectively).

Reactogenicity generally decreased from first to second MVA-BN dose, including mild/moderate discomfort symptoms to, in very rare cases, fever, and was similarly observed across PLWHIV and PrEP user groups. Less than a quarter of those vaccinated experienced any systemic or severe systemic complaint.

Self-reported data on sexual behaviour showed a significant overall decrease in the number of sexual partners and the frequency of condom usage before vaccination, compared to the period post-vaccination, suggesting substantial alterations in behaviour surrounding vaccination. Cluster analyses revealed various distinct behavioural patterns, with a particular subgroup of participants with moderate-to-high number of sexual partners emerging as the strongest contributor to the observed changes.

Discussion: This prospective non-interventional study aimed to assess the safety and effectiveness of the MVA-BN vaccine against mpox. The study revealed a favourable safety profile. The study was designed, reviewed and initiated during a public health emergency. However, owing to regulatory requirements especially due to individual ethical approvals being required for multiple regions and study centres in Germany as well as logistical hurdles, recruitment of participants was not fully rolled out before the epidemiological situation changed and reported cases of mpox declined. Owing to the epidemiological situation during the study period, reliable VE could not be estimated in SEMVAc. Therefore, a complementary daughter study, using retrospective data from SEMVAc study centres and an emulated target trial design (TEMVAc) was initiated to calculate VE. SEMVAc (with TEMVAc) represents a key European study in the context of the public health emergency caused by the mpox outbreak. The TEMVAc results demonstrated a VE of 54.15 (95% CI 21.09 to 73.36) after one dose of MVA-BN in this population of MSM, in line with other recent VE studies. SEMVAc/TEMVAc provides evidence on the benefits and safety of MVA-BN vaccination against mpox, as well as on changes in sexual behaviour of the MSM population during the deployment of mpox vaccination. Altogether, evidence from SEMVAc/TEMVAc supports other studies evaluating vaccine safety and effectiveness, may inform regulatory decisions, and contribute to improving future mpox outbreak preparedness and response.

2. Amendments

Number	Date	Section of study report	Amendment or update	Reason
1	26/06/2024	Version 1.1 of Study Report, Results Section	TEMVAc	Additional analysis

3. Milestones

Milestone	Planned date	Actual date	Comments
Date approval IEC	33 IECs approved the study for different study centres throughout Germany. The different approval dates are collected in Supplementary Table 1		
Start of data collection	01.07.2022	07.07.2023	
End of data collection	31.12.2023	31.12.2023	
Registration in the former EU PAS register (now HMA-EMA Catalogue of real-world studies)	15.12.2022	15.12.2022	
1st data extraction	06.01.2023	12.01.2023	
Interim report 1	07.02.2023	15.02.2022	
Interim report 2	20.03.2023	27.03.2022	
Interim report 3	15.05.2023	24.05.2023	
Interim report 4	06.07.2023	14.07.2023	
Interim report 5	15.09.2023	19.09.2023	
Interim report 6	16.11.2023	23.11.2023	
Final study results SEMVAc (Final study report v.1)	08.04.2024	08.04.2024	
Final study results SEMVAc (Final study report v.2)	30.04.2024	26.04.2024	
Addition of TEMVAc analyses - Final study report integrating SEMVAc/TEMVAc, v.3	30.06.2024	01.07.2024	
Discussion content – v.4	07.08.2024	19.08.2024	

4. Background and rationale

Mpox disease is a viral infection caused by the human mpox virus (hMPXV), which belongs to the same genus (Orthopox) as the variola virus, the causative agent of smallpox, but disease manifestations of mpox are generally less severe and less transmissible in the general population compared to smallpox (1). Transmission of mpox virus (MPXV) typically occurs upon human contact with infected animal hosts, however, human-to-human transmission can occur via close contact, especially upon contact with mpox skin lesions, bodily fluids, respiratory secretions, or contaminated surfaces (2–4). The global mpox outbreak in 2022 was of particular concern due to novel transmission patterns observed in non-endemic countries in Europe and in the Americas, associated with extensive human-to-human transmission, specifically in men having sex with men (MSM) (4–6). Molecular epidemiology of the 2022 MPXV outbreak indicated a previously undetected spread of hMPXV clade II in humans, followed by adaptation to the human host. Characteristic modalities of transmission appeared as primarily sexual transmission, close skin and mucosal contacts, or multiple sexual partners. On 23 July 2022, the World Health Organization declared the current outbreak a "Public Health Emergency of International Concern" (PHEIC). At the time of writing, the 2022 outbreak of MPXV clade II resulted in 93,921 total confirmed infections worldwide, mostly (97.4%) outside the endemic areas in Western and Central Africa (7), including over 3,800 confirmed mpox cases in Germany, with a predominance of infections among MSM (8).

At the onset of the 2022 MPXV outbreak, no mpox vaccine was licensed for use in Europe. However, previous smallpox vaccination had been previously shown to offer limited cross-protection against mpox (9). EMA's Committee for Medicinal Products for Human Use (CHMP) therefore recommended extending the indication of the available smallpox vaccine, the Modified Vaccinia Ankara vaccine from Bavarian Nordic (MVA-BN, Imvanex®) to include protecting adults from mpox disease. On 21 June 2022, the German Standing Committee on Vaccination (STIKO) recommended the use of MVA-BN as an indication for vaccination against mpox. STIKO recommends vaccination for all MSM with changing sexual partners, as well as for staff in diagnostic laboratories with contact to MPXV, and as post-exposure prophylaxis (10). According to estimates and surveys, approximately 786.000 people in Germany identify as MSM (11). A smaller proportion belong to the at-risk population with a significantly increased risk of sexually transmitted infections (STI) due to frequently changing contacts. Most of these individuals are seeking regular care at specialised infectious diseases (ID) offices or specialised HIV clinics. In particular, this includes individuals who are taking pre-exposure prophylaxis (PrEP) medication for HIV prevention. In 2022, there was high demand for MVA-BN vaccination against mpox within the MSM community, especially among PrEP users. It was assumed that the number of persons in the high-risk group and the demand for MVA-BN significantly exceeded the available number of vaccine doses at the start of the vaccine campaign. To use the available vaccine as effectively as possible to contain the 2022 outbreak, the vaccine was administered to persons with the highest risk of infection (high-risk group). In Berlin, estimates of 30,000 individuals were MSM or transgender persons who belonged to the high-risk group with frequently changing sexual partners, however, only 7,500 doses of MVA-BN were initially available in the city of Berlin. Therefore, the vaccine was only distributed to ID offices and HIV clinics with the aim to ensure coverage of the high-risk group, including PrEP users.

Due to the public health emergency, the vaccine was authorised despite very limited data on safety and effectiveness of MVA-BN against MPXV in humans, with the clear aim to review any new information as it was obtained (12). In the subsequent months, few studies have shown that a single subcutaneous dose of the MVA-BN smallpox vaccine was associated with a lower risk of MPXV infection when used in high-risk, close contacts as pre and post exposure prophylaxis (13). Furthermore, the recent outbreaks of clade IIb mpox in South Africa and Rio de Janeiro and the large outbreak of clade I MPXV in the Democratic Republic of Congo, and the African CDC and WHO August 2024 PHEIC declaration emphasise the need for clinical evidence on safety and effectiveness of MVA-BN vaccination against mpox (14–16). In particular, information pertaining to vaccine safety and effectiveness among those with pre-existing medical conditions or medication use (i.e., HIV and PreP) is scarce.

To obtain effectiveness and safety data for pre-exposure vaccination with MVA-BN, the EMA supported an mpox vaccine monitoring programme comprising three related studies. The SEMVAc study and TEMVAc analyses aim to generate data on MVA-BN for vaccination to prevent mpox disease in a Germany-based multicentric study (SEMVAc, EUPAS50093) and also includes a retrospective target trial emulation analysis on the effectiveness of MVA-BN vaccination against confirmed mpox (TEMVAc). SEMVAc/TEMVAc are further complemented by the USMVAc study (17) (EUPAS104386), which provides insights into the characteristics of the at-risk population in the United States. USMVAc used secondary healthcare data to generate real-world evidence for MVA–BN vaccine effectiveness and safety to prevent mpox disease in men who have sex with men (MSM) and transgender women, the most affected population during the 2022 mpox outbreak. Fully vaccinated subjects (two doses ≥ 28 days apart) were initially matched with five unvaccinated subjects on calendar date, age, US region, and insurance type. Study results showed a VE of 89% (95% CI: 12%, 99%) among those fully vaccinated. This approach of three complementary studies was aimed at generating RWE on the safety and effectiveness of the MVA-BN vaccine.

5. Research questions and objectives

The aim of the current study is to address the following scientific questions:

- Does vaccination with MVA-BN reduce the likelihood of infection with MPXV and symptomatic mpox disease compared to non-vaccinated individuals?
- Do pre-existing medical conditions and medication influence the risk of contracting mpox as a vaccinated person?
- What is the safety profile of MVA-BN in high-risk populations?

Primary Objective (Vaccine Effectiveness (VE)): To assess VE, i.e. whether and to which extent vaccination with MVA-BN reduces the likelihood of MPXV infection compared to non-vaccinated participants. This objective is met by comparing the incidence of symptomatic Polymerase chain reaction (PCR) confirmed MPXV infection among participants vaccinated with one and two doses of MVA-BN to a matched unvaccinated population. VE is defined as reduction in risk of MPXV infection in vaccinated versus unvaccinated participants.

Secondary Objective (Safety, Sexual Behaviours, Pre-existing Conditions): To describe the incidence of safety events (SAR, AR, AESIs), reactogenicity (tolerability), changes in sexual behaviour, and the influence of pre-existing medical conditions and medications (HIV and PrEP use) and history of previous smallpox vaccination (HSMV) on reactogenicity, safety events and the risk of contracting mpox for study participants who received MVA-BN vaccination.

6. Research methods

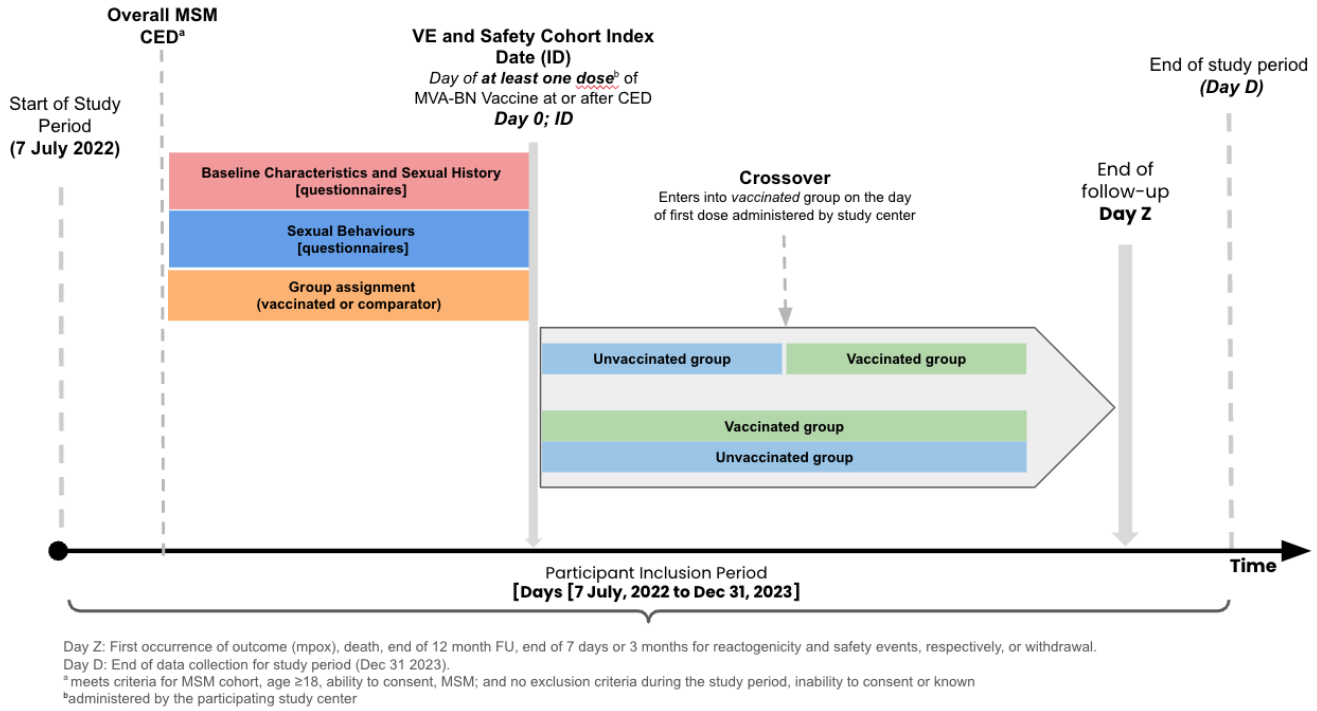
6.1. Study Design

SEMVAc is a **prospective, non-interventional, multicentric cohort study** of the **safety and effectiveness** of the MVA-BN vaccine in a population of MSM and transgender persons (18). TEMVAc is a retrospective, emulated target trial component of SEMVAc and further addresses the primary objective of VE (see below Section 6.1.2).

Given the observational, *non-interventional* nature of SEMVAc, administration of the MVA-BN vaccine was not part of the study and vaccination was administered as part of routine medical practice. To participate, *study centres* were required to have experience in the treatment of MSM and HIV patients, and regularly treat patients who meet the inclusion criteria (see Section 6.3.1). At the participant level, the decision for or against MVA-BN vaccination was not influenced by study participation, but entirely based on the decision of the person seeking care at the treating physician at the participating study centre. Moreover, all medical

examinations were performed as part of routine clinical practice. The campaign to assign study centres and to recruit participants into the SEMVAc study began in Berlin, Germany on 7 June 2022, when Charité began contacting candidate study centres via email and phone calls. The study team utilised direct contacts with ID offices and HIV clinics and used flyers and web content to raise awareness about the study. Study participants were screened and recruited from persons who sought medical care at the participating study centres between 7 July 2022 and 31 December 2023. A person was invited to participate in the study and enrolled based on the criteria described below (see "CED"). Enrolment in the study was only possible until September 2023, but the observation period extended until 31 December 2023.

Figure 1: Study design schema for primary objective assessment.



Day Z: First occurrence of outcome (mpox), death, end of 12 month FU, end of 7 days or 3 months for reactogenicity and safety events, respectively, or withdrawal.
Day D: End of data collection for study period (Dec 31 2023).
[a] meets criteria for MSM cohort, age ≥18, ability to consent, MSM; and no exclusion criteria during the study period, inability to consent or known
[b] administered by the participating study center

6.1.1 Schedule of Assessments

Study participants' data were collected in the described intervals for SEMVAc (see Table 1 for details).

During study visits:

- **At enrolment** (i.e. cohort entry date (CED)): Upon informed consent and agreeing to study participation, all study participants were asked to fill in questionnaires (electronic or paper-based) at the first visit (*inclusion)* for the collection of baseline characteristics, sexual behaviour, and mpox exposure status.
- **Quarterly visits** (as part of routine medical care) for the duration of study participation.
- **For vaccination with MVA-BN**:
  - At the first vaccination.
  - At the second vaccination (approximately 4 weeks after the first vaccination)

Through multiple questionnaires administered at the following intervals:

- All study participants were asked to fill in monthly electronic questionnaires on sexual behaviour.
- MPXV infection was assessed via monthly questionnaire and confirmed with a PCR test and documented in the eCRF by a physician.
- All participants who received the first or second or both vaccinations during the study period, starting at CED received electronic questionnaires regarding any symptoms of reactogenicity within the 7 days following a vaccination and their sexual behaviour in the 4 weeks before and after each vaccination.

Safety events were reported by the study centre physician for up to 3 months following administration of the MVA-BN vaccine.

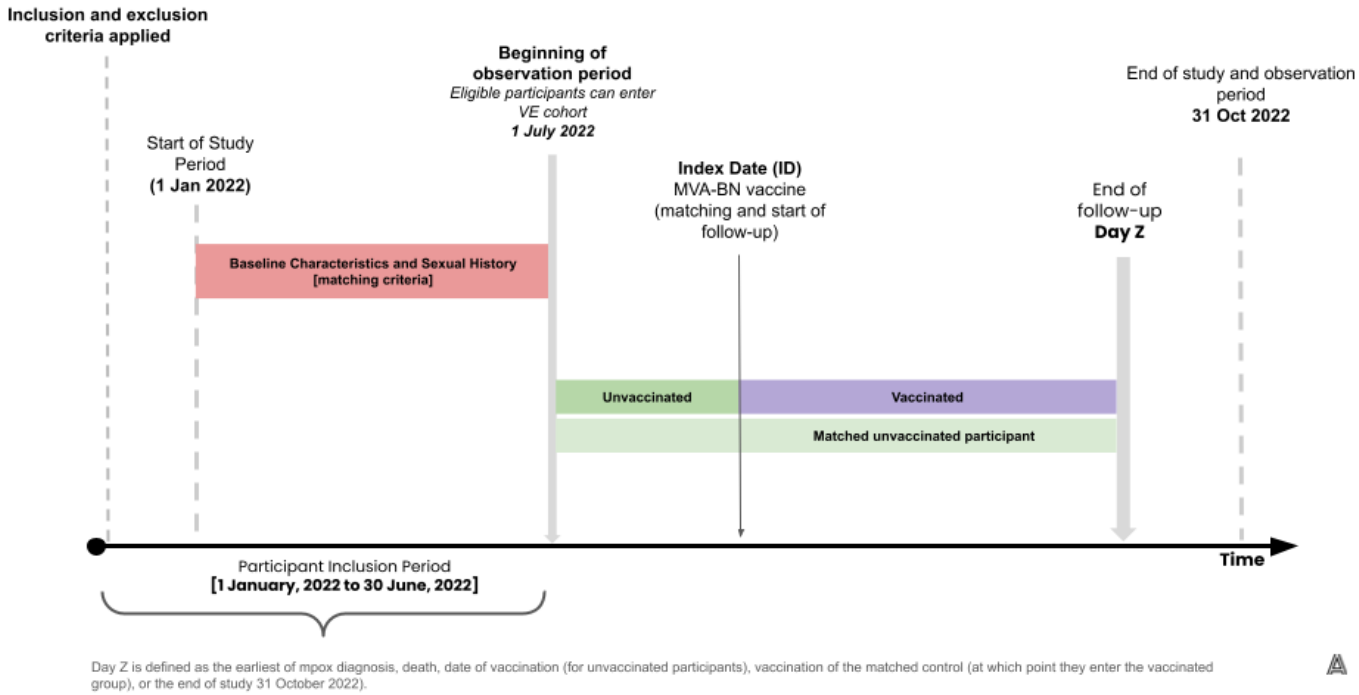
Table 1: Questionnaire content and frequency for baseline, exposure, and outcome variables.

Questionnaire	Study visit	Frequency	Information obtained
Enrolment Questionnaire - Participant	Enrolment	Once at CED	Baseline characteristics Sexual history mpox exposure
Enrolment Questionnaire - Physician	Enrolment	Once at CED	Baseline characteristics
Vaccination and Infection - Physician	1st or 2nd dose of MVA-BN, follow-up visit	At CED, quarterly, at 1st and/or 2nd vaccination	Vaccination status mpox status
Sexual Behaviour - Participant	Enrolment	- At inclusion - 1x per month - 4 weeks after 1st and/or 2nd MVA-BN vaccination (in the vaccinated cohort)	Information on sexual history and sexual practices of the participant
Vaccination Tolerability - Participant	1st or 2nd dose of MVA-BN	7 days after vaccination	Reactogenicity
AR / SAR - Physician	1st or 2nd dose of MVA-BN	Once (if applicable within 3 months after each vaccination)	Safety event deemed to be related to the vaccine by the study centre physician
Infection Participant	After confirmed positive PCR	Once after confirmed positive PCR	Symptoms and outcome of infection

6.1.2 Target Trial Emulation (TEMVAc)

TEMVAc is a retrospective, non-interventional, multicentric target trial emulation study. Data from the electronic medical records (EMRs) of eligible patients of the participating study centres fulfilling the inclusion criteria were collected and manually entered into the eCRF by study centre physicians, (see Appendix TEMVAc SAP for details). All eligible patients that met inclusion criteria and did not meet any exclusion criteria (see Sections 6.3.2.1 and 6.3.2.2 for more details) from the participating centres within the study period from 1 January 2022 to 31 October 2022 were included. Baseline covariates were obtained from 1 January 2022 until 30 June 2022, the day before the start of the observation period. Start of the observation period began on 1 July 2022 at which point information regarding the vaccination status and endpoints, i.e. diagnosis of mpox of subjects, began. Index date (ID) was defined as the day a subject was vaccinated and matched to an unvaccinated control subject. Follow-up for each matched pair started with the ID and continued until Day Z, defined as the earliest date of mpox diagnosis, death, date of vaccination (for unvaccinated subjects), vaccination of the matched control, or end-of-follow-up (end of study 31 October 2022). Each vaccinated subject was matched to an unvaccinated subject at any time during the observation period (Figure 2).

Figure 2: Study design schematic for data collected in TEMVAc.



Day Z is defined as the earliest of mpox diagnosis, death, date of vaccination (for unvaccinated participants), vaccination of the matched control (at which point they enter the vaccinated group), or the end of study 31 October 2022).

6.2. Setting

Data for the **SEMVAc** study was prospectively collected by 31 participating study centres (at offices and healthcare clinics) in Germany (Supplementary Table 1). The selection of study centres and participating physicians was based on previous experience with other relevant studies, likelihood of reaching recruitment goals, and feasibility aspects (e.g. capacity to vaccinate participants on site). All study centres were selected based on their experience in the treatment of MSM, infectious disease, sexually transmitted infections (STI) and HIV patients (e.g. membership of the German Association of Outpatient Physicians for Infectious Diseases and HIV Medicine DAGNAE). The participating study physicians were required to comply with the study procedures specified in the schedule of assessments.

Data for **TEMVAc** were retrospectively collected by 7 of the 31 study centres that participated in SEMVAc, including centres in five major German cities with the largest MSM populations (Berlin, Hamburg, Cologne, Frankfurt, and Munich). Additionally, these study centres are the largest in their respective regions and had long-term, consistent contact and EMR data from participating patients prior to 2022. This inclusion criterion for selection of the centres was applied to TEMVAc to ensure the completeness of baseline information. Therefore, while some overlap exists between SEMVAc and TEMVAc, the majority of subjects in TEMVAc represent overall long-term clients or patients of the study centres, while the SEMVAc population more broadly captured at-risk individuals.

As part of SEMVAc and TEMVAc participation, study centre physicians were compensated for patient recruitment, the medical chart review and data extraction.

6.3. Subjects

6.3.1. Recruitment of subjects

Recruitment for **SEMVAc** was carried out in specialised infectious disease offices or HIV clinics. Potential participants were informed via flyers, websites, and/or by study centre physicians (written and oral). The background of the study, as well as the study procedures, risks, benefits, and protection of personal data were explained to the study participant. Potential participants were given sufficient time to consider participation and to give their informed consent (usually up to 24 hours). If they had any questions regarding

study participation, they could contact the study team. Participants who met the inclusion criteria and who did not meet the exclusion criteria were invited to participate in the study.

TEMVAc subjects qualified for enrolment if they were a client or patient at one of the participating study centres in SEMVAc and fulfilled all inclusion and no exclusion criteria. From the selection of study centres participating in TEMVAc, physicians reviewed the medical charts of potential subjects. Since data in TEMVAc is based on medical chart review (secondary use of data) and anonymised, there was no direct involvement of the subjects in study enrolment and therefore no need for informed consent to study participation (as confirmed by the respective IRBs). All medical records were reviewed and selected for TEMVAc enrolment based on inclusion and exclusion criteria of TEMVAc (see Section 6.3.2.1).

6.3.2 Cohort Entry Date

The ***Cohort Entry Date (CED)*** is defined as the date of first visit at the study centre when the participant meets the inclusion criteria for the overall study population, provides consent (SEMVAc specific, not applicable to TEMVAc), and does not meet exclusion criteria. The CED represents the start of the follow-up period in SEMVAc, whereas CED in TEMVAc signifies the entry into the observation period (see Figure 2). Inclusion and exclusion criteria are described below:

6.3.2.1 Inclusion criteria

SEMVAc inclusion criteria
The following criteria was applied to all persons recruited for the study who were patients of the participating study centres and provided informed consent. Those meeting the inclusion criteria and not meeting any of the exclusion criteria were included in the overall MSM cohort prior to the group assignment.

Persons were included in the study if they:

- Were age ≥ 18 years at the time of recruitment,
- Had the ability to consent,
- Identified as a man or trans person who has sex with changing male partners (MSM) and/or a trans person (in accordance with the STIKO vaccine recommendation).

TEMVAc inclusion criteria:
The following additional criteria were applied for TEMVAc inclusion:

- Individual is treated at the participating study centre:
    - for a minimum of 6 months prior to the beginning observation period (1 July 2022), i.e. ≥1 contact to study centre between 1 January 2021 and 31 December 2021 and;
    - after the end of follow-up, i.e. ≥1 contact with the study centre in the period of Q4/2022, Q1/2023, Q2/2023, or Q3/2023.

6.3.2.2 Exclusion criteria

In **SEMVAc**, persons were excluded from the study if they:

- Did not consent or were unable to consent
- Had known exposure to MPXV before vaccination (post-exposure prophylaxis)

In **TEMVAc**, subjects were excluded if there was a documented diagnosis of mpox or MVA-BN vaccination in the EMR prior to the start of observation period for TEMVAc (1 July 2022).

6.3.3. Index Date

The **SEMVAc *Index Date (ID)*** is defined as the date that a participant enters the analytical cohorts, either for the Vaccine Effectiveness (VE) or Safety cohort (see Section 6.3.4).

- The index date for *vaccinated participants* in both the VE and Safety cohorts is the date of the MVA-BN vaccination administered by a participating study centre at or after CED (inclusion).
- For participants who enter the study at CED via the receipt of a second MVA-BN vaccination (*vaccinated prior to entering the SEMVAc study*), the ID is the date that the participant received the second MVA-BN vaccination administered by the participating SEMVAc study centre.
- For participants enrolled in the VE cohort as *unvaccinated,* the index date refers to CED.

For the primary objective of effectiveness, the *index date (ID_E) for exposed participants* was defined as the date of administration of one dose of MVA-BN (either first or second dose) *by the study centre*, at or after CED or at crossover. For safety, the *index date (ID_S)* was the date of the first or second MVA-BN administration *by the study centre*, at or after CED, or at crossover. Please note that in practice, ID_E and ID_S are identical but the outcomes measured in the VE and Safety cohorts differ.

Vaccination status for all participants was determined at CED (inclusion) and included the following categories: one dose prior CED, two doses prior CED, first dose at CED, second dose at or after CED, unvaccinated (see Table 3). Those initially categorised as unvaccinated at CED were allowed to crossover to the vaccinated category upon receipt of a MVA-BN vaccination during the follow-up period. Four **follow-up periods** of interest were defined based on distinct outcomes between the VE and Safety cohorts:

For the primary objective of effectiveness, follow-up ended at the earliest of: occurrence of the outcome, withdrawal from the study, death, the end of data acquisition (31 December), or the end of the prespecified 12-month follow-up period starting from CED. In the **TEMVAc analysis**, follow-up began at ***index date (ID)*** and was defined as the beginning of observation for vaccination or outcome of mpox diagnosis. Follow-up ended at the earliest date of mpox diagnosis, death, date of vaccination (for unvaccinated subjects), vaccination of the matched control, or end of the study period, 31 October 2022.

For the secondary objective of safety assessment, follow-up ended at the earliest of: occurrence of the outcome, 3 months after the 1st or 2nd vaccination administered by the participating study centre, withdrawal from the study, death, the end of data acquisition (31 December), or the end of the prespecified 12-month follow-up period starting from CED. For effectiveness and safety calculations, follow-up after first vaccination was additionally restricted until the receipt of second vaccination. Reactogenicity was assessed within the 7 days following vaccination. Information on sexual behaviour was collected at CED, after each vaccination, and in monthly intervals for the duration of the study. See Table 2 below for more information.

Table 2: Study Objectives, Measures and Endpoint Definitions (SEMVAc and TEMVAc).

Objective	Measure	Endpoints(s)	Applies to	Assessment period
Primary Objective	**Vaccine Effectiveness**	**Primary Endpoint:** Mpox disease - report of MPXV infection with positive polymerase chain reaction (PCR) test result as documented in the corresponding electronic case report form (eCRF).	SEMVAc and TEMVAc	ID through 31 December 2023 (31 October 2022 in TEMVAc) or after a maximum of 12 months starting from CED in SEMVAc.

Objective	Measure	Endpoints(s)	Applies to	Assessment period
Secondary Objective	**Safety**	**Safety Endpoints:** - Adverse Reaction (ARs) - Serious Adverse Reaction (SARs) - AESI's (pericarditis, myocarditis, encephalitis) Report of AR/SAR by participant and confirmed as associated with the vaccine by study centre physician following WHO causality criteria and documented by ICD10 and MedDRA diagnosis codes on the clinical record and corresponding electronic case report form (eCRF).	SEMVAc only	IDs until the end of 3 months after 1st and/or 2nd vaccination
Secondary Objective	**Reactogenicity**	Reactions to the vaccine such as rash, fever, pain etc. (see Section 6.4.2) recorded by the participant on the questionnaire	SEMVAc only	IDs until 7 days following vaccination
Secondary Objective	**Sexual Behaviour**	**Baseline and monthly** sexual behaviours and changes in behaviour surrounding vaccination* (see Section 6.4.2).	SEMVAc only	At CED, after each vaccination, and monthly for the duration of the study**

**Responses regarding MPXV exposure refer to the previous 4 weeks*
***Sexual behaviour questionnaires for unvaccinated group were only collected at CED and monthly*

6.3.4 Study cohort definitions

The **overall MSM cohort in SEMVAc** (Figure 3a, b) included participants enrolled at the study centres, fulfilling the inclusion criteria and not meeting any exclusion criteria and who were not excluded after enrolment (CED). Possible exclusion criteria after enrolment included: occurrence of exclusion criteria after recruitment or withdrawal of consent.

The **overall MSM cohort in TEMVAc** (Figure 3c) included subjects enrolled retrospectively by the participating centres fulfilling the inclusion criteria and not meeting any exclusion criteria for the selection of centres (Section 6.3.2.1). Participating study centres were provided with the specified TEMVAc inclusion and exclusion criteria after agreeing to participate in SEMVAc. Physicians reviewed charts of all potential subjects and provided data if a potential subject met the inclusion and no exclusion criteria (Section 6.3.4.1). Then information on relevant variables was manually extracted by the physician based on the TEMVAc analysis protocol (i.e., history of STIs, PrEP use, etc.) and entered into the eCRF provided by the study investigators (see Appendix SAP for more details).

The **VE cohorts** (Figure 3a) are subsets of the overall MSM cohorts. For **SEMVAc**, participants who entered

the study at CED and were classified by the study physician as either 'unvaccinated' (comparator group) or 'vaccinated' (received the first dose or second dose administered by the study centre at CED). Participants who enter as unvaccinated at CED and were subsequently vaccinated (crossover) were included in the VE cohort and contribute follow-up time for each respective group (unvaccinated and vaccinated). Participants who received the second dose at CED (i.e. ID for the VE cohort) and the first dose prior to CED were included in the VE cohort, however, only contributed follow-up time starting from the second MVA-BN vaccination. Participants who received both vaccinations prior to CED were excluded from the VE analysis. MVA-BN exposure is defined as the documented receipt of the vaccine in the eCRF. For **TEMVAc**, the VE cohort is a subset of the overall MSM cohort in TEMVAc, that includes only those individuals that were selected by the matching algorithm and matched (see Appendix SAP for details).

The VE cohort includes participants who have entered the overall MSM cohort and meet the following criteria:

Participants were included in the vaccinated *exposure group* if they:

- Received a first dose of an MVA-BN vaccine by the participating study centre at CED. Participants who received a second dose by the study centre after receiving the first dose at CED remained in the vaccinated group.
- Received a second dose of an MVA-BN vaccine by the participating study centre at or after CED.
- Received a first dose of MVA-BN vaccine during follow-up after entering the overall MSM cohort as an unvaccinated participant. These participants were the crossover group and contributed to the vaccine effectiveness cohort only after receiving vaccination. Follow-up continued in the event of a second dose after the initial crossover event.

Participants were included in the *unvaccinated group* if they:

- Met the overall MSM cohort criteria and have not received a vaccination prior to CED.
- Participants who entered the study and were assigned to the unvaccinated group, but then received the first MVA-BN dose during the study period (crossover participant) contributed time as an unvaccinated participant *prior* to vaccination. These participants were censored from the unvaccinated group upon vaccination.

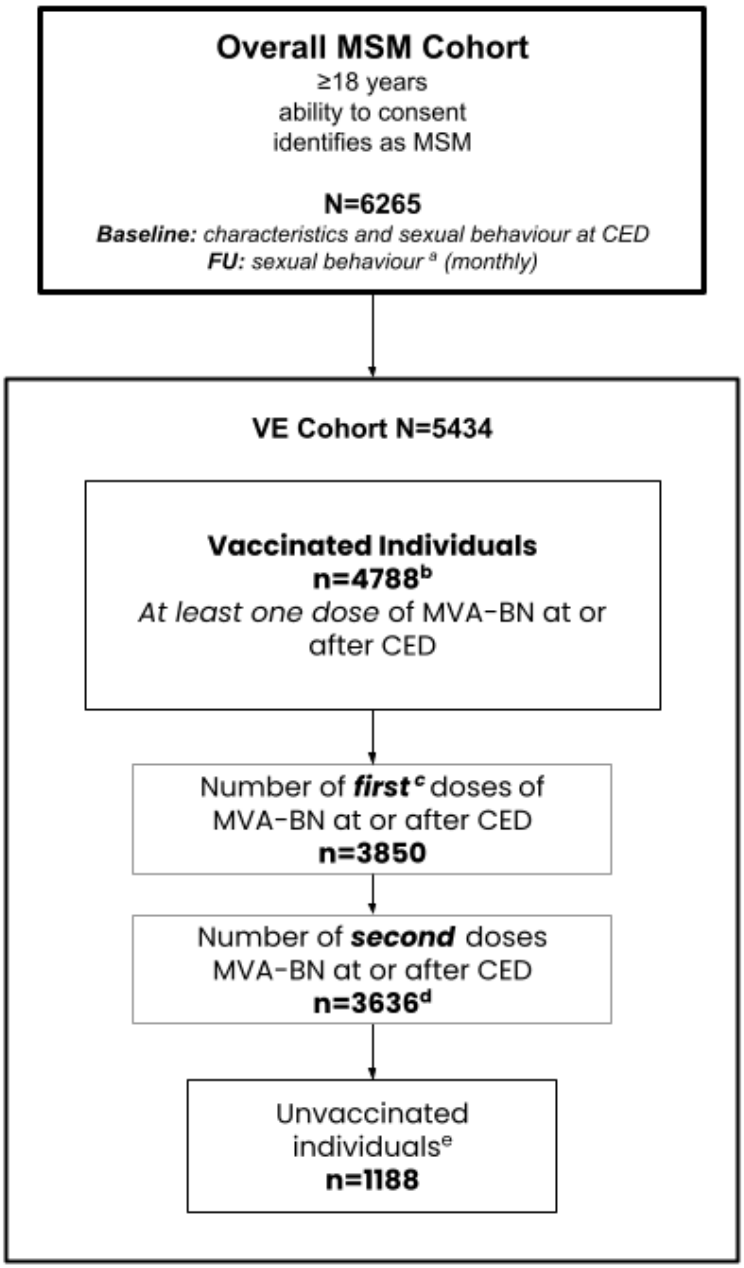
The **Safety cohort** (Figure 3b) includes all participants who were enrolled and received at least one confirmed MVA-BN vaccination at or after CED.

Participants were included in the Safety Cohort if they:

- Received a first dose of an MVA-BN vaccine by the participating study centre at CED. Participants who received a second dose by the study centre during follow-up after receiving the first dose at CED also contributed information regarding safety after the second vaccination.
- Participants who received a second dose by the participating study centre at or after CED. Those participants only contributed information regarding safety after the second vaccination.
- Participants who received a first and/or second dose of MVA-BN vaccine during follow-up after entering the overall MSM cohort as an unvaccinated participant. These participants are the crossover group and contributed to the safety cohort at vaccination.
- Overall, participants contributed information after the receipt of first and/or second vaccination at or after CED. Safety analyses were stratified by dose of MVA-BN vaccine. The number of participants which were included in the safety analysis for the first and second vaccination may, therefore, be distinct.

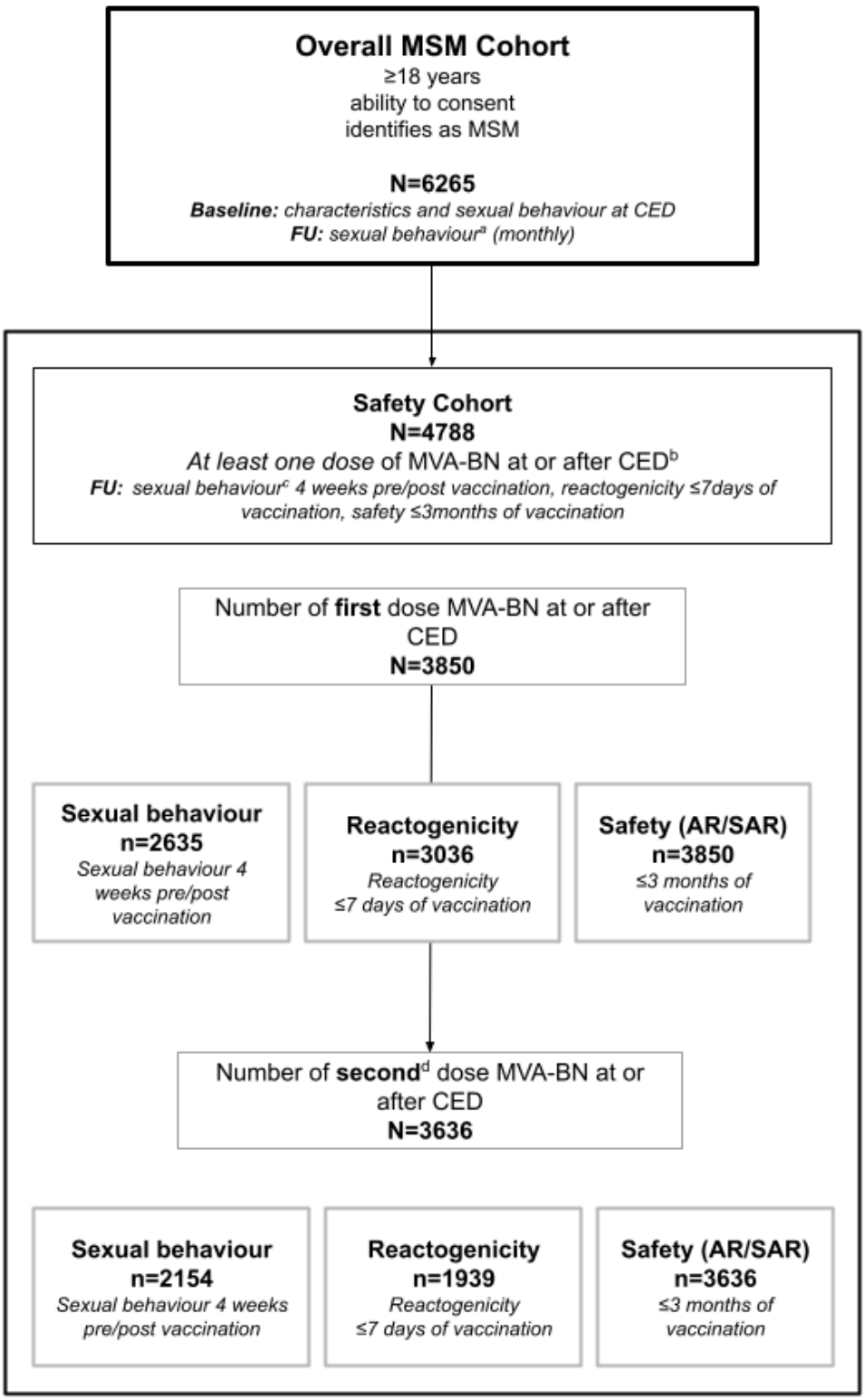
Figure 3: Attrition diagrams of MSM participants included in a) SEMVAc Vaccine Effectiveness (VE) cohort b) SEMVAc Safety cohort, and c) TEMVAc analyses, describing the data collected for each of the respective cohorts.

a)



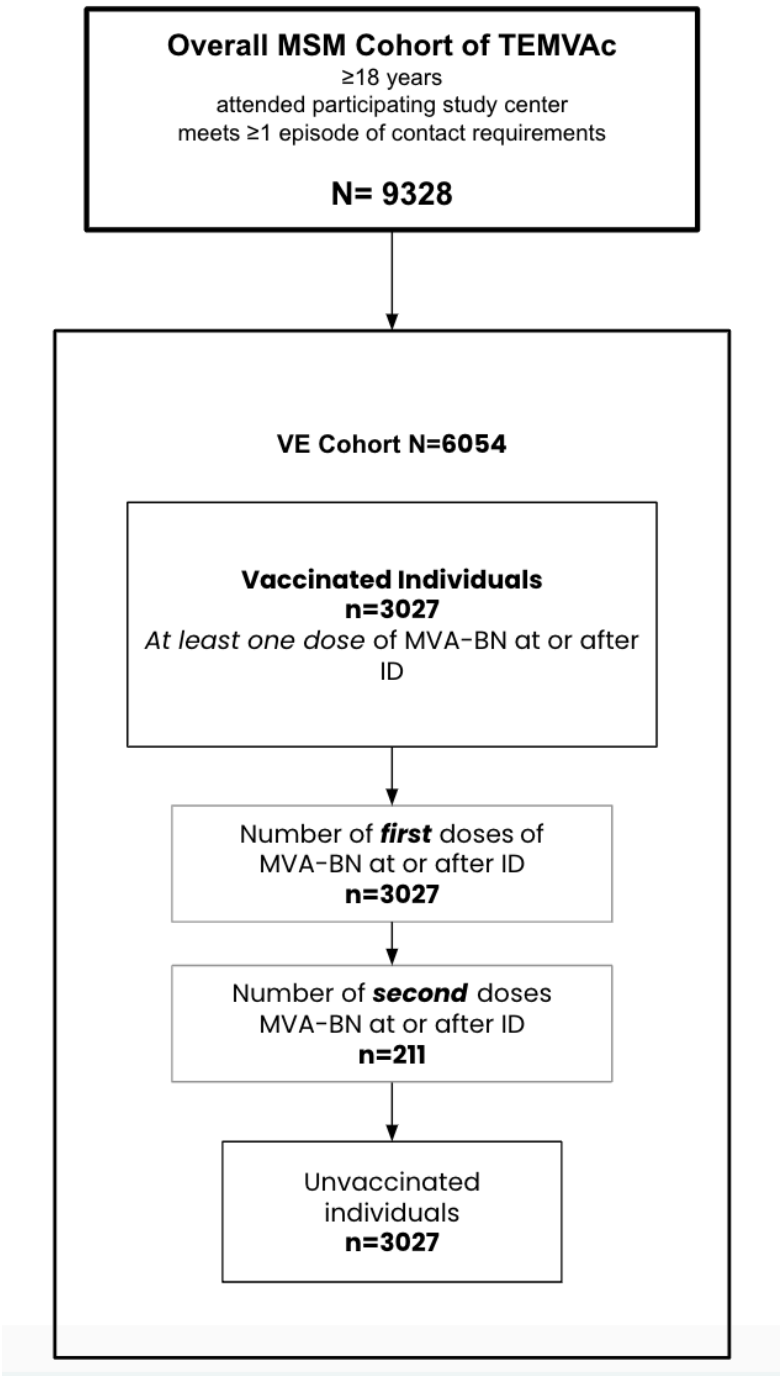
[a] answered questionnaire at least once
[b] includes those participants with the first dose prior to CED. Crossover participants (n=542), 1st dose at CED (n=3308), 2nd dose at CED (n=938).
[c] includes those participants with 1st dose at CED (n=3308) at CED and crossover participants (n=542), initially unvaccinated
[d] includes those with 2nd dose at CED (n=938) and 2nd dose during follow up (n=2312 with 1st vaccination at CED and n=386 from crossover participants).
[e] initially unvaccinated participants who received the first dose during follow-up (crossover participants, n=542)
Note: Individual participants and doses were counted separately. Participants who have a follow-up time of 0 were excluded from VE and safety effect estimate calculations, however, were included in the attrition diagram and baseline tables.

b)



[a] answered questionnaire at least once
[b] Includes those who may have been vaccinated with second dose during follow up
[c] *sexual behaviour data is in addition to data collected in the MSM cohort*
[d] *includes those participants with first dose prior to CED*

c)



Participants who were reported as having received either the **1st or 2nd dose of MVA-BN vaccine prior to CED** and no subsequent vaccination during the study period were followed up but not included in the VE or Safety and reactogenicity cohorts. However, baseline characteristics (demographics and sexual behaviour), monthly sexual behaviour, mpox status and vaccination status questionnaires were collected for all these participants and are described separately.

6.3.4.1 Exposure and comparator groups for primary (vaccine effectiveness) objective

SEMVAc

Participants were included in the **exposure (vaccinated) group** if they:

- Received a first dose of an MVA-BN vaccine by the participating study centre at CED. Participants who received a second dose by the study centre after the first dose by the study centre at CED remained in the vaccinated group.
- Received a first dose of MVA-BN vaccine during follow-up after entering the overall MSM cohort as an unvaccinated participant. These participants were the *crossover* group and contributed to the VE cohort at vaccination.
- Received a second dose at or after CED and the first dose prior CED (index date is the day of the second dose at or after CED)

Participants were included in the **unvaccinated group** if they:

- Meet the overall MSM cohort criteria and have not received a vaccination prior to CED or during follow-up.

- Participants who enter the study and are assigned to the unvaccinated group, but then receive the first MVA-BN dose during the study period (crossover participant) contributed follow-up time first as an unvaccinated participant prior to vaccination and then switch to the contribution to the vaccinated group on the day of vaccination. These participants are censored from the unvaccinated group upon vaccination.

TEMVAc

Subjects were included in the **vaccinated** group if they:
- Met the overall MSM cohort criteria in TEMVAc (Section 6.3.2.1);
- Had documentation of receiving the MVA-BN vaccine during the observation period in the electronic medical record and on the electronic CRF, recorded by the participating medical physician;
- Were matched (1:1) to an unvaccinated individual based on matching criteria of the covariates at baseline on the same calendar date as the unvaccinated pair (as required by the matching algorithm).

Subjects were included in the **unvaccinated (comparator)** group if they:
- Fulfilled the matching criteria of the covariates at baseline, were unvaccinated and did not have a documented PCR confirmed MPXV infection on the electronic medical record prior to the same calendar date as the vaccinated pair on the same calendar date (as required by the matching algorithm);
- Did not have a documented PCR confirmed MPXV infection on the electronic medical record at any time prior to the observation period;
- Did not have documentation of receiving the MVA-BN vaccine prior to the observation period in the electronic medical record.

6.3.4.2 Exposure group for secondary (Safety, Reactogenicity, Sexual Behaviour) objectives

All participants who were enrolled and received at least one confirmed MVA-BN vaccination at or after CED were included in the Safety cohort.

Participants were included in the safety **exposure** group if they:

- Received a first dose or second dose of an MVA-BN vaccine by the participating study centre at CED. Participants who received a second dose by the study centre after a first dose prior to CED remained in the exposed safety group.
- Received a first and/or second dose of MVA-BN vaccine during follow-up after entering the overall MSM cohort as an unvaccinated participant. These participants were the *crossover* group and contributed to the Safety cohort at vaccination.

6.3.4.3 Cohort subgroup analyses

To assess the influence of pre-existing medical conditions (e.g., HIV) and medications (e.g., HIV pre-exposure prophylaxis [PrEP]) and history of smallpox vaccination prior CED on vaccine effectiveness and tolerability of the vaccination, outcomes were be assessed in the following subgroups:

- Diagnosis of HIV at baseline
- Use of PrEP to prevent HIV at baseline
- History of smallpox vaccination (HSMV) prior to CED
    - Defined as at least one smallpox vaccination prior to CED. In case of missing data, a proxy of birth at or after 1975 (no smallpox vaccination) and birth prior to 1975 (smallpox vaccination) were used.

6.4 Variables

6.4.1 Exposure

In **SEMVAc**, MVA-BN exposure is defined as the documented receipt of a vaccine dose either at the study centre or reported by the participant as indicated on the *Vaccination and Infection* intake form for inclusion in the study. Five types of exposure status were captured for all participants who enter the overall MSM cohort and were assigned to the vaccinated group (see Table 3 for a detailed description of the operational definitions for each exposure status).

For the primary outcome measure of mpox, only participants who were vaccinated with the first dose or second dose of MVA-BN (including crossover participants initially unvaccinated) at or after CED were included in the VE analysis. Participants who received two doses prior to CED, or one dose prior to CED and received no second dose during their study participation were excluded from the VE analysis. Participants who enter into the study with their second dose as part of study enrolment were included in the VE analysis as exposed.

Similarly, for safety outcomes and reactogenicity, a participant is considered exposed if they received one or two doses of the MVA-BN vaccine *by the participating study centre* at or after CED (inclusion). Safety information was collected and reported for all persons who received the vaccine prospectively during SEMVAc enrolment.

In TEMVAc, exposure was defined as documented administration of the MVA-BN vaccine in the EMR at any time from 1 July 2022 to 31 October 2022. See Appendix SAP Section 4.2 for further details on exposure definition details.

Table 3: Description of exposure to MVA-BN vaccination status.

Variable	Operational Definition	Applies to
1st Dose MVA-BN at CED	First dose of MVA-BN is administered to the participant and documented by the study centre physician in the *Vaccination and Infection* form. If relevant*, safety events are documented in the AR / SAR Report by the physician (SEMVAc only).	SEMVAc and TEMVAc
2nd dose MVA-BN at or after CED	Second dose of MVA-BN administered to the participant and documented by the study centre physician in the *Vaccination and Infection* form. If relevant*, safety events are documented in the AR / SAR Report by the physician. Participants may have received the first dose prior to CED.	SEMVAc and TEMVAc

Variable	Operational Definition	Applies to
Crossover	Only for those participants initially included in the unvaccinated group and then, upon receipt of the first dose of MVA-BN and documented by the study centre physician in the Vaccination and Infection form. (Second dose for crossover participants is also included)	SEMVAc only
Vaccinated 1 dose only prior to CED	Participants reported a first dose of MVA-BN prior to CED and did not receive their second dose as part of study participation. Documentation of the prior dose by the study centre physician in the *Vaccination and Infection* intake form.	SEMVAc only
Vaccinated 2 doses prior to CED	Participants reported two previous doses of MVA-BN prior to CED. Documentation of the prior dose by the study centre physician in the *Vaccination and Infection* intake form.	SEMVAc only

*Safety information was only collected for SEMVAc

6.4.2 Outcomes

Study outcomes were defined and collected in questionnaires administered to the study participants or via eCRF completed by the physician and a report of a positive PCR test. Table 2 describes the outcomes by study objective and their definitions.

Primary objective: Vaccine Effectiveness outcome

The primary outcome of VE is mpox, defined as the confirmation of a positive orthopoxvirus PCR laboratory test result indicating MPXV infection and reported by the study centre physician on the electronic case report form (eCRF) for SEMVAc, or in the medical charts and subsequently on the eCRF for TEMVAc (Table 4).

In SEMVAc, mpox disease status was evaluated and documented at every study centre visit and documented by the study centre physician. Study participants were informed about possible symptoms (e.g., skin changes, rash, fever, muscle pain, swelling of lymph nodes) of an infection at enrolment and instructed to notify their study centre for confirmation by PCR as soon as possible (PCR may be performed by any other physician if the study centre is not immediately available). If participants reported an infection in their monthly questionnaire, they were also instructed to visit their study site for PCR confirmation. In case they could not receive a PCR test at the study site, participants could visit any other physician. Participants were asked to present the result at the next visit to the study site for it to be documented in the eCRF. Reported infections by participants that were not confirmed by PCR by study centres (e.g., only reporting via monthly questionnaires) were reported separately from PCR-confirmed infections.

For **TEMVAc**, MPXV infections were defined as the confirmation of a positive PCR laboratory test result for mpox and reported by the study centre physician on the electronic case report form (eCRF). See Appendix SAP, Section 4.2 Table 2 for further details.

Table 4: Primary outcome: operational definition of mpox disease in SEMVAc and TEMVAc.

Variable	Operational Definition	Assessment period
SEMVAc - Mpox (physician reported and PCR confirmed)	Physician reports positive PCR test for MPXV infection and documents infection in the eCRF (includes calendar date).	ID to end of follow-up (earliest occurrence of the outcome, withdrawal, death, end of study period (31st December 2023) or after a maximum of 12 months follow-up starting from CED).

TEMVAc - Mpox	Medical charts report a positive PCR test for MPXV infection and physician documents infection in the eCRF.	Start of observation period to end of follow-up (earliest occurrence of the outcome, death, end of study period (31st October 2022))

Secondary Objectives: Safety, Reactogenicity, and Sexual Behaviour outcomes

Safety outcomes: The safety outcomes included any event that classifies as adverse reaction, severe adverse reaction, and the pre-specified AESIs myo- and pericarditis and encephalitis (as per the Risk Management Plan of Imvanex). The determination of causality is defined by the pharmacovigilance reporting system and is reported by the study centre. The study centre physician completes an AR / SAR questionnaire to report any vaccine related event and describes the severity and level of association with the vaccine. Safety events are reported for up to 3 months after any vaccination received as part of SEMVAc study participation. The primary causality assessment (Table 5) of adverse reactions was conducted by the study centre physician and accounts for the patient's full medical history and physical examination. Only adverse events that have at least a possible causal relationship with the studied vaccine were reported and were thus classified as adverse reactions in accordance with WHO-UMC guidelines (19). Below is a summary of the criteria used to assess causality:

Assessment of intensity

The following definitions according to the Common Terminology Criteria for Adverse Events (20) grading in Version 5 were applied:

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL (Activities of daily living).
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

Table 5: Relationship to MVA-BN vaccination (causality) using WHO causality criteria.

Certain	• Plausible temporal correlation with drug administration • AE cannot be explained by disease or another drug • AE can be definitively explained by pharmacology or phenomenology
Probable	• Sufficient temporal correlation with drug administration • Unlikely, that the AE is caused by disease or another drug
Possible	• Sufficient temporal correlation with drug administration • Disease or another drug may also cause the AE

Table 6 below describes the operational definitions and period of assessments for safety outcomes from physician and participant questionnaires.

Table 6: Operational definitions and period of assessment of safety outcomes.

Variable	Operational Definition	Assessment Period
Level of causality	Categorical: Certain, Probably, Possible	Date of vaccination to end of 3 months follow-

System Organ Class	Categorical: Blood and lymphatic system, Cardiac, Congenital, familial and genetic, Ear and labyrinth, Endocrine, Eye, Gastrointestinal, General and administration site conditions, Hepatobiliary, Immune system, Infections and Infestations, Injury, poisoning and procedural complications, Metabolism and nutrition, Musculoskeletal and connective tissue, Neoplasms benign, malignant and unspecified (incl cysts and polyps), Nervous system, Pregnancy, puerperium and perinatal conditions, Psychiatric, Renal and urinary, Reproductive system and breast, Respiratory, thoracic and mediastinal, Skin and subcutaneous tissue, Social circumstances, Surgical and medical procedures, Vascular	up[1], earliest occurrence of a safety outcome, death, end of study period 31st December 2023 or after a maximum of 12 months follow-up starting from CED.
Duration	Number of days that the event occurs as documented by the physician on the AR/SAR report	
Intensity	Categorical: mild, moderate, severe, life-threatening, death	
Serious Reaction	Categorical: yes, no	
Classification of event	Categorical: death, life-threatening, hospitalization, permanent/severe disability, congenital birth defect	
Resolution of event	Categorical: recovered, recovered with consequences, in recovery, continuous, death, unknown	

[1]For participants with first vaccination, follow-up was restricted until the receipt of second vaccination, and only if both vaccinations were less than 3 months apart

Reactogenicity outcomes: participants completed reactogenicity questionnaires regarding symptoms experienced within 7 days of receiving the vaccination. Table 7 describes the reactogenicity variables, operational definitions, and assessment period.

Table 7: Reactogenicity of MVA-BN vaccination.

Variable	Operational Definition	Assessment Period
Local symptoms at injection site	Categorical: yes, no	At CED for those vaccinated by study centre or day of vaccination for crossover participants or receipt of second vaccination during follow-up
Pain	Categorical: No pain, mild, moderate, severe	
Tenderness with pressure/movement	Categorical: No pain, mild, moderate, severe	
Redness	Categorical: No or <2cm, 2-5cm, 5.1-10cm, >10cm	
Swelling	Categorical: No or <2cm, 2-5cm, 5.1-10cm, >10cm	
General Symptoms	Categorical: Fever and/or chills, Fatigue and/or tiredness, New onset of muscle pain or worsening of preexisting muscle pain, New onset of joint pain or worsening of preexisting joint pain, headache, nausea and/or vomiting, diarrhoea, other	
Symptom severity	Categorical: Mild, Moderate to severe, Very severe	
Highest temperature	Categorical: not measured, <37.5ºC, 37.5°C to 37.9°C, 38°C to 38.4°C, 38.5°C to 38.9°C, 39°C to 40°C, <40º	
Took medication (for pain/fever)	Categorical: yes, no	
Prophylactic medication	Categorical: yes, no	

6.4.3 Covariates

In **SEMVAc**, all variables were mandatory to collect for all the participating centres, however, at the individual participant level, some of them might be missing due to participants not completing the questionnaires or not disclosing certain variables. Tables 8 and 9 below lists the operational definition and assessment period **for**

baseline information collected regarding demographics, sexual history, and sexual behaviour of participants. **In TEMVAc**, covariates were defined similarly and were collected via retrospective medical chart review. Baseline period for collection of covariates in TEMVAc is from 1 January 2022 to 30 June 2022. See Appendix SAP Section 4.1 for further details.

Table 8: Baseline Characteristics collected for all participants in the overall MSM cohort at CED.

Variable	Operational Definition	Assessment period
Age	Continuous: Years Categorical: 18-35, 36-49, and ≥50 years	At CED
Height	Continuous: Centimetres (cm)	At CED
Weight	Continuous: Kilograms (kg)	At CED
BMI	Continuous: Kilograms per meter^2 (kg/m2)	
Medications	Categorical: None, PrEP users, Antiretroviral therapy (ART), Systemic immunosuppressive therapy (e.g. systemic glucocorticoids)	At CED
CD4 count	Continuous: Cells per microliter	At CED
HIV status	Continuous: Viral copies per mL	At CED
HIV viral copies under detection limit	Categorical: No, Yes	At CED
Pre-existing conditions	Categorical: None, Immunosuppression (non-HIV), HIV, STI infection, Rheumatological disease, Tumor/malignancy, Hematological disease, Chronic Cardiovascular disease, Chronic lung disease, Chronic kidney disease, Chronic liver disease, Diabetes mellitus, Atopic dermatitis	At CED
Sex at birth	Categorical: Male, Female, Not specified	At CED
Current Gender	Categorical: Male, Female, Trans-male, Trans-female, Non-binary, Not specified	At CED
Smallpox Vaccination	Categorical: None, Yes, (vacc. certificate, once), Yes (vacc certificate twice), Yes, probably (one scar/participant history), Yes, probably (two scars/participant history), Not specified	At CED
Calendar week	Week of the calendar year as documented on the inclusion form	At CED
Federal region	Federal region of Germany to which the study centre pertains	At CED

Sexual behaviour:

In addition to sexual behaviours collected at baseline, participants who were vaccinated during the study period and are included in the Safety cohort completed sexual behaviour questionnaires monthly and after each vaccination (first or second dose) in reference to the sexual behaviour four weeks prior and post vaccination. Sexual behaviours were assessed on a monthly basis in the overall MSM cohort, including for those vaccinated prior to CED. Table 9 describes the variables and operational definitions of sexual behaviour variables.

Table 9: Sexual behaviour in participants vaccinated prior to the study or during the study period.

Variable	Operational Definition	Assessment Period
Sexual activity within the last month*	Categorical: No, Yes, Not specified	Monthly after CED until Dec 31st, 2023.
Number of male (including trans men) sexual partners	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	

Variable	Operational Definition	Assessment Period
Number of female (including trans female) sexual partners	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of non-binary sexual partners	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of sexual partners without using a condom	Categorical: Always used condoms, 1, 2, 3, etc. or not specified	
Sexual practices	Categorical: Oral (passive, active), Anal (passive, active), Vaginal (passive, active), Other, Not specified	
Sexual behaviour of sex partner	Categorical: Unknown, Does not use condoms, Intravenously injects drugs, Sex worker, None of the above, Not specified	
STI	Categorical: No, Yes, Yes - several, Uncertain - with symptoms and not tested, Not specified	
PEP Use	Categorical: No, Yes, Yes - twice, Yes ≥3, Not specified	
PrEP Use	Categorical: No, Yes - regularly & daily, Yes - regularly, but not currently, Yes - irregularly and several times a week, Yes - but only when I needed it, Not specified	
Contact with mpox infected person	Categorical: No, Yes, Not specified	
Skin lesions	Categorical: No, Yes, Not specified	
Asked only in reference to the first or second vaccination only for those vaccinated in the Safety cohort		
Sex in the 4 weeks immediately before vaccination	Categorical: No, Yes, Not specified	Four weeks before and after vaccination for those vaccinated in the Safety Cohort
Number of male (including trans men) sexual partners within the 4 weeks immediately before first vaccination	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of female (including trans female) sexual partners within the 4 weeks immediately before first vaccination	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of non-binary sexual partners within the 4 weeks immediately before first vaccination	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number sexual partners within the 4 weeks immediately before your first vaccination without using a condom.	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Sex in the 4 weeks after the vaccination	Categorical: No, Yes, Not specified	
Number of male (including trans male) sexual partners the 4 weeks immediately after vaccination	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of female (including trans female) sexual partners the 4 weeks immediately after vaccination	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of nonbinary sexual partners within the 4 weeks immediately after vaccination	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of sexual partners the 4 weeks after vaccination without using a condom	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Sexual behaviour questionnaires administered to the overall MSM cohort at CED		
Sexual attraction of participant	Categorical: Men, Women, Non-binary person, asexual, not specified	At CED

Variable	Operational Definition	Assessment Period
Sexual activity*	Categorical: No, Yes, Not specified	
Number of male (including trans) sexual partners *	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of female (including trans) sexual partners*	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of non-binary sexual partners*	Categorical 1, 2, 3, 4, 5, 6-10 or >10, Not specified	
Number of sexual partners without using a condom?	Categorical: Always used condoms, 1, 2, 3, etc. or not specified	
Sexual practices*	Categorical: Oral (passive, active), Anal (passive, active), Vaginal (passive, active), Other, Not specified	
History of sex partner*	Categorical: Unknown, Does not use condoms, Intravenously injects drugs, Sex worker, None of the above, Not specified	
STI*	Categorical: No, Yes, Yes - several, Uncertain - with symptoms and not tested, Not specified	
PEP Use*	Categorical: No, Yes, Yes - twice, Yes ≥3, Not specified	
PrEP Use*	Categorical: No, Yes - regularly & daily, Yes - regularly, but not currently, Yes - irregularly and several times a week, Yes - but only when I needed it, Not specified	
Contact with mpox infected person in the past month	Categorical: No, Yes, Not specified	
Skin lesions in the past month	Categorical: No, Yes, Not specified	

* These questions were asked in reference to the time period within the previous month and 3 months from the day of completing the questionnaire

6.5 Data sources

Primary data collection in SEMVAc

Each participant received a participant number (pseudonym) that was unique for the individual person. All study participant-related data was stored under this pseudonym. Each study centre created a confidential list which linked this pseudonym to the full name of the study participant. This list was accessible only to the study team at the study centre and the monitor. Data entry was performed using electronic CRFs (eCRF). The data collection was facilitated through the utilisation of the application REDCap (Research Electronic Data Capture, version 13.7.31). REDCap is a secure software platform that includes audit trails to track data manipulation and export procedures, ensuring data integrity and security throughout the study process. At the end of the study after all entries have been finalised, and data validation and querying processes were completed, the database was locked.

Trained study personnel at each study centre were responsible for collecting and validating the data. Enrolment of participants, ensuring they met inclusion criteria and had no exclusion criteria, as well as the collection of baseline data, was conducted by a study physician. Assessment of potential adverse reactions and cases of mpox was carried out by a study physician.

Case Report Form (CRF)
The data collected from the study participants is documented electronically in an eCRF. Personal data were pseudonymized before being transmitted. The data is stored within the Charité server system of the study department and is only accessible through the study team. Paper documents are stored in a safe location within the study centres.

Questionnaires
Study participants were asked to complete questionnaires during their participation in the study including the following items: Baseline characteristics (including age, sex, height, body weight, previous smallpox vaccinations, comorbidities including previous sexually transmitted infections (STIs), intake of selected medications (i.e. antiretroviral therapy, immunosuppression, HIV pre-exposure prophylaxis), exposure risk to MPXV infection (i.e. sexual behaviour), tolerability (reactogenicity) of the vaccination, and symptoms that may indicate monkeypox disease. For this purpose, participants must consent that their email address is transmitted to the lead investigating team.

Data on exposure status, outcomes, and covariates were defined a priori and collected using questionnaires administered by the study centres. Outcomes pertaining to safety events are defined by International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) codes and the Medical Dictionary for Regulatory Activities (MedDRA). Vaccination status for those participants vaccinated before the study entry was directly available in the electronic health record (EHR) and/or checked in the vaccination passport that is used in Germany. Table 1 describes the information collected by which questionnaires at which time points throughout follow-up.

Data privacy
Participants were asked to provide their personal email address, personal health information (PHI) and sexual behaviour. All attempts were made to keep this PHI confidential according to regulatory requirements and national law. However, the risk of unauthorised persons gaining access to PHI cannot be fully ruled out.

Risk mitigation measures
All study records with identifying information were kept in a locked file cabinet or locked room at the participating study site. Electronic files and databases were password protected. The database has only pseudonymized data without any reference to real name, address, date of birth or place of birth. Only participants involved in conducting, monitoring, or evaluating the study had access to the non-aggregated data. The study lead team had only access to pseudonymized data within the electronic case report form (eCRF) without any access to the source participant data at the study centres. Variables were itemised and only study data related to the study goals documented in the eCRF to protect participant data and prevent identification.

Data is published in aggregated form so that inferences about the identity of study participants can be ruled out. Publications and aggregated data do not include information that identifies study participants by name, nor includes exact age of participants, exact dates such as vaccination dates, date of MPXV infection, or date of death. Regarding narratives detailing safety events required for pharmacovigilance, no identifiable characters were included (e.g., name, date of birth, age, study centre, or city). Exact vaccination date was not specified, but rather only the month of vaccination. Only relevant pre-existing conditions or medications were retained and all rare diseases or complete medical history (an exhaustive listing of all pre-existing conditions or medications) was not included in the narratives.

Secondary use of data in TEMVAc

Data used for the emulation of the target trial in TEMVAc were collected from the EMRs of eligible subjects by study centre physicians, via manual chart review. Data manually extracted from the EMR was subsequently entered into the eCRF using the software Redcap. During the chart review process, study

physicians were asked to select all eligible subjects independently of the outcome of MPXV infection to ensure inclusion and exclusion criteria were applied equally and to avoid selection bias (see Section 6.3). The study centres already participating in SEMVAc were contacted and offered participation in TEMVAc.

6.6 Bias

SEMVAc was a non-interventional study in which data was collected via questionnaires, which can introduce personal or social desirability bias regarding the sensitive information around sexual history and behaviours. It is possible that participants were hesitant to fully disclose their sexual practices and history given the stigma that mpox or identifying as MSM may carry. Furthermore, participants were asked to refer to the previous month and three months period of activity and some recall bias may exist. The participants were selected based on being attended to by healthcare clinics that specialised in HIV treatment and prevention, thus selecting for a population that may not be representative of the general population as they had a higher risk of contracting mpox disease, and thus more likely to be vaccinated early and under consistent care of medical professionals.

The mpox vaccination campaign was developed to prevent spread of mpox disease in those most at risk in the context of a vaccine shortage. Therefore, a potential bias exists when evaluating VE in the most at risk populations, specifically given that the most at risk were vaccinated early in the outbreak. This group is not only at higher risk, but those who were vaccinated early on entered the current study with some immunity if they were vaccinated prior to CED and then entered the study with their second vaccination. VE estimates may be affected by this selection bias, however this is limited and adjusted using multistate models, which adequately incorporate the time-dependency of vaccination status at study enrolment into the analysis. Additional selection bias may exist regarding the missing information on participants who do not attend ID clinics but may live in the surrounding study area. The demographic characteristics of participants in this study may not fully represent the diverse communities of Berlin or greater Germany. Time-varying confounding may be introduced given the timing of the mpox outbreak in relation to vaccine uptake. The outbreak showed rapid spread of disease early on, with a subsequent drop in cases just as the vaccine rollout was fully executed. It is necessary to recognize the varying natural immunity versus vaccine induced immunity given the changes to the epidemiological curve in tandem with the vaccine rollout. To date, there is lack of information on potential waning immunity, which could affect future VE studies.

A well-designed target trial emulation can mitigate bias related to measured confounders. As a difference to SEMVAc, TEMVAc leverages use of secondary data without the ability to supplement the patient information available in the medical charts with additional surveys. Thus, potential for unmeasured confounding exists due to the inability to ascertain information for recognised confounding variables (i.e., sexual behaviour). Additional bias may arise due to the exclusion of subjects who did not meet the criterion of ≥1 episode of contact in TEMVAc, meaning that potential subjects who did not seek care at a participating centre more than once during the required time window remained unobserved due to this exclusion criteria, but may exist in the source population as vaccinated, unvaccinated, or an mpox case. Therefore, while the source population was defined as all patients attending the participating infectious disease or HIV clinics across Germany, the final sample population only included subjects who consistently received care from the participating study centres. These excluded subjects may be characteristically different from the study sample (i.e., healthier at baseline, less care-seeking behaviour) than those included. Notably, subjects who consistently seek care and develop a relationship with the healthcare practitioner may be more likely to engage in preventative measures (i.e., vaccination, receive PrEP). However, given that all subjects included in the study were seen by one of the participating centres, some healthcare seeking behaviour is implied and substantial differences in health seeking behaviour between vaccinated and unvaccinated subjects is unlikely. Lastly, because TEMVAc is based on secondary data, the information collected regarding sexual behaviours were a proxy to the survey data on sexual behaviours collected in SEMVAc. Therefore, there is some uncertainty regarding the associated

level of risk at baseline, given that history of sexually transmitted infections (STIs), number of STI tests, and number of STI diagnoses does not fully represent the number of sexual partners or sexual behaviours that may increase the risk of mpox.

Nevertheless, target trial emulation offers several distinct advantages. First, the matching algorithm in a rolling cohort emulates the randomisation process of a randomised control trial, ensuring a balance in covariates, thus significantly adjusting for potential confounding. Second, unlike many studies that use secondary data, TEMVAc utilised the in-depth patient knowledge of the practising physicians in the participating study centres to ensure accurate selection and assessment of the study population. These physicians manually reviewed charts and determined who fit the inclusion and exclusion criteria. This personalised approach ensured that the sample of patients closely approximates the target population at risk, thereby increasing the generalizability of the results. Lastly, the target trial emulation design of TEMVAc allowed researchers to select data from a rich source of secondary data and thus achieve a larger sample size from the target population, bolstering power and overall confidence in the effect estimates.

6.7. Study size

The following sample size calculations were conducted before the beginning of the study. After an extensive consultation with multiple experts, including the Robert Koch Institute (RKI) - Department 33 Vaccine Prevention, the sample size was estimated based on incidence data from the months of May and June 2022, particularly the RKI reporting data from calendar weeks 23-25 in various German cities. Using the formulas of Fleiss with continuity correction for cohort studies on the grounds of a significance level alpha of 0.05, a power of 0.8, a proportion of non-exposed persons with outcome of 0.5% and a proportion of exposed persons with outcome of 0.15% (corresponding to an estimated VE of 70%), a **sample size of approx. 5000 participants per study arm was calculated**, which was used as a foundation for further sample size planning.

In anticipation of group changes in the control group due to increased vaccination supply, expected loss-to-follow-up (discontinuation or exclusion in case of e.g., post-exposure prophylaxis) and regarding the necessity of a statistical adjustment by means of propensity score matching, we assumed an initial necessary **number of participants of 10,000 for the unvaccinated group**. For the analysis of the outcome measures, it was assumed that this sample size would provide sufficient information for the comparison of the two groups.

6.7.1 Study size for VE analysis using TEMVAc

Assuming a VE of approx. 0.8 (13) after one dose, a proportion of unvaccinated with mpox of 0.5%, a proportion of vaccinated with mpox of 0.1%, and a drop-out rate to account for loss to follow-up in controls that get vaccinated of 20%, it was estimated that approx. 3600 subjects should be recruited into each study arm to achieve a power of 0.8 (significance level alpha: 0.05, log-rank test of survival in two groups, nQuery version 8.4.1.0.). These assumptions were based on reports of cases from study centres and information provided by the Robert Koch Institute (RKI) - Department 33 Vaccine Prevention.

This estimation was used to guide the selection of study centres of the SEMVAc network for the TEMVAc analyses, but a larger number of retrospective datasets were included. In particular, all eligible patients from the selected study centres were included to avoid selection bias and to increase the sample size needed to obtain VE results while ensuring sufficient power when accounting for dropout due to censoring when controls were vaccinated. In addition, the higher sample size was expected to serve as a “buffer” for the matching procedure to have a larger pool of matching controls.

6.8. Data transformation

Descriptive statistics of baseline characteristics were used to describe the overall MSM cohort, which was categorised by vaccination status at CED into the following distinct subgroups: two doses MVA-BN prior to CED, one dose MVA-BN prior to CED, second MVA-BN vaccination at or after CED, first MVA-BN vaccination at CED, unvaccinated at CED. Baseline characteristics for participants who receive an MVA-vaccination during follow-up (crossover from unvaccinated to vaccinated) are presented alongside.

For continuous variables, we report measures of central tendency such as mean with standard deviation or median with interquartile range, depending on the distribution of the data.

Categorical variables were reported as counts with percentages. Data was not transformed to a different scale (e.g., log transformation). Clinically meaningful categories to aggregate the raw data for sexual behaviour were established to provide a better overview within the tables. A more granular reporting of the categories of sexual behaviour is provided in the appendix.

6.9 Statistical methods

6.9.1. Main summary measures

The baseline characteristics and sexual behaviours of participants were reviewed and described prior to propensity score (PS) matching in the overall MSM cohort in the fully vaccinated and comparator groups for the primary VE objective. Categorical variables were reported as the count and percentage of participants within each category. Continuous variables were reported as the mean and standard deviation. Participants with missing values were retained and missing values were quantified and reported.

6.9.2 Main statistical methods

6.9.2.1 Primary objective: Vaccine Effectiveness analysis

VE was estimated by comparing the occurrence of the outcome, mpox confirmed by positive PCR, in vaccinated versus unvaccinated participants. Reported infections by participants that were not confirmed with PCR by study centres (e.g., only reported via monthly questionnaires) were reported separately from PCR confirmed infections.

Initially, the analysis of the primary endpoint VE was defined as reduction in risk of infection/disease in vaccinated versus unvaccinated participants by VE = 1- Relative Risk, RR defined using cumulative incidences (i.e. attack rate) or hazard ratios. The number of vaccinations at ID was accounted for in the analyses. Propensity score matching was planned at a ratio of 1:2 for vaccinated and unvaccinated, using a calliper of 0.1, to reduce bias in results and ensure comparability and balance in baseline characteristics across vaccinated and comparator groups. However, no mpox cases were observed in the unvaccinated group at the time of the SEMVAc final analyses, requiring the implementation of TEMVAc, implementing the planned VE calculations (Risk Ratios, HRs) in a matched rolling cohort design.

For the SEMVAc analysis, frequency of participants in the VE cohort were reported as n (%). The number of confirmed mpox disease events were reported alongside cumulative incidences after the 1st and 2nd vaccination with 95% confidence intervals. The occurrence of MPXV infection after the receipt of vaccination were additionally accounted for by applying the following strata:

- MPXV infection within 0-13 days after the first vaccination
- MPXV infection at least 14 days after the first vaccination
- MPXV infection within 0-13 days after the second vaccination
- MPXV infection at least 14 days after the second vaccination

TEMVAc: Vaccine Effectiveness analysis

Time-dependent MVA-BN vaccination was used within the observation period from 1 July 2022 to 31 October 2022. Individuals enrolled as unvaccinated and who received a vaccine dose during follow-up were censored in the unvaccinated cohort alongside with their matched vaccinated individual and re-enrolled in the vaccinated cohort (rolling cohort, Figure 2).

Matching was performed based on a rolling cohort, thereby, beginning on 1 July 2022 and advancing daily, eligible persons receiving the first MVA-BN vaccination on that day were matched to controls that were not previously recruited at a ratio 1:1. Exact matching was performed based on the variables listed below. If exact matching was not feasible, matching rules for each variable were adapted accordingly or variables were excluded from the matching algorithm.

Matching variables: exposed were matched to unexposed based on the following potential variables

- Age ([18-35], [36-49], and ≥50 years)
- HIV infection (yes/no)
- PrEP intake (yes/no)
- History of smallpox vaccination (yes/no)
- Number of STI diagnoses in past 6 months prior to index date before study observation period (categorical: 0, ≥1)
- Federal region of study centre (Berlin, Hamburg, Bavaria, North Rhine-Westphalia, Hessia)

VE was calculated as (1 - RR)*100. VE was reported for both groups, those with one dose and two doses of MVA-BN

6.9.2.2 Secondary Objective (Safety, Sexual Behaviours, Pre-existing Conditions)

Vaccine safety (SEMVAc)

All vaccinated participants were interviewed about potential adverse events by the study centre physician. The causal relationship between the MVA-BN vaccination and the SARs/ARs reported by the participant was assessed by the physician at the study centre and standard pharmacovigilance reporting procedures were followed. The study centre physician used the WHO causality guidelines to make the assessment and any adverse reaction was recorded in a case narrative. Only events whose causal relationship with the vaccine was assessed as certain, probable, or possible, and which occurred within 3 months after the last MVA-BN vaccination were recorded in the eCRF as an adverse reaction (AR). Those who completed less than 3 months of follow-up for safety events were censored accordingly related to: death, end of study period, maximum of 12 months follow-up, and for participants with first vaccination, follow-up was restricted until the receipt of second vaccination, however, only if both vaccinations were less than 3 months apart.

Safety endpoints SARs and ARs were described via absolute frequencies of participants. Time-to-event analysis was used for time to first AR/SAR for each safety endpoint via cumulative incidences using the Kaplan-Meier estimator. In the presence of competing events (e.g. death), cumulative incidences would be estimated via Aalen-Johansen estimator. Given only two reported deaths during study observation, death is not considered as a competing event. Each AR was counted once for a given participant and graded using the highest intensity and relationship to MVA-BN vaccination. ARs were coded using the current version of the Medical Dictionary for Regulatory Activities (MedDRA) and presented by system organ class (SOC) and preferred term (PT), timing of occurrence after vaccination and outcome.

Reactogenicity

Reactogenicity was summarised using descriptive statistics (absolute and relative frequencies) with 95% CI and influence of relevant covariables on any local and any systemic reaction was analysed using univariable and multivariable logistic regression models. ORs with 95% CIs are presented. Relevant covariables used for the logistic regression model included: age (per 10 years), HIV+ status according to CD4 counts (according to CDC classification of CD4 counts as <200, ≥200 and <500, ≥500 CD4+ cells/µl), PrEP use, and previous smallpox vaccination.

Change in sexual behaviour following vaccination

Change in sexual practice was analysed in a descriptive manner. Longitudinal cluster analysis was performed using k-means clustering for longitudinal data of pre- and post- vaccine sexual practice questionnaires (21). The number of clusters was determined by assessing variants of the nonparametric quantitative metric such as Calinski-Harabatz based on between-cluster and within-cluster covariance matrices (22).

6.9.3. Missing data

Results include the categories "unspecified" and NA (missing). Unspecified is not equivalent to missing data, because the participant actively answered and confirmed that they would not disclose this information. Some participants answered parts of the enrolment questionnaire, however, did not answer all items. This explains the varying values of missing information for the baseline and sexual behaviour questionnaires. Some of the missing data applies to the dropouts.

On participant questionnaires, which were filled out by the participants themselves, no query management could be performed, and missing values had to be accepted based on participant self-documentation. For questions and information that was provided by the study centres, data validation checks were conducted according to an established data validation plan. The data validation plan was developed in collaboration between the database management team and the study leading team at Charité, and missing values were queried. Queries were generated and were imported into the study database to allow for rectification by the study centres. If queries were insufficiently answered, further clarification was sought. Study centres were regularly reminded, and communication was established to allow for timely answers to all queries.

6.9.4. Sensitivity analyses

6.9.4.1 Sensitivity analyses in SEMVAc

The following sensitivity analyses were planned during the protocol development phase and executed after completion of the analyses for the primary and secondary objectives.

To assess the incidence of MPXV infection using different definitions of ‘vaccinated’, crude IRs per 1000 person-years were calculated for the following groups and compared to the unvaccinated:

- Participants who were ‘fully vaccinated’, meaning a requirement of 2 doses after CED, with 14 days after the receipt of the second dose and at least 28 days and a maximum of 35 days apart (the receipt of the second dose 28 days after the first dose as per recommended vaccination regime plus one additional week to account for delays in vaccine administration). Follow-up started at least 14 days after the receipt of the second vaccination.
- Vaccinated participants stratified by the first and second vaccination.
- Vaccinated participants stratified by the occurrence of MPXV infection after the receipt of vaccination:
    - MPXV infection within 0-13 days after the first vaccination
    - MPXV infection at least 14 days after the first vaccination
    - MPXV infection within 0-13 days after the second vaccination
    - MPXV infection at least 14 days after the second vaccination

6.9.4.2 Sensitivity analyses in TEMVAc

Sensitivity Analyses for the Primary Outcome
Cause-specific Cox proportional hazards (PH) models will be used to study the association between vaccine administration and the risk of MPXV infection. For the main association, the results will be presented as adjusted matched hazard ratios (aHRs) with 95% confidence intervals (CIs) to account for differences in covariates not included in the matching process. Vaccine administration will be modelled as time-dependent in the same way as in the Kaplan-Meier estimator. The assumption of proportional hazards will be checked using scaled Schoenfeld residuals (23). If the assumption does not hold, we will consider alternative models, i.e., Landmarking, proportional subdistribution hazards (24) or Aalen's additive model (25).

Secondary Analyses
Analysis of symptoms of PCR-confirmed MPXV infection will be presented descriptively. Interval and ratio variables will be reported by mean and standard deviation (SD), or if the variable is not normally distributed, with median, interquartile range, minimum and maximum. Categorical variables by absolute and relative frequencies. Symptoms of MPXV infection will be stratified by vaccination status.

6.9.5. Amendments to the statistical analysis plan

None.

7. Quality control

Throughout the study period, the Clinical Research Associates (CRA) of the Charité – Clinical Trial Office (CTO) conducted interim monitoring visits at all SEMVAc study sites to ensure equal data quality in all study sites. Thereby, the CRAs checked adherence to the observation plan of all participating study sites and the performance of study procedures. Furthermore, the correct enrolment process was monitored to ensure participant rights and data protection.

During the whole study period the CRAs performed on average two Interim Monitoring Visits per site. High recruiting sites were visited more often and additionally, phone or mail contacts were performed to track and resolve open issues with the sites. The quantity and frequency of the visits were performed depending on the number of enrolled participants, the data quality, and open issues.

The CRAs performed the following tasks during the Interim Monitoring Visits:

- Verify the existence of study participants
- Check signed consent forms for both initial and amended ICFs
- Perform source data verification for selected participants (see "Source Data Verification")
- Ensure compliance with the observation plan based on reviewed key data
- Address any outstanding questions from previous visits
- Verify the completeness of the study folder (new study documents should be promptly provided by the study leadership team)
- In case of staff changes at the study centre, verify correct documentation
- Discuss any issues with the study site personnel

Throughout the monitoring process, the CRAs checked the informed consent forms for 100% of the study participants. Additionally, they performed Source Data Verification (SDV) for at least 10-25% of the enrolled participants on the following data: inclusion and exclusion criteria, vaccinations, and the end of the study.

Furthermore, all infections as well as adverse reaction (AR) and serious adverse reaction (SAR) occurred in this study were verified by the CRAs.

After each Interim Monitoring Visit the CRA prepared a monitoring visit report, which was sent to the Study lead team at Charité to inform them about the progress, possible issues and protocol deviations of the study sites. In case of urgent issues, the principal investigators were directly informed by the CRA. Each site received a follow-up letter of the Interim Monitoring Visit with outstanding tasks, which needed to be processed and rectified by the study site personnel.

8. Results

8.1. Enrolment

A total of 6,459 participants were initially recruited in **SEMVAc** between 7th July 2022 and 31st of December 2023 prior to exclusions or dropouts. After sequential exclusion criteria were applied, a total of 6,265 participants were included in the overall MSM cohort. A total of 94 participants were excluded for various reasons as described in Table 11, the majority due to withdrawal of the declaration of consent. Most participants resided in highly populated cities (i.e. 59.8% of participants were in Berlin). Eight of the 12 cities had only one active study site, with Berlin having the most active recruitment sites (Table 10). The majority of participants were recruited between July and December 2022 (Figure 4 and Supplementary Table 2 show the distribution of recruited participants by study month).

Table 10: Distribution of participants in study sites by city.

	Number of active sites per city	**Number of included participants by city**
		n (%)
Aachen	1 (3.23)	34 (0.53)
Augsburg	1 (3.23)	3 (0.05)
Berlin	13 (41.94)	3803 (59.81)
Bochum	1 (3.23)	124 (1.95)
Bonn	1 (3.23)	24 (0.37)
Essen	1 (3.23)	51 (0.81)
Frankfurt/M.	1 (3.23)	246 (3.84)
Hamburg	2 (6.45)	891 (13.92)
Köln	3 (9.68)	587 (9.17)
Leipzig	1 (3.23)	19 (0.3)
Lübeck	1 (3.23)	8 (0.12)
München	5 (16.13)	569 (9.04)
Total	**31 (100)**	**6359 (100)**

Table 11: Study enrolment characteristics.

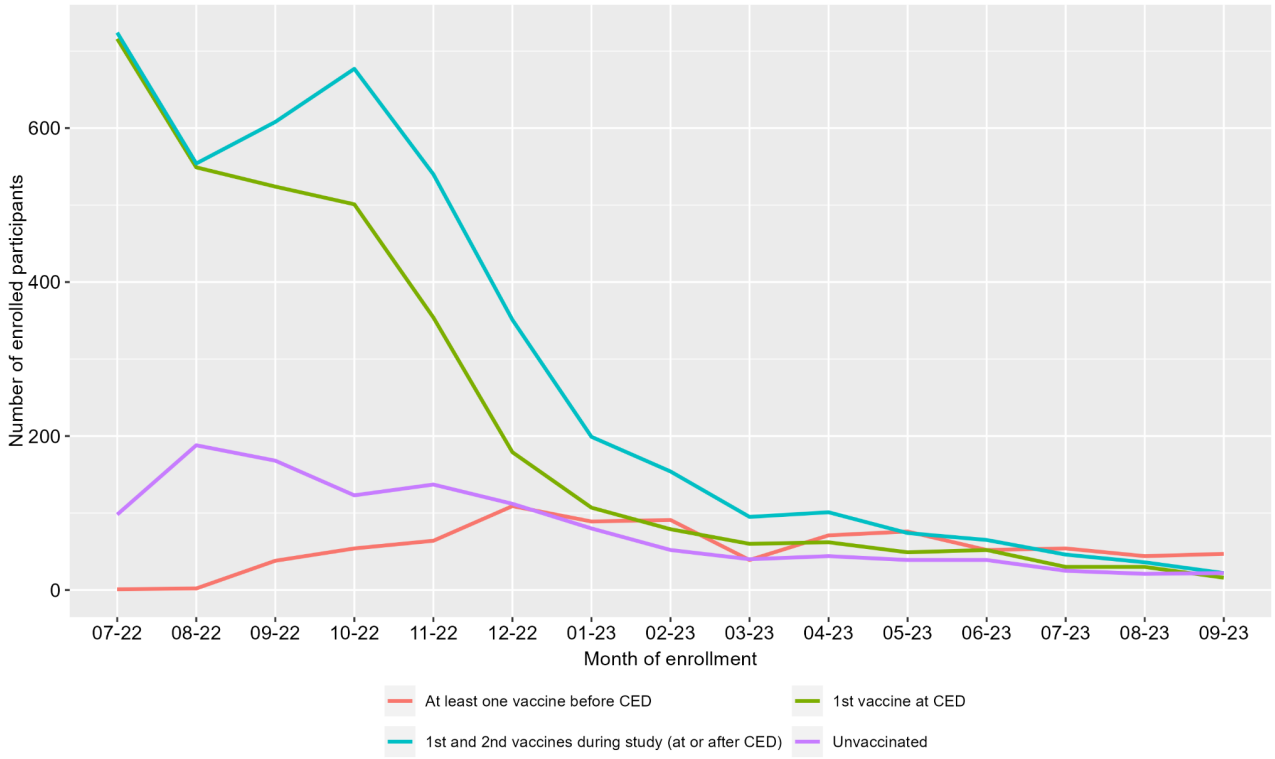
	Total
Total recruited participants	**6459**
Reasons of exclusion[1]	
Subsequent occurrence of exclusion criteria (after recruitment)	13
Withdrawal of the declaration of consent	48
Known exposure to MPXV as unvaccinated prior to CED	12
Drop-out at Enrolment	21
Total excluded participants	**94**
Participants after exclusion	**6359**
Deleted participants by study centre[2]	100

Group Totals	
Vaccination group	5077
Comparator group (unvaccinated)	1188
Missing	0
Total included participants	**6265**
Drop-out	48
Participant deceased	2

[1] More than one possible reason for exclusion could apply during the study period

[2] Reasons for deletion by the study centres included withdrawal of study consent with participant's wish to have their data deleted, significant violation of study protocol, and incorrect study consent.

Figure 4: Count of SEMVAc study participants recruited by month and vaccination status.



8.2. Baseline characteristics of the study cohort

8.2.1. SEMVAc study cohort baseline characteristics

Table 12 describes the number of participants included in the study period and the total reported number of MPXV infections (patient reported and/or PCR confirmed, and physician reported), stratified by vaccination status. There was a total of 14 MPXV infections PCR confirmed and physician reported, the majority (n=11) occurring in the 1st MVA-BN at CED group. In some cases, the MPXV infection was only reported by the physician on the eCRF and not necessarily by the participant on the mpox questionnaire. More details on the MPXV infection by study subgroups and evolution by month can be found in Section 8.3 Outcome Data.

Table 12: Evolution of study participant selection and MPXV infections, stratified by vaccination status.

	All included	2nd dose MVA-BN prior to CED	1st dose MVA-BN prior to CED	1st dose MVA-BN at CED[1]	2nd dose MVA-BN at or after CED[2]	Unvaccinated	NA[3]
N	6265	531	300	3308	938	1188	0
Crossover[4]	542	0	0	0	0	542	0
Drop-out[5]	48	4	2	26	6	10	0
Deceased[6]	2	0	0	2	0	0	0
MPXV infection (PCR confirmed, reported by physician)[7]	14	1	0	11	0	2	0
MPXV infection (participant-reported)	11	2	0	8	0	1	0
Total MPXV infections reported	15	2	0	11	0	2	0

1 Out of 3308 participants with the first dose at CED, 2312 received a second vaccination during follow-up.
2 participant received first vaccination prior to CED
3 Exposure group not provided by study centre
4 participants who were first included in the unvaccinated group and were vaccinated later
5 Includes drop-outs mentioned in Table Summary Enrolment
6 participant 1 died of hypertensive crisis 3 months after the 1st dose, participant 2 died 2.5 months after the 2nd dose from lung cancer.
7 MPXV infection in the unvaccinated cohort at baseline was reported for the first participant 15 days after first vaccination and for the second participant 294 days after the first vaccination and 264 days after the second vaccination.

Baseline characteristics are summarized in Table 13 below. Participants were of an average of 41 years of age, had a BMI of 25, and the majority had no chronic disease (81.4%). Persons living with HIV (PLWHIV) participating in the study were slightly older than PrEP users, (48 versus 37 years of age) and those with a history of smallpox vaccination were the oldest of the study population (54 years of age). PLWHIV, PreP users, and HMSV subgroups had similar BMI at baseline (25.05 m^2/kg, 24.51m^2/kg, 25.55m^2/kg, respectively). PrEP users had slightly more frequent STIs (13.4%) when compared to PLWHIV (11.2%) and HSMV (11.3%) subgroups, while those in the HSMV subgroup were 54% PLWHIV. Those in the HSMV subgroup had the highest frequency of chronic cardiac (16.4%) and lung disease (4.9%). Those in the subgroup of PLWHIV had higher frequency of chronic cardiovascular (11.1%) and lung disease (3.7%) when compared to PrEP users (cardiovascular, 5.5%; lung 3.4%). With regards to vaccination status

at baseline, the PrEP users subgroup was more frequently vaccinated with two doses of MVA-BN prior to (11.8%) and at CED (16.3%), while the HSMV subgroup was most frequently vaccinated with one dose prior to CED (9.0%). PLWHIV and HSMV subgroups were more frequently vaccinated with the first dose at CED (53.9%, 53.8%) than the PrEP user subgroup (45.7%). Frequency of participants from each subgroup were represented fairly equally in the unvaccinated group (PLWHIV, 20.1%; PrEP, 20.6%; HSMV 18.2%).

Table 13: Baseline characteristics of the overall MSM cohort and PLWHIV, PreP users and HSMV subgroups.

	MSM cohort (N=6265)	**PLWHIV (N=1920)**	**PrEP users (N=3009)**	**HSMV (N=1739)**
	Mean (SD) or n (%)			
Age	41.06 (11.55)	47.95 (10.85)	37.21 (9.86)	53.61 (8.74)
Height	180.35 (7.14)	180.11 (7.04)	180.44 (7.16)	180.38 (6.93)
Weight	80.40 (14.73)	81.20 (14.14)	79.79 (15.02)	83.25 (14.80)
BMI	24.72 (4.84)	25.05 (4.73)	24.51 (5.17)	25.55 (4.14)
Antiretroviral therapy (ART)	1901 (30.3)	1901 (99.0)	0 (0.0)	937 (53.9)
CD4 count cells per microliter	781.91 (307.05)	781.91 (307.05)	NaN (NA)	768.38 (297.31)
HIV viral copies per mL	1979.31 (18275.20)	1979.31 (18275.20)	NaN (NA)	2375.66 (21195.81)
HIV viral copies under detection limit	1433 (22.9)	1433 (74.6)	0 (0.0)	709 (40.8)
PrEP users	3009 (48.0)	0 (0.0)	3009 (100.0)	466 (26.8)
Systemic immunosuppressive therapy (e.g. systemic glucocorticoids)	16 (0.3)	3 (0.2)	3 (0.1)	6 (0.3)
No pre-existing conditions	3377 (53.9)	0 (0.0)	2263 (75.2)	501 (28.8)
Immunosuppression (non-HIV)	22 (0.4)	3 (0.2)	12 (0.4)	7 (0.4)
HIV infection	1920 (30.6)	1920 (100.0)	0 (0.0)	944 (54.3)
STI infection	676 (10.8)	215 (11.2)	403 (13.4)	196 (11.3)
Rheumatological disease	41 (0.7)	15 (0.8)	19 (0.6)	15 (0.9)
Tumor/malignancy	80 (1.3)	45 (2.3)	22 (0.7)	49 (2.8)
Hematological disease	29 (0.5)	13 (0.7)	14 (0.5)	14 (0.8)
Chronic Cardiovascular disease	468 (7.5)	213 (11.1)	166 (5.5)	286 (16.4)
Chronic lung disease	216 (3.4)	71 (3.7)	103 (3.4)	86 (4.9)
Chronic kidney disease	46 (0.7)	31 (1.6)	7 (0.2)	33 (1.9)
Chronic liver disease	142 (2.3)	105 (5.5)	26 (0.9)	74 (4.3)
Diabetes mellitus	97 (1.5)	43 (2.2)	38 (1.3)	60 (3.5)

	MSM cohort (N=6265)	PLWHIV (N=1920)	PrEP users (N=3009)	HSMV (N=1739)
Atopic dermatitis (neurodermatitis)	46 (0.7)	12 (0.6)	19 (0.6)	10 (0.6)
MVA vaccinations				
2 doses MVA-BN prior to CED	531 (8.5)	150 (7.8)	356 (11.8)	110 (6.3)
1 dose MVA-BN prior to CED	300 (4.8)	94 (4.9)	167 (5.6)	156 (9.0)
2nd MVA-BN at CED	938 (15.0)	256 (13.3)	490 (16.3)	221 (12.7)
1st MVA-BN at CED	3308 (52.8)	1034 (53.9)	1375 (45.7)	936 (53.8)
Unvaccinated	1188 (19.0)	386 (20.1)	621 (20.6)	316 (18.2)
Missing vaccination status	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gender (at birth) *Not specified*	11 (0.2)	2 (0.1)	6 (0.2)	2 (0.1)
Male	5663 (90.4)	1774 (92.4)	2695 (89.6)	1614 (92.8)
Female	19 (0.3)	2 (0.1)	6 (0.2)	1 (0.1)
Missing	572 (9.1)	142 (7.4)	302 (10.0)	122 (7.0)
Gender Identity *Not specified*	15 (0.2)	2 (0.1)	9 (0.3)	4 (0.2)
Male	5543 (88.5)	1750 (91.1)	2631 (87.4)	1599 (91.9)
Female	7 (0.1)	6 (0.3)	0 (0.0)	4 (0.2)
Trans male	11 (0.2)	1 (0.1)	4 (0.1)	1 (0.1)
Trans female	14 (0.2)	2 (0.1)	5 (0.2)	1 (0.1)
Non-binary	103 (1.6)	17 (0.9)	58 (1.9)	8 (0.5)
Missing	572 (9.1)	142 (7.4)	302 (10.0)	122 (7.0)
Smallpox vaccine *Not specified*	782 (12.5)	240 (12.5)	333 (11.1)	347 (20.0)
None	4091 (65.3)	904 (47.1)	2299 (76.4)	0 (0.0)
Yes, vacc. Certificate, once	409 (6.5)	194 (10.1)	130 (4.3)	409 (23.5)
Yes, vacc. certificate twice	270 (4.3)	160 (8.3)	61 (2.0)	270 (15.5)
Yes, probably (one scar/participant history)	500 (8.0)	286 (14.9)	136 (4.5)	500 (28.8)
Yes, probably (two scars/participant history)	213 (3.4)	136 (7.1)	50 (1.7)	213 (12.2)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

The baseline characteristics stratified by vaccination status of the overall MSM cohort and subgroups PLWHIV, PrEP users, and HSMV are described below in Table 14 and Supplementary Tables 3, 4, and 5, respectively.

Approximately 30% were PLWHIV and 48% were PrEP users (Table 14). When compared at baseline to participants who received the first dose of MVA-BN, those who received the second dose at or after CED were more frequently PrEP users (41.6% vs. 52.2%), diagnosed with chronic cardiovascular (5.3% vs 9.7%) or lung disease (2.4% vs 5.8%), and less frequently diagnosed with HIV (31.3% vs 27.3%) and STIs (10.7% vs 7.4%) (Table 14 Baseline characteristics of the overall MSM cohort by vaccination status). Unvaccinated participants were 52.3% PrEP users, 32.5% PLWHIV, and had similar frequencies of chronic disease and STIs (cardiac, 9.2%; lung, 3.5%; STIs 7.7%). Overall, most participants were assigned male gender at birth across all vaccination groups and identified as male.

Table 14: Baseline characteristics of the overall MSM cohort *by vaccination status*.

	All included (N=6265)	**1st dose MVA-BN at CED (N=3308)**	**2nd dose MVA-BN at or after CED (N=938)**	**Unvaccinated (N=1188)**	**Crossover (N=542)**	**1st dose MVA-BN prior to CED (N=300)**	**2nd dose MVA-BN prior to CED (N=531)**
	Mean (SD) or n (%)						
Age	41.06 (11.55)	41.21 (11.63)	39.56 (10.75)	40.95 (12.19)	41.44 (11.53)	45.04 (11.81)	40.76 (10.21)
Height	180.35 (7.14)	180.39 (7.06)	180.35 (7.19)	179.92 (6.87)	180.14 (6.69)	180.64 (6.76)	180.86 (8.14)
Weight	80.40 (14.73)	79.83 (14.41)	79.83 (13.67)	81.32 (15.60)	81.65 (15.78)	81.48 (14.01)	82.13 (16.41)
BMI (m^2/kg)	24.72 (4.84)	24.51 (4.21)	24.49 (3.71)	25.09 (4.44)	25.12 (4.50)	24.93 (3.93)	25.38 (9.09)
Antiretroviral therapy (ART)	1901 (30.3)	1026 (31.0)	253 (27.0)	379 (31.9)	148 (27.3)	93 (31.0)	150 (28.2)
CD4 count cells per microliter	781.91 (307.05)	760.86 (288.73)	777.46 (301.42)	819.60 (350.74)	812.52 (347.44)	799.27 (321.34)	821.41 (294.51)
HIV viral copies per mL	1979.31 (18275.20)	1228.14 (8531.12)	423.73 (2493.69)	5549.18 (35926.11)	14105.85 (62820.39)	891.41 (5332.06)	24.70 (9.44)
HIV viral copies under detection limit	1433 (22.9)	794 (24.0)	198 (21.1)	286 (24.1)	121 (22.3)	51 (17.0)	104 (19.6)
PrEP users	3009 (48.0)	1375 (41.6)	490 (52.2)	621 (52.3)	302 (55.7)	167 (55.7)	356 (67.0)
Systemic immunosuppressive therapy	16 (0.3)	12 (0.4)	2 (0.2)	2 (0.2)	2 (0.4)	0 (0.0)	0 (0.0)
No pre-existing conditions	3377 (53.9)	1796 (54.3)	539 (57.5)	644 (54.2)	319 (58.9)	133 (44.3)	265 (49.9)
Immunosuppression (non-HIV)	22 (0.4)	19 (0.6)	1 (0.1)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
HIV infection	1920 (30.6)	1034 (31.3)	256 (27.3)	386 (32.5)	148 (27.3)	94 (31.3)	150 (28.2)
STI infection	676 (10.8)	354 (10.7)	69 (7.4)	109 (9.2)	42 (7.7)	54 (18.0)	90 (16.9)
Rheumatological disease	41 (0.7)	29 (0.9)	5 (0.5)	5 (0.4)	3 (0.6)	1 (0.3)	1 (0.2)
Tumour/malignancy	80 (1.3)	35 (1.1)	16 (1.7)	16 (1.3)	8 (1.5)	7 (2.3)	6 (1.1)
Haematological disease	29 (0.5)	16 (0.5)	5 (0.5)	3 (0.3)	1 (0.2)	2 (0.7)	3 (0.6)

	All included (N=6265)	1st dose MVA-BN at CED (N=3308)	2nd dose MVA-BN at or after CED (N=938)	Unvaccinated (N=1188)	Crossover (N=542)	1st dose MVA-BN prior to CED (N=300)	2nd dose MVA-BN prior to CED (N=531)
Chronic Cardiovascular disease	468 (7.5)	175 (5.3)	91 (9.7)	109 (9.2)	48 (8.9)	37 (12.3)	56 (10.5)
Chronic lung disease	216 (3.4)	79 (2.4)	54 (5.8)	41 (3.5)	20 (3.7)	23 (7.7)	19 (3.6)
Chronic kidney disease	46 (0.7)	17 (0.5)	8 (0.9)	12 (1.0)	7 (1.3)	5 (1.7)	4 (0.8)
Chronic liver disease	142 (2.3)	62 (1.9)	27 (2.9)	33 (2.8)	12 (2.2)	9 (3.0)	11 (2.1)
Diabetes mellitus	97 (1.5)	45 (1.4)	16 (1.7)	22 (1.9)	10 (1.8)	3 (1.0)	11 (2.1)
Atopic dermatitis (neurodermatitis)	46 (0.7)	28 (0.8)	7 (0.7)	5 (0.4)	1 (0.2)	3 (1.0)	3 (0.6)
Gender (at birth) not specified	11 (0.2)	6 (0.2)	3 (0.3)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Male	5663 (90.4)	3000 (90.7)	835 (89.0)	1044 (87.9)	493 (91.0)	279 (93.0)	505 (95.1)
Female	19 (0.3)	10 (0.3)	2 (0.2)	5 (0.4)	2 (0.4)	2 (0.7)	0 (0.0)
Missing	572 (9.1)	292 (8.8)	98 (10.4)	137 (11.5)	47 (8.7)	19 (6.3)	26 (4.9)
Gender Identity Not specified	15 (0.2)	8 (0.2)	2 (0.2)	3 (0.3)	1 (0.2)	1 (0.3)	1 (0.2)
Male	5543 (88.5)	2930 (88.6)	815 (86.9)	1025 (86.3)	486 (89.7)	273 (91.0)	500 (94.2)
Female	7 (0.1)	4 (0.1)	0 (0.0)	2 (0.2)	0 (0.0)	1 (0.3)	0 (0.0)
Trans male	11 (0.2)	8 (0.2)	1 (0.1)	2 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)
Trans female	14 (0.2)	8 (0.2)	2 (0.2)	2 (0.2)	0 (0.0)	2 (0.7)	0 (0.0)
Non-binary	103 (1.6)	58 (1.8)	20 (2.1)	17 (1.4)	7 (1.3)	4 (1.3)	4 (0.8)
Missing	572 (9.1)	292 (8.8)	98 (10.4)	137 (11.5)	47 (8.7)	19 (6.3)	26 (4.9)
Smallpox vaccine Not specified	782 (12.5)	348 (10.5)	207 (22.1)	105 (8.8)	40 (7.4)	31 (10.3)	91 (17.1)
None	4091 (65.3)	2201 (66.5)	577 (61.5)	817 (68.8)	384 (70.8)	135 (45.0)	361 (68.0)
Yes, vacc. certificate, once	409 (6.5)	196 (5.9)	67 (7.1)	61 (5.1)	37 (6.8)	59 (19.7)	26 (4.9)
Yes, vacc certificate twice	270 (4.3)	110 (3.3)	46 (4.9)	60 (5.1)	32 (5.9)	21 (7.0)	33 (6.2)
Yes, probably (one scar/participant history)	500 (8.0)	302 (9.1)	35 (3.7)	96 (8.1)	28 (5.2)	49 (16.3)	18 (3.4)
Yes, probably (two scars/participant history)	213 (3.4)	151 (4.6)	6 (0.6)	49 (4.1)	21 (3.9)	5 (1.7)	2 (0.4)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Baseline sexual behaviour (assessed at CED) of the overall MSM cohort stratified by study subgroups can be found in Supplementary Table 6. The stratification by vaccination status, is described in Table 15 (and with the detailed categories of number of sexual partners in Supplementary Table 7).

Of the overall MSM cohort, (n=6,265), most participants reported being sexually attracted to men (89.8%), sexually active in the three months previous to study enrolment (83.4%), and the majority reported ≥5 sexual partners (38.2%) in the last three months (Table 15 and Supplementary Table 7). In the overall MSM cohort, less than half (45.1%) reported taking PrEP in the last 3 months. Those in the unvaccinated group took PrEP regularly and daily (30.7%), while those vaccinated with two doses prior to CED reported higher frequency of PrEP use regularly and daily (50.1%). Baseline sexual behaviour stratified by vaccine status for the PLWHIV, PreP users and HSMV can be found in Supplementary Tables 8 to 10.

Table 15: Baseline Sexual Behaviour in the overall MSM cohort, by vaccination status.

	All included (N=6265)	**1st dose MVA-BN at CED (N=3308)**	**2nd dose MVA-BN at or after CED (N=938)**	**Unvaccinated (N=1188)**	**Crossover (N=542)**	**1st dose MVA-BN prior to CED (N=300)**	**2nd dose MVA-BN prior to CED (N=531)**
	Mean (SD) or n (%)						
Sexually attracted to:							
Men	5629 (89.8)	2981 (90.1)	837 (89.2)	1032 (86.9)	489 (90.2)	276 (92.0)	503 (94.7)
Women	371 (5.9)	214 (6.5)	44 (4.7)	75 (6.3)	32 (5.9)	13 (4.3)	25 (4.7)
Non-binary	338 (5.4)	184 (5.6)	57 (6.1)	61 (5.1)	29 (5.4)	12 (4.0)	24 (4.5)
None	12 (0.2)	8 (0.2)	0 (0.0)	3 (0.3)	2 (0.4)	1 (0.3)	0 (0.0)
Not specified	23 (0.4)	14 (0.4)	1 (0.1)	4 (0.3)	1 (0.2)	3 (1.0)	1 (0.2)
Missing	576 (9.2)	294 (8.9)	99 (10.6)	138 (11.6)	48 (8.9)	19 (6.3)	26 (4.9)
Sexually active last 3 months							
Not specified	8 (0.1)	6 (0.2)	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
No	462 (7.4)	251 (7.6)	49 (5.2)	131 (11.0)	50 (9.2)	16 (5.3)	15 (2.8)
Yes	5223 (83.4)	2759 (83.4)	791 (84.3)	918 (77.3)	445 (82.1)	265 (88.3)	490 (92.3)
Missing	572 (9.1)	292 (8.8)	98 (10.4)	137 (11.5)	47 (8.7)	19 (6.3)	26 (4.9)
# of male sexual partners last 3 months							
Not specified	35 (0.6)	20 (0.6)	6 (0.6)	4 (0.3)	2 (0.4)	3 (1.0)	2 (0.4)
0	494 (7.9)	269 (8.1)	51 (5.4)	143 (12.0)	56 (10.3)	16 (5.3)	15 (2.8)
1	847 (13.5)	495 (15.0)	107 (11.4)	173 (14.6)	63 (11.6)	31 (10.3)	41 (7.7)
2-4	1914 (30.6)	1021 (30.9)	276 (29.4)	382 (32.2)	201 (37.1)	79 (26.3)	156 (29.4)
≥5	2393 (38.2)	1205 (36.4)	399 (42.5)	346 (29.1)	172 (31.7)	152 (50.7)	291 (54.8)
Missing	582 (9.3)	298 (9.0)	99 (10.6)	140 (11.8)	48 (8.9)	19 (6.3)	26 (4.9)
# of female sexual partners last 3 months							
Not specified	179 (2.9)	93 (2.8)	20 (2.1)	48 (4.0)	21 (3.9)	8 (2.7)	10 (1.9)
0	5291 (84.5)	2806 (84.8)	786 (83.8)	952 (80.1)	456 (84.1)	262 (87.3)	485 (91.3)
1	123 (2.0)	68 (2.1)	20 (2.1)	21 (1.8)	6 (1.1)	8 (2.7)	6 (1.1)
2-4	69 (1.1)	32 (1.0)	10 (1.1)	21 (1.8)	10 (1.8)	2 (0.7)	4 (0.8)
≥5	17 (0.3)	7 (0.2)	3 (0.3)	6 (0.5)	1 (0.2)	1 (0.3)	0 (0.0)

	All included (N=6265)	1st dose MVA-BN at CED (N=3308)	2nd dose MVA-BN at or after CED (N=938)	Unvaccinated (N=1188)	Crossover (N=542)	1st dose MVA-BN prior to CED (N=300)	2nd dose MVA-BN prior to CED (N=531)
Missing	586 (9.4)	302 (9.1)	99 (10.6)	140 (11.8)	48 (8.9)	19 (6.3)	26 (4.9)
# many sexual partners you had sex without a condom last 3 months							
Not specified	89 (1.4)	52 (1.6)	5 (0.5)	18 (1.5)	9 (1.7)	7 (2.3)	7 (1.3)
0	1088 (17.4)	635 (19.2)	125 (13.3)	243 (20.5)	99 (18.3)	38 (12.7)	47 (8.9)
1	1161 (18.5)	643 (19.4)	160 (17.1)	241 (20.3)	114 (21.0)	45 (15.0)	72 (13.6)
2-4	1621 (25.9)	828 (25.0)	250 (26.7)	326 (27.4)	167 (30.8)	76 (25.3)	141 (26.6)
≥5	1724 (27.5)	852 (25.8)	299 (31.9)	220 (18.5)	105 (19.4)	115 (38.3)	238 (44.8)
Missing	582 (9.3)	298 (9.0)	99 (10.6)	140 (11.8)	48 (8.9)	19 (6.3)	26 (4.9)
In what ways have you had sex in the last 3 months?							
Oral - passive	4490 (71.7)	2372 (71.7)	697 (74.3)	766 (64.5)	384 (70.8)	232 (77.3)	423 (79.7)
Oral - active	4489 (71.7)	2364 (71.5)	694 (74.0)	775 (65.2)	389 (71.8)	227 (75.7)	429 (80.8)
Anal - passive	3444 (55.0)	1784 (53.9)	559 (59.6)	584 (49.2)	280 (51.7)	174 (58.0)	343 (64.6)
Anal - active	3618 (57.7)	1878 (56.8)	564 (60.1)	604 (50.8)	303 (55.9)	192 (64.0)	380 (71.6)
Vaginal - passive	29 (0.5)	14 (0.4)	4 (0.4)	9 (0.8)	4 (0.7)	1 (0.3)	1 (0.2)
Vaginal - active	175 (2.8)	96 (2.9)	23 (2.5)	39 (3.3)	15 (2.8)	4 (1.3)	13 (2.4)
Other	530 (8.5)	308 (9.3)	65 (6.9)	88 (7.4)	49 (9.0)	22 (7.3)	47 (8.9)
Not specified	46 (0.7)	29 (0.9)	4 (0.4)	9 (0.8)	0 (0.0)	1 (0.3)	3 (0.6)
In the last 3 months did you knowingly have sex with a person who							
You did not know before	3628 (57.9)	1855 (56.1)	590 (62.9)	597 (50.3)	310 (57.2)	200 (66.7)	386 (72.7)
Did not use condoms	3146 (50.2)	1580 (47.8)	511 (54.5)	521 (43.9)	263 (48.5)	174 (58.0)	360 (67.8)
Intravenously takes drugs ("injects")	245 (3.9)	141 (4.3)	37 (3.9)	31 (2.6)	16 (3.0)	13 (4.3)	23 (4.3)
Sex worker	262 (4.2)	130 (3.9)	45 (4.8)	39 (3.3)	18 (3.3)	18 (6.0)	30 (5.6)
None of the special partner group	777 (12.4)	445 (13.5)	99 (10.6)	165 (13.9)	65 (12.0)	31 (10.3)	37 (7.0)
Not specified	173 (2.8)	107 (3.2)	17 (1.8)	28 (2.4)	14 (2.6)	10 (3.3)	11 (2.1)
Have you had an STI in the last 3 months?							
Not specified	35 (0.6)	16 (0.5)	6 (0.6)	9 (0.8)	3 (0.6)	2 (0.7)	2 (0.4)
No	4695 (74.9)	2524 (76.3)	681 (72.6)	886 (74.6)	415 (76.6)	217 (72.3)	387 (72.9)
Yes, one	766 (12.2)	385 (11.6)	114 (12.2)	128 (10.8)	62 (11.4)	46 (15.3)	93 (17.5)
Yes, several	129 (2.1)	56 (1.7)	32 (3.4)	12 (1.0)	6 (1.1)	12 (4.0)	17 (3.2)
Uncertain/Unclear (I had symptoms but did not get tested)	57 (0.9)	28 (0.8)	6 (0.6)	13 (1.1)	8 (1.5)	4 (1.3)	6 (1.1)
Missing	583 (9.3)	299 (9.0)	99 (10.6)	140 (11.8)	48 (8.9)	19 (6.3)	26 (4.9)

	All included (N=6265)	1st dose MVA-BN at CED (N=3308)	2nd dose MVA-BN at or after CED (N=938)	Unvaccinated (N=1188)	Crossover (N=542)	1st dose MVA-BN prior to CED (N=300)	2nd dose MVA-BN prior to CED (N=531)
Have taken HIV post exposure prophylaxis (PEP) in the last 3 months							
Not specified	116 (1.9)	72 (2.2)	19 (2.0)	15 (1.3)	6 (1.1)	4 (1.3)	6 (1.1)
No	5342 (85.3)	2831 (85.6)	790 (84.2)	983 (82.7)	466 (86.0)	265 (88.3)	473 (89.1)
Yes, once	48 (0.8)	24 (0.7)	3 (0.3)	13 (1.1)	5 (0.9)	3 (1.0)	5 (0.9)
Yes, twice	10 (0.2)	7 (0.2)	0 (0.0)	1 (0.1)	1 (0.2)	2 (0.7)	0 (0.0)
Yes, three times or more	165 (2.6)	74 (2.2)	27 (2.9)	36 (3.0)	16 (3.0)	7 (2.3)	21 (4.0)
Missing	584 (9.3)	300 (9.1)	99 (10.6)	140 (11.8)	48 (8.9)	19 (6.3)	26 (4.9)
Have taken HIV PrEP in the last 3 months							
Not specified	94 (1.5)	55 (1.7)	14 (1.5)	13 (1.1)	5 (0.9)	6 (2.0)	6 (1.1)
No	2828 (45.1)	1649 (49.8)	362 (38.6)	522 (43.9)	227 (41.9)	126 (42.0)	169 (31.8)
Yes, regularly and daily	2039 (32.5)	947 (28.6)	352 (37.5)	365 (30.7)	186 (34.3)	109 (36.3)	266 (50.1)
Yes, regularly, but not currently	114 (1.8)	61 (1.8)	19 (2.0)	20 (1.7)	9 (1.7)	4 (1.3)	10 (1.9)
Yes, irregularly and several times a week	120 (1.9)	49 (1.5)	24 (2.6)	23 (1.9)	14 (2.6)	9 (3.0)	15 (2.8)
Yes, but only when I needed it (on demand PrEP /event based dosing).	496 (7.9)	253 (7.6)	69 (7.4)	108 (9.1)	54 (10.0)	27 (9.0)	39 (7.3)
Missing	574 (9.2)	294 (8.9)	98 (10.4)	137 (11.5)	47 (8.7)	19 (6.3)	26 (4.9)
Have you had contact with a person with mpox in the last 4 weeks?							
Not specified	106 (1.7)	63 (1.9)	12 (1.3)	17 (1.4)	7 (1.3)	4 (1.3)	10 (1.9)
No	5393 (86.1)	2817 (85.2)	811 (86.5)	1002 (84.3)	471 (86.9)	272 (90.7)	491 (92.5)
Yes	190 (3.0)	132 (4.0)	17 (1.8)	32 (2.7)	17 (3.1)	5 (1.7)	4 (0.8)
Missing	576 (9.2)	296 (8.9)	98 (10.4)	137 (11.5)	47 (8.7)	19 (6.3)	26 (4.9)
Have you had reddish skin lesions (rash/bubbles/pustules) that were painful in the last 4 weeks?							
Not specified	31 (0.5)	20 (0.6)	1 (0.1)	5 (0.4)	1 (0.2)	1 (0.3)	4 (0.8)
No	5494 (87.7)	2921 (88.3)	813 (86.7)	1004 (84.5)	472 (87.1)	268 (89.3)	488 (91.9)
Yes	165 (2.6)	72 (2.2)	26 (2.8)	42 (3.5)	22 (4.1)	12 (4.0)	13 (2.4)
Missing	575 (9.2)	295 (8.9)	98 (10.4)	137 (11.5)	47 (8.7)	19 (6.3)	26 (4.9)

8.2.2. TEMVAc analysis cohort baseline characteristics

Baseline characteristics of the TEMVAc subjects are summarised in Table 16 below. Subjects were of an average of 44 years of age and had a BMI of 25.0. More than half of TEMVAc subjects were PLWHIV (51.5%). 66.6% had a diagnosis of STI documented in their EHR prior to the start of the observation period, mainly diagnosis of syphilis (41.8%) or gonorrhoea, chlamydia or mycoplasma infection (41.3%). The majority of subjects did not have a STI diagnosis during the baseline period (85.8%). Less than a quarter of the population had chronic cardiovascular disease (17.6%) or history of acute or chronic viral hepatitis (16.6%).

Subjects in the vaccinated group were comparable to the subjects in the unvaccinated group on almost all characteristics; however, those in the vaccinated group had a slightly higher frequency of STI diagnosis when compared to the unvaccinated group (previous STI diagnosis of 69.3% vs 63.2%). Similarly, the number of STI tests during the baseline period was slightly higher for those in the vaccinated group.

The subjects in the PLWHIV subgroup were slightly older compared to the overall MSM cohort (as seen in SEMVAc) with a mean age of 48.5 years, which remained similar in the VE cohort for both vaccinated and unvaccinated (Supplementary Table 30). Most PLWHIV subgroup subjects were aged 50 or older (51.1%) and had a BMI of 25.2. The PLWHIV subgroup experienced more pre-existing health conditions than the overall MSM cohort: 74.1% had a previous of STI infection documented in their EHR prior to the start of the observation period, approximately one quarter had chronic cardiovascular disease (24.8%) or history of acute/chronic viral hepatitis (27.7%), and 9% had a tumour or malignancy. Syphilis accounted for the majority of STI diagnoses (57.9%) and 13% of this subgroup had at least 1 STI test during the baseline period. When comparing the vaccinated and unvaccinated groups in PLWHIV, subjects were mostly comparable at baseline, save for slight differences in history of STI frequency and acute/chronic hepatitis diagnosis. Approximately 88% were not diagnosed with an STI during the baseline period.

Subjects taking PrEP medication were on average 39.2 years old (slightly younger than the overall MSM cohort) and almost half of these were aged 18-35 (43%) (Supplementary Table 31). PrEP users on average had a BMI of 24.7 and similar history of STI infection (62.5%) when compared to the overall MSM cohort. PrEP users were generally healthier at baseline when compared to the PLWHIV subgroup. Pre-existing conditions included chronic cardiovascular disease (8.9%), chronic lung disease (5.2%), and history of acute/chronic viral hepatitis (5.1%) at much lower frequencies. PrEP users were also more frequently tested for STIs with approximately 72% of the subgroup having at least 1 STI test during the baseline period. Additionally, PrEP users were less frequently diagnosed with syphilis (26.7%). When comparing vaccinated and unvaccinated groups, those vaccinated had slightly higher frequency of STI infection in their medical history (62.1% vs 57.3%), chronic cardiovascular disease (11.1% vs 9.1%), chronic lung disease (6.6% vs 5.3%), and STI testing (% vs% 2 or more tests during baseline).80% were not diagnosed with an STI during the baseline period.

Those with HSMV were on average older (mean age 55.0) than both the PLWHIV and PrEP subgroups and the overall MSM cohort (Supplementary Table 32). Most subjects were ≥50 years of age (82.5%) and had a BMI of 25.5. In general, this subgroup was less healthy than the overall cohort and other subgroups due to comorbidities associated with age. They frequently were PLWHIV (71.5%) and had a history of STI infection (68.9%). Most frequent pre-existing conditions included chronic cardiovascular disease (32.2%), history of acute/chronic viral hepatitis (27.6%), chronic lung disease (10.6%), tumour or malignancy (9.7%) and diabetes mellitus (6.2%). Syphilis diagnosis

accounted for the majority of historical STI diagnoses (51.9%), though most subjects were not tested for an STI in the baseline period (71.1%).

Table 16: Baseline characteristics of the overall MSM cohort and the VE cohort for matched vaccinated and comparator groups in TEMVAc.

	Overall MSM cohort (N=9328)	**Vaccinated group (VE cohort) (N=3027)**	**Comparator group (VE cohort) (N=3027)**
Mean (SD) or n (%)			
Age in years, *mean (SD)*	44.04 (11.71)	44.00 (10.85)	43.92 (11.36)
Age categories in years			
18-35	2633 (28.2)	1010 (33.4)	1010 (33.4)
36-49	3358 (36.0)	776 (25.6)	776 (25.6)
≥50	3337 (35.8)	1241 (41.0)	1241 (41.0)
Height	179.64 (6.90)	179.76 (6.71)	179.93 (6.89)
Weight	82.22 (16.00)	82.48 (15.56)	84.43 (17.96)
BMI (in kg/m2)	25.02 (4.19)	24.80 (4.08)	25.28 (4.39)
Gender			
Male	9267 (99.3)	3007 (99.3)	3010 (99.4)
Trans persons (trans male, trans female)	61 (0.6)	20 (0.6)	17 (0.5)
Existing preconditions			
None	1424 (15.3)	495 (16.4)	541 (17.9)
Immunosuppression (non-HIV)	20 (0.2)	0 (0.0)	0 (0.0)
HIV infection	4802 (51.5)	1417 (46.8)	1417 (46.8)
History of STI infection	6211 (66.6)	2099 (69.3)	1914 (63.2)
Rheumatological disease	166 (1.8)	66 (2.2)	57 (1.9)
Tumour/malignancy	499 (5.3)	149 (4.9)	159 (5.3)
Haematological disease	213 (2.3)	67 (2.2)	80 (2.6)
Chronic Cardiovascular disease	1641 (17.6)	516 (17.0)	547 (18.1)
Chronic lung disease	705 (7.6)	241 (8.0)	244 (8.1)
Chronic kidney disease	198 (2.1)	64 (2.1)	62 (2.0)
Chronic liver disease	293 (3.1)	100 (3.3)	86 (2.8)
Diabetes mellitus	309 (3.3)	81 (2.7)	101 (3.3)
Antiretroviral therapy (ART)	4690 (98.3)	1390 (98.6)	1389 (98.7)
CD4 count cells per microliter			

	Overall MSM cohort (N=9328)	Vaccinated group (VE cohort) (N=3027)	Comparator group (VE cohort) (N=3027)
No data	4526 (48.7)	1610 (53.3)	1610 (53.3)
< 200	56 (0.6)	11 (0.4)	12 (0.4)
(≥200 and <500)	649 (7.0)	176 (5.8)	218 (7.2)
≥500	4068 (43.7)	1222 (40.5)	1182 (39.1)
HIV viral copies per mL			
< 50	4605 (96.5)	1378 (97.8)	1373 (97.4)
50-199	105 (2.2)	24 (1.7)	22 (1.6)
200-1000	19 (0.4)	5 (0.4)	6 (0.4)
>1000	42 (0.9)	2 (0.1)	9 (0.6)
Systemic immunosuppressive therapy (e.g. systemic glucocorticoids)	0 (0.0)	0 (0.0)	0 (0.0)
PrEP users	3455 (37.0)	1312 (43.3)	1312 (43.3)
History of acute/chronic viral hepatitis	1551 (16.6)	523 (17.3)	477 (15.8)
History of STI *(until 30/06/2022)*			
Syphilis	3895 (41.8)	1349 (44.6)	1172 (38.7)
Gonorrhoea, Chlamydia, or Mycoplasma infection	3849 (41.3)	1349 (44.6)	1168 (38.6)
Other infection (e.g., sexually transmitted hepatitis A or Shigella, condylomas)	1535 (16.5)	585 (19.3)	495 (16.4)
Number of STI tests during baseline period (01/01/22-30/06/22)			
0	5553 (59.5)	1654 (54.6)	1843 (60.9)
1	1839 (19.7)	663 (21.9)	596 (19.7)
≥2	1936 (20.8)	710 (23.5)	588 (19.4)
Number of STI diagnoses during baseline period (01/01/22-30/06/22)			
0	8004 (85.8)	2542 (84.0)	2542 (84.0)
1	1266 (14.2)	485 (16.0)	485 (16.0)
Smallpox vaccination			
None	3803 (40.8)	1353 (44.7)	1293 (42.7)
One smallpox vaccination (anamnestic, scar or vaccination certificate)	815 (8.7)	375 (12.4)	226 (7.5)
Two smallpox vaccinations (anamnestic, scar or	437 (4.7)	162 (5.4)	153 (5.1)

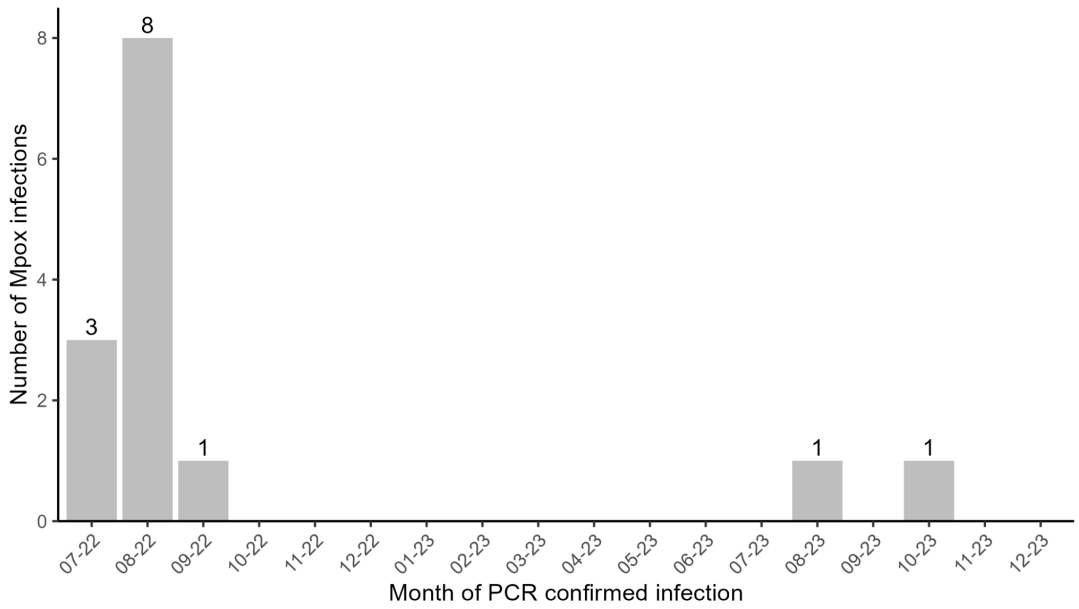
	Overall MSM cohort (N=9328)	Vaccinated group (VE cohort) (N=3027)	Comparator group (VE cohort) (N=3027)
vaccination certificate)			
Unknown	4273 (45.8)	1137 (37.6)	1355 (44.8)
Vaccinated with ACAM2000 before 2022	108 (1.2)	52 (1.7)	33 (1.1)
Region			
Berlin	2473 (26.5)	875 (28.9)	875 (28.9)
Frankfurt	1995 (21.4)	270 (8.9)	270 (8.9)
Hamburg	2037 (21.8)	818 (27.0)	818 (27.0)
Cologne	2629 (28.2)	965 (31.9)	965 (31.9)
Munich	194 (2.1)	99 (3.3)	99 (3.3)

8.3. Outcome Data

Outcome data for SEMVAc

No cases of mpox were reported in the unvaccinated group in the VE cohort. Of all participants vaccinated (n=4788), 11 mpox cases were reported in those administered a first MVA-BN dose during the study period (n=3617). In participants who were administered a second MVA-BN dose (including those who may have previously been vaccinated with a first dose) during the study period (n=3126), 2 cases were reported. One mpox case was reported in a participant that was vaccinated with 2 doses prior to CED (Table 12). Figure 5 describes the evolution of total PCR confirmed mpox cases by month within the study period. The majority of infections were reported early on in the study period between July and September 2022.

Figure 5: Number of SEMVAc participants with PCR confirmed MPXV infection per month.



Of those who reported an MPXV infection (n=14), all reported skin changes or pox lesions (100%), and almost half reported accompanying systemic symptoms such as fever and fatigue (45%).

Approximately half (51%) reported mild to moderate fever (≥ 38.5ºC), (38% moderate, 13% mild). Genital or anal areas were the most affected by skin lesions (45%) and 40% of lesions were reported to be painful. Two participants (14%) were hospitalised during their infection and no participants were admitted to the ICU or died due to mpox during the study period. The majority of infected participants experienced symptomatic disease and did not require hospitalisation (77%) (Table 17).

Table 17: Symptoms and treatment of PCR confirmed MPXV infections reported by study physicians and participants.

	PCR confirmed infection n (%)
Clinician reported	n=14
Prodromal/accompanying symptoms occur (general symptoms such as fever, fatigue)	5/11 (45%)
Skin changes/pox lesions	12/12 (100%)
Hospitalisation during infection	2/14 (14%)
Progression of disease:	
Asymptomatic course of disease, treatment without hospitalisation	1/13 (8%)
Symptomatic course of disease, treatment without hospitalisation	11/13 (77%)
Treatment in hospital	2/13 (15%)
Treatment in hospital in an intensive care unit (ICU)	0/13 (0%)
Death	0/13 (0%)
Therapy with Tecovirimat	0/13 (0%)
Number of skin lesions (Median [IQR])*	2 (1-3.25)
Confluence of several smallpox-like skin lesions	0/6 (0%)
Healing of skin lesions, in days after first occurrence (Median [IQR])*	16 (11.5-30)
Occurrence of scars after healed pox lesions	1/7 (14%)
Participant reported	n=11/14
Body region affected with skin lesions	
Genital	5/11 (45%)
Anal	5/11 (45%)
Mouth or throat	2/11 (18%)
Face area incl. neck	4/11 (36%)
Torso	2/11 (18%)
Arms/hands or legs/feet	3/11 (27%)
Start of skin lesions	
Genital	3/11 (27%)
Anal	4/11 (36%)
Mouth or throat	1/11 (9%)
Face area incl. neck	3/11 (27%)
Torso	0/11 (0%)
Arms/hands or legs/feet	0/11 (0%)
Rash scattered over body	
No	7/10 (70%)
Yes, but only isolated scattered rash	3/10 (30%)
Yes, rash all over the body with many scattered skin lesions	0/10 (0%)
Itching of skin lesions	3/10 (30%)
Body region of itching skin lesions	
Genital	1/11 (9%)
Anal	3/11 (27%)
Mouth or throat	0/11 (0%)
Face area incl. neck	0/11 (0%)
Torso	1/11 (9%)
Arms/hands or legs/feet	1/11 (9%)
Painful skin lesions	4/10 (40%)
Severity of pain (scale 0-10) (Median [IQR])*	7.5 [4.75 -10]

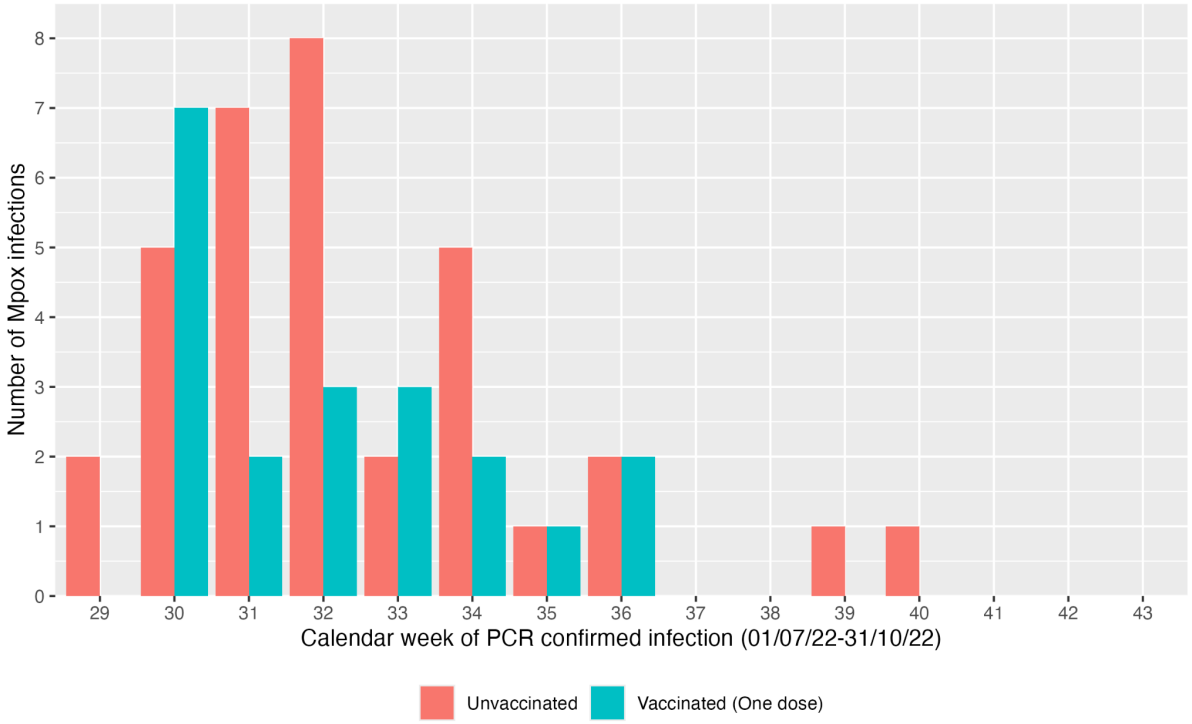
	PCR confirmed infection n (%)
Body region of painful skin lesions	
Genital	1/11 (9%)
Anal	3/11 (27%)
Mouth or throat	1/11 (9%)
Face area incl. neck	0/11 (0%)
Torso	0/11 (0%)
Arms/hands or legs/feet	0/11 (0%)
Other symptoms (e.g., fever, fatigue, muscle pain, swollen lymph nodes)	9/11 (82%)
General symptoms (e.g. fatigue, muscle pain, exhaustion)	
No complaints	1/9 (11%)
Mild (no restriction of daily activities)	4/9 (44%)
Moderate (restriction of daily activities)	2/9 (22%)
Severe (daily activities barely possible)	2/9 (22%)
Very severe (daily activities no longer possible)	0/9 (0%)
Fever (≥38.5°C)	
No complaints	4/8 (50%)
Mild (no restriction of daily activities)	1/8 (13%)
Moderate (restriction of daily activities)	3/8 (38%)
Severe (daily activities barely possible)	0/8 (0%)
Very severe (daily activities no longer possible)	0/8 (0%)
Swollen lymph nodes	
No complaints	2/9 (22%)
Mild (no restriction of daily activities)	3/9 (33%)
Moderate (restriction of daily activities)	2/9 (22%)
Severe (daily activities barely possible)	2/9 (22%)
Very severe (daily activities no longer possible)	0/9 (0%)
Body region of swollen lymph nodes	
Neck	4/11 (36%)
Armpits	1/11 (9%)
Groins/inguinal	4/11 (36%)
Other region	0/11 (0%)
Days of fever pain medication intake during infection	
No medication at all	5/11 (50%)
1 day	1/11 (9%)
2 days	0/11 (0%)
3 days	1/11 (9%)
4-5 days	1/11 (9%)
>5 days	3/11 (27%)

**Of those who responded to the question.*

Outcome data for TEMVAc

In the TEMVAc analyses, a total of 48 mpox cases were reported, 32 of them in the unvaccinated group and 16 in the vaccinated group; all cases in the vaccinated group occurred after the first dose of MVA-BN. No mpox cases were reported in subjects who received two doses of MVA-BN during follow-up (Table 18). Figure 6 shows the number of total PCR confirmed mpox cases by month, in vaccinated and unvaccinated groups. Most infections were reported early on in the study period between July and September 2022.

Figure 6: Number of TEMVAc subjects with PCR confirmed MPXV infection per month in the vaccinated and unvaccinated groups.



Of those who reported an MPXV infection (n=48), 68.8% reported prodromal/accompanying symptoms (81.2% in the unvaccinated, contrasting with a 43.8% in those vaccinated with a first dose). Symptoms of fever (18.8% vaccinated vs 53.1% unvaccinated) and fatigue (25% vaccinated vs 37.5% unvaccinated) were less frequent in the vaccinated group. The majority of mpox cases also experienced skin changes or pox lesions (83.3%), with a frequency of 93.8% in the vaccinated and 78% in the unvaccinated groups (genital or anal areas were the most affected by skin lesions (33.3% and 35.4%, respectively). In the vaccinated and unvaccinated, a similar percentage of painful lesions (40% and 36%, respectively) were reported. Most infected subjects experienced symptomatic disease and did not require hospitalisation (89.6%). Only 1 subject (unvaccinated) was hospitalised during their infection and no subjects were admitted to the ICU or died due to mpox during the study period (Table 18). The hospitalised patient was a PLWHIV with CD4+ count ≥ 500 and was hospitalised for three days due to the mpox infection.

Table 18: Symptoms and treatment of PCR confirmed MPXV infections* reported by clinicians in the VE cohort and by vaccination status.

	PCR confirmed MPXV infection in VE cohort (N=48)	**PCR confirmed MPXV infection in Unvaccinated (N=32)**	**PCR confirmed MPXV infection after first dose MVA-BN (N=16)**
	Mean (SD) or n (%)		
Therapy with tecovirimat	1 (2.1)	1 (3.1)	0 (0.0)
Prodrome/accompanying symptoms occur (general symptoms such as fever, fatigue)			
Yes	33 (68.8)	26 (81.2)	7 (43.8)
No	10 (20.8)	2 (6.2)	8 (50.0)
Unknown	5 (10.4)	4 (12.5)	1 (6.2)
Fever	21 (43.8)	17 (53.1)	4 (25.0)

	PCR confirmed MPXV infection in VE cohort (N=48)	PCR confirmed MPXV infection in Unvaccinated (N=32)	PCR confirmed MPXV infection after first dose MVA-BN (N=16)
Fatigue	15 (31.2)	12 (37.5)	3 (18.8)
Night sweats	2 (4.2)	2 (6.2)	0 (0.0)
Lymph node swelling	11 (22.9)	9 (28.1)	2 (12.5)
Other	15 (31.2)	12 (37.5)	3 (18.8)
Skin changes/pox lesions			
Yes	40 (83.3)	25 (78.1)	15 (93.8)
No	3 (6.2)	3 (9.4)	0 (0.0)
Unknown	5 (10.4)	4 (12.5)	1 (6.2)
Genital	16 (33.3)	8 (25.0)	8 (50.0)
Anal	17 (35.4)	12 (37.5)	5 (31.2)
Mouth or throat	3 (6.2)	1 (3.1)	2 (12.5)
Face area incl. neck	5 (10.4)	4 (12.5)	1 (6.2)
Torso	5 (10.4)	3 (9.4)	2 (12.5)
Arms/hands or legs/feet	11 (22.9)	8 (25.0)	3 (18.8)
Unknown	1 (2.1)	0 (0.0)	1 (6.2)
Painful skin lesions			
Yes	15 (37.5)	9 (36.0)	6 (40.0)
No	15 (37.5)	9 (36.0)	6 (40.0)
Unknown	10 (25.0)	7 (28.0)	3 (20.0)
Number of skin lesions/pox lesions			
None	15 (37.5)	9 (36.0)	6 (40.0)
1-3	15 (37.5)	9 (36.0)	6 (40.0)
4-10	0 (0.0)	0 (0.0)	0 (0.0)
11-20	0 (0.0)	0 (0.0)	0 (0.0)
21-50	0 (0.0)	0 (0.0)	0 (0.0)
>50	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	10 (25.0)	7 (28.0)	3 (20.0)
Days with healing of skin lesions/pox lesion after onset (Median [IQRI])	13.50 [10.00, 20.00]	17.00 [10.75, 20.25]	11.50 [10.00, 13.75]
Missing data for days with healing of skin lesions	22/46	9/16	13/32
Occurrence of scars after healed pox lesions			
Yes	1 (2.1)	0 (0.0)	1 (6.2)
No	22 (45.8)	15 (46.9)	7 (43.8)
Unknown	25 (52.1)	17 (53.1)	8 (50.0)

	PCR confirmed MPXV infection in VE cohort (N=48)	PCR confirmed MPXV infection in Unvaccinated (N=32)	PCR confirmed MPXV infection after first dose MVA-BN (N=16)
Progression of disease			
Asymptomatic course of disease, treatment without hospitalisation	2 (4.2)	2 (6.2)	0 (0.0)
Symptomatic course of disease, treatment without hospitalisation	43 (89.6)	27 (84.4)	16 (100.0)
Treatment in hospital	1 (2.1)	1 (3.1)	0 (0.0)
Treatment in hospital in an intensive care unit (ICU)	0 (0.0)	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	2 (4.2)	2 (6.2)	0 (0.0)
Number of days in hospital (Median [IQRI])	3.00 [3.00, 3.00]	3.00 [3.00, 3.00]	NA [NA, NA]
Intake of fever/pain medication			
Yes	15 (31.2)	11 (34.4)	4 (25.0)
No	15 (31.2)	9 (28.1)	6 (37.5)
Unknown	18 (37.5)	12 (37.5)	6 (37.5)

*Symptoms after the second MVA-BN vaccination were not described as no mpox cases were reported after the second MVA-BN vaccination.

8.4. Main results

8.4.1 Primary objective: Vaccine Effectiveness

Primary objective: Vaccine effectiveness analysis in SEMVAc

Cumulative incidence was estimated for the vaccinated groups in the VE cohort and is shown in Table 19. Of 5434 participants originally included in the VE cohort, 61 unvaccinated, 233 vaccinated with a first dose, and 510 vaccinated with a second dose were lost to follow-up (no clinic visits were reported after CED) and therefore, not included in the cumulative incidence calculations for initial SEMVAc results. A total of 3850 participants received a first dose at or after CED, and 3636 participants were included in the 2nd dose group (2nd dose at CED n=938, 2nd dose during follow up with n=2312 with 1st vaccination at CED and n=386 from crossover participants). Given that as of 31 December 2023, there were no cases in the unvaccinated group, propensity score matching was not performed, and risk ratios were not estimated to examine VE.

Table 19: Cumulative incidence and VE in the VE cohort, before and after PS-matching.

	Overall unmatched population		Overall PS-matched population	
	Unvaccinated	***Vaccinated***	***Unvaccinated***	***Vaccinated***
1st MVA-BN				
Participants	1127	3617	-	-
Mpox events	0	11	-	-

	Overall unmatched population		Overall PS-matched population	
Cumulative incidence	0.00	0.0034 (95% CI 0.0014-0.0054)	-	-
VE with 1 dose	-	-	-	-
2nd MVA-BN				
Participants	1127	3126		
Mpox events	0	2		
Cumulative incidence	0.00	0.0016 (95% CI 0.00-0.0041)	-	-
VE with 2 doses	-	-	-	-

A lower estimated cumulative incidence of mpox was seen in participants who were vaccinated with 2 doses of MVA-BN (0.0016 [95% CI 0.00-0.0041]), while the cumulative incidence after one dose of MVA-BN resulted in a slightly higher estimate of 0.0034 [95%CI 0.0014-0.0054]. In PLWHIV, there was also a decrease in cumulative incidence when participants received a second dose (first dose 0.0066 [95% CI 0.0017-0.0115] versus second dose 0.0038 [95% CI 0.00-0.0114]). A similar decrease in cumulative incidence was seen in participants taking PrEP (1st dose, 0.003 [95% CI 0.0004-0.0059], 2nd dose, 0.0011 [95% CI 0-0.0034]). In those with a history of smallpox vaccine, no cases were reported after the second dose of MVA-BN and a similar cumulative incidence was reported after the first dose (0.0022 [95% CI 0.00-0.0053]) (Table 19). See Supplementary Tables 11 to 13 for further details.

Cumulative incidence of mpox within 0-13 days after the first dose was higher (0.0016 [95% CI 0.0002-0.003] when compared to the same period after the second dose, 0.0011 [95% CI 0-0.0034]) (Table 20). The cumulative incidence of mpox 14 days or more days after the first dose was similar to the 0–13-day period (0.0018 [95% CI 0.0004-0.0033]). The lowest cumulative incidence was observed 14 or more days after the second dose (0.0005 [95% CI 0-0.0015]).

Similarly, in the PLWHIV subgroup, a lower cumulative incidence of mpox was observed for 0-13 days after the first dose (0.0019 [95% CI 0-0.0046]) when compared to the same period after the second dose 0.0038 (95% CI 0-0.0114). During the ≥14 days after the first dose, cumulative incidence was 0.0047 [95% CI 0.0006-0.0088]) and no MPXV infection occurred ≥14 days after the second dose in PLWHIV subgroup. In contrast, cumulative incidence decreased for those in the PrEP user subgroup after the first dose: within 0-13 days the cumulative incidence was 0.0023 (95% CI 0-0.0048), and for at least 14 days 0.0007 (95% CI 0-0.0022). No MPXV infection occurred within 0-13 days after the second dose and the cumulative incidence for at least 14 days after the second dose was 0.0011 (95% CI 0-0.0034). In the HSMV subgroup, the cumulative incidence of mpox within 0-13 days after the first dose was 0.0011 (95% CI 0-0.0034), and 0.0011 (95% CI 0-0.0032) 14 days or more after the first dose. No mpox cases were reported after the second vaccine dose. Supplementary Tables 14 to 16 provide further details on the subgroups.

Table 20: Cumulative incidence and VE stratified by time period after vaccination in the VE Cohort, before and after PS-matching.

	Overall unmatched population		Overall PS-matched population	
	Unvaccinated	**Vaccinated**	**Unvaccinated**	**Vaccinated**
1st MVA-BN				
Participants	1127	3617	-	-

	Overall unmatched population		Overall PS-matched population	
Mpox events 0-13 days	0	5	-	-
Cumulative Incidence 0-13 days	0.00	0.0016 (95% CI 0.0002-0.003)	-	-
VE with 1 dose 0-13 days	-	-	-	-
Mpox events ≥14 days	0	6	-	-
Cumulative Incidence ≥14 days	0.00	0.0018 (95% CI 0.0004-0.0033)	-	-
VE with 1 dose ≥14 days	-	-	-	-
2nd MVA-BN			-	-
Participants	1127	3126	-	-
Mpox events 0-13 days	0	1	-	-
Cumulative Incidence 0-13 days	0.00	0.0011 (95% CI 0-0.0034)	-	-
VE with 2 doses 0-13 days	-	-	-	-
Mpox events ≥14 days	0	1	-	-
Cumulative Incidence ≥14 days	0	0.0005 (95% CI 0-0.0015)	-	-
VE with 2 doses ≥14 days	-	-	-	-

Primary objective: Vaccine effectiveness analysis in TEMVAc

In the VE cohort of TEMVAc, the overall median follow-up time was 52 days. In the vaccinated group, median follow-up time was 53 days (after the first vaccination) and 53 days in the unvaccinated group. Of the 3027 matched subjects in the VE cohort (n=6054), the estimated cumulative incidence in those unvaccinated was higher than those who received one dose of MVA-BN (Table 21). The pattern was similar in the subgroups. In the PLWHIV subgroup, cumulative incidence was almost two times higher in the unvaccinated versus the vaccinated (1.497 [95% CI 0.909-2.461] versus 0.88 [95% CI 0.472-1.637]). Figure 7 (left top panel) shows the cumulative incidence differences between vaccinated and unvaccinated individuals in the overall VE cohort and in the subgroups. Overall, the number of mpox cases in all subgroups was lower in vaccinated compared to unvaccinated individuals (Figure 7). Across age categories, the highest cumulative incidence was among the unvaccinated subjects aged 36-49 (2.009 [95% CI 1.225-3.286]) and the lowest in those vaccinated that were aged ≥50 (0.374 (95% CI 0.12-1.164) and HSMV (0.32 (95% CI 0.103-0.998)). VE in the overall cohort was 54.15 (95% CI 21.09 to 73.36) when comparing those with one dose to the unvaccinated. Given that no mpox cases were reported in those subjects who received two doses of MVA-BN, VE was not estimated in the fully vaccinated group. Among subgroups, VE was similar to the overall cohort with PrEP subgroup having the highest VE (63.64 [95% CI 14.92 to 84.46]) followed by those with HSMV (60.71 [95% CI -33.37 to 88.42]) and PLWHIV (41.25 [95% CI -20.88 to 71.45]), though the latter two VE estimates were non-significant. In the age groups, the greatest effectiveness was in the subjects aged 18-35 (60.16 [95% CI -14.09 to 86.09]) and aged 36-49 (53.28 [95% CI 3.21 to 77.44]), while the lowest VE was observed in those aged ≥50 (47.24 [95% CI -90.92 to 85.42]), though results for age categories 18-35 and ≥50 years were non-significant.

Table 21: Estimated VE* against PCR confirmed mpox for the three exposure groups of the VE cohort and for each subgroup (PLWHIV, PrEP users, HSMV), and for each age groups (i.e. [18-35], [36-49], and ≥50 years) of the VE cohort.

	No. at risk at start of follow-up (N)	PCR confirmed mpox (N)	Cumulative incidence (95% CI) in %	Risk ratio (95% CI)	VE (95% CI) in %
Overall VE cohort					
Unvaccinated	3027	32	1.441 (95% CI 1.008-2.059)	-	-
After first MVA-BN vaccination	3027	16	0.661 (95% CI 0.403-1.082)	0.46 (95% CI 0.27-0.79)	54.15 (95% CI 21.09 to 73.36)
PLWHIV					
Unvaccinated	1417	16	1.497 (95% CI 0.909-2.461)	-	-
After first MVA-BN vaccination	1417	10	0.88 (95% CI 0.472-1.637)	0.59 (95% CI 0.29-1.21)	41.25 (95% CI -20.88 to 71.45)
PrEP					
Unvaccinated	1312	14	1.455 (95% CI 0.841-2.511)	-	-
After first MVA-BN vaccination	1312	6	0.529 (95% CI 0.238-1.174)	0.36 (95% CI 0.16-0.85)	63.64 (95% CI 14.92 to 84.46)
HSMV					
Unvaccinated	1178	6	0.815 (95% CI 0.361-1.836)	-	-
After first MVA-BN vaccination	1178	3	0.32 (95% CI 0.103-0.998)	0.39 (95% CI 0.12-1.33)	60.71 (95% CI -33.37 to 88.42)
Age 18-35					
Unvaccinated	776	10	1.517 (95% CI 0.815-2.813)	-	-
After one dose MVA-BN vaccination	776	4	0.604 (95% CI 0.227-1.602)	0.4 (95% CI 0.14-1.14)	60.16 (95% CI -14.09 to 86.09)
Age 36-49					
Unvaccinated	1241	17	2.009 (95% CI 1.225-3.286)	-	-
After one dose MVA-BN vaccination	1241	9	0.939 (95% CI 0.485-1.813)	0.47 (95% CI 0.23-0.97)	53.28 (95% CI 3.21 to 77.44)
Age ≥50					
Unvaccinated	1010	5	0.709 (95% CI 0.292-1.717)	-	-
After one dose MVA-BN vaccination	1010	3	0.374 (95% CI 0.12-1.164)	0.53 (95% CI 0.15-1.91)	47.24 (95% CI -90.92 to 85.42)

*Estimates after the second MVA-BN vaccination were not included in the table as no mpox cases were reported after second MVA-BN vaccination.

Cumulative incidence of mpox from 0 to 13 days after the first vaccination in the overall VE cohort was 0.218 (95% CI 0.098-0.486) which translates to a VE of 44.55 (95% CI -38.4 to 77.79). When VE was assessed after at least 14 days after the first vaccination, both cumulative incidence (0.444 (95% CI 0.238-0.827)) and the VE estimate increased (57.83 (95% CI 17.03 to 78.56)), similar to what was observed in the overall VE across all of follow-up (Table 22). For PLWHIV, an initially higher VE was present during the 0-13 day period (60.63 (95% CI -74.4 to 91.11)) compared to at least 14 days after the first vaccination (34.87 (95% CI -49.17 to 71.56)). PrEP users were similar to

the overall cohort in that the initial VE estimate (30.63 (95% CI -128.43 to 78.94)) increased 14 days following vaccination (80.78 (95% CI 21.25 to 95.31)). In those with HSMV, mpox cases in the strata were reduced and VE increased 14 days after first vaccination (68.12 (95% CI -36.69 to 92.56)). Regarding differences across age groups stratified by time period, the greatest increase in VE was observed in those aged 18-35 from 0-13 days after vaccination (14.26% [95% CI -210.64 to 76.33]) to at least 14 days after vaccination (89.7% [95% CI 26.24 to 98.56]). VE for the 0-13 day period in the remaining age groups was not estimated due to insufficient mpox cases in each corresponding strata. VE in those aged 36-49 at least 14 days after vaccination was comparable to the overall VE cohort during the same time period (67.22% [95% CI 23.3 to 85.99]). In those aged ≥50, the lowest VE was observed, though mpox cases were minimal in this group at least 14 days after vaccination (22.27% [95% CI -168.97 to 77.54]).

Table 22: Estimated VE* against mpox during the two time periods after MVA-BN vaccination in the VE cohort,for each subgroup (PLWHIV, PrEP users, HSMV) and for each age groups (i.e. [18-35], [36-49], and ≥50 years) of the VE cohort.

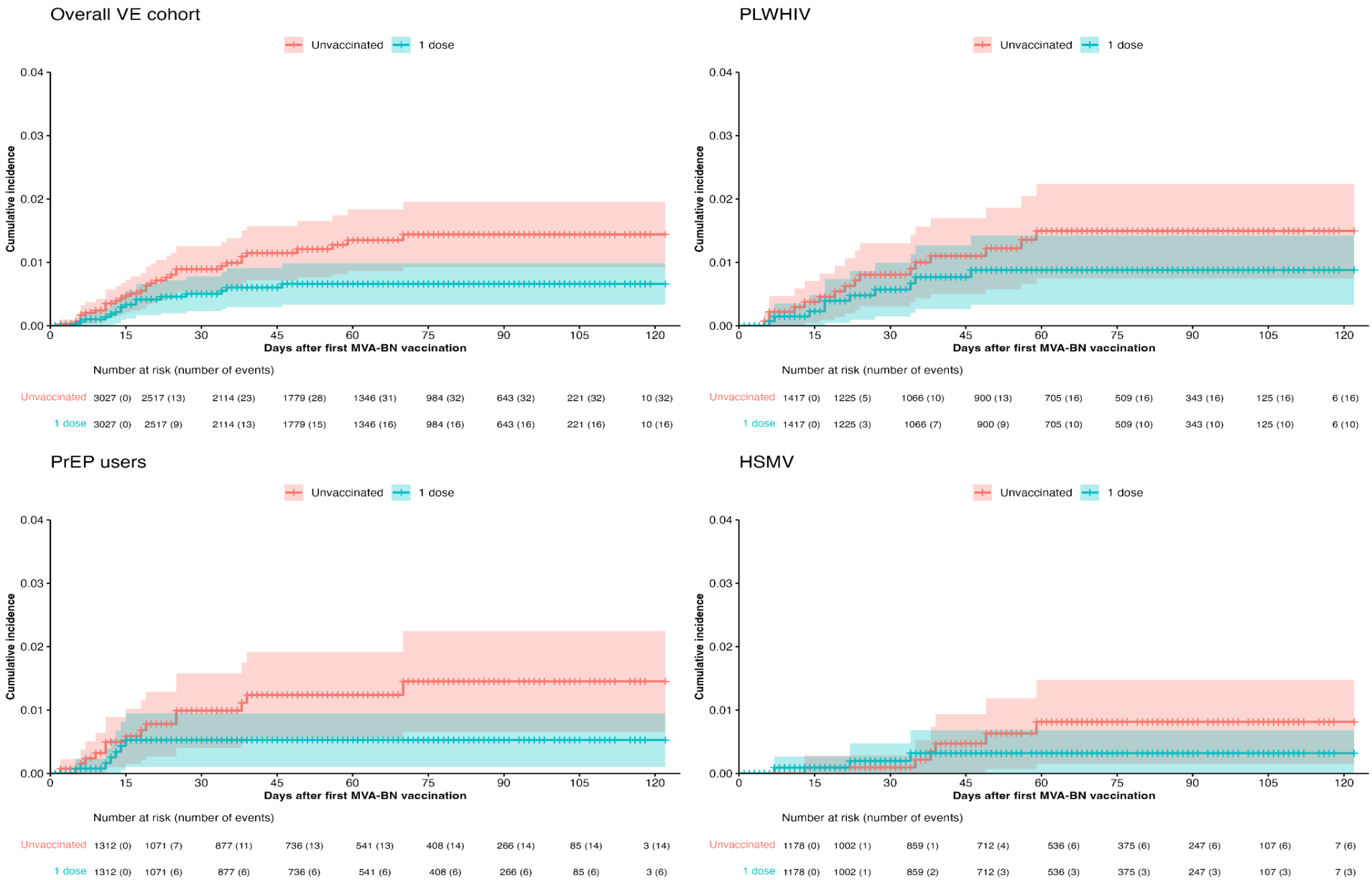
	No. at risk at start of follow-up (N)	PCR confirmed mpox (N)	Cumulative incidence (95% CI) in %	Risk ratio (95% CI)	VE (95% CI) in %
Overall VE cohort					
Within 0-13 days					
Unvaccinated	3027	11	0.394 (95% CI 0.361-0.71)	-	-
After first vaccination	3027	6	0.218 (95% CI 0.098-0.486)	0.55 (95% CI 0.22-1.38)	44.55 (95% CI -38.4 to 77.79)
≥14 days					
Unvaccinated	2551	21	1.052 (95% CI 0.677-1.631)	-	-
After first vaccination	2551	10	0.444 (95% CI 0.238-0.827)	0.42 (95% CI 0.21-0.83)	57.83 (95% CI 17.03 to 78.56)
PLWHIV					
Within 0-13 days					
Unvaccinated	1417	5	0.377 (95% CI 0.222-0.905)	-	-
After first vaccination	1417	2	0.149 (95% CI 0.037-0.593)	0.39 (95% CI 0.09-1.74)	60.63 (95% CI -74.4 to 91.11)
≥14 days					
Unvaccinated	1238	11	1.124 (95% CI 0.617-2.043)	-	-
After first vaccination	1238	8	0.732 (95% CI 0.365-1.465)	0.65 (95% CI 0.28-1.49)	34.87 (95% CI -49.17 to 71.56)
PrEP					
Within 0-13 days					
Unvaccinated	1312	6	0.496 (95% CI 0.617-1.102)	-	-
After first vaccination	1312	4	0.344 (95% CI 0.129-0.916)	0.69 (95% CI 0.21-2.28)	30.63 (95% CI -128.43 to 78.94)

≥14 days					
Unvaccinated	1089	8	0.963 (95% CI 0.467-1.981)	-	-
After first vaccination	1089	2	0.185 (95% CI 0.046-0.738)	0.19 (95% CI 0.05-0.79)	80.78 (95% CI 21.25 to 95.31)
HSMV					
Within 0-13 days					
Unvaccinated	1178	1	0.097 (95% CI 0.467-0.683)	-	-
After first vaccination	1178	1	0.091 (95% CI 0.013-0.645)	0.94 (95% CI 0.06-13.98)	5.56 (95% CI -1297.69 to 93.62)
≥14 days					
Unvaccinated	1012	5	0.719 (95% CI 0.297-1.74)	-	-
After first vaccination	1012	2	0.229 (95% CI 0.057-0.916)	0.32 (95% CI 0.07-1.37)	68.12 (95% CI -36.69 to 92.56)
Age 18-35					
Within 0-13 days					
Unvaccinated	776	5	0.677 (95% CI 0.282-1.619)	-	-
After first vaccination	776	3	0.447 (95% CI 0.144-1.379)	0.66 (95% CI 0.17-2.55)	34 (95% CI -155.39 to 82.94)
≥14 days					
Unvaccinated	644	21	1.052 (95% CI 0.677-1.631)	-	-
After first vaccination	644	0	0	-	-
Age 36-49					
Within 0-13 days					
Unvaccinated	1241	5	0.446 (95% CI 0.186-1.07)	-	-
After first vaccination	1241	2	0.17 (95% CI 0.043-0.678)	0.38 (95% CI 0.09-1.68)	61.92 (95% CI -67.58 to 91.35)
≥14 days					
Unvaccinated	1037	12	1.57 (95% CI 0.873-2.812)	-	-
After first vaccination	1037	7	0.77 (95% CI 0.365-1.622)	0.49 (95% CI 0.21-1.13)	50.94 (95% CI -12.63 to 78.63)
Age ≥50					
Within 0-13 days					
Unvaccinated	1010	1	0.112 (95% CI 0.016-0.795)	-	-
After first vaccination	1010	1	0.106 (95% CI 0.015-0.75)	0.94 (95% CI 0.06-13.95)	5.62 (95% CI -1295.4 to 93.62)
≥14 days					

Unvaccinated	870	4	0.597 (95% CI 0.222-1.6)	-	-
After first vaccination	870	2	0.268 (95% CI 0.067-1.071)	0.45 (95% CI 0.1-2.05)	55.09 (95% CI -105.4 to 90.18)

*Estimates after second MVA-BN vaccination were not estimated as no mpox cases were reported after second MVA-BN vaccination.

Figure 7: Cumulative incidence of PCR-confirmed mpox for vaccinated (one and two doses) vs. unvaccinated individuals in the VE cohort and for each subgroup.



8.4.2 Secondary objectives: Safety (AR, SAR, AESIs) (SEMVAc)

The Safety cohort included 4788 participants with the first and/or second of MVA-BN administered at or after CED of which 3850 participants were followed up after first vaccination and 3636 participants were followed up after second vaccination and provided safety information. Of those who responded to the reactogenicity questionnaires, 3036 participants were vaccinated with the first dose of MVA-BN at or after CED by the study centre, and 1939 participants were vaccinated with a second dose at or after CED. Only those participants who responded to the questionnaires administered contributed to the reactogenicity data.

Participants in the Safety cohort were followed up for 3 months for safety events, however not all participants completed the full follow up and were censored, accordingly, in the cumulative incidence results. Moreover, there were participants who have a follow up time of 0, meaning there were no visits at a study centre reported after CED, and these participants were consequently excluded from the cumulative incidence of AR and SAR analysis (accounting for 233 participants with the first dose of MVA-BN and 510 with second dose of MVA-BN).

A total of 13 and 5 ARs were reported after the first and second MVA-BN doses, respectively. No SAR, nor AESIs (pericarditis, myocarditis, encephalitis) were reported during the follow up period. The most common AR categories were gastrointestinal disorders (n=5), skin and subcutaneous tissue disorders (n=4) and general disorders and administration site conditions (n=3). Table 23 shows the number of adverse reactions and cumulative incidence of adverse reactions by system organ class after the 1st and 2nd MVA-BN. The cumulative incidence of any adverse reaction, classified by System Organ Class, during the study period was approximately 0.35% (95% CI 0.16-0.54%) to 0.14% (95% CI 0.02-0.26%), in those with one or two doses of MVA-BN, respectively. The highest cumulative incidence was observed for skin and subcutaneous tissue disorders (0.11% (95% CI 0-0.21)) in those with one dose. A detailed narrative of selected ARs is provided in Section 8.6.

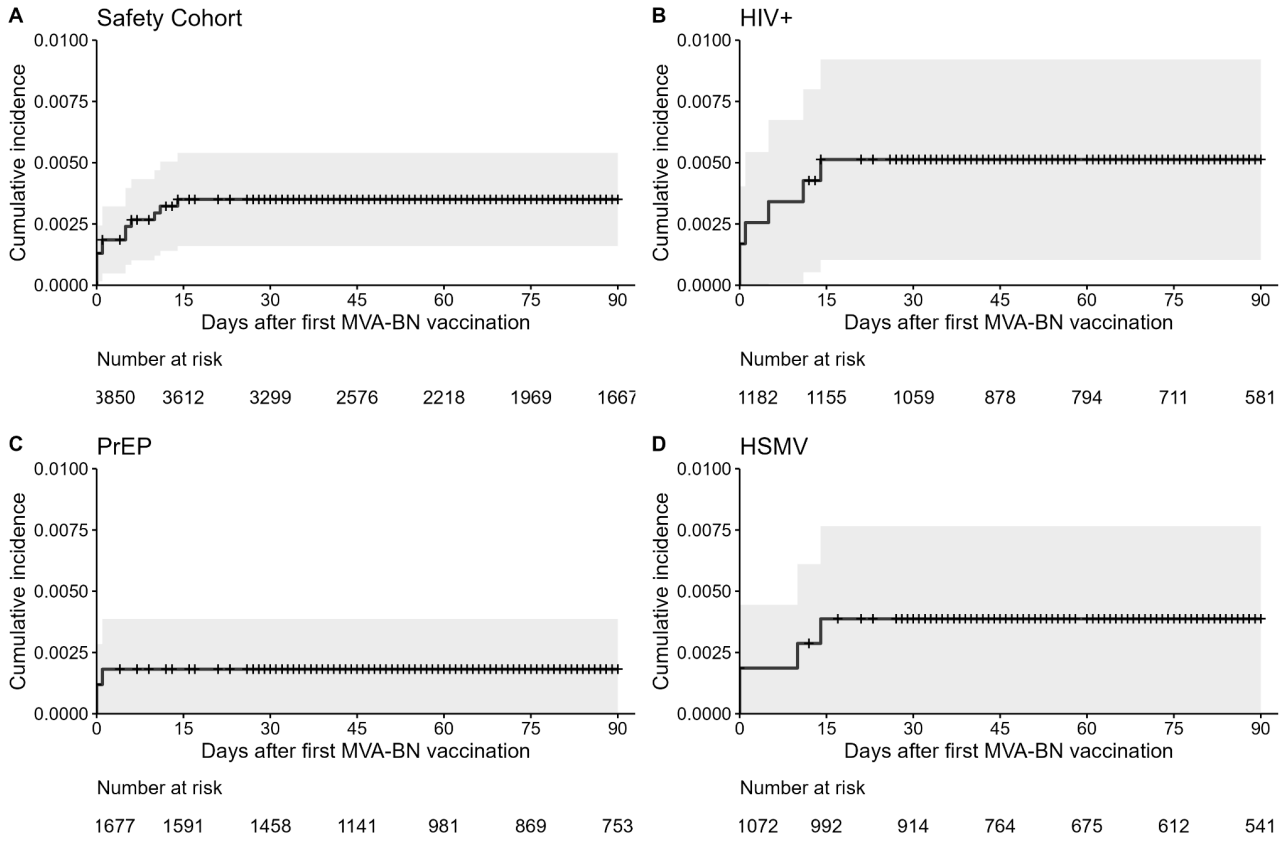
A graphical depiction of the cumulative incidence of AR and SAR in the Safety cohort and in the PLWHIV, PreP and HSMV subgroups is shown in Figure 8. Supplementary Tables 17-19 describe the cumulative incidence of AR/SAR in the PLWHIV, PreP and HSMV subgroups.

Table 23: Total n and cumulative incidence of Adverse or Severe Adverse reactions (AR/SAR) by System Organ Class in the Safety cohort who received the first and second dose of MVA-BN.

		1st MVA-BN		2nd MVA-BN
Systemic Organ Class	**n**	**Cumulative Incidence (95% CI)**	**n**	**Cumulative Incidence (95% CI)**
Any	13	0.0035 (95% CI 0.0016-0.0054)	5	0.0014 (95% CI 0.0002-0.0026)
Gastrointestinal disorders	3	0.0008 (95% CI 0..00-0.0017)	2	0.0006 (95% CI 0-0.0013)
Ear and labyrinth disorders	1	0.0003 (95% CI 0.00-0.0008)	-	-
Cardiac disorders	1	0.0003 (95% CI 0.00-0.0008)	-	-
Skin and subcutaneous tissue disorders	4	0.0011 (95% CI 0.00-0.0021)	-	-
Infections and Infestations	1	0.0003 (95% CI 0.00-0.0008)	-	-
General disorders and administration site conditions	3	0.0008 (95% CI 0.00-0.0018)	-	-
Nervous system disorders	-	-	1	0.0003 (95% CI 0-0.0008)
Hepatobiliary disorders	-	-	1	0.0003 (95% CI 0-0.0008)
Immune system disorders	-	-	1	0.0003 (95% CI 0-0.0008)

Figure 8: Cumulative incidence of AR/SAR in the Safety cohort and PLWHIV, PreP and HSMV subgroups after a) first and b) second vaccination.

a)



b)

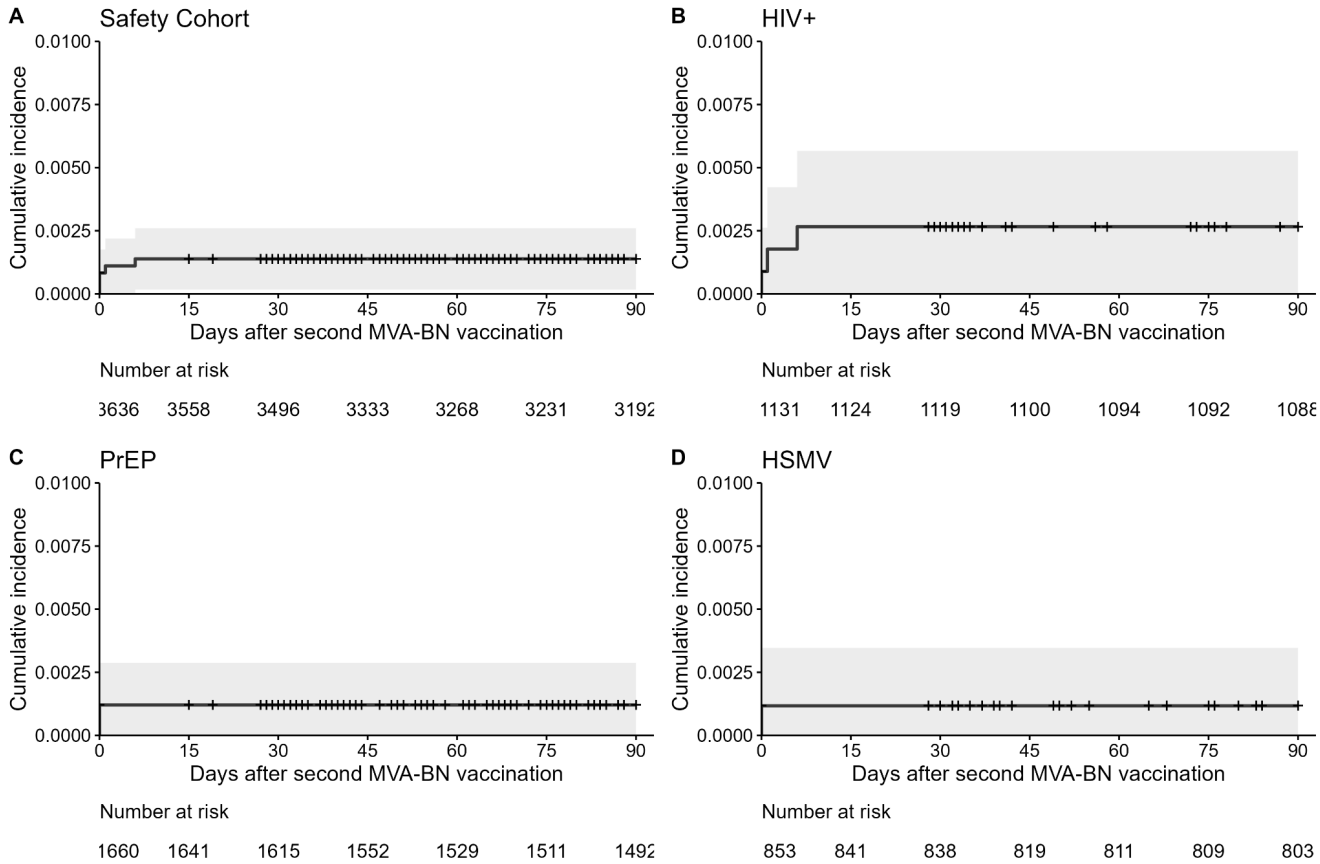


Table 24 (Safety cohort) and Tables 25, 26 and 27 (per study subgroups) describe the ARs observed in the participants that received one vaccination (either first or second dose) classified by ICD-10

and MedDRA diagnosis codes, as well as the duration, causality and severity of the reactions. The majority of the identified ARs were classified as mild (n=10) or moderate (n=9). Ten events were 'possibly' associated with the vaccination, whereas only 7 and 3 were 'likely' and 'confirmed' to be associated with the vaccination, respectively. One event was classified as severe (10000269 Abscess,10024784 Localised superficial swelling, mass, or lump) with confirmation of causality associated with vaccination. The average duration of an adverse reaction was 42.25 days, with the longest lasting events being those related to the injection site (Table 24). Two adverse reactions related to liver function studies and palpitations respectively, were 79 and 110 days in duration. The evolution of these events is explained in the participant narratives, Section 8.6.1. Overall, the PLWHIV subgroup experienced more adverse reactions, with more frequent moderate and severe levels of reaction, and more often associated with the vaccination, when compared to the PrEP users and HSMV subgroups (Table 25 vs. Table 26 and Table 27).

Table 24: Adverse reactions in participants that received one vaccination (either first or second dose) in the Safety cohort by ICD-10 diagnosis and MedDRA code.

MedDRA Diagnosis code	ICD-10 Diagnosis code	Duration	Causality	Severity
10021959 Inflammation localised, 10022102, Injection site tenderness 10060708, Induration	T88.1	274	Likely	Mild
10021959 Inflammation localised, 10022102, Injection site tenderness 10060708, Induration	T88.1	291	Likely	Moderate
10000269 Abscess, 10024784 Localised superficial swelling, mass, or lump	L02.4	17	Confirmed	Severe
10015587 Exanthema	R21	6	Possible	Mild
10021959 Inflammation localised, 10016558, Fever, 10013573, Dizziness, 10028813, Nausea, 10022102, Injection site tenderness	T88.1	7	Confirmed	Moderate
10010914 Convulsions, 10015037 Epilepsy	G40.9	0	Possible	Moderate
10033985 Paresis, 10025482 Malaise, 10033425 Pain in extremity, 10019211 Headache, 10043890 Tiredness	G83.0	1	Likely	Moderate
10015587 Exanthema, 10038198 Redness, 10023084 Itching, 10071701 Pain in upper extremities	R21	13	Confirmed	Moderate
10012735 Diarrhoea	K52.9	2	Likely	Mild
10012735 Diarrhoea	K52.9	2	Possible	Mild
10016059 Facial pain	M79.28	2	Possible	Mild
10012735 Diarrhoea	K52.9	10	Possible	Moderate
10064880 Post procedural dizziness	R42	0	Possible	Moderate
10077692 Liver function test increased	R94.5	79	Likely	Mild
10037500 Pulsus bigeminus, 10033557 Palpitations	I49.4	110	Possible	Mild
10047700 Vomiting	R11	10	Possible	Moderate
10012735 Diarrhoea	K52.9	10	Possible	Moderate
10012735 Diarrhoea	K52.9	2	Likely	Mild
10047700 Vomiting	R11	2	Likely	Mild

MedDRA Diagnosis code	ICD-10 Diagnosis code	Duration	Causality	Severity
10016558, Fever	T88.1	7	Possible	Mild
Total average days		42.25		
Total causality by level		*Possible*	10	
		Likely	7	
		Confirmed	3	
Total severity by level			*Mild*	10
			Moderate	9
			Severe	*1*

Table 25: Adverse reactions in participants that received one vaccination (either first or second dose) in the PLWHIV subgroup of the Safety cohort by ICD-10 diagnosis and MedDRA code.

MedDRA Diagnosis code	ICD-10 Diagnosis code	Duration	Causality	Severity
10000269 Abscess, 10024784 Localised superficial swelling, mass, or lump	L02.4	17	Confirmed	Severe
10021959 Inflammation localised, 10016558, Fever, 10013573, Dizziness, 10028813, Nausea, 10022102, Injection site tenderness	T88.1	7	Confirmed	Moderate
10010914 Convulsions, 10015037 Epilepsy	G40.9	0	Possible	Moderate
10033985 Paresis, 10025482 Malaise, 10033425 Pain in extremity, 10019211 Headache, 10043890 Tiredness	G83.0	1	Likely	Moderate
10015587 Exanthema, 10038198 Redness, 10023084 Itching, 10071701 Pain in upper extremities	R21	13	Confirmed	Moderate
10012735 Diarrhoea	K52.9	10	Possible	Moderate
10064880 Post procedural dizziness	R42	0	Possible	Moderate
10077692 Liver function test increased	R94.5	79	Likely	Mild
10012735 Diarrhoea	K52.9	2	Likely	Mild
10047700 Vomiting	R11	2	Likely	Mild
Total average days		13.1		
Total causality by level		*Possible*	3	
		Likely	4	
		Confirmed	3	
Total severity by level			*Mild*	3
			Moderate	6
			Severe	1

Table 26: Adverse reactions in participants that received one vaccination (either first or second dose) in the PrEP users subgroup of the Safety cohort by ICD-10 diagnosis and MedDRA code.

MedDRA Diagnosis code	ICD-10 Diagnosis code	Duration	Causality	Severity
10021959 Inflammation localised, 10022102, Injection site tenderness 10060708, Induration	T88.1	274	likely	Mild
10012735 Diarrhoea	K52.9	2	likely	Mild
10012735 Diarrhoea	K52.9	2	possible	Mild
10016059 Facial pain	M79.28	2	possible	Mild
10016558, Fever	T88.1	7	possible	Mild
Total average days		57.4		
Total causality by level		*Possible*	3	
		Likely	2	
		Confirmed	0	
Total severity by level			*Mild*	5
			Moderate	0
			Severe	0

Table 27: Adverse reactions in participants that received one vaccination (either first or second dose) in the HSMV subgroup of the Safety Cohort by ICD-10 diagnosis and MedDRA code.

MedDRA Diagnosis code	ICD-10 Diagnosis code	Duration	Causality	Severity
10021959 Inflammation localised, 10022102, Injection site tenderness 10060708, Induration	T88.1	274	likely	Mild
10021959 Inflammation localised, 10022102, Injection site tenderness 10060708, Induration	T88.1	291	likely	Moderate
10012735 Diarrhoea	K52.9	10	possible	Moderate
10064880 Post procedural dizziness	R42	0	possible	Moderate
10012735 Diarrhoea	K52.9	2	likely	Mild
10047700 Vomiting	R11	2	likely	Mild
Total average days		96.5		
Total causality by level		*Possible*	2	
		Likely	4	
		Confirmed	0	
Total severity by level			*Mild*	3
			Moderate	3
			Severe	0

8.4.3 Secondary Objectives: Reactogenicity

Reactogenicity (tolerability) of the MVA-BN vaccine was assessed during seven days after the first and second administration (Table 28) for the Safety cohort. Most participants experienced discomfort or a localized reaction (70.2% [95% CI 68.5 - 71.8]) after the first vaccination, which decreased in frequency after the second vaccination. The most common symptom was mild pain at the injection site with pressure or movement (46.7% [95% CI 44.9 - 48.5]) 1st dose, 40.6% [95% CI 38.4 - 42.8] 2nd dose). Less than 10% of all participants vaccinated as part of the study experienced myalgia (muscle pain), arthralgia (joint pain), headache, nausea or diarrhoea. Mild to moderate fatigue was common after both 1st and 2nd dose. Reactogenicity in PLWHIV, PrEP users, and HSMV subgroups (Supplementary Tables 20 to 22) was similar in that participants in these subgroups experienced discomfort or a local reaction at the injection site, though with slightly more frequency than the Safety cohort (first dose, 61.5% [58.3 - 64.6]; 73.3% [70.8 - 75.7]; 63.7% [60.3 - 66.9], and slightly less after the second dose (52.0% [47.8 - 56.2]; 57.1% [53.6 - 60.4]; 55.7% [51.2 - 60.2]). In line with the Safety cohort, the most common symptom in all subgroups was mild pain at the injection site with pressure or movement.

Less than a quarter of the Safety cohort experienced any systemic or severe systemic complaint after the first dose (22.3% [20.9 - 23.9], 2.5% [2.0 - 3.1]) and less often with the second dose (17.6% [15.9 - 19.4], 1.9% [1.3 - 2.6]. The covariates that affected the odds of experiencing any local or systemic reaction are described in Tables 29 and 30 and Figure 9. For every 10-year increase in participants' age, the odds of experiencing any *local* reaction after the first dose were decreased by 18% or 9% respectively (OR 0.82 [0.74-0.90]; 0.91 [0.82-1.01]). No association was seen when examining differences between age in the odds of a local reaction from the second dose or a systemic reaction from the first or second dose.

Table 28: Reactogenicity as reported by participants in the Safety cohort in the 7 days following first or second MVA-BN dose vaccination.

	MVA 1st dose at/after CED n=3036	**MVA 2nd dose at/after CED n=1939**
	% (95%CI)	
Any discomfort or a local reaction at the injection site	70.2% (68.5 - 71.8)	56.8% (54.6 - 59)
Any severe discomfort or a local reaction at the injection site	1.6% (1.2 - 2.1)	1.9% (1.4 - 2.6)
Pain in the area of the puncture site at rest:		
Mild	40.9% (39.2 - 42.7)	34.1% (32 - 36.3)
Moderate	8.2% (7.3 - 9.3)	6.2% (5.2 - 7.4)
Severe	0.4% (0.2 - 0.7)	0.3% (0.1 - 0.6)
Pain at the injection site with pressure and/or movement:		
Mild	46.7% (44.9 - 48.5)	40.6% (38.4 - 42.8)
Moderate	17.7% (16.4 - 19.1)	10.8% (9.5 - 12.3)
Severe	0.9% (0.6 - 1.3)	0.4% (0.2 - 0.8)
Size of redness:		
2 - 5 cm	21.9% (20.4 - 23.4)	19.3% (17.6 - 21.2)
5.1 - 10 cm	4.4% (3.7 - 5.2)	5.3% (4.4 - 6.4)
> 10 cm	0.4% (0.2 - 0.8)	1.3% (0.9 - 2)
Size of swelling:		
2 - 5 cm	30.9% (29.3 - 32.6)	24.7% (22.8 - 26.6)
5.1 - 10 cm	3.8% (3.1 - 4.5)	3.1% (2.4 - 4)
> 10 cm	0.5% (0.3 - 0.9)	0.6% (0.3 - 1.1)
Any systemic complaints	22.3% (20.9 - 23.9)	17.6% (15.9 - 19.4)

	MVA 1st dose at/after CED n=3036	MVA 2nd dose at/after CED n=1939
Any severe systemic complaints	2.5% (2 - 3.1)	1.9% (1.3 - 2.6)
Fever present:		
Mild	1.8% (1.4 - 2.4)	1.9% (1.3 - 2.6)
Moderate	2.1% (1.6 - 2.7)	2.1% (1.5 - 2.9)
Severe	0.7% (0.4 - 1)	0.4% (0.2 - 0.8)
Fatigue:		
Mild	6.2% (5.4 - 7.2)	5.7% (4.8 - 6.9)
Moderate	8.3% (7.4 - 9.4)	7.2% (6.1 - 8.5)
Severe	1.3% (0.9 - 1.8)	0.9% (0.6 - 1.5)
Myalgia:		
Mild	2.7% (2.1 - 3.3)	1.9% (1.3 - 2.6)
Moderate	3.5% (2.9 - 4.2)	2.8% (2.2 - 3.7)
Severe	0.4% (0.2 - 0.8)	0.4% (0.2 - 0.8)
Arthralgia:		
Mild	0.7% (0.5 - 1.1)	0.9% (0.5 - 1.4)
Moderate	1.5% (1.2 - 2.1)	1.4% (0.9 - 2)
Severe	0.3% (0.2 - 0.6)	0.3% (0.1 - 0.6)
Headache:		
Mild	3.7% (3.1 - 4.4)	2.8% (2.1 - 3.6)
Moderate	3.8% (3.2 - 4.5)	2.7% (2.1 - 3.6)
Severe	0.7% (0.5 - 1.1)	0.6% (0.3 - 1)
Nausea:		
Mild	0.7% (0.5 - 1.1)	0.6% (0.3 - 1)
Moderate	0.4% (0.2 - 0.7)	0.2% (0.1 - 0.6)
Severe	0.3% (0.2 - 0.6)	0.3% (0.1 - 0.6)
Diarrhoea:		
Mild	0.8% (0.5 - 1.2)	0.9% (0.6 - 1.5)
Moderate	0.6% (0.4 - 1)	0.5% (0.3 - 1)
Severe	0.4% (0.2 - 0.7)	0.3% (0.1 - 0.6)
Intake of analgesics, antipyretics	9.8% (8.8 - 10.9)	6.9% (5.8 - 8.1)
Intake of prophylactic analgesics, antipyretics	1.4% (1.1 - 2)	1.2% (0.8 - 1.8)

Note: the overlap of those who received both 1st and 2nd MVA-BN doses within the study period is n=1779

CD4 counts in PLWHIV somewhat influenced the likelihood of a local or systemic reaction after the first and second dose in those with increasing CD4 counts (≥200). Results showed a protective effect with increasing CD4 levels and lower odds of experiencing a local or systemic reaction (Tables 29 and 30, Figure 9). No significant associations were found in those with CD4 counts <200 except a significantly increased odds of systemic reactions after the first vaccination (OR 14.98 [95% CI 1.74-129.16]). However, as the event count in this group was very low (n=5/6 experienced a systemic reaction), the corresponding confidence intervals indicate a high degree of uncertainty. PrEP use did not influence the likelihood of local or systemic reaction after the first or second dose. Having a previous smallpox vaccination showed a protective effect against any systemic reaction after the second dose (OR 0.65 [0.44-0.95]). Figure 9 shows the ORs for each influencing variable of local or systemic reaction.

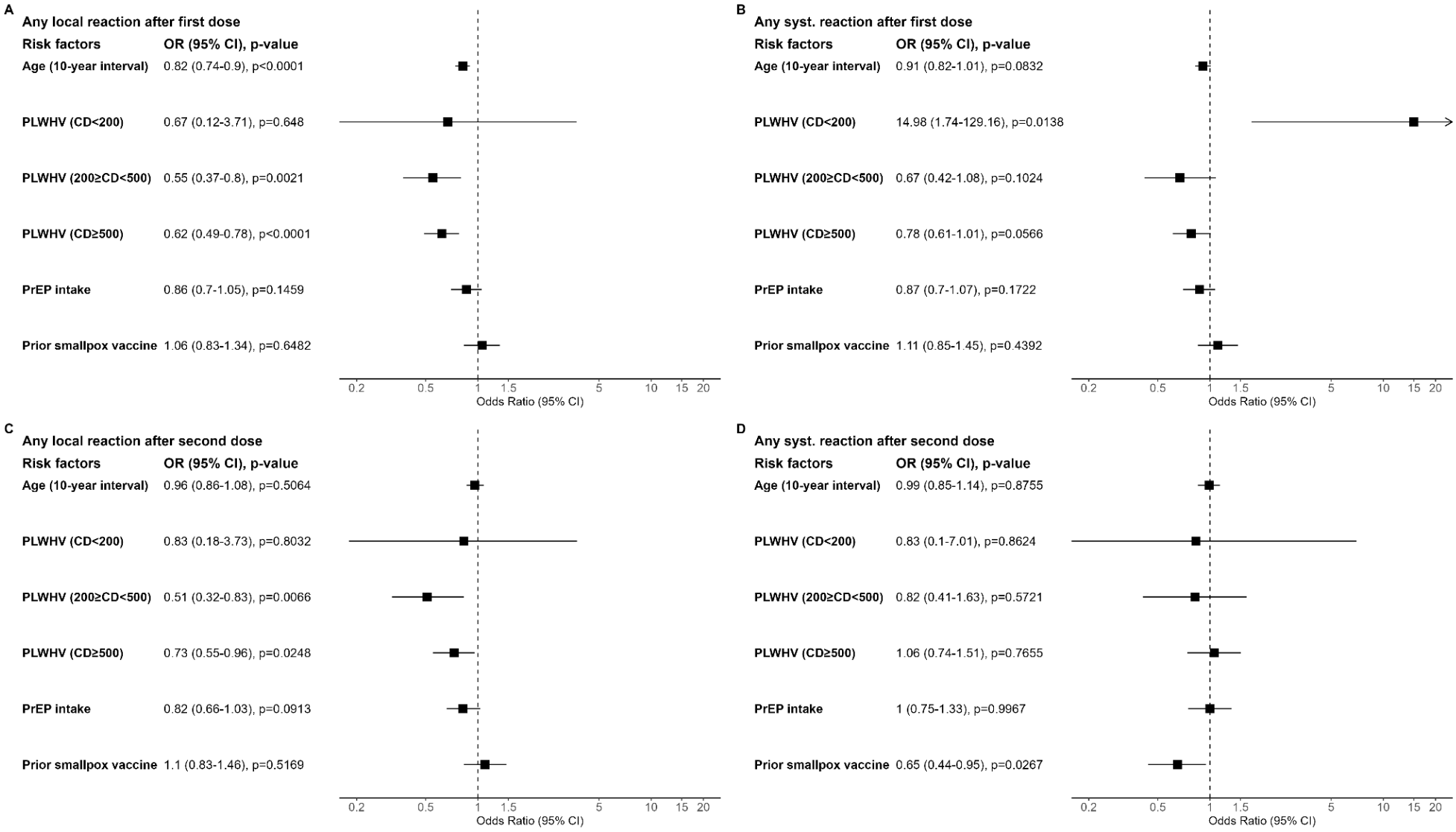
Table 29: Effect of covariables on reactogenicity for any local reaction and any systemic reaction after first and second MVA-BN vaccination in the Safety cohort. Effects presented using multivariable logistic regression reporting ORs (95% CI).

	Any local reaction		Any systemic reaction	
	MVA-BN 1st OR (95% CI)	**MVA-BN 2nd OR (95% CI)**	**MVA-BN 1st OR (95% CI)**	**MVA-BN 2nd OR (95% CI)**
Age (per 10 years unit)	0.82 (0.74-0.9)	0.96 (0.86-1.08)	0.91 (0.82-1.01)	0.99 (0.85-1.14)
HIV+ with CD4 counts < 200	0.67 (0.12-3.71)	0.83 (0.18-3.73)	14.98 (1.74-129.16)	0.83 (0.1-7.01)
HIV+ with CD4 counts ≥ 200 and < 500	0.55 (0.37-0.8)	0.51 (0.32-0.83)	0.67 (0.42-1.08)	0.82 (0.41-1.63)
HIV+ with CD4 counts ≥ 500	0.62 (0.49-0.78)	0.73 (0.55-0.96)	0.78 (0.61-1.01)	1.06 (0.74-1.51)
PrEP	0.86 (0.7-1.05)	0.82 (0.66-1.03)	0.87 (0.7-1.07)	1 (0.75-1.33)
Previous smallpox vaccination	1.06 (0.83-1.34)	1.1 (0.83-1.46)	1.11 (0.85-1.45)	0.65 (0.44-0.95)

Table 30: Effect of covariables on reactogenicity for any local reaction and any systemic reaction after first and second MVA-BN vaccination in the Safety cohort. Effects presented using univariable logistic regression reporting ORs (95% CI).

	Any local reaction		Any systemic reaction	
	MVA-BN 1st OR (95% CI)	**MVA-BN 2nd OR (95% CI)**	**MVA-BN 1st OR (95% CI)**	**MVA-BN 2nd OR (95% CI)**
Age (per 10 years unit)	0.78 (0.73-0.83)	0.94 (0.87-1.02)	0.9 (0.84-0.97)	0.88 (0.79-0.98)
HIV+ with CD4 counts < 200	0.7 (0.13-3.82)	0.93 (0.21-4.18)	16.27 (1.9-139.58)	0.73 (0.09-6.1)
HIV+ with CD4 counts ≥ 200 and < 500	0.5 (0.35-0.71)	0.57 (0.36-0.9)	0.68 (0.44-1.08)	0.7 (0.36-1.34)
HIV+ with CD4 counts ≥ 500	0.57 (0.48-0.68)	0.82 (0.66-1.01)	0.81 (0.66-0.99)	0.88 (0.66-1.17)
PrEP	1.3 (1.11-1.53)	1.02 (0.85-1.22)	1.02 (0.86-1.22)	1.15 (0.91-1.46)
Previous smallpox vaccination	0.66 (0.55-0.78)	0.94 (0.77-1.16)	0.88 (0.73-1.07)	0.63 (0.47-0.84)

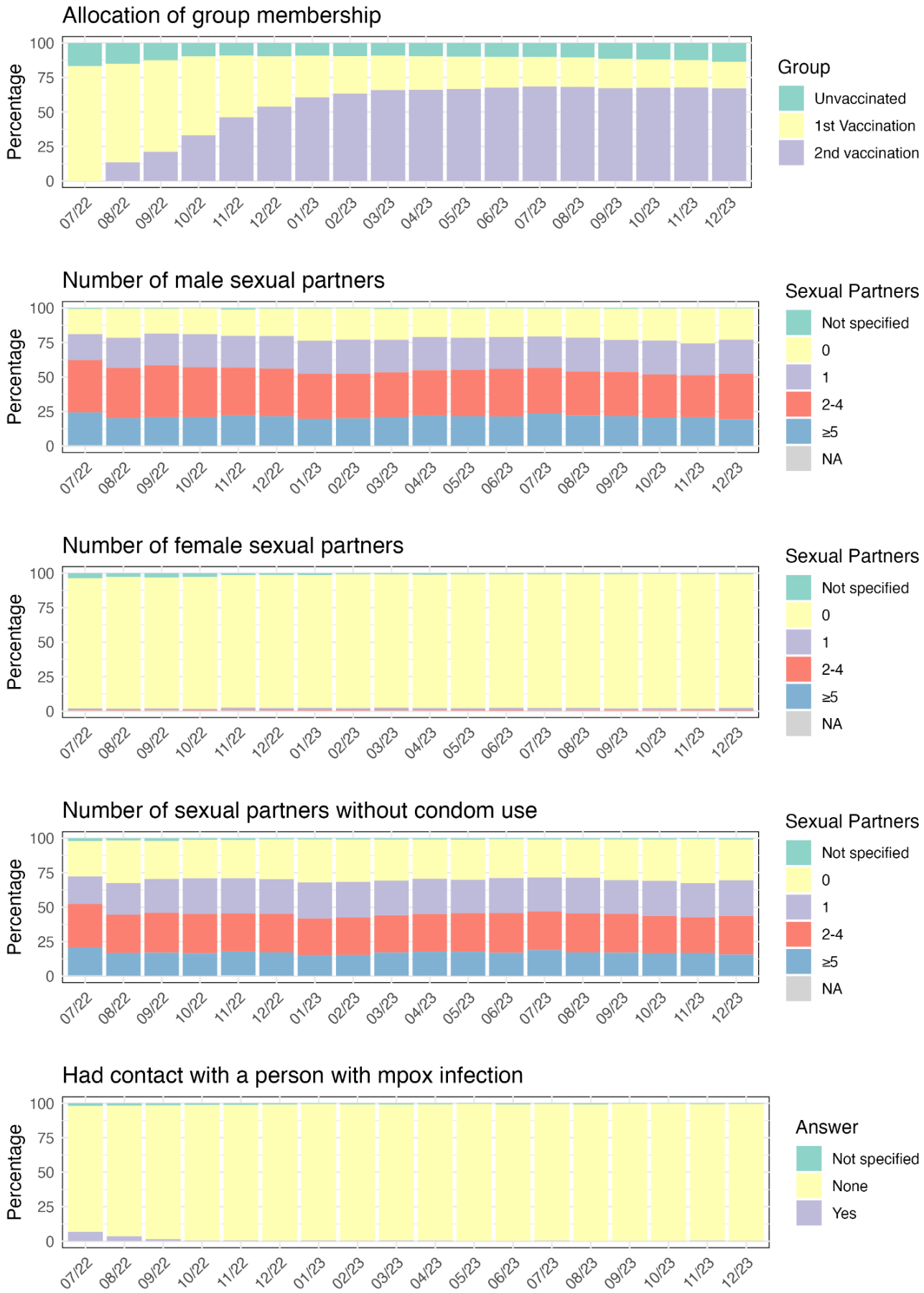
Figure 9: Forest plot showing results of multivariable logistic regression analysis of any local and any systemic reactogenicity.



8.4.4 Secondary Objectives: Sexual Behaviour Follow-up

Sexual behaviours that were assessed monthly after CED for all participants in the overall MSM cohort are displayed in (Figure 10) and described in Supplementary Table 23 for the overall MSM cohort, and Supplementary Tables 24, 25 and 26 for the PLWHIV, PreP and HSMV subgroups, respectively. Sexual behaviour was assessed in all the participants regardless of MVA-BN vaccination occurring prior or at/after to CED. Each month is analysed separately given that the rate of response from participants varied across the study period. The majority of participants responded in May of 2023 (n=4158), while only 647 participants could be assessed in the first month of the study period (July 2022) reflecting ongoing enrolment and individual observation periods of 12 months. Across the study period, those who were vaccinated with a minimum of one dose by the participating study centre responded more frequently to the questionnaires when compared to the unvaccinated group.

Figure 10: Sexual Behaviour (number of male/female sexual partners, number of sexual partners without condom use, contact with person with MPXV infection) in the overall MSM cohort reported by participants monthly during follow-up.



A specific assessment of sexual behaviour in the 4 weeks prior to and 4 weeks after vaccination in the Safety cohort showed a significant slight overall increase in the number of sexual partners (Table 31, Figures 11a and 11b). A more detailed description of the cluster analyses can be found in Supplementary Tables 27 and 28, after administration of first and second dose MVA-BN doses, respectively. To further group the individual patterns a cluster analysis was conducted that resulted in four distinct patterns of sexual behaviour in relation to vaccination ("low-constant sexual behaviour", "moderate-constant sexual behaviour", "moderate-to-high variable sexual behaviour", and "high-constant sexual behaviour"). Cluster C with "moderate-to-high variable sexual behaviour" was notably different from the other three clusters in that there was a distinct increase in sexual partners (approx. 4 – 6 partners) after the first dose of MVA-BN. Those that had 0-1 (cluster A, "low-constant sexual behaviour") and >10 (cluster D, "high-constant sexual behaviour") sexual partners did not show an overall change in number of sexual partners given the vaccination, while those with approximately 2 partners also remained stable but showed a very slight decrease after the first dose of MVA-BN (cluster B, "moderate-constant sexual behaviour"). Most participants in the cluster analysis were included in cluster A (46.5%) (Figure 12a). A similar, though less prominent, pattern was observed in the change in number of sexual partners after the second dose of MVA-BN (Figure 12b).

Table 31: Sexual behaviour 4 weeks prior and post vaccination of participants in the VE cohort vaccinated at or after CED.

	4 weeks before 1st vaccination (N=2635)	**4 weeks after 1st vaccination (N=2635)**	**4 weeks before 2nd vaccination (N=2154)**	**4 weeks after 2nd vaccination (N=2154)**
	Mean (SD) or n (%)			
Sexually active: Yes	1989 (75.5)	2055 (78.0)	1635 (75.9)	1687 (78.3)
# of sexual partners				
0	687 (26.1)	624 (23.7)	559 (26.0)	502 (23.3)
1	597 (22.7)	616 (23.4)	472 (21.9)	526 (24.4)
2	461 (17.5)	460 (17.5)	401 (18.6)	368 (17.1)
3	262 (9.9)	253 (9.6)	215 (10.0)	222 (10.3)
4	194 (7.4)	178 (6.8)	177 (8.2)	172 (8.0)
5	152 (5.8)	134 (5.1)	109 (5.1)	108 (5.0)
6-10	177 (6.7)	240 (9.1)	150 (7.0)	169 (7.8)
>10	105 (4.0)	130 (4.9)	71 (3.3)	87 (4.0)
# of male sexual partners				
0	680 (25.8)	612 (23.2)	547 (25.4)	497 (23.1)
1	606 (23.0)	630 (23.9)	477 (22.1)	533 (24.7)
2	457 (17.3)	452 (17.2)	397 (18.4)	369 (17.1)
3	255 (9.7)	249 (9.4)	211 (9.8)	213 (9.9)
4	190 (7.2)	182 (6.9)	174 (8.1)	170 (7.9)
5	151 (5.7)	130 (4.9)	108 (5.0)	103 (4.8)
6-10	161 (6.1)	227 (8.6)	141 (6.5)	161 (7.5)
>10	102 (3.9)	120 (4.6)	66 (3.1)	82 (3.8)

	4 weeks before 1st vaccination (N=2635)	4 weeks after 1st vaccination (N=2635)	4 weeks before 2nd vaccination (N=2154)	4 weeks after 2nd vaccination (N=2154)
Missing	33 (1.3)	33 (1.3)	33 (1.5)	26 (1.2)
# of female sexual partners				
0	2564 (97.3)	2571 (97.6)	2101 (97.5)	2094 (97.2)
1	43 (1.6)	31 (1.2)	30 (1.4)	29 (1.3)
2	12 (0.5)	11 (0.4)	9 (0.4)	12 (0.6)
3	3 (0.1)	3 (0.1)	1 (0.0)	2 (0.1)
4	1 (0.0)	2 (0.1)	2 (0.1)	1 (0.0)
5	1 (0.0)	1 (0.0)	1 (0.0)	1 (0.0)
6-10	1 (0.0)	1 (0.0)	2 (0.1)	2 (0.1)
>10	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)
Missing	10 (0.4)	15 (0.6)	8 (0.4)	12 (0.6)
# of non-binary sexual partners				
0	2516 (95.5)	2503 (95.0)	2054 (95.4)	2053 (95.3)
1	52 (2.0)	51 (1.9)	42 (1.9)	40 (1.9)
2	6 (0.2)	9 (0.3)	9 (0.4)	9 (0.4)
3	6 (0.2)	11 (0.4)	2 (0.1)	0 (0.0)
4	2 (0.1)	6 (0.2)	3 (0.1)	5 (0.2)
5	5 (0.2)	2 (0.1)	1 (0.0)	0 (0.0)
6-10	2 (0.1)	5 (0.2)	0 (0.0)	3 (0.1)
<10	1 (0.0)	2 (0.1)	1 (0.0)	1 (0.0)
Missing	45 (1.7)	46 (1.7)	42 (1.9)	43 (2.0)
# sexual partners you had sex without a condom				
0	948 (36.0)	936 (35.5)	757 (35.1)	771 (35.8)
1	649 (24.6)	641 (24.3)	514 (23.9)	513 (23.8)
2	357 (13.5)	352 (13.4)	322 (14.9)	303 (14.1)
3	181 (6.9)	179 (6.8)	141 (6.5)	155 (7.2)
4	142 (5.4)	133 (5.0)	155 (7.2)	107 (5.0)
5	114 (4.3)	100 (3.8)	72 (3.3)	91 (4.2)
6-10	127 (4.8)	159 (6.0)	111 (5.2)	122 (5.7)
>10	82 (3.1)	100 (3.8)	52 (2.4)	65 (3.0)
Missing	35 (1.3)	35 (1.3)	30 (1.4)	27 (1.3)

Figure 11a. Number of sexual partners 4 weeks before/after the first dose.

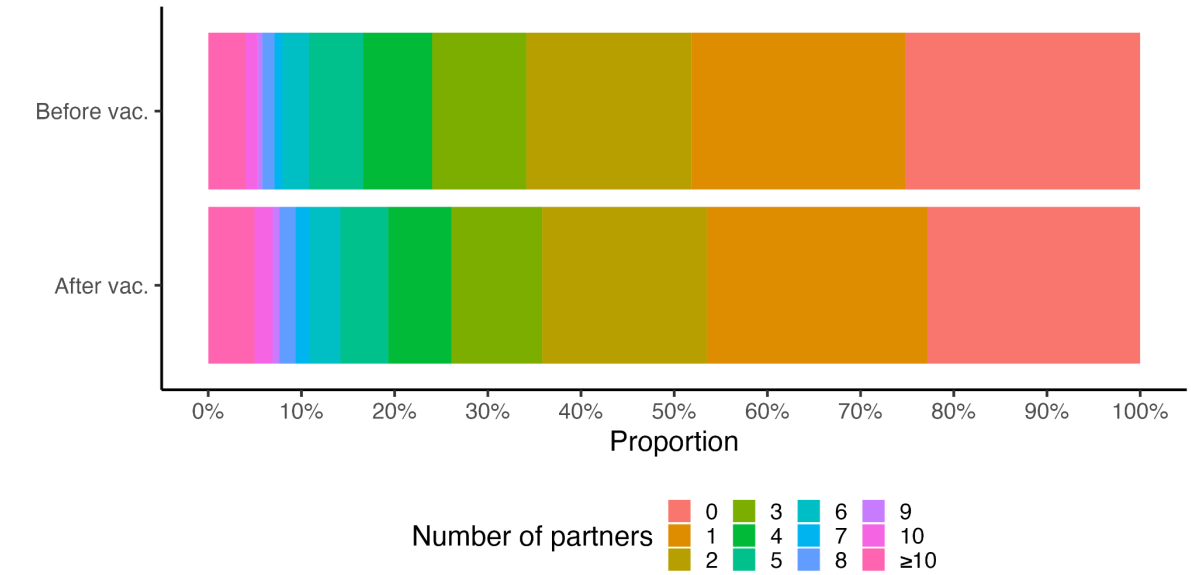
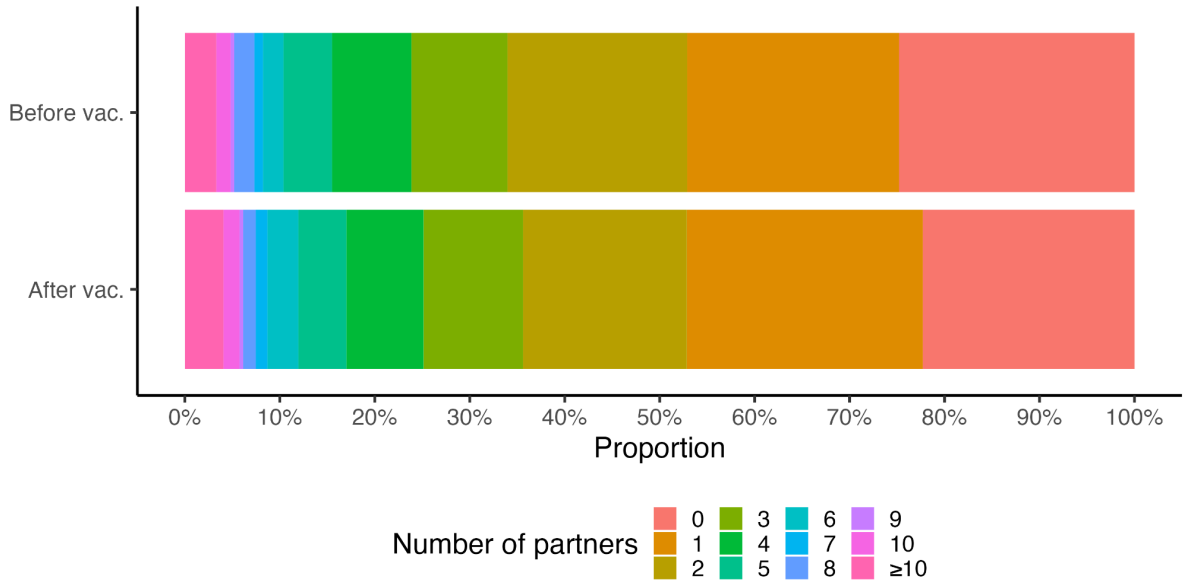


Figure 11b. Number of sexual partners 4 weeks before/after the second dose.



Alphabetic naming of clusters and corresponding description: Cluster A –"low-constant sexual behaviour", Cluster B – "moderate-constant sexual behaviour", Cluster C - "moderate-to-high variable sexual behaviour", and Cluster D - "high-constant sexual behaviour". Single small lines denote individual changes coloured in the respective cluster colour. The four thick lines denote the averages for each cluster.

Figure 12a. Change in number of sexual partners with cluster analysis prior to and after the first dose of MVA-BN.

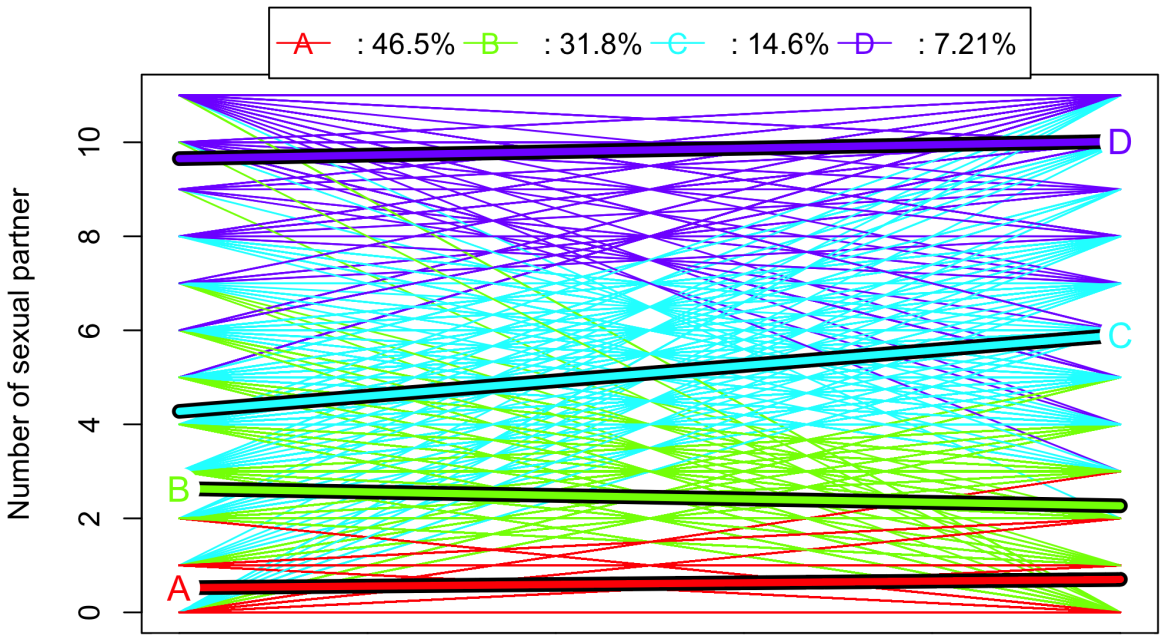
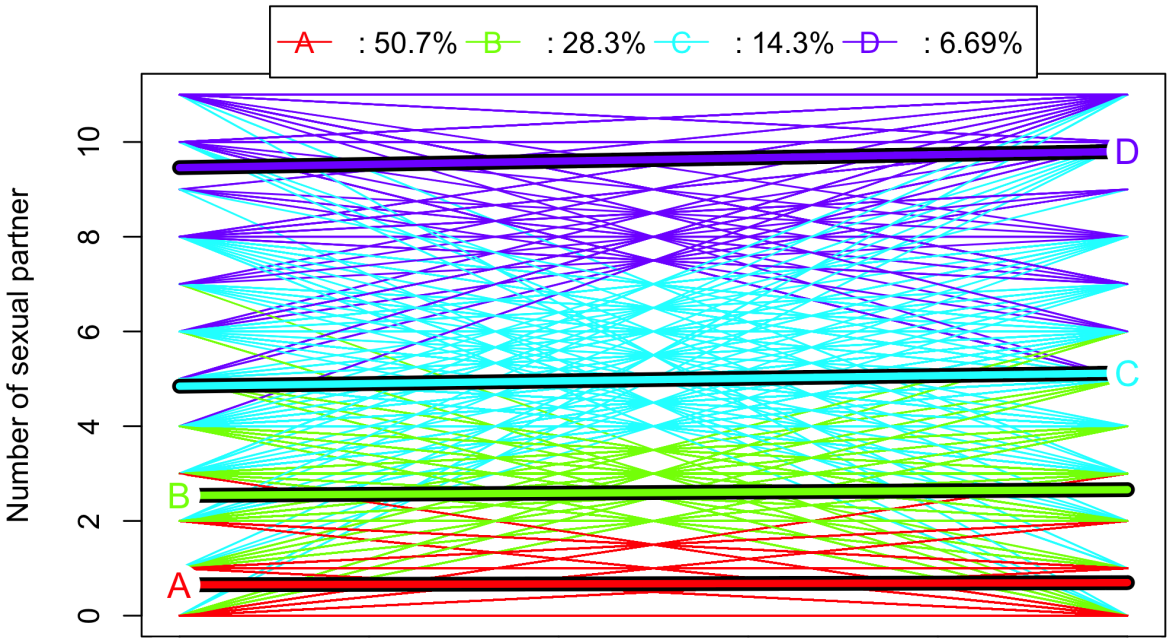


Figure 12b. Change in number of sexual partners with cluster analysis prior to and after the second dose of MVA-BN.



8.5. Sensitivity analyses

A first sensitivity analysis was performed applying the 'fully vaccinated' exposure definition. By applying the 'fully vaccinated' definition (see Section 6.9.4), one case of mpox was observed during the follow-up period (mean follow-up time 269.68 days). The IR per 1000 person years in those who met the criteria for 'fully vaccinated' (n=635) was 2.13 (95% CI 0.12-9.38). Table 32 describes the incidence of mpox when the exposure definition was modified to 'fully vaccinated'.

The 'fully vaccinated' exposure definition was also applied in the PLWHIV, PrEP users, and HSMV subgroups, and no cases were observed for those defined as fully vaccinated in the PLWHIV and HSMV subgroups, whereas an IR of 4.73 [95% CI 0.27-20.82]) was obtained for PrEP users. Supplementary Table 29 displays the sensitivity analysis of 'fully vaccinated' status definition in the PLWHIV, PreP users and HSMV subgroups.

As a second sensitivity analysis, the IR per 1000 person-years in those vaccinated with 1 dose was 8.88 (95% CI 4.61-15.21), while in those who received the second dose was 0.91 (95% CI 0.15-2.8). When follow-up for vaccinated participants was restricted to 0-13 and ≥14 days, slightly more than half of total MPXV infections (n=7) occurred ≥ 14 days of either dose of vaccination administration (Table 32). IRs across different time periods after vaccination varied (one dose, IR for mpox within 0-13 days after first MVA-BN vaccination 38.84 [95% CI 13.93-83.47]; second dose, IR for mpox within 0-13 days after second MVA-BN vaccination 9.01 [95% CI 0.51-39.61]). IR were lower for the strata of at least 14 days after first MVA-BN vaccination 5.41 (95% CI 2.15-10.96) and after the second MVA-BN vaccination 0.48 (95% CI 0.03-2.1).

Supplementary Tables 14-16 provide further details in the PLWHIV, PrEP users and HSMV subgroups. In the PLWHIV subgroup, the IR for those vaccinated with one dose was much higher than those with a second dose (16.51 (95% CI 7.09-31.92), 1.27 (95% CI 0.07-5.59)). When follow-up time was restricted, the highest IR (48.33 (95% CI 8.03-149.15)) was detected in 0-13 days after the first (48.33 (95% CI 8.03-149.15)) and second doses (25.91 (95% CI 1.48-113.96)). For the period of ≥14 days after the first dose, the IR was significantly reduced (13.06 (95% CI 4.68-28.08)) and no cases were detected in the PLWHIV subgroup ≥14 days after the second dose. In PrEP users, a similar pattern was observed in IRs, one dose versus a second dose (7.52 (95% CI 2.33-17.46), 0.98 (95% CI 0.06-4.33)). The IR was further increased in 0-13 days after the first dose (53.05 (95% CI 13.19-137.52)), as seen with PLWHIV participants, and dropped significantly in the 14 days after the first dose (2.1 (95% CI 0.12-9.25)). No cases were reported 0-13 days after the second dose, and an IR of 1.04 (95% CI 0.06-4.56) was observed ≥14 days after the second dose. In the HSMV subgroup, IRs were the lowest of all subgroups in those vaccinated with one dose (4.68 (95% CI 0.78-14.45)), and no cases were reported in those with a second dose. Across restricted follow-up windows, IR was highest in 0-13 days after the first dose (28.25 (95% CI 1.61-124.26)) and similar to PrEP users, but significantly lower than in PLWHIV subgroup in ≥14 days after the first dose (2.55 (95% CI 0.15-11.23)). No mpox cases were observed after the 2nd dose in the HSMV subgroup.

Table 32: IR of MPXV infections by vaccination status in VE cohort.

	N of participants	Mpox cases	Mean follow-up time (days)	Total Person-time (days)	Total Person-time (years)	IR per 1000 Person-years	IR (95% CI)
Unvaccinated	1127	0	169.83	191401	524.39	0	-
Vaccinated with one dose[1]	3617	11	124.94	451925	1238.15	8.88	8.88 (95% CI 4.61-15.21)
Vaccinated with second dose[2]	3126	2	257.15	803856	2202.35	0.91	0.91 (95% CI 0.15-2.8)
MPXV infection within 0-13 days after 1st MVA-BN	3617	5	12.99	46990	128.74	38.84	38.84 (95% CI 13.93-83.47)
MPXV infection ≥ 14 days after 1st MVA-BN	3607	6	112.26	404935	1109.41	5.41	5.41 (95% CI 2.15-10.96)
MPXV infection within 0-13 days after 2nd MVA-BN	3126	1	12.97	40529	111.04	9.01	9.01 (95% CI 0.51-39.61)

	N of participants	Mpox cases	Mean follow-up time (days)	Total Person-time (days)	Total Person-time (years)	IR per 1000 Person-years	IR (95% CI)
MPXV infection ≥14 days after 2nd MVA-BN	3114	1	245.13	763327	2091.31	0.48	0.48 (95% CI 0.03-2.1)
Fully vaccinated	635	1	269.68	171247	469.17	2.13	2.13 (95% CI 0.12-9.38)

[1]Includes all participants with first vaccination at CED and crossover participants who received their first vaccination.

[2]Includes all participants with second vaccination at or after CED and crossover participants who received the first and second doses during the study period.

Sensitivity Analyses in TEMVAc

To understand the hazard of mpox over the entire follow-up time and to account for covariates not included in the matching process, Cox proportional hazards models were used to calculate VE. The overall aHR for those vaccinated with a first MVA-BN dose was 0.47 (95% CI 0.26-0.86) translating to a VE of 53% in the overall VE cohort (52.82 (95% CI 13.91 to 74.15)) (Table 33). Regarding subgroups, the highest VE was observed in PrEP users (58.15 (95% CI -9.05 to 83.94)) while the lowest was observed in the PLWHIV (44 (95% CI -23.88 to 74.69)). Regarding VE across age groups, similar VE estimates were observed across age groups, 18-35 (61.97 (95% CI -21.52 to 88.1)), 36-49 (49.93 (95% CI -12.55 to 77.72)), and aged ≥50 (42.96 (95% CI -139.71 to 86.43)).

Table 33: VE sensitivity analysis*, using matched hazard ratios (aHRs) from cause-specific Cox proportional hazards ratios to estimate VE in the VE cohort and for each subgroup and age group analysis (in the overall VE cohort) using three exposure groups.

	No. at risk at start of follow-up (N)	PCR confirmed mpox (N)	aHR (95% CI)	VE (95% CI) in %
Overall VE cohort				
Unvaccinated	3027	32	-	-
After first MVA-BN vaccination	3027	16	0.47 (95% CI 0.26-0.86)	52.82 (95% CI 13.91 to 74.15)
PLWHIV				
Unvaccinated	1417	16	-	-
After first MVA-BN vaccination	1417	10	0.56 (95% CI 0.25-1.24)	44 (95% CI -23.88 to 74.69)
PrEP				
Unvaccinated	1312	14	-	-
After first MVA-BN vaccination	1312	6	0.42 (95% CI 0.16-1.09)	58.15 (95% CI -9.05 to 83.94)
HSMV				
Unvaccinated	1178	6	-	-
After first MVA-BN vaccination	1178	3	0.46 (95% CI 0.11-1.84)	54.11 (95% CI -84.06 to 88.56)
Age 18-35				
Unvaccinated	776	10	-	-
After first MVA-BN vaccination	776	4	0.38 (95% CI 0.12-1.22)	61.97 (95% CI -21.52 to 88.1)

Age 36-49				
Unvaccinated	1241	17	-	-
After first MVA-BN vaccination	1241	9	0.5 (95% CI 0.22-1.13)	49.93 (95% CI -12.55 to 77.72)
Age ≥50				
Unvaccinated	1010	5	-	-
After first MVA-BN vaccination	1010	3	0.57 (95% CI 0.14-2.4)	42.96 (95% CI -139.71 to 86.43)

**Estimates after the second MVA-BN vaccination were not estimated as no mpox cases were reported after second MVA-BN vaccination.*

When the same model was applied across restricted time periods of follow-up (Table 34), the overall VE within 0 to 13 days after the first vaccination was 48.91 (95% CI -38.4 to 81.14) and increased to 54.84 (95% CI 3.93 to 78.78) when estimated after at least 14 days. Similar patterns were observed across subgroups (increase in VE after at least 14 days of vaccination except for PLWHIV). The largest increase in VE was observed in HSMV between the time period 0-13 days of first vaccination (2.78 (95% CI -1461.28 to 93.95)) and at least 14 days after first vaccination (64.03 (95% CI -85.99 to 93.05)).

Table 34: VE sensitivity analysis*, using matched hazard ratios (aHRs) from cause-specific Cox proportional hazards ratios in the VE cohort, for each subgroup and age group (in the overall VE cohort) using the two time periods after MVA-BN vaccination.

	No. at risks start of follow-up (N)	**PCR confirmed mpox (N)**	**aHR (95% CI)**	**VE (95% CI) in %**
Overall VE cohort				
Within 0-13 days				
Unvaccinated	3027	11	-	-
After first vaccination	3027	6	0.51 (95% CI 0.19-1.38)	48.91 (95% CI -38.4 to 81.14)
≥14 days				
Unvaccinated	2551	21	-	-
After first vaccination	2551	10	0.45 (95% CI 0.21-0.96)	54.84 (95% CI 3.93 to 78.78)
PLWHIV				
Within 0-13 days				
Unvaccinated	1417	5	-	-
After first vaccination	1417	2	0.37 (95% CI 0.07-1.92)	62.95 (95% CI -92.26 to 92.86)
≥14 days				
Unvaccinated	1238	11	-	-
After first vaccination	1238	8	0.64 (95% CI 0.26-1.61)	35.68 (95% CI -60.65 to 74.25)
PrEP				
Within 0-13 days				

	No. at risks start of follow-up (N)	PCR confirmed mpox (N)	aHR (95% CI)	VE (95% CI) in %
Unvaccinated	1312	6	-	-
After first vaccination	1312	4	0.63 (95% CI 0.18-2.25)	36.55 (95% CI -125.12 to 82.12)
≥14 days				
Unvaccinated	1089	8	-	-
After first vaccination	1089	2	0.25 (95% CI 0.05-1.19)	74.73 (95% CI -19.26 to 94.64)
HSMV				
Within 0-13 days				
Unvaccinated	1178	1	-	-
After first vaccination	1178	1	0.97 (95% CI 0.06-15.61)	2.78 (95% CI -1461.28 to 93.95)
≥14 days				
Unvaccinated	1012	5	-	-
After first vaccination	1012	2	0.36 (95% CI 0.07-1.86)	64.03 (95% CI -85.99 to 93.05)
Age 18-35				
Within 0-13 days				
Unvaccinated	776	5	-	-
After first vaccination	776	3	0.55 (95% CI 0.13-2.33)	44.56 (95% CI -132.58 to 86.78)
≥14 days				
Unvaccinated	644	21	-	-
After first vaccination	644	0	-	-
Age 36-49				
Within 0-13 days				
Unvaccinated	1241	5	-	-
After first vaccination	1241	2	0.38 (95% CI 0.07-1.98)	61.61 (95% CI -98.49 to 92.57)
≥14 days				
Unvaccinated	1037	12	-	-
After first vaccination	1037	7	0.55 (95% CI 0.22-1.4)	45.15 (95% CI -39.65 to 78.46)
Age ≥50				
Within 0-13 days				
Unvaccinated	1010	1	-	-
After first vaccination	1010	1	0.96 (95% CI 0.06-15.41)	4.2 (95% CI -1440.81 to 94.04)
≥14 days				

	No. at risks start of follow-up (N)	PCR confirmed mpox (N)	aHR (95% CI)	VE (95% CI) in %
Unvaccinated	870	4	-	-
After first vaccination	870	2	0.47 (95% CI 0.09-2.6)	52.59 (95% CI -160.24 to 91.36)

** Estimates after the second MVA-BN vaccination were not estimated as no mpox cases were reported after second MVA-BN vaccination.*

8.6 Adverse Reactions

The primary causality assessment of adverse reactions in the SEMVAc study was conducted by the study centres as only the study centres have access to the full participant's data and can examine the participant. It is important to note that as per study protocol study centres only report adverse events that have at least a possible causal relationship with the studied vaccine and were thus classified as adverse reactions in accordance with WHO-UMC guidelines (19). A detailed narrative of selected ARs of interest is provided below.

8.6.1 Narratives of ARs of interest

Each incident was discussed with the study centres in relation to its causal linkage to the vaccine.

Before describing the narratives for the ARs of interest it is worth to note that a severe gastrointestinal disorder, namely vomiting and diarrhoea, was reported previously (Interim Report 6) for a single participant. Initially, these symptoms were classified as severe by a study physician. However, during the query process, further investigation into this case was conducted, and the study physician was consulted to reassess the classification according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5. In this context, 'severe' refers to cases where hospitalisation may be indicated. Upon reassessment, the study physician determined that both complaints of the single participant should be reclassified as moderate. Accordingly, the classification of these gastrointestinal disorders has been updated in the database by the respective study centre.

The selected ARs of interest for which narratives are provided include (SOC, MedDRA, ICD-10):

1. Cardiac disorders, 10033557 Palpitations, I49.4:

History: The patient reported an episode of palpitations with recorded rhythm abnormality on a wearable device (Apple watch), which recorded arrhythmia, ventricular extrasystoles, Pulsus bigeminus. The symptoms occurred 5 days after the first vaccination and were judged to be possibly related to vaccination by the study centre. The episodes of arrhythmia lasted mostly 1 minute, occasionally up to 2-3 minutes. Initially, the episodes were observed daily for a week, they only occurred sporadically, approximately 2-3 times per month. In total, symptoms lasted for 110 days and have subsided at the time of writing. No treatment was required. The participant is a healthcare professional but did not seek further diagnostic workup. No further clinical and/or cardiological consultations of the reported episode of palpitation were performed.
Diagnosis / Symptoms: Palpitations
AR/SAR: AR
System Organ Class: Cardiac disorders
Causal relationship with vaccine: Possible
Intensity: Mild

Time delta to 1st vaccination (days): 5 days after first vaccination
AR duration in days: 110 days
ICD: I49.4
MedDRA: 10033557 Palpitations
Age group: 35-39 years
Relevant medical conditions: None
Discussion of the case: The participant reported palpitations. There was no ECG examination, the rhythm abnormality was only confirmed by an Apple watch. Although a link to the vaccine cannot be ruled out, it is not clear, since the participant did not seek further cardiological diagnostic work-up, and alternative causes remain possible.

2. Gastrointestinal disorders, 10047700 Vomiting, 10012735 Diarrhoea, R11

History: The patient presented with enteritis, characterised by diarrhoea, and vomiting that started after receiving the first MVA-BN vaccination. The symptoms lasted for 10 days and subsided completely (complete reconstitution of health). The patient reported later that his partner may also have had diarrhoea during the same time period.
Diagnosis: Enteritis
AR/SAR: AR
System Organ Class: Gastrointestinal disorders
Causal relationship with vaccine: Possible
Intensity: Moderate
Time delta to 1st vaccination (days): 0 days after first vaccination
AR duration in days: 10
ICD: R11
MedDRA: 10047700 Vomiting, 10012735 Diarrhoea
Age group: 35-39 years
Relevant medical conditions: None
Discussion of the case: the patient reported vomiting and diarrhoea for 10 days, as reported by the study centre. The severity was classified as "moderate" in accordance with the CTCAE version 5 guidelines. The patient reported likely diarrhoea of the partner in the same time period, therefore an infectious cause of gastroenteritis cannot be ruled out and appears likely.

3. Gastrointestinal disorders, 10047700 Vomiting, 10012735 Diarrhoea, R11

History: The patient presented with diarrhoea that lasted for 10 days and subsided completely (complete reconstitution of health). No laboratory test abnormalities were reported.
Diagnosis: Enteritis
AR/SAR: AR
System Organ Class: Gastrointestinal disorders
Causal relationship with vaccine: Possible
Intensity: Moderate
Time delta to 1st vaccination (days): 12 after first vaccination
AR duration in days: 10
ICD: R11
MedDRA: 10047700 Vomiting, 10012735 Diarrhoea
Age group: 55-59 years
Relevant medical conditions: HIV under ART with >500 CD4+ cells and virus below detection limit, HBV, HDV
Discussion of this case: The patient had diarrhoea for 10 days, as reported by the study centre. Diarrhoea and GI complaints were listed as potential side effects after MVA-BN vaccination. However, the symptoms only started 12 days after vaccination and the study centre reported that

infections with HDV and HBV could also have played a causative role in this patient. The patient in question has a history of chronic hepatitis B and HIV infection, which were diagnosed several years ago. However, during the assessment for diarrhoea after vaccination, it was discovered that the patient had anti-HDV antibodies, and HDV PCR qualitative testing confirmed the presence of viral replication. While many infections with HDV are asymptomatic, the treating physician at the study centre concluded that the recent diagnosis of HDV co-infection could have also contributed to the episode of diarrhoea. Therefore, it is possible that both the recent HDV co-infection and the timing of symptom onset, which occurred 12 days after vaccination, played a causative role in this adverse event.

4. Infections and infestations, 10000269 Abscess, 10024784 Localised superficial swelling, mass, or lump, L02.4

History: The patient presented to the emergency department 11 days after the first vaccination due to swelling and warmth in the area of the injection site. An abscess 3 cm in diameter in the middle of the right upper arm above the deltoid muscle was diagnosed. It was incised and debridement of the abscess was performed. No microbiological culture or further diagnostics concerning the pathogen were performed.
Diagnosis: Abscess right upper arm
ICD: L02.4
AR/SAR: AR
MedDRA: 10000269 Abscess, 10024784 Localised superficial swelling, mass, or lump
System Organ Class: Infections and Infestations
Causal relationship with vaccine: Certain
Intensity: Severe
Time delta to 1st vaccination (days): 11
AR duration in days: 17
Age group: 35-39 years
Relevant medical conditions: HIV under ART with >500 CD4+ cells and virus below detection limit
Discussion of this case: the most likely explanation appears to be related to the injection. However, in its assessment, the study centre reported handling of the vaccine according to the manufacturer's instructions, administration in a sterile manner and into the correct location, and a correct handling of the cold chain. This incident was unique and occurred as the only case among several thousand vaccine doses administered at this study site. An alternative explanation may be a sterile abscess, a rare adverse reaction that has been reported following vaccinations (26).

5. Nervous system disorders, 10010914 Convulsions, 10015037 Epilepsy, G40.9

History: The patient has a history of epilepsy with approx. 3 seizures per year. It was suspected that the vaccination may have triggered the convulsion.
Diagnosis: Convulsion
AR/SAR: AR
System Organ Class: Nervous system disorders
Causal relationship with vaccine: Possible
Intensity: Moderate
Time delta to 2nd vaccination (days): 1 day after second vaccination
AR duration in days: 0
ICD: G40.9
MedDRA: 10010914 Convulsions, 10015037 Epilepsy.
Age group: 40-44 years.
Relevant medical conditions: Epilepsy, HIV under ART with >500 CD4+ cells and virus slightly above detection limit.

Discussion of this case: given the history of epilepsy and the occurrence of a convulsion one day after the second vaccination, a potential causal relationship cannot be ruled out.

6. General disorders and administration site conditions, 10033985 Paresis, 10025482 Malaise, 10033425 Pain in extremity, 10019211 Headache, 10043890 Tiredness, G83.0

History: The patient reported fatigue, tiredness, body aches, headaches, weakness, and pain in both hands. The symptoms occurred on the day after the first vaccination and persisted until the following day. The patient did not present to a physician during the symptoms, therefore no physical examination or laboratory tests were performed.
Diagnosis: Fatigue and incomplete temporary hand paresis
AR/SAR: AR
System Organ Class: General disorders and administration site conditions
Causal relationship with vaccine: Likely
Intensity: Moderate
Time delta to 1st vaccination (days): 1 day after first vaccination
AR duration in days: 1
ICD: G83.0
MedDRA: 10033985 Paresis, 10025482 Malaise, 10033425 Pain in extremity, 10019211 Headache, 10043890 Tiredness
Age group: 30-34 years
Relevant medical conditions: HIV under ART with >500 CD4+ cells and virus below detection limit, chronic lung disease
Discussion of this case: The patient reported an incomplete temporary paralysis of both hands that lasted for one day and that spontaneously resolved. As the patient did not present to a physician with the complaints, no validation of any presumed temporary neurological motoric and/or sensory deficit was possible. The patient has not had similar complaints since the initial incident. In summary, given the timing of these complaints directly after the vaccination a causal link cannot be ruled out. However, a neurological deficit was not confirmed by examination, which makes it difficult to conclude and leaves open the possibility that painful sensations and general fatigue may have been misinterpreted as weakness of the hands.

7. Gastrointestinal disorders, 10012735 Diarrhoea, R11

History: The patient reported mild diarrhoea that lasted for 2 days after both the first and second vaccination. Both episodes subsided completely (complete reconstitution of health). No further abnormalities were reported. This AR was actually reported twice after both the first and second vaccination by the same patient.
Diagnosis: Diarrhoea
AR/SAR: AR, reported by study centre
System Organ Class: Gastrointestinal disorders
Causal relationship with vaccine: Possible
Intensity: Mild
Time delta to 1st vaccination (days): 0 days after first vaccination and 0 days after second vaccination
AR duration in days: 2 days
ICD: R11
MedDRA: 10012735 Diarrhoea
Age group: 30-34 years
Relevant medical conditions: No chronic medical conditions, intake of PrEP medication
Discussion of this case: The patient reported mild diarrhoea lasting for 2 days following both the initial and second vaccination. Onset of symptoms occurred on the day of vaccination. The patient

has no relevant medical conditions and adheres to a regular PrEP medication regimen. It is worth noting that diarrhoea and gastrointestinal complaints were documented as potential side effects following MVA-BN vaccination.

8. Gastrointestinal disorders, 10047700 Vomiting, 10012735 Diarrhoea, R11

History: The patient presented with diarrhoea and vomiting that lasted for 2 days and subsided completely (complete reconstitution of health). No laboratory test abnormalities were reported.
Diagnosis: Diarrhoea
AR/SAR: AR, reported by study centre
System Organ Class: Gastrointestinal disorders
Causal relationship with vaccine: Possible
Intensity: Mild
Time delta to 1st vaccination (days): 0 days after second vaccination
AR duration in days: 2
ICD: R11
MedDRA: 10047700 Vomiting, 10012735 Diarrhoea
Age group: 50-54 years
Relevant medical conditions: HIV under ART with >450 CD4+ cells and virus below detection limit, gastroesophageal reflux disease
Discussion of this case: The patient had diarrhoea and vomiting for 2 days, as reported by the study centre. Diarrhoea and GI complaints were listed as potential side effects after MVA-BN vaccination. The symptoms started on the day of vaccination. The patient has a well-controlled HIV infection and gastroesophageal reflux disease and did not experience any GI complaints following the first MVA-BN vaccination.

9. Discussion

9.1. Key results

Baseline characteristics

Overall, a total of 6265 participants met the study eligibility criteria and were included in the final SEMVAc analysis, of which 3308 (53%) and 938 (15%) participants received the first and second MVA-BN vaccination at CED, respectively. 1188 participants entered the study as unvaccinated at CED, of which 542 received a first vaccination during the study period. Participants were of an average of 41 years of age, had a mean BMI of 25kg/m2, and the majority had no chronic disease (81.4%). Approximately 30% were PLWHIV (99% of whom were on ART) and 48% were PrEP users. Overall, the majority of participants were assigned male gender at birth and identified as male, secondarily as non-binary.

In general, PrEP users were younger, healthier, and not vaccinated against smallpox. PLWHIV participating the study were overall older than other cohort participants, (48 versus 37 y.o.a.) similar to the HSMV subgroup (average 54 y.o.a). PrEP users had a slightly higher frequency of STIs (13.4%) when compared to the PLWHIV subgroup (11.2%). Those in the HSMV subgroup were 54% PLWHIV and 11.3% reported other STIs. Those in the HSMV subgroup had the highest frequency of chronic cardiac (16.4%) and lung disease (4.9%). More PrEP users (who tend to be younger) were not vaccinated against smallpox, while approximately 40% of PLWHIV participants have been vaccinated at least once against smallpox, which could be related to the more advanced age.

Regarding comparisons between vaccinated and unvaccinated at baseline, those vaccinated with a first or second dose prior to CED, reported STIs slightly more frequently. Those vaccinated prior to CED also tended to be diagnosed more often with cardiovascular disease. In general, the majority

of participants were not vaccinated for smallpox (65.3%), though slightly more so in the unvaccinated group (68.8%).

Focusing on the MVA-BN vaccination status across study subgroups, 31% PLWHIV, 41% PreP users and 28% participants with HSMV received a first dose of MVA-BN at CED. Among the participants that received 2 vaccinations before CED, 67% were PreP users, 28% were PLWHIV and 21% had a history of smallpox vaccination. Half of the participants receiving a second dose of MVA-BN at or after CED were PreP users, while 28% were PLWHIV and 23% had a HSMV. Out of all participants who were unvaccinated at CED, slightly more than half were PrEP users (52.3%), but the frequency of unvaccinated participants across subgroups was represented equally (PLWHIV 20.1%; PrEP 20.6%; HSMV 18.2%).

Notably, baseline characteristics may influence high-risk or likelihood of vaccination between groups. For example, PLWHIV and MSM with changing sexual partners (often also PrEP users) were given priority in this vaccination campaign; however, study centre physicians assessed each individual's risk, based on sexual behaviours likely to increase risk or risk of severe illness if infected. In the case that PLWHIV did not engage in sexual behaviours that increased risk of infection, a study centre physician may have delayed vaccination, or prioritised PLWHIV participants based on viral count. In SEMVAc, PLWHIV participants who received vaccination at or prior to CED were majority participants with HIV viral copies under the detection limit. The group with the fewest persons with HIV viral copies under the detection limit was vaccinated with 1st dose prior to CED (54.3%). Other comorbid groups (i.e. cardiovascular disease) were those vaccinated with their second or first dose prior to CED. In addition to the prioritisation of comorbid participants or those engaging more frequent sexual behaviours that increase risk, it is possible that the differences in vaccinated and unvaccinated groups in SEMVAc are due to the temporality of joining the study. The delay in recruitment of participants given the regulatory approval process meant that some study centres had already vaccinated a larger proportion of their patients at the point of study participation.

Baseline characteristics in the TEMVAc and SEMVAc overall MSM study populations, and VE cohorts were comparable, suggesting that the TEMVAc VE analysis is generalisable to the at-risk population of the 2022-2023 mpox outbreak in Germany and as described in the literature (27,28). Overall, a total of 9328 subjects were eligible for inclusion in TEMVAc at the start of the observation period, of which 6054 were included and matched (1:1) in the VE cohort. Age, BMI, and pre-existing conditions were similar across the overall MSM cohort, the vaccinated subjects, and their matched controls (Table 16). When compared to SEMVAc, TEMVAc subjects were slightly older at inclusion (44 versus 41 years of age), as well as when comparing vaccinated vs unvaccinated subjects. There may have been a tendency for younger subjects to delay vaccination, given the patterns observed across the two recruitment periods. In terms of health status, approximately half of TEMVAc subjects (52%) were PLWHIV whereas only 31% of SEMVAc subjects were PLWHIV. Slightly fewer subjects in the vaccinated group of TEMVAc were PLWHIV (47%), whereas in SEMVAc PLWHIV were evenly distributed across unvaccinated and vaccinated groups (31% vs 33%). In TEMVAc, vaccinated and unvaccinated groups were comparable, however subgroups were distinct at baseline, drawing special attention to the differences we see in VE estimates in each subgroup: PLWHIV were slightly less healthy when compared to the overall cohort and PrEP users, while HSMV had much higher frequency of comorbidities related to an overall increased age, when compared to the overall MSM cohort.

Chronic diseases among TEMVAc subjects were mainly attributed to chronic cardiovascular disease (18% in the overall MSM cohort). Chronic cardiovascular disease was more frequent in the TEMVAc population when compared to SEMVAc (8%), however, this may be related to the more advanced age of subjects in TEMVAc. The frequency of STI diagnosis during the baseline period was comparable in TEMVAc and SEMVAc. In TEMVAc, 14% of subjects in the VE cohort had a history

of STI diagnosis in the 6 months prior to the observation period; in SEMVAc 14.3% of subjects reported at least one or more STI diagnoses within the previous 3 months before enrolment into the study period, and only 10.8% had a current STI diagnosis at CED. Recent US and European studies estimate that a reduction in sexual contacts and increased awareness likely contributed to the rapid decrease in cases of mpox during the 2022-2023 outbreak (27–29). Given the timeframe of each analysis, these differences in sexual behaviour may account for the differences in baseline STIs between SEMVAc and TEMVAc subjects, more than differences in baseline health between the two cohorts.

Baseline sexual behaviour

The sexual behaviour and characteristics of the SEMVAc study overall MSM cohort (n=6265) were evaluated at baseline and showed that most participants are attracted to men (89.8%). PrEP users reported being attracted to women and non-binary persons more often (12.6%) than PLWHIV participants (6.7%).

The majority of participants were sexually active within the last three months (83.4%). Those who were unvaccinated report the least amount of sexual activity in the last 3 months. Moreover, most participants had either between 2 and 4 (30.6%) or 5 or more (38.2%) male sexual partners in the last 3 months. Notably, those who were unvaccinated reported no or one sexual partner more often, in the overall MSM cohort and across the PLWHIV and HSMV study subgroups.

The proportion of participants reporting ≥5 sexual partners without a condom in the last 3 months was the largest in participants who were fully or partially vaccinated prior to CED (41.6%) compared to those vaccinated at or after CED (28.9%) and to unvaccinated participants (18.5%). This is observed in the MSM study cohort, but also consistently across subgroups. This might be associated with the perception of lower risk among those already vaccinated.

PrEP users reported to be more sexually active than PLWHIV (86.5% versus 79.1%), had a higher proportion of participants with 5 or more partners than PLWHIV (45.8% versus 34.6%), and reported a higher number of sexual partners without the use of condoms (58.2% versus 51%) likely due to the higher overall number of sexual partners and a possibly lower perceived risk of STIs in PreP users. PLWHIV reported more sexual contacts with intravenous drug users compared to PrEP users (7.1% versus 3%).

Approximately 58-68% of study participants vaccinated prior to CED reported sexual contacts with a person who did not use condoms, or whom they did not know. Slightly less, 44%, of unvaccinated participants reported sexual contacts with a person who did not use condoms, or whom they did not know. Again, this might be associated with the perception of lower risk among participants who were already vaccinated.

75% of study participants reported no STI in the last three months, and more specifically, approximately 3% reported contact with a person with mpox within the previous 4 weeks. The majority who reported contact with a person with mpox were those that were vaccinated with the first dose at CED (4% versus 1-3%). While PrEP users reported having at least one STI or several slightly more than the PLWHIV participants (17.2% versus 13.1%), PLWHIV participants reported being in contact with a person with mpox slightly more often than PrEP users and all participants (4% versus 2.9%). A possible explanation could be that PLWHIV were prioritised during the vaccination campaign, and many of them received their vaccinations during months of high incidence.

Primary objective: vaccine effectiveness

SEMVAc

In this prospective cohort of MSM in Germany, no estimates of effectiveness of the MVA-BN vaccine could be provided, given the lack of reported cases among unvaccinated participants. Therefore, in the current study report, we examined cumulative incidence and IRs as a sensitivity analysis of mpox in vaccinated participants. Due to the observational nature of the study, enrolment into the vaccination and unvaccinated cohort was not performed randomly, thus remaining bias should be recognized in the interpretation of results.

A lower estimated cumulative incidence of mpox was seen in participants who were vaccinated with 2 doses of MVA-BN when compared to those vaccinated with only one dose, which translates to a lower risk of mpox following the full vaccination schedule. A similar decrease in cumulative incidence was seen across subgroups of PLWHIV, as well as PrEP users. These results may support previous findings in similar populations for a stronger protective effect of two vaccinations (13,30). However, of note, the overall incidence of mpox in Germany was highly variable during the study period with highest incidence during the first months of the study, when the first MVA-BN doses were administered. The lower cumulative incidence in participants with 2 doses was, therefore, also influenced by the sharp decreased risk of infection at later time points in the study, when second doses were administered. In those with a prior history of smallpox vaccination, no cases were reported after the second dose of MVA-BN, therefore, no conclusion could be made concerning decrease in risk as related to number of doses for this subgroup. Significantly fewer unvaccinated persons were recruited for the study. The under sampled unvaccinated groups infers a decreased opportunity to detect mpox occurrence.

Regarding the timing of occurrence, the majority of mpox cases occurred ≥14 days after the first dose (n=6), however, an almost equal number of cases (n=5) occurred between 0-13 days after the first dose, and cumulative incidence was similar between the two time periods. Reasons to explain this are various. First, it is possible that those who were more at risk for mpox were encouraged to get vaccinated early on in the outbreak and may have been engaging in behaviours that increased their risk at the time of vaccination (i.e. they were exposed very shortly before or after vaccination). A decreasing IR could explain the consistent decrease in cumulative incidence (0-13 to ≥14 days) after the second dose, assuming that some immunogenicity was retained after one dose. Although behaviours that increased risk were continuous, it is important to note that second MVA-BN vaccinations were mostly administered in months marked by lower mpox incidence. Secondly, it may indicate that stronger protection is only conferred after sufficient time to mount an immunological response.

The IR per 1000 person years in those who met the criteria for ‘fully vaccinated’ (n=635) in the sensitivity analysis was lower (2.13 ([95% CI 0.12-9.38)] when compared to the IRs of the stratified time periods of 0-13 and ≥14 days. Notably, the IR in the group vaccinated with two doses, n=3126, showed the lowest IR, 0.91 (95% CI 0.15-2.8), however, the person time contributed to this group was greater than the ‘fully vaccinated’. Results from the IR estimates were similar to the patterns observed in the cumulative incidence, however, were not comparable given the distinct model parameters. It is important to note that results from multistate models (cumulative incidence) and IRs cannot be compared directly. While multistate models give probability estimates, IRs were an estimator for the hazard. Moreover, crude IRs incorporate the mean follow-up time, which is truncated by 13 days in the group “within 0-13 days”. This results in the high IR in that group, which is not reflected by the results of cumulative incidences, mainly because the calculation of cumulative incidences does not depend on mean follow-up time, rather than on number at risk at each event time.

TEMVAc

TEMVAc was designed to overcome the limitations of estimating VE in the SEMVAc study, in which cases were observed as recruitment progressed after the peak outbreak, and the lack of mpox cases in the unvaccinated cohort necessary to make a relative comparison prevented the ability to estimate VE. The retrospective nature of TEMVAc allowed the capture of the epidemiologically relevant population during the months of highest incidence of mpox in Germany. After target trial emulation, a total of 48 mpox cases were reported during the study period, 32 in the unvaccinated group, and 16 in those having received a first dose of MVA-BN. In subjects having received a second MVA-BN dose during the study period, no mpox cases were reported, therefore, VE could not be estimated for this group. In TEMVAc, the majority of infections were reported early on in the study period, between July and September 2022, corresponding to the epidemic peak of mpox in Germany (Figure 6), whereas at the time of high recruitment in SEMVAc, the number of cases had already begun to decline.

VE of the MVA-BN vaccine to prevent PCR-confirmed MPXV infection was 54.15 (95% CI 21.09 to 73.36) in the overall TEMVAc VE cohort and 63.64 (95% CI 14.92 to 84.46) in the subgroup of individuals taking PrEP. These estimations show the robustness of the VE results in the TEMVAc analyses.

In contrast, the analysis of VE within the PLWHIV and HSMV subgroups presents some challenges, given the smaller number of mpox cases in each stratum and the wide CIs that include zero, making the VE estimates less conclusive. PLWHIV showed lower VE compared to the overall, PrEP, and HSMV groups (PLWHIV 41.25 [95% CI -20.88 to 71.45]; HSMV 60.71 [95% CI -33.37 to 88.42]). This reduction of VE in PLWHIV could partially be explained by higher age, increased comorbidities, or an impaired immune response (possibly due to increased age) after vaccination with varying degrees of lower T-cell counts in PLWHIV when compared to younger, healthy individuals on PrEP. Previous studies have demonstrated an impaired vaccine response in PLWHIV specifically in those with a decreased ability to mount an adequate immune response. A reduced T-cell function as a result of HIV infection can impair vaccine responses. Lower antibody and neutralizing antibody levels in addition to accelerated waning of antibody titres after vaccination may interfere with vaccine effectiveness in PLWHIV (31,32). In general, it is challenging to discern the differential impact of HIV-associated immune dysfunction, comorbidities, or age, on the observed reduced VE in PLWHIV in the current study due to the low case count observed in the PLWHIV subgroup. The HSMV subgroup showed a slightly increased VE when compared to the overall cohort which is in line with previous efficacy literature (33). Of note is the considerable overlap between those with HSMV and those aged ≥50, however, less than 10% of the study population had confirmed previous smallpox vaccination as per EHR while more than one third of the study population was aged ≥50. Given that smallpox vaccination was compulsory in West Germany until 1976 and in East Germany until 1982 and is typically not documented in EHR due to the time since vaccination, overlap between the two groups is likely. The close association of age and likelihood of smallpox vaccination may influence the VE in the ≥50 age group. Many studies have demonstrated the long lasting effects of the smallpox vaccine, supporting the fact that it remains effective even into advanced age (34). The smallpox vaccine likely provides a previously existing degree of immunity, meaning that persons not vaccinated with MVA-BN are not truly representative of ‘vaccine naive’ (persons without any protection) which could lead to a reduction in an observable difference in infection rates and therefore, and underestimation of VE (bias towards the null). In contrast, advanced age may also lead to a reduced immune response to vaccination as has been demonstrated in several studies (35,36). In the USMVAc study (17) there was no evidence of VE observed among those aged >50 years who were assumed to have received a smallpox vaccine and had received at least one MVA-BN dose (VE, 0% [-242%, 71%]). Most likely is that these VE estimates with wide CIs inclusive of zero were primarily due to the small number of mpox cases observed in the subgroups. It is difficult to estimate VE in those with a history of smallpox vaccination given the lower observed number of mpox cases and further potential confounding factors (age, chronic disease). Few current studies

provide VE solely in HSMV, but rather exclude them to better isolate a population that is vaccine naive and most likely at highest risk of mpox (37,38).

When examining VE across the other age groups, the data show that younger participants exhibited higher VE. For individuals aged 18-35 and 36-49, VE was 60.16 (95% CI -14.09 to 86.09) and 53.28 (95% CI 3.21 to 77.44), respectively. Similar VE was observed in an Israeli population of PLWHIV and PrEP users, aged 18–42 years, estimated at 86% (95% CI 59–95%), and in a MSM population of those <50 years of age in the UK (78% [95% CI 54 to 89]) (13,39).

Regarding the vaccination status and symptomatic mpox, vaccinated subjects reported accompanying symptoms less frequently than the unvaccinated (81% in the unvaccinated vs. 44% in the vaccinated with a first dose of MVA-BN). Notably, although the present results are descriptive, they indicate less symptomatic disease after vaccination and are aligned with previous studies that assessed the association between vaccination and a resulting decrease in symptomatic mpox disease (40). In TEMVAC, the only subject that was hospitalised was unvaccinated.

Cox proportional hazards regression models were used as alternative methods to estimate VE with aHRs (Table 34). Hazard ratios provide an averaged comparison of the rates of mpox between vaccinated and unvaccinated populations over the follow-up time (41). aHRs were used to calculate VE in the sensitivity analysis, and the results closely mirrored those obtained from the cumulative incidence-based analysis.

The overall aHR for those vaccinated with one dose of MVA-BN was 0.47 (95% CI 0.26-0.86) translating into a VE of 52% in the overall VE cohort (52.82 (95% CI 13.91 to 74.15)) and suggests a protective effect against mpox in those with vaccination, similar to the results from the primary VE analysis. Regarding subgroups, the highest VE was observed in those diagnosed with PrEP intake (58.15 (95% CI -9.05 to 83.94)) while the lowest was in PLWHIV (44 (95% CI -23.88 to 74.69). Regarding VE across age groups, similar VE estimates were observed across age groups, 18-35 (61.97 (95% CI -21.52 to 88.1)) and 36-49 (49.93 (95% CI -12.55 to 77.72)), and aged ≥50 (42.96 (95% CI -139.71 to 86.43)). When the same model was applied across restricted time periods of follow-up (Table 34), the overall VE within 0 to 13 days after the first vaccination was 48.91 (95% CI -38.4 to 81.14) and increased to 54.84 (95% CI 3.93 to 78.78) when estimated after at least 14 days. The largest increase in VE - despite the large CI obtained - was observed in HSMV between the time period 0-13 days of first vaccination (2.78 (95% CI -1461.28 to 93.95)) and at least 14 days after first vaccination (64.03 (95% CI -85.99 to 93.05)).

Secondary objective: safety

Regarding safety of the MVA-BN vaccine, the current study observed no more than 18 total adverse reactions, no severe adverse reactions and no AESIs (pericarditis, myocarditis, encephalitis) during the follow up period. Our results were similar to what has previously been reported in completed preclinical and clinical trials of MVA-BN (42,43). Similarly to SEMVAc, in a Canadian prospective safety surveillance study there were no cases of myocarditis reported following 7 or 30 days after MVA-BN vaccination (44). The cumulative incidence for those in the PLWHIV subgroup to experience an AR was slightly higher when compared to the overall Safety cohort (0.35% [95% CI 0.16-0.54] compared to (0.51% [95% CI 0.10-0.92]) with the first dose of MVA-BN. However, these estimates should be interpreted with caution due to the low number of events. Regarding factors that influence the likelihood of an adverse reaction, the likelihood of experiencing an adverse event was extremely low. The likelihood of experiencing and the severity of an adverse reaction decreased in those participants who received the second dose. Additionally, the majority of adverse reactions occurred in participants between 40 and 59 years old, indicating that age may be a relevant factor associated with safety events. The second most frequent occurrence of adverse reactions was in

the age group 30-39 and the mean age of those unvaccinated and crossover participants was approximately 42. Thus, it is also possible that both age and health status influence the likelihood of adverse reaction, potentially more so in those persons immunosuppressed (i.e., HIV+).

Secondary objective: reactogenicity

Reactogenicity was also evaluated during seven days after the first and second administration of the MVA-BN vaccine in the Safety cohort participants, in those participants that responded to the questionnaires. It generally decreased from first to second MVA-BN dose, including mild/moderate discomfort symptoms to, in very rare cases, fever. Reactogenicity was reported similarly across PLWHIV and PrEP user groups.

Most participants experienced discomfort or a localised reaction (70.2%) after the first vaccination, which decreased in frequency after the second vaccination (56.8%). The most common symptom was mild pain at the injection site with pressure or movement (46.7%) after the first dose and reduced to 40.6% after the second dose. Only around 5% of participants reported fever, and in those participants that did report fever, no participants reported fever >40ºC and very few >39ºC. The majority of participants did not report fatigue, myalgia, arthralgia, headache, nausea, or diarrhoea. In those that did, <1.5% across groups and vaccination status reported severe symptoms. Less than 3% of participants reported any severe systemic complaints.

CD4 counts in PLWHIV participants were associated with the likelihood of a local or systemic reaction after the first and second vaccination. Results showed a protective effect with increasing CD4 levels and a lower odds of experiencing a local or systemic reaction, which might be explained by a more robust immune response in participants with higher CD4 counts, improving tolerability. Study participants with CD4 counts of less than 200/µl only exhibited significantly increased odds of systemic reactions after the first vaccination (OR 14.98 [95% CI 1.74-129.16]). In this case the value was associated with a very wide confidence interval (Table 29 and Figure 9 panel B) and consequently a high degree of uncertainty, as the occurrence of mpox in this group was very low (5/6 experienced a systemic reaction).

Use of PrEP did not significantly impact the likelihood of local or systemic reaction after the first or second dose. In those participants with a previous smallpox vaccination, a protective effect against any systemic reaction was observed only after the second vaccination. Possibly, these participants were less likely to experience reactogenicity due to previous smallpox vaccination, given that serum anti-vaccinia virus neutralising antibody responses are detected decades after smallpox vaccination, though the relationship between vaccine reactogenicity and immunogenicity is, in general, unclear (31,45,46). A recent prospective, observational study found that in those with HSMV, reactogenicity increased after the second dose. However, when examining differences specifically among those participants who were not reactive after the first dose versus those who became reactive after two doses, the two groups did not differ by previous smallpox vaccination. Thus, it is likely that reactogenicity is specific to the health of the individual. A randomised, double-blind, placebo-controlled trial of the MVA-BN vaccine in 56- to 80-year-old vaccinia-experienced subjects found that safety and reactogenicity were similar to those seen in younger, healthy participants suggesting that in general, MVA-BN vaccine is well tolerated in healthy participants, across HSMV status and age group (46). Nevertheless, given that the HSMV groups and PLWHIV groups were not mutually exclusive, we cannot be assured that the HSMV subgroup is overall healthier.

The abovementioned results were indicative of a low reactogenicity and good tolerability of the MVA-BN vaccine in the overall MSM population and across study subgroups, with improved tolerability after the second dose. These results were congruent with other clinical trials that

examine tolerability of the smallpox vaccine (47,48).

Secondary objective: sexual behaviour during follow-up

To the best of our knowledge, this is the first prospective examination of MVA-BN vaccinated and unvaccinated participants that repeatedly captures sexual behaviour throughout the follow-up period. Changes in sexual behaviour, such as reduced number of sexual partners prior to receiving the initial vaccine dose, particularly during periods of high transmission rates, were likely to have played a role in decreasing mpox case numbers. This analysis enables a comprehensive assessment of any shifts in behaviour following both first and second MVA-BN vaccinations. A noticeable overall decrease in the count of sexual partners and increase in the frequency of condom usage before vaccination, compared to the period post-vaccination, suggests alterations in behaviour related to vaccination.

Cluster analyses revealed four distinct behavioural patterns in relation to vaccination, and the majority of participants (46.5%) were clustered as low-constant sexual behaviour. Interestingly, a particular subgroup (cluster C “moderate-to-high variable sexual behaviour”) emerged as the primary contributor to the observed changes. For instance, participants in the cluster C exhibited a distinct increase in sexual partners (approx. 4 – 6 partners) after the first dose of MVA-BN. It is important to consider such increases in sexual activity post-vaccination when assessing the broader public health implications of vaccination against infections transmitted by direct contact like mpox.

The sexual behaviour shifts described above likely played a role in mitigating infections, contributing to the observed decline in IRs. Understanding these behavioural changes is crucial for future public health vaccination initiatives targeting sexually transmitted infections in populations with changing sexual partners (e.g., MSM). Given these associations, focusing on the effects of sexual behaviour’s influence on risk of infection with MPXV is key to accurately estimating vaccine efficacy and effectiveness.

9.2. Limitations

The following limitations and their potential impact have been considered when interpreting the study results:

Specificity of the case definition for exposure (MVA-BN vaccination).

Several participants reported their vaccination/s before CED and vaccinations were confirmed by documentation (e.g., vaccine passport, medical records). Those receiving first and second MVA-BN vaccination before CED were part of the descriptive baseline and sexual behaviour assessment in the overall MSM cohort, but not part of the VE or Safety cohorts. The exclusion of those participants has limited the available sample size for the VE and safety objectives. Participants receiving the first vaccination before CED but a second vaccination at or after CED were allowed to enter the VE and safety cohorts for the second vaccine. However, this presents some limitations in that prospective vaccinations outside of participating study centres were permitted (i.e., no indication of location of vaccination on the eCRF). In most cases, vaccinations were administered within the participating study centres and the monitoring of safety and MPXV infections was mandatory for all study centres. Study centre physicians verified any mpox vaccination outside of participating study centres via documentation in the vaccination passport (as is common practice in Germany).

Specificity of the case definition for effectiveness outcomes (mpox)

SEMVAc primary outcome was VE, based on the occurrence of confirmed MPXV infection by a positive PCR result, in vaccinated and unvaccinated participants. Thus, confirmation of the outcome was partially relying on the sensitivity and specificity characteristics of the PCR, which could lead to potential misclassification of the outcomes. This limitation is partially overcome by the clinician-based confirmation based on symptoms, which are very distinctive for mpox. Moreover, reported infections by participants that were not confirmed with PCR by study centres (e.g., only reported via monthly questionnaires) were reported separately from PCR confirmed infections.

Limited number of mpox cases

Estimation of VE was initially challenging given the lack of mpox cases reported in the unvaccinated group of the VE cohort in SEMVAc. Successful recruitment of the first SEMVAC participants began on 7 July 2022, however, several large study centres started later with their recruitment due to regulatory delays in obtaining approvals for different federal regions and cities in Germany. Additionally, around the time of study initiation, the MPXV epidemic curve in Germany and worldwide quickly and unexpectedly started to decline. Consequently, only 14 mpox cases in the vaccinated and no cases in the unvaccinated group were detected by 31 December 2023 (end of SEMVAc study period). The limitations in the number of mpox cases, especially in the unvaccinated group, required the implementation of the TEMVAc analyses to obtain VE estimates. TEMVAc was a retrospective, complementary sub-study conducted within the study centre framework of SEMVAc. TEMVAc successfully overcame the limitation of identifying mpox cases in the unvaccinated group (resulting in the identification of a total of 48 cases in this cohort) and enabled estimation of the planned VE calculations (Risk Ratios, aHRs). TEMVAc implemented a matched rolling cohort design using retrospectively collected data in eligible persons receiving the first MVA-BN vaccination on the same day as controls that were not previously matched into the VE cohort. Therefore, TEMVAc was able to address the limitations of SEMVAc. Given the similarities in characteristics of SEMVAc and TEMVAc populations, VE results from TEMVAc were considered generalisable to the source population, MSM patients of the participating infectious disease clinics across Germany. However, TEMVAc is not without limitations of its own, which are discussed below.

Differences in total follow-up time between the vaccinated and unvaccinated groups

Follow-up time between the vaccinated and unvaccinated groups is notable. Total follow-up time was approximately double in those vaccinated with a second dose when compared to those who received the first dose, and four times more when compared to the unvaccinated group, making the IRs difficult to compare. Multiple factors contribute to these differences in follow-up time. First, risk of MPXV infection at baseline is related to the prioritisation of first dose administration to at-risk groups at the beginning of the outbreak, and to the time frame of the study (variable IR during study period). Secondly, certain aspects of the study design limit the ability to accurately account for these differences in follow-up time and changing risks. Participants in the VE cohort were permitted to enter on a second dose of MVA-BN, thus, follow-up time prior to the first dose is not observable. Therefore, it is important to consider that this heterogeneity can lead to uncertainty in the estimation of VE between 1 versus 2 doses. Variability in follow-up time between 1st dose, 2nd dose and unvaccinated groups contributes to a higher proportion of participants being censored in the group with shorter follow-up time. This is likely the case, given the shortened time between mpox vaccination and occurrence of mpox. Those at higher risk were followed-up for a shorter amount of time due to censoring, while simultaneously, the sample size of unvaccinated was significantly reduced (less follow-up time). Longer follow-up and follow-up that is comparable between groups (unvaccinated, and 1 versus 2 doses of MVA-BN) could be considered to increase study power (49). Lastly, follow-up time is influenced by the nature of the statistical models used: participants with first vaccination were "censored" for follow-up time once they received their second vaccination, there is a lower number of unvaccinated participants in

comparison to vaccinated, and several participants that were unvaccinated at CED received a vaccination during follow-up (crossover), which consequently reduced the follow-up time. It is important to note that results from multistate models (cumulative incidence) and incidence rates differ and cannot be compared directly. While multistate models give probability estimates, incidence rates were an estimator of the hazard. Moreover, crude incidence rates incorporate the mean follow-up time, which is truncated at 13 days in the '0-13 days' stratum after vaccination. This results in a high incidence rate, which is not reflected by the results of cumulative incidences (i.e., the calculation of cumulative incidence does not depend on mean follow-up time, but rather on the number of persons at risk at each event time).

Bias related to TEMVAc

While TEMVAc successfully addressed the limitation of lack of cases in the unvaccinated group in SEMVAc, there are other limitations inherent to the TEMVAc analytic design. In the TEMVAc analytic population there may have been individuals with a higher risk of mpox and who therefore sought vaccination early in the vaccination campaign. This initial early uptake of the MVA-BN vaccine in those with a potentially higher risk (e.g., due to sexual behaviours, physician advice, limited vaccine availability) may have left subjects who were less susceptible to mpox infection to remain as potential controls. The first PCR confirmed reported case of mpox in Germany was on 20 May 2022 and cumulative incidence reached 12.64 per million by 1 July 2024 (50). Relatedly, the overall estimated magnitude of the VE may be affected by the varying risk between vaccinated and unvaccinated groups across follow-up time.

Similarly, differential misclassification bias may exist related to the time restriction for incident HIV diagnoses. Investigators examined VE in those diagnosed with HIV as of the start of the observation period, however, potentially undiagnosed PLWHIV may have entered into the study and remained in the overall cohort. This may lead both to bias away from the null in the overall VE cohort, or towards the null in the PLWHIV subgroup, though the number of undiagnosed HIV patients is most likely to be extremely low during the observation period, because HIV testing is routinely administered in these study centres which are specialised in treatment of HIV and STIs.

Furthermore, use of medical records versus participant reported data may introduce potential information bias related to baseline characteristics and sexual behaviours. Gender identity and specific sexual behaviours cannot be validated by electronic medical records and are related to both the identification of the at-risk population (i.e., MSM) and risk of mpox infection. Nevertheless, subject selection and data entry was performed by study physicians who know their regular patients who were included in TEMVAc. TEMVAc further attempted to account for potential bias by using and matching to proxy variables (i.e., number of STI diagnoses at baseline).

By manually reviewing charts and determining who fitted the inclusion and exclusion criteria, the study centres ensured that the sample of subjects closely approximates the target population at risk. Despite all efforts to mitigate selection into the study by adding all eligible patients of the study centres, some selection bias may exist given that inclusion was based on criteria according to information available in the medical chart. Those with information on inclusion criteria may differ from those who were excluded, leading to some differences between the study population and the target population (i.e., MSM).

Despite the possibility of bias, the large sample size in the TEMVAc analysis, along with the matching algorithm in a rolling cohort, aims to emulate randomisation and achieve a balance in covariables and to minimise bias.

Study enrolment in specific health clinics as a source of selection bias in SEMVAc

Given the enrolment of participants in specific infectiology/sexual health clinics, rather than in general healthcare settings, **selection bias** could be a limiting factor to take into consideration when interpreting SEMVAC results:

- It might lead to a *more limited representation*. Participants who visit ID/sexual health clinics may not be representative of the broader MSM population. They may have different demographics, health behaviours, and risk factors compared to participants who seek healthcare in more general settings. For instance, they might be more proactive about their sexual health or have a higher likelihood of engaging in risky behaviours. However, it is important to note that although these study centres specialise in infectious diseases, their inclusive approach toward the MSM community results in a diverse participant population representing participants with varying sexual behaviours. Additionally, they often function as primary care providers for a significant portion of the MSM community. Therefore, the selection of MSM participants from the multiple study centres is likely to be a representative good sample of the overall MSM population in Germany.
- The MSM community may feel more comfortable seeking care in specialised clinics due to the *sensitive nature of infectiology or sexual health issues*. However, this also means that participants who attend these clinics may be more open about their conditions or behaviours compared to those who visit general healthcare providers. This openness can influence reporting and behaviour in ways that may not reflect the broader MSM population.
- Enrolling participants exclusively from specialised clinics may *exclude participants who lack access to such facilities* due to various barriers, such as geographic location, financial constraints, or social stigma (which is especially important in a country with a high proportion of immigrants such as Germany). Consequently, the study results may not be applicable to these underserved populations.
- Finally, if the enrolment process at these clinics is not random but rather based on certain criteria (e.g., willingness to participate in research), it can *introduce bias into the sample*. This sampling bias can affect the generalizability of study findings.

Potential for residual confounding

Despite efforts to control for confounders through best epidemiological practices, some variables may remain unmeasured or inaccurately measured, leading to residual confounding. This is an inherent characteristic of any observational cohort analysis. Residual confounding can distort the observed associations between exposure and outcome, potentially leading to erroneous conclusions about causality. Residual confounding has been addressed to the extent possible through careful consideration of the study design, rigorous adjustment for measured confounders, sensitivity analyses, and interpretation of results with caution, emphasising the need for complementary evidence from experimental studies to corroborate findings.

9.3. Interpretation

The VE results from SEMVAc suggest a potential reduction in risk of acquiring mpox with vaccination, which was further explored through the TEMVAc analyses. Previous preclinical, clinical, and recent observational studies support that two doses of the MVA-BN vaccine reduce risk of MPXV infection, while 1 dose also conveys significant protection in the context of at-risk populations (13,39,47,48,51).

Overall in TEMVAc,the MVA-BN vaccine demonstrated significant VE in younger MSM and general populations, however, the results for subgroups have increased variability and wide CIs due to fewer cases with mpox, thus further research in different populations is necessary. TEMVAc results contribute to the totality of evidence regarding MVA-BN VE against mpox, in line with the results

from the SEMVAc and USMVAc studies of this EMA supported mpox program. While VE could not be estimated in SEMVAc, USMVAc results showed that cumulative incidence in those vaccinated with two doses of MVA-BN (0.0016 (95% CI 0.00-0.0041)) was lower than those with a single dose (0.0034 (95% CI 0.0014-0.0054)) (17). VE could not be estimated in those with two doses in TEMVAc, however, in a matched cohort of those that received at least one dose and unvaccinated subjects, VE was similar to estimates in the US population studied in USMVAc (89% (95% CI 12%-99%)). Source populations for all three studies had similar frequency of PLWHIV and PrEP users, age range 18-49, and health conditions (i.e., chronic disease, STIs) (17).

A recent, retrospective, observational study in a Spanish population of persons receiving HIV-PrEP (majority aged 30-49) compared 5660 vaccinated and matched unvaccinated participants and found an overall estimated VE of 37.9% (95% CI, −24.4 to 69.1) from at least one dose of MVA-BN (52). Cumulative incidence in the vaccinated population was 3.46 per 1000 persons. Most cases occurred during the first 6 and 13 days of vaccination, resembling the results in SEMVAc. While this study observed cases in the unvaccinated group (not seen in SEMVAc), estimated VE during the first 6 and 13 days showed a non-statistically significant higher risk of MPXV infection in the vaccinated group. As follow-up time increased post-vaccination, a protective effect and increase in VE was observed (79.3% (95% CI, 33.3 to 100.0) at ≥14 days), similar to the decrease in the IR seen in the sensitivity analysis results in SEMVAc. Other studies also considered the temporal occurrence of mpox in relation to vaccination. Bertran et al (39) found the majority of cases (32 of 40) in those vaccinated occurred within 0–13 days after vaccination and estimated VE at least 14 days after a single dose as 78% (95% CI 54 to 89). This UK case-coverage study took place between July 4 and Oct 9, 2022, when a sharp increase in incidence was reported in the UK and included a total of 363 cases, 323 cases in the unvaccinated group. It is therefore likely that the lack of cases in the unvaccinated group in SEMVAc is related to the delay in recruitment of participants in relation to the epidemiological curve in Germany and that when estimating VE for MVA-BN, it is important to consider that the strategy for most countries during the peak of cases, priority was given to those at highest risk of infection. A US case–control study of EHR records in PLWHIV and PrEP users, wherein 89.2% of the participants identified as men, defined partial vaccination as the receipt of one dose plus 14 days (51). VE was estimated after adjustment for age, race or ethnic group, SVI score, and the presence or absence of immunocompromising conditions, VE was 35.8% (95% CI, 22.1 to 47.1) for partial vaccination and 66.0% (95% CI, 47.4 to 78.1) for full vaccination. However, it is notable that more case participants than control participants were immunocompromised and had lower CD4 cell counts (<200 per cubic millimetre), meaning these VE estimates were specific to an immunocompromised study population. Studies examining VE related to the 2022-2023 outbreak confirm that those at highest risk for mpox were MSM with changing sexual partners; however, selection of high-risk groups such as those targeted for SEMVAc enrolment can bias VE results in the context of generalizability.

SEMVAc demonstrates high tolerability and safety of the MVA-BN vaccination in populations with potential immunocompromised status (e.g., HIV+) and generally healthy participants aged ≥18. Safety outcomes identified as potential risks for the MVA-BN vaccine (pericarditis, myocarditis, and encephalitis) at the time of the initial EU Risk Management Plan approval were not observed during follow-up. Although there were no confirmed cases of myocarditis or pericarditis in completed clinical trials for IMVANEX, smallpox vaccines were associated with a rare risk of myocarditis and pericarditis among healthy adult vaccines (573 per 100,000 primary vaccines)(38). As in other previous studies, CD4 count in PLWHIV was associated with the likelihood of a local or systemic reaction after the first and second vaccination, showing a protective effect with increasing CD4 levels and a lower odds of experiencing a local or systemic reaction. Age of participants was associated with the odds of experiencing any local reaction and decreased with every 10-year increase in age; this effect was not seen after the second dose.

These results should be confirmed in future studies with a larger sample size.

While cumulative incidence and IRs of mpox observed in the vaccinated group were coherent with other studies, the absence of reported cases in unvaccinated participants did not allow for the estimation of risk ratios and VE. However, as part of the complementary approach of the EMA to obtain VE and safety information, SEMVAc results contribute to recent findings from USMVAc (17). As in SEMVAc, mpox cases were overall few, with only one and 12 cases in the fully vaccinated and unvaccinated groups, respectively. VE results showed that full vaccination (2 doses after ≥14 days) was associated with a decreased risk of mpox disease that corresponded to adjusted VE of 89% (95% CI 12%, 99%). Given the nature of secondary data in USMVAc, the lack of gender identity and sexual behaviour was a limitation for which SEMVAC provided a greater insight on this aspect. Initially, to adjust for confounding and ensure comparability across treatment groups, propensity score matching was planned. Due to a lack of cases in the unvaccinated group, matching was not feasible via SEMVAc alone. TEMVAc was able to overcome these limitations, by obtaining data corresponding to the epidemiological peak and estimated a similar VE of 62.21 (95% CI 35.98 to 77.69) and 61.78 (95% CI 32.02 to 78.51) in the sensitivity analysis, resembling recent findings that compare VE across studies (38). The complementary approach of these three studies has provided data contributing to the overall body of evidence on the effectiveness and safety of MVA-BN vaccination.

9.4. Generalisability

The generalisability of study results conducted solely within one country is often subject to limitations. While such studies provide valuable insights into the specific context and population of that country, in this case Germany, their applicability to other regions or populations may be uncertain. Thus, the present results may be partially generalisable to the adult, MSM European population, and more so to central European countries. To enhance the generalisability of findings, conducting multi-country studies that include younger age groups (i.e., paediatrics) and those of female gender assigned at birth, or replicating studies in different cultural and socio-economic contexts is essential.

Furthermore, participants who visited ID/sexual health clinics during the study period, fulfilled the inclusion/exclusion criteria and consented to participate in the study may not be fully representative of the broader MSM population. They may have different demographics, health behaviours, and risk factors compared to participants who seek healthcare in more general settings. It is possible that the current study's population might be more proactive about their sexual health or have a higher likelihood of engaging in sexual behaviours that include changing partners.

10. Other information

None

11. Conclusion

Results from this prospective, multi-centre, real-world study conducted in Germany complement the results of the USMVAc study and suggest that completing the second dose according to the MVA-BN vaccine schedule is associated with a lower estimated cumulative incidence of mpox disease among MSM within the study period. However, mpox incidence in Germany also declined during the study observation period and coincided with the administration of second vaccinations, which must be considered when interpreting the current results. Given the limitations when estimating VE in SEMVAc, a sub-study, TEMVAc, collected retrospective data in unvaccinated subjects to supplement the sample and when possible, complement the SEMVAc data to facilitate

the estimation of VE. VE in TEMVAc overall MSM population (54.15 (95% CI 21.09 to 73.36)) after 1 dose of MVA BN) was comparable to that of USMVAc (64% (95% CI: 40%, 78%) among those with any dose of MVA BN, but 89% (95% CI: 12%, 99%) among those fully vaccinated with 2 doses of MVA BN), with the advantage of a larger sample size and mpox events in TEMVAc, leading to more precise VE estimates.

Overall, the vaccine was well tolerated and results from SEMVAc supported a favourable safety profile. A low number of adverse reactions, and no serious adverse reactions were reported. AESIs such as pericarditis, myocarditis, or encephalitis events were not reported. Reactogenicity generally decreased from first to second MVA-BN dose, including mild/moderate discomfort symptoms to, in very rare cases, fever, and was similarly observed across PLWHIV and PrEP user groups.

Changes in sexual behaviour, such as reduced number of sexual partners prior to receiving the initial vaccine dose, particularly during periods of high transmission rates, were likely to have played a role in decreasing mpox cases. It is important to consider the observed increases in sexual activity post-vaccination when assessing the broader public health implications of vaccination against infections transmitted via direct contact.

SEMVAc represents a key European study in the context of the mpox public health threat and complemented with TEMVAc and USMVAc will provide crucial evidence on the benefit/risk of mpox vaccination, as well as the trends in sexual behaviour of the MSM population during the deployment of the mpox vaccination programme, particularly in Germany. Altogether, SEMVAc/TEMVAc/USMVAc will inform regulatory decisions and support future mpox outbreak preparedness and response.

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Appendices

Annex 1. List of stand-alone documents

Number	Document reference number	Date	Title
1	*SEMVAc_SAP_V3.0*	*15 February 2024*	*Statistical Analysis Plan for the Safety and Effectiveness of MVA-BN vaccination against MPXV infection in at-risk individuals in Germany (SEMVAc) study*
2	*TEMVAc_SAP_V3.0*	*30 May 2024*	*Statistical Analysis Plan - Emulated Target trial for Effectiveness of MVA-BN Vaccination against MPXV infection in at-risk individuals (TEMVAc)*
3	*TEMVAc_target_trial_protocol*	*29 May 2024*	*Emulated Target trial for Effectiveness of MVA-BN Vaccination against Mpox infection in at-risk individuals (TEMVAc)*

Annex 2. Supplementary Material (see attached)