OFFICIAL TITLE: Morbidity, Mortality And Risk Factors of Mpox in HIV negative high risk Sexual health clinic attenders and People Living With HIV -MASH-1 Study-

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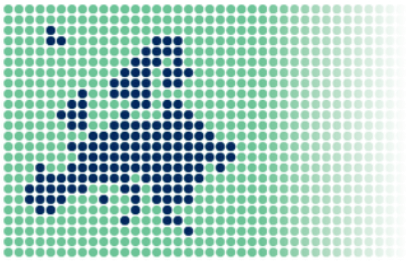

neatid

The European treatment network for HIV, hepatitis and global infectious diseases

Morbidity, Mortality And Risk Factors of Mpox in HIV negative high risk Sexual health clinic attenders and People Living With HIV
- MASH-1 Study-

Version:	3.0 dated 01 NOV 2023
Sponsor Protocol ID:	606
SPONSOR:	NEAT ID Foundation

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Compliance Statement:

This study will be conducted in compliance with the protocol, the ethical principles for medical research as specified by the Declaration of Helsinki, the International Society for Pharmacoepidemiology (ISPE) guidelines of Good Pharmacoepidemiology Practice (GPP), the GDPR and other applicable country legislation.

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SPONSOR AND CHIEF INVESTIGATOR SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the ISPE guidelines of Good Pharmacoepidemiology Practice (GPP) and Heads of Medicines Agencies (HMA), the GDPR and other applicable Data Protection legislation, the Sponsor's SOPs, and other regulatory requirements as applicable or as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the study sponsor:

Signature:	Date:
Name: (please print):	

Chief Investigator:

Signature:	Date:
Name: (please print):	

STATISTICIAN OR STUDY ANALYST SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Statistician or Study Analyst agrees to conduct the study in compliance with the approved protocol, Statistical Principles for Clinical Trials, ICH E10 and will adhere to the principles outlined in the ISPE guidelines of Good Pharmacoepidemiology Practice (GPP) and Heads of Medicines Agencies (HMA), the GDPR and other applicable Data Protection legislation, the Sponsor's SOPs and other regulatory requirements as applicable or as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Statistician or Study Analyst:

Signature:	Date:
Name: (please print):	

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

I agree to conduct the study in accordance with principles outlined in the ISPE guidelines of Good Pharmacoepidemiology Practice (GPP) and Heads of Medicines Agencies (HMA), the GDPR and other applicable Data Protection legislation, the applicable regulatory requirements and with the approved protocol.

I agree to comply with the procedures for data recording/reporting.

I agree to permit monitoring, auditing and inspection at this site and to retain all study related essential documentation for the duration of the study as necessary.

Principal Investigator:

Signature:	Date:
Name: (please print):	

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KEY STUDY CONTACTS

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FUNDER

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https://verdiproject.org/

COLLABORATORS/KEY CONTRIBUTORS

VERDI consortium partners.
VERDI project is coordinated by:
Fondazione Penta ETS
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STUDY SYNOPSIS

Full study Title:	Morbidity, Mortality And Risk Factors of Mpox in HIV negative high-risk Sexual health clinic attenders and People Living With HIV - MASH Study	
Short title/Acronym	MASH	
Clinical Phase	Observational Retrospective Cohort Study	
Study Design	Case-descriptive study	
Study Population	PLWHIV (people living with HIV) who are currently sexually active and HIV negative high-risk Sexual Health Clinic attenders (PrEP users) with Mpox	
End of study data collection	Date of clinical resolution e.g. lesion resolution, hospital discharge or death (up to 3 months)	
Planned Sample Size	Approximately 2000	
Eligibility Criteria	at least 18 years of age with either documented HIV-1 infection or PrEP clinic attenders, and confirmed MPX infection by documented result on PCR testing on skin and/or mucosal and/or any anatomical site.	
Indications	HIV & MPX; PrEP patients & MPX	
Methodology	Only real-world, routinely collected, retrospective data will be used for this study collected from patients who during the study period have had confirmed Mpox. Pseudonymised data will be transcribed into the eCRF (electronic Case Report Form) system from source data at each clinical site or from existing databases, where appropriate. No personal identifiable data will be transmitted to NEAT ID.	
Number of sites	Up to 12 sites in up to 7 countries	
Objectives	Primary 1. To describe and compare outcomes of MPX in PLWHIV and an HIV-negative population of people using pre-exposure HIV prophylaxis (PrEP users) 2. To identify risk factors for specific MPX outcomes (including CD4 cell count nadir and current CD4 count in PLWHIV)	Secondary 1. To estimate the approximate prevalence of MPX in PLWHIV and PrEP users in this retrospective cohort 2. To describe clinical manifestation of MPX and response to MPX treatment in PLWHIV and PrEP users 3. To describe the co-morbidities in PLWHIV and PrEP users

Outcome Measures	Primary 1. Severity of lesions, clinical complications, percentage of hospitalisations, death. 2. Differences in the clinical manifestation of MPX in PLWHIV and PrEP users 3. Differences in the clinical manifestation of MPX in PLWHIV by CD4 and VL	Secondary • Number of MPX patients attending as a percentage of total number of patients the sites have seen over a set period of time (first Mpox patient to last Mpox patient at site) • time to lesion resolution (if known) • Length of stay in hospital • Differences in the clinical manifestation of MPX in PLWHIV and PrEP users by co-morbidities.

LIST OF ABBREVIATIONS

Acronym	Description
ACCI	Age-adjusted Charlson Comorbidity Index
ART	Antiretroviral Therapy/Treatment
ARV	Antiretroviral
CCI	Charlson Comorbidity Index
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
EC	European Commission
ECMO	Extracorporeal Membrane Oxygenation
eCRF	Electronic Case Report Form
EMEA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
GDPR	General Data Protection Regulation
GDocP	Good Documentation Practice
GPP	Good Pharmacoepidemiology Practice
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.
ISF	Investigator Site File
ISPE	International Society for Pharmacoepidemiology
MS	Member State
MPX	MPox (previously known as Monkeypox)
MSM	Men who have sexual activity with men
PI	Principal Investigator
PLWHIV	People living with HIV
PrEP	Pre-exposure prophylaxis
PrEPHRA	HIV negative, high risk sexual health clinic attenders
R&D	Research & Development
REC	Research Ethics Committee
SOP	Standard Operating Procedure
TMF	Trial Master File
VFD(s)	Ventilator-Free Day(s)
WHO	World Health Organisation

1 BACKGROUND

Mpox (or MPX) (previously known as Monkeypox) is an acute febrile rash illness caused by the mpox virus, an orthopox virus related to variola virus (causative agent of smallpox), cowpox virus and the vaccine strain vaccinia virus.

Since the first identification of a mpox infection in humans in 1970, the majority of human cases have been reported from Africa, including sporadic outbreaks that usually resulted from contact with wildlife reservoirs, in particular rodents. Animal-to-human (zoonotic) transmission can result from direct contact with the blood, bodily fluids, or cutaneous or mucosal lesions of infected animals. There are two distinct genetic clades of the mpox virus: the central African or Congo Basin clade (Clade 1) and the west African clade (Clade 2). The case fatality ratio of mpox has historically ranged from 0 to 11 % in the general population and has been higher among young children. In recent times, the case fatality ratio has been around 3–6%. The central African (Congo Basin) clade has historically caused more disease and was thought to be more transmissible. (WHO fact sheet) Human-to-human transmission can result from close contact with respiratory secretions, skin lesions of an infected person or recently contaminated objects. Transmission via droplet respiratory particles usually requires prolonged face-to-face contact. (WHO fact sheet) Cross-immunity exists between different orthopox viruses, and, in the past, vaccination against smallpox was protective against mpox infection. However, the global smallpox vaccination campaigns were discontinued after eradication of the disease, likely leaving the population below 40 to 50 years of age (depending on the country) more susceptible to mpox.

Mpox is usually a self-limited disease with the symptoms lasting from 2 to 4 weeks. Severe cases occur more commonly among children and are related to the extent of virus exposure, patient health status and nature of complications. Underlying immune deficiencies may lead to worse outcomes. Complications of mpox can include secondary infections, bronchopneumonia, sepsis, encephalitis, and infection of the cornea with ensuing loss of vision.

Until recently, there have been very few travel-associated cases in non-African countries, with very limited onward transmission. However, following notification from the United Kingdom to WHO on the 15th of May 2022, of two confirmed and one probable case of mpox, person-to-person transmission of mpox has been increasingly observed in countries outside of Africa. By August 5th, 2022, 28,220 cases of mpox infection have been reported in 88 locations worldwide, 27,875 of whom in countries that have not historically reported mpox. The largest numbers of cases have been reported in Europe. (Joint ECDC-WHO Regional Office for Europe Mpox Surveillance Bulletin (europa.eu)) The viruses associated with these cases are most closely related to clade 2 but have some genetic differences and has been tentatively named Clade 3.

An early report indicates that patients in the epidemic outside Africa may present with a clinical picture that differs from the usual presentation. 95% of patients had a rash, 73% had anogenital lesions and 41% had mucosal lesions. 13% of the patients were hospitalized. Reasons for hospitalization included pain management (mostly severe anorectal pain), soft-tissue superinfection, pharyngitis limiting oral intake, eye lesions, acute kidney injury, myocarditis and/or infection-control purposes. In this report, 98% were Men who have sexual activity with men (MSM) and 41% had Human Immunodeficiency Virus (HIV) infection – these groups are currently considered at a higher epidemiological risk to contract the infection. (Thornhill e.a. NEJM 2022)

A vaccine campaign with vaccinia virus (smallpox vaccine) has been started for specific risk groups; this may impact the transmission of mpox virus, but clinical evidence is still lacking and available dosages are limited. Furthermore, it is feared that the epidemic may extend outside of the currently defined risk groups.

Currently, antiviral medication for mpox that has been properly evaluated in clinical trials is not available, but several antivirals have demonstrated activity in vitro and in animal models. The most promising is tecovirimat, an oral anti-viral drug that inhibits the cytopathic effect of mpox virus on cell cultures and reduces plaque formation. (Smith, AAC 2009) Furthermore, tecovirimat has been found to protect non-human primates, when administered at three days post challenge with lethal doses of mpox virus. (Jordan *et al*. AAC 2009).

2 RATIONALE

We have few data to suggest whether or not People Living with HIV (PLWHIV) are at an increased risk of serious illness or death or there is an impact of being HIV positive on outcomes or clinical presentation or if there is any dependence on CD4 cell count and viral load.

The most affected countries in Europe include Spain, the United Kingdom, Germany, France and the Netherlands. The majority of the cases identified to date have been among men who have sexual activity with men (MSM). There has been a preponderance of genital, perianal and mucosal lesions, rather than the centrifugal rash classically described for mpox. An early report indicates that 95% of patients had a rash, 73% had anogenital lesions and 41% had mucosal lesions. Some of the lesions persist for a long period and some patients have required hospitalisation for pain management or treatment of secondary skin infections. 13% of the patients were hospitalized. Reasons for hospitalization included pain management (mostly severe anorectal pain), soft-tissue superinfection, pharyngitis limiting oral intake, eye lesions, acute kidney injury, myocarditis and/or infection-control purposes. In this report, 98% were MSM and 41% had HIV infection. (Thornhill e.a. NEJM 2022)

Rapid data collection and analysis about MPX in PLWHIV will depend on international multi-centre collaborations, for which NEAT ID has a strong expertise. The majority of PLWHIV in Europe are in routine clinical care, allowing for a comprehensive data collection about MPX and studying the impact of HIV drugs and specific risk factors. The NEAT ID MPX Coinfection Dashboard was established in August 2022 to collect data on the number of PLWHIV co-infected with MPX and anonymysed, aggregated data from this dashboard on 249 PLWHIV who attended Chelsea and Westminster Hospital between May 15th and December 15th 2022 were recently presented at the CROI 2023 conference (Girometti *et al*, CROI 2023). It has provided evidence that a significant number of PLWHIV were suffering from MPX in Europe at that time and that these individuals can be enrolled into an Epidemiology and Real-World- Data study.

A feasibility study of HIV negative patients receiving Pre-exposure prophylaxis (PrEP) across the NEAT ID network has identified 45 sites that provide PrEP services for a total of 27,416 PrEP users with 1,361 new PrEP initiators each month.

There are two central questions to address:

1. Do PLWHIV have a more severe disease course and outcomes from MPX as compared to MPX in PrEP user?

2. Are there particular factors, (e.g. CD4 T-cell nadir, current CD4 T-cell count, co-morbidities) within the group of PLWHIV, which put them at risk for a more severe MPX disease course?

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

It is hypothesised that HIV infection could be a risk factor for more severe MPX outcomes and that advanced immunodeficiency as defined by current CD4 T-cell count or CD4 T-cell nadir is a risk factor for more severe MPX outcomes in PLWHIV.

3.1 Primary objective

1. To describe and compare outcomes of MPX in People Living with HIV (PLWHIV) and a HIV-negative population of people using pre-exposure HIV prophylaxis (PrEP users)
2. To identify risk factors for specific MPX outcomes (including CD4 cell count nadir and current CD4 count in PLWHIV)

3.2 Secondary objectives

1. To estimate the approximate prevalence of MPX in PLWHIV and PrEP users in this retrospective cohort
2. To describe clinical manifestation of MPX and response to MPX treatment in PLWHIV and PrEP users
3. To describe the co-morbidities in PLWHIV and PrEP users

3.3 Primary endpoint/outcome

- Severity of lesions, clinical complications, percentage of hospitalizations*, death.
- Differences in the clinical manifestation of MPX in PLWHIV and PrEP users
- Differences in the clinical manifestation of MPX in PLWHIV by CD4 and VL.

3.4 Secondary endpoints/outcomes

- Number of MPX patients attending as a percentage of total number of patients the sites have seen over a set period of time (from first Mpox patient to last Mpox patient**)
- Length of stay in hospital*** for inpatients treated for MPX.
- Time to lesion resolution (if known)
- Differences in the clinical manifestation of MPX in PLWHIV and PrEP users by co-morbidities.

*Hospitalisations for clinical reasons only (i.e. not for precautionary measures or quarantine).
** This is regardless of if these first and last patients were included in the collected data
***in case of multiple hospitalisations, this will be the sum of the length of all stays.

4 STUDY DESIGN

This is an observational retrospective cohort study.

In total, approximately 2000 cases from PLHIV and PrEP users will be analysed

Only real-world retrospective, routine data will be used for this study. Deidentified data will be transcribed into the Electronic Case Report Form (eCRF) system from source data at each clinical site or from existing cohort databases, where appropriate.

Case data will be collected until the Date of clinical resolution e.g. lesion resolution, hospital discharge or death (up to 3 months)

5 ELIGIBILITY CRITERIA

The study population will consist of adult PLWHIV and PrEP users who have had confirmed MPX and are followed up at the study sites for routine clinical care.
Cases (PLWHIV + PrEP users) will be at least 18 years of age, with confirmed MPX infection by documented, positive result on PCR testing of lesions from 1st May 2022 to 1st December 2023.

5.1 Inclusion Criteria

- Diagnosis of MPX was more than 90 days prior to data collection
- Confirmed MPX infection by documented PCR testing on skin and/or mucosal and/or any anatomical site between 1st May 2022 to 1st December 2023
- At least 18 years of age
- Cases (PLWHIV + MPX)
    - Documented HIV-1 infection
- Cases (PrEP users + MPX)
    - Attended a clinic to receive PrEP or self-procured PrEP at time of Mpox diagnosis.

5.2 Exclusion Criteria

- MPX diagnosed based on clinical criteria only
- MPX diagnosis was within the last 90 days

6 STUDY PROCEDURES

6.1 Participant Identification

Most People living with HIV (PLWHIV) and HIV negative PrEP users will be identified from sites in the NEAT ID network. PLWHIV are routinely followed-up at specialised outpatient clinics with regular follow up visits as standard of care and many of these centres take care of patients on PrEP. Patients are taking PrEP to prevent contraction of HIV as they perceive themselves to be at risk due to frequent sexual activity with different partners.

In July 2022, NEAT ID opened a dashboard to 150 sites from 20 countries to provide basic information about the number of PLWHIV with MPX and disease outcomes at their centres (https://www.NEAT-ID.org/). Data were provided from patients' cases from April 2022 until November 2022 from 31 global sites, 26 sites from 16 European countries and 5 site in Latin America. The dashboard provided a first estimate about the total numbers of PLWHIV with MPX and where these patients are in continued care. As of the end of November 2022, 907 PLWHIV with MPX were reported in care, with 53 hospitalisations and 1 death from an estimated number of 85,279 PLWHIV in routine care at these sites (Girometti *et al*, EACS 2023). These case numbers strongly suggested feasibility to recruit sufficient numbers of PLWHIV with MPX and that patients have been identified at their centres already.

A feasibility study of patients receiving PrEP across the NEAT ID network have identified 45 sites that provide PrEP services for a total of 27,416 PrEP users with 1,361 new PrEP initiators each month.

6.2 Data collection

Only real-world retrospective data will be used for this study. Patients who have had mpox and fulfil the entry criteria will be enrolled after 90 days from date of diagnosis to allow for follow up data to be collected. Pseudonymised retrospective data will be transcribed into the eCRF system from source data at each clinical site or from existing cohort databases, where appropriate. No personal identifiable data will be transmitted to NEAT ID. The data elements will be transcribed from source data according to the data collection list below. Mechanisms to ensure data quality and integrity will be deployed as per applicable standard operating procedures and in line with Good Documentation Practice (GDocP) and Good Pharmacoepidemiology Practice (GPP). Patients will be identified and documented by participating sites.

For all patients with MPX the following information will be collected where routinely available:

- Age (year of birth)
- Sex and gender identity
- Ethnicity (where available)
- HIV status
- HIV pre-exposure prophylaxis (PrEP) use
- Comorbidities and Medical history (diabetes mellitus, other immunosuppression (e.g. solid organ transplant), known chronic kidney or liver disease, mental health conditions)
- Recent sexual history (3 months) – number of recent partners
- Recent and/or concomitant sexually transmitted infections
- Orthopoxvirus vaccination history (if available)
- Symptom onset date (fever onset, lesion onset, rectal pain)

- Date of positive PCR test result (documented)
- MPX severity -Reported clinical signs and symptoms of mpox (e.g. rash, mouth lesions, rectal pain, breathlessness)
- Time to resolution of lesions, if known
- Mortality as a consequence of MPX including cause
- Hospitalisation for MPX and length of hospitalisation
- Drug treatment for MPX (Tecovirimat, other)
- Drug for complications associated with MPX (laxatives, antibiotics, analgesia)
- recent travel to/living in endemic country or country with outbreak
- contact with suspected, probable, or confirmed case (if known)
- contact with infected animal (if known)

For PLWHIV the following additional information will be collected:

- Date of HIV diagnosis
- Current Antiretroviral Therapy/Treatment (ART)
- If not on ART, reason for not being on ART
- Last CD4 cell count (including CD4% and CD4/CD8 ratio) and
- Last HIV-RNA before MPX diagnosis

Skin lesion at peak severity score:

- Not presenting with skin lesions (0 skin lesions)
- Mild (<25 lesions)
- Moderate (25-99 skin lesions)
- Severe (100-250 skin lesions)
- Very severe (>250 skin lesions)

Site of lesion will also be collected as part of the severity assessment:

- Genital lesions (defined as involvement of either vulva or vaginal mucosa or penis, pubic area)
- Ano-rectal/perianal lesions
- Oral mucosa (lips/gums/oral/pharynx) lesions
- Face
- Trunk (chest/torso/abdomen/back)
- Limbs (arms/forearms/legs/hands/feet)

Complications which will be collected are as follows:

- Severe rectal and/or perianal pain (i.e. due to perianal/anal abscess, proctitis)
- Tonsillitis and/or dysphagia
- Secondary bacterial infection on affected skin
- Urological complications (genital oedema, urinary retention)
- Ocular involvement (conjunctivitis, corneal involvement, periorbital cellulitis)
- Central nervous system involvement (encephalitis, meningitis, focal neurology signs)
- Pneumonia/pulmonary abscess or necrotizing involvement
- Myocarditis
- Diarrhoea

The definition of a mental health condition will be that reported by the patient and recognized in the ICD-10 classification.

In the UK, severe disease is defined as:

- Adults with severe clinical illness (e.g. National Early Warning Score [NEWS] 2 score of ≥5), which may include significant lower respiratory symptoms, confusion/encephalitis, and other complications (e.g. secondary bacterial infection, sepsis)
- Widely disseminated lesions and very many in number (≥100)
- Suspected infection of the cornea
- Severe, refractory pain from lesions requiring hospitalisation to achieve symptomatic control
- Lesions associated with complications due to pain or swelling (e.g., constipation, urinary retention, inability to swallow)
- Confirmed infection, regardless of severity, in immunocompromised people, pregnant women, or children aged ≤16 years.

7 STATISTICS AND DATA ANALYSIS

7.1 Planned recruitment rate

We believe that approximately 12 sites from 7 countries globally could participate. Therefore, we estimate that an average of 170 patients would need to be recruited per site to reach the total number of subjects required. Recruitment however will be competitive with no upper limit on the number of patients per site.
The patients should be entered into the eCRF in chronological order (i.e. first Mpox patient who was diagnosed with MPX and who meets the criteria entered first etc.)

7.2 Statistical analysis plan

This is primarily a descriptive retrospective data cohort study but will compare MPX severity between 1) PLWHIV and PrEP users, to show whether HIV infection is associated with more severe MPX; 2) PLWHIV with different characteristics, to identify factors associated with severe MPX within HIV infected population.
All enrolled participants who meet the eligibility criteria will be included in the analysis population (full analysis population). All primary and secondary endpoints will be performed with the full analysis population.

7.3 Summary of baseline data and flow of patients

All continuous variables will be summarised using the following descriptive statistics: n (non-missing sample size), mean, standard deviation (SD), 1st quartile, median, 3rd quartile, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical variables. The number of patients and the flowchart of the study will be presented.

7.4 Primary outcome analysis

The first primary outcome analysis will be to describe outcomes of MPX in PLWHIV and in PrEP users in terms of severity of lesions, time to lesion resolution, hospitalization, death.
The rate of serious lesions will be estimated by dividing the number of participants with serious lesions by the total number of participants in the full analysis population. Unadjusted and adjusted odds ratios (ORs) will be calculated using the logistic regression model to assess the impact of HIV on the occurrence of serious lesions compared to PrEP users.
Because some participants' lesion may not be healed by the end of the study period and, therefore, the duration of resolution would not be known, and thus right-censored, survival analysis methods such as the Kaplan-Meier and Cox model will be used to assess the time to lesion resolution. Kaplan-Meier estimates will be used to estimate the median time to lesion resolution. Unadjusted and adjusted hazard ratios (HR) will be estimated using the Cox proportional hazard model to assess the impact of HIV-infection on the time to lesion resolution compared with PrEP users.
The hospitalization rate will be estimated by dividing the number of participants hospitalized for mpox infection by the total number of participants in the total analysis population. Unadjusted and adjusted odds ratios (ORs) will be calculated using the logistic regression model to compare hospitalization rates between PLWHIV and PrEP users.
The mortality rate will be estimated using the Kaplan-Meier Method. Unadjusted and adjusted hazard ratios (HR) will be calculated using the Cox proportional hazard model to compare the rates of mortality between PLWHIV and PrEP users.
Adjustment variables will include the following potential confounders: Age (<30 vs 30-40 vs >40 years); Sex at birth (female vs male); Comorbidities; multiple sexual partners (yes vs no); number of sexual partners in the last 3 months prior to mpox diagnosis; and smallpox vaccination history (yes vs no).

The second primary outcome analysis will be performed between PLWHIV with MPX versus PrEP users with MPX. The endpoint is to assess differences in clinical manifestations of MPX between the groups. Unadjusted and adjusted odds ratios (ORs) will be calculated using the logistic regression models to compare the rates of clinical manifestations between PLWHIH and PrEP users. The adjusted variables will be the following (potential confounders): Age (<30 vs 30-40 vs >40 years); Sex at birth (female vs male); Comorbidities; multiple sexual partners (yes vs no); number of sexual partners in the last 3 months prior to MPX diagnosis; and smallpox vaccination history (yes vs no).
The third primary outcome analysis will be performed within the PLWHIV group. The endpoint is to evaluate the characteristics of HIV infection associated with severe mpox. Univariable and multivariable logistic regression models will be used to assess factors associated with severe mpox in PLWHIV. The following variables will be evaluated: time since HIV diagnosis (years); current ART; CDC disease stage; CD4 cell nadir; last CD4 cell count/HIV-RNA before MPX diagnosis (including CD4% and CD4/CD8 ratio). Variables achieving P <0.20 in the univariable analysis will be retained for the multivariable analysis.

7.5 Secondary outcome analysis

All secondary outcome analysis will be performed on PLWHIV with MPX compared to PrEP users with MPX.
The prevalence of MPX per site during the study period will be estimated by the number of MPX patients attending the site divided by the total number of patients the sites saw during the study period. The 95%

Confidence Interval of the prevalence will be calculated with the exact Clopper-Pearson method. Fisher exact test will be used to compare the prevalence of MPX in HIV-infected individuals to that of PrEP users.

The length of stay in hospital will be estimated using competing risk models with death as a competing event. Gray's test will be used to compare the duration of hospitalisation between PLWHIV and PrEP users. Unadjusted and adjusted sub-distribution hazard ratios (sHR) will be calculated using competing risk models with death as a competing event. The duration of hospitalization will be defined by the time (days) from hospitalization to hospital discharge or last observation. Adjustment variables will include: Age (<30 vs 30-40 vs >40 years); Sex at birth (female vs male); Comorbidities; multiple sexual partners (yes vs no); number of sexual partners in the last 3 months prior to mpox diagnosis; and Orthopoxvirus vaccination history (yes vs no).
Subgroup analyses for clinical manifestation in MPX will be conducted to assess the difference between PLWHIV and PrEP users across comorbidities. The following comorbidities will be assessed: diabetes mellitus, immunosuppression (e.g. solid organ transplant), chronic kidney or liver disease, mental health illness. Adjusted odds ratio will be calculated to compare PLWHIV and PrEP users within subgroup using logistic regression models. Adjustment variables will include: Age (<30 vs 30-40 vs >40 years); Sex at birth (female vs male); multiple sexual partners (yes vs no); number of sexual partners in the last 3 months prior to mpox diagnosis; and smallpox vaccination history (yes vs no).
Heterogeneity in the impact of HIV infection on clinical manifestations based on comorbidities will be assessed by including terms for interactions between patient status (PLWH and PrEP users) and the comorbidity variable in multivariable logistic regression models. All variables described above for adjustments will be included in the multivariable models.

7.6 Subject population

All eligible subjects will be included in the analysis population (full population analysis).

7.7 Procedure(s) to account for missing or spurious data

Multiple imputation using Chained Equations approach (MICE) will be used to fill in missing data. Ten imputations (M=10) will be chosen to obtain valid inference and reduce sampling variability resulting from the imputation process. Variables with a missing rate above 20% will be excluded and the outcomes will be included in the imputation model. All 10 datasets will be analyzed and combined using Rubin rules. A complete case analysis will also be performed as a sensitivity analysis to assess the robustness of the results.

8 Consent

Due to the nature of this data collection study, consent will not be sought.
In order to maintain confidentiality, the participant will be identified only by subject number. Participant data will be collected from electronic health records by appropriately trained and authorised member(s) of the study team who must be identified and authorised in writing by the Principal Investigator (PI). A delegation of responsibility log will be updated accordingly.
As data collection is entirely retrospective, participants will not need to attend any additional visits.
Participants will not have undergone any procedures above their routine standard of care.

9 DATA HANDLING

9.1 Dissemination of results, authorship eligibility guidelines and any intended use of professional writers

Data from the study will be published independently by PENTA and NEAT ID. All contributing PI investigators will have the opportunity to participate in publication (abstracts and manuscripts) on the analyses.

This study will be reported in line with the STROBE Statement for reporting observational studies. Details of this study and the results will be available on a freely accessible clinical trial registry (ISRCTN, clinicaltrials.gov or equivalent) and will be presented/disseminated at appropriate conferences.

9.2 Data Protection

All data collected during the trial will be processed in agreement with the requirements set forth in applicable national data protection laws and in compliance with the General Data Protection Regulation (Regulation EU 2016/679) (GDPR), the UK Data Protection Act 2018 (DPA 2018), and the UK GDPR. All investigators and investigator site staff will comply with the data protection requirements applicable to the country in which they are conducting study-related activities.

The security of personal data collected in the study will be upheld using the following technical and organisational measures:

- Participant data will be pseudo-anonymised by replacing the information that identifies the participant with a unique identifier (pseudonym) at the point of enrolment. Datasets sent to data processors and third parties authorised by the Sponsor to process personal data will no longer be attributable to a specific participant without the use of additional information.
- The link between the unique identifier and the participant's identity will be securely maintained at the investigator site, accessible by authorised personnel only. The link may be made available to regulatory agencies for the purposes of audits or inspections, or to study monitors authorised by the Sponsor to monitor the trial.
- It is the responsibility of the investigator to protect the identity of the participant, and the security of personal data held at site should be maintained per local technical and organisational measures.
- Study data will be stored in a secure electronic data capture system (EDC) overseen by the Contract Research Organisation (CRO). The EDC system is protected by high levels of physical and cyber security. The EDC meets the requirements for the storage of personal data. The data will be stored on a secure / backed up network accessed by authorised personnel only. A log of authorised personnel with access permissions is maintained centrally.
- Only sufficiently trained and delegated personnel will have access to the EDC.
- Data will continue to be processed in a pseudonymised format until there is no longer a requirement for data subject identification. At this point, the data will be anonymised for further use.
- Data collection will be kept to the minimum required to answer the objectives of the study.

In the event of a data security breach, the investigator or site staff will report the incident to the Data Protection Officer of the Sponsor (dpo@neat-id.org). The severity of the risk will be assessed to determine reporting requirements per relevant data protection legislation. It remains the responsibility of the Sponsor to report applicable data breaches to the relevant Data Protection Authority(ies). Depending on the result of this assessment, the participant(s) to whom the breach relates may be informed. A Corrective and Preventative Action review may also be performed to reduce the risk of future data security breaches.

9.3 Data Retention

Patient data may be retained for up to two (2) years after the last publication of the results of research or, in the absence of publication, until the signing of the final research report.

10 ETHICAL AND REGULATORY CONSIDERATIONS

10.1 Research Ethics Committee (REC) review & reports

- Before the start of the study, approval will be sought from applicable bodies for the trial protocol and other relevant documents.
- Where applicable a notification will be submitted to a Competent Authority in accordance with the country regulations
- Substantial amendments that require review by relevant authorities will not be implemented until there is a favourable opinion for the study (note that amendments may also need to be reviewed and accepted by the Competent Authority and/or local Research & Development (R&D) departments before they can be implemented in practice at sites)
- All correspondence with respect to submissions and approvals will be retained in the Trial Master File/Investigator Site File
- An annual progress report (APR) will be submitted to the authorities within 30 days of the end of the reporting period, and annually until the study is declared ended
- It is the Chief Investigator's responsibility to produce the annual reports as required.
- The Chief Investigator will notify the authorities of the end of the study
- If the study is ended prematurely, the Chief Investigator will notify the REC(s), including the reasons for the premature termination
- Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC(s)

10.2 Peer review

This protocol has undergone peer review as per NEAT ID's standard peer review process. Members of the VERDI consortium have also reviewed late-stage drafts of the protocol.

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APPENDICIES

12 APPENDIX 1 – Comorbidity Variables & Definitions

VARIABLE	DEFINITION
Myocardial Infarction (heart attack)	History of definite or probable MI (EKG changes and/or enzyme changes)
Congestive Heart Failure	Exertional or paroxysmal nocturnal dyspnoea and has responded to digitalis, diuretics, or afterload reducing agents
Peripheral Vascular Disease	Intermittent claudication or past bypass for chronic arterial insufficiency, history of gangrene or acute arterial insufficiency, or untreated thoracic or abdominal aneurysm (≥6 cm)
Cerebrovascular Accident (stroke) or Transient Ischemic Attack	History of a cerebrovascular accident with minor or no residua and transient ischemic attacks
Dementia	Chronic cognitive deficit
Chronic Obstructive Pulmonary Disease	-
History of Pneumonia	-
Connective Tissue Disease • Rheumatologic Disease • Any Other	-
Peptic Ulcer Disease	Any history of treatment for peptic ulcer disease or history of peptic ulcer bleeding
Liver Disease • Mild • Moderate • Severe	Mild = chronic hepatitis (or cirrhosis without portal hypertension) Moderate = cirrhosis and portal hypertension but no variceal bleeding history Severe = cirrhosis and portal hypertension with variceal bleeding history
Diabetes • None or Diet-Controlled • Uncomplicated • With Complications	-
Hemiplegia	-
Paralysis of Arm(s) or Leg(s)	-
Chronic Kidney Disease • Moderate	Moderate = creatinine >3 mg/dL (0.27 mmol/L) Severe = on dialysis, status post kidney transplant, uraemia

• Severe	
Current or History of Cancer • Localised • Metastatic	-
Leukaemia	-
Lymphoma	-
AIDS	-

13 APPENDIX 2 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
N/A	1.0	15th June 2023	Anton Pozniak, Deborah Roberts. Nicolo Girometti	Initial version
1.0	2.0	20 Sept 2023	Deborah Roberts, Nicolo Girometti	The inclusion criteria were widened slightly to include participants who were diagnosed with PCR test not only on lesions. Also, to those who received PrEP from other sources than the clinical site.
2	3.0	01 November 2023	Deborah Roberts, Nicolo Girometti	The end of data collection time point was added: Date of clinical resolution e.g. lesion resolution, hospital discharge or death (up to 3 months)

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