| ICECREAM Interventions to curb hepatitis C reinfections among MSM | INTERVENTIONS TO CURB HEPATITIS C REINFECTIONS AMONG MEN WHO HAVE SEX WITH MEN | Version (EN) | |
|---|---|---|---|
| **STATISTICAL ANALYSIS PLAN** | | 1.0 | 01/05/2023 |

# ICECREAM TRIAL
## *Interventions to curb hepatitis C reinfections among men who have sex with men*

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## CONTENTS

## ABBREVIATIONS

| | |
|---|---|
| CeGIDD | Les Centres gratuits d'information, de dépistage et de diagnostic |
| CI | Confidence Interval |
| CSH | Centre of Sexual Health |
| DBS | Dried Blood Spots |
| HCV | Hepatitis C Virus |
| HIV | Human Immunodefiency Virus |
| ICECREAM | Intervention to Curb hEpatitis C REinfections among Men who have sex with men |
| ITT | Intention To Treat |
| M | Month |
| MOSAIC | Men who have sex with men Observational Study of Acute Infection with hepatitis C |
| MSM | Men who have Sex with Men |
| OR | Odds ratio |
| PP | Per Protocol |
| PrEP | Pre-Exposure Prophylaxis |
| RNA | Ribonucleic Acid |
| RT | Randomised trial |
| STI | Sexually Transmitted Infection |

## DEFINITIONS

The statistical analysis plan is a document that contains a more detailed and technical description of the main aspects of the analysis outlined in the protocol, and defines the terms of the statistical analysis conducted for primary and secondary endpoints as well as for other data.

## SCOPE

This document applies to the main statistical analysis of the ICECREAM trial.

# 1. TRIAL SYNOPSIS

## 1.1. TRIAL OBJECTIVES

### 1.1.1. Primary objective

The primary objective is to investigate whether a behavioural and testing intervention, alone or in combination, has an effect on self-reported behaviours associated with hepatitis C virus (HCV) acquisition in men who have sex with men (MSM) with cured or spontaneously cleared HCV infection.

### 1.1.2. Secondary objectives

Secondary objectives are to evaluate the effect of a behavioural intervention and testing intervention, alone or in combination, on:

- HCV reinfection incidence
- Other HCV-related behaviours
- Sexually transmitted infection (STI) incidence
- Sexual wellbeing

## 1.2. TRIAL DESIGN

### 1.2.1. Study design and methods

This study is an international, multi-centre, phase 2, 3-arm, randomized trial (RT) comparing run-in and intervention periods to evaluate the effect of an online behavioural intervention, a home-based sampling for testing intervention, or both on self-reported behaviours associated with HCV acquisition. The first 6 months of the trial includes follow-up with no intervention (termed the "run-in" period) during which participants receive standard care. At month (M) 6, participants are randomly assigned, with an allocation ratio of 1:1:1, to one of the following three arms: (1) a targeted, online behavioural intervention; (2) a participant-initiated, home-based HCV-RNA self-sampling test service using dried blood spots (DBS); (3) consists of a combination of intervention I and II. Participants continue the intervention for 18 months (termed the "intervention" period) in addition to standard care. During both the run-in and intervention periods, five online questionnaires measuring risk behaviour over the past 6 months are administered (i.e., at M0, M6, M12, M18 and M24 of the study).

## 1.3. RANDOMISATION

Randomisation takes place via an online, centralized system. Lists of randomly permuted blocks assigning six interventions (i.e., two from each of the three arms) are generated. Research staff do not have access to the randomisation sequence nor do they know the arms randomly assigned to individuals before a given participant is recruited.

## 1.4. SAMPLE SIZE

We aim to have a minimum of 78 individuals per arm (total of 234) who complete at least one study questionnaire during the intervention period. Assuming a maximum drop-out rate of 5% during the run-in period, 246 participants in total are needed to be enrolled. We simulated power under varying proportions at risk of HCV infection during the run-in period and absolute risk reduction during the intervention. With a sample size of 78 in each arm, we would have 80% power to demonstrate a statistically significant difference, at a type 1 error of 0.05, of a >22% reduction in the primary endpoint from a 60% proportion at risk of HCV infection during the run-in period.

# 2. STATISTICAL ASPECTS

## 2.1. ANALYTICAL STRATEGIES AND STUDY POPULATIONS

Inclusion in the analysis may be discussed for participants who meet, in particular, one of the following conditions:

- Participants who have never received one of the two interventions;
- Participants who withdrew their consent;
- Participants who were wrongly enrolled due to major eligibility criterion(a) not being met

The decision to exclude a participant from the analysis is determined by the Scientific Committee, blinded to intervention group and to participant follow-up after enrolment or randomisation. Research staff analysing the endpoints will remain blinded to allocation to the intervention arm until all analyses are completed.

### 2.1.1. Primary endpoints

- **Definition**

The primary endpoint is the proportion at risk of HCV infection, as determined by the previously developed and validated HCV-MOSAIC risk score [1], which is compared between the run-in and intervention periods, within each arm.

- **Strategy for the main analysis**

Analyses are performed:

- By intent-to-treat (ITT): including all observations and analysed according to the intervention to which the participants were originally assigned, regardless of which intervention they received. This analysis assumes that any individual who was lost to follow-up did not achieve the primary endpoint (i.e., are at risk of HCV infection). Participants who are mistakenly included due to major eligibility criterion(a) not being met are removed from the analysis.
- By per protocol (PP): including all available observations and analysed according to the intervention to which the participants were originally assigned. This analysis excludes all observations after an individual has been lost to follow-up. Participants who are mistakenly included due to major eligibility criterion(a) not being met are removed from the analysis as well as participants who did not receive the intervention to which they were originally assigned.

- **Sensitivity analysis**

The following sensitivity analyses will be performed;

- Including only MSM who used the assigned intervention(s) (both ITT and PP).
- Including only MSM who were at risk of HCV infection at randomization, as determined by the HCV-MOSAIC score (both ITT and PP).
- Correction for issues in adherence and differential loss to follow-up (only PP): identifying pre-randomisation and post-randomisation covariates that predict adherence to the intervention. Depending on the mechanism of inadequate adherence and loss to follow-up, adjustment for confounding by adherence, g-methods or both will be entertained [2].

### 2.1.2. Secondary endpoints

- **Definition**

Secondary endpoints are as follows:

- Incidence rate of HCV reinfections during both the run-in (i.e., M0 and M6) and intervention period (i.e., M12, M18, M24), both self-reported and laboratory-confirmed, defined as the number of cases divided by the total person-years of follow-up at risk for reinfection
- Incidence rate of any STI during the run-in and intervention period, both self-reported and laboratory-confirmed, defined as the number of chlamydia, gonorrhoea, genital herpes and/or syphilis infections divided by the total person-years of follow-up
- Other HCV-related risk behaviour during run-in versus intervention period, more specifically:
    - Changes in the mean or median number of sex partners (depending on the distribution of the variable of interest)
    - Changes in the mean or median number of condomless anal sex acts with casual partners (depending on the distribution of the variable of interest)
    - Changes in the proportion of participants reporting any individual behaviours that are included in the HCV-MOSAIC risk score
    - Changes in mean or median sexual wellbeing score (depending on the distribution of the variable of interest)
- The proportion of participants achieving an HCV-MOSAIC risk score ≥2.0 at the end of follow-up compared between intervention arms (in both ITT and PP populations)

## 2.2. STATISTICAL METHODS

### 2.2.1. Descriptive analysis

Descriptive statistical analyses are performed to describe study population characteristics at inclusion, intervention-related outcomes at M12 (i.e., 6 months after randomisation) and at the end of follow-up (e.g., use of services, acceptability and usability) and the number of tests, home-based or otherwise, performed during the intervention period.

- Descriptive analysis is performed overall and per intervention group
- Frequencies of missing data are described for each variable
- Quantitative variables are described in terms of frequency, average, standard deviation, median, minimum, maximum and interquartile range. They can also be presented in class (e.g., age groups) and described in the form of categorical variables, based on medians and quartiles
- Additional descriptive analyses not mentioned in this document might be added to the statistical report

### 2.2.2. Comparative analysis

The primary endpoint is compared between the run-in period (i.e., at M0, M6) and at each time point during the intervention period (i.e., at M12, M18, M24) using a mixed-effect logistic regression model. In this model, each individual serves as their own control and between-individual differences at baseline are accounted for using a random-intercept. Odd ratios (OR) comparing the odds of run-in versus intervention time points, along with their 95% confidence intervals (CI), are estimated and stratified on study arm. These stratified analysis are performed by including an interaction term between arm and study time point (along with their individual covariate) in the model, while study time points are statistically tested within strata of the study arms. Comparisons between run-in and intervention time points are systematically conducted as follows:

- Unadjusted;
- Adjusted on the randomisation strata (country and, if sufficient numbers of individuals in each intervention arm are available, inclusion centre [3]);
- Adjusted on covariates that could potentially affect the evaluation of the primary outcome

Incidence rates of HCV reinfections and STIs are calculated during the run-in and interventions periods, separately. Considering that few HCV reinfections are likely to occur,

we intend to analyse the relative difference in incidence rates between periods using a Bayesian exponential survival regression model with non to weakly informative *a priori* distributions [4, 5]. The relative difference in incidence rates of STIs are compared between periods using a Poisson regression model with ln of person years of observation as an offset. These models are run on both the overall study population and stratified by treatment arm (using the same model configuration of fixed-effect covariates as in the primary endpoint).

Changes are described for HCV related risk behaviour and sexual wellbeing (see paragraphs 3.5.3 and 3.5.4) during run-in (i.e,, M0 and M6) versus intervention period (i.e., M12, M18, M24). We use logistic (for binary outcomes) and linear (for continuous outcomes) regression models, corrected for repeated measurements within individuals using a random-intercept for participant. The relative changes in each endpoint are compared between run-in and intervention periods. These models are run on both the overall study population and stratified by treatment arm (using the same model configuration of fixed-effect covariates as in the primary endpoint).

## 2.3. CALCULATION CONVENTIONS

### 2.3.1. Convention for defining follow-up time

- Follow-up during the run-period is defined as the amount of participation time for each participant enrolled from moment of inclusion (following sign inform consent) until the moment of randomisation
- Follow-up during the intervention period is defined as the amount of participation time for each participant enrolled from moment of randomisation until last study visit

### 2.3.2. Convention for time calculations

- Time between two dates in months or years is estimated as the number of months between two dates by subtracting the later date to the earlier date
- Calculation of age in years at enrolment = $\frac{date\ of\ enrollment - date\ of\ birth}{365.25}$
- Date of HCV reinfection is estimated using the mid-date between the last HCV-RNA negative and first HCV-RNA positive test result
- Calculated time at risk for HCV risk is defined as the amount of participation time for each participant enrolled from moment of inclusion until estimated date of HCV reinfection or the last recorded HCV-RNA negative test date

- STI period prevalence is estimated using the proportion of participants that have a positive STI test result (self-reported) at any study visit during the past 6 months

### 2.3.3. Convention for HCV-MOSAIC risk score calculations

The HCV-MOSAIC risk score consists of six self-reported risk behaviours and is calculated by summing the beta coefficients of the following factors, when present:

- Condom less receptive anal intercourse (β = 1.1)
- Sharing of sex toys (β = 1.2)
- Unprotected fisting (β = 0.9)
- Injecting drug use (β = 1.4)
- Sharing of straws during nasally-administered drug use (β = 1.0)
- Having an ulcerative STI (β = 1.4)

A score of 2.0 or higher indicates that the person is at risk of HCV infection.

### 2.3.4. Other calculation conventions

- Categorical variables:
  - Age in years: <35, 35-44, ≥45
  - Ethnicity: Dutch, French, Other
  - Educational level: low, middle, high
  - HIV status: with HIV, without HIV
  - PrEP use: no PrEP use, daily prep use, event-driven prep use

## 2.4. SOFTWARE

Analyses are performed using STATA Intercooled software (version 15.0 or higher) and R software (version 4.0.0 or higher) if it is impossible to perform certain analysis or produce figures with STATA.

# 3. ANALYSIS PLAN

## 3.1. DESCRIPTION OF ENROLMENT AND FOLLOW-UP

Treating physicians and nurses at the study centres propose the study to individuals meeting study inclusion criteria. Only until verbal and written informed consent is obtained are individuals enrolled in the study. Only one site visit is required at the start of the study (i.e., to sign informed consent), all further study procedures take place online or at home. Participants are invited to complete the study questionnaire every six months by email (five in total).

### 3.1.1. Flowchart

According to the CONSORT diagram [6]:

- Participants assessed for eligibility (n)
- Participants assessed for eligibility and not enrolled (n, %) – the denominator of the proportion is the number of participants assessed for eligibility
- Participants enrolled (n, %) – the denominator of the proportion is the number of participants assessed for eligibility
- Participants having completed the baseline study visit (n, %) – the denominator of the proportion is the number of participants enrolled
- Participants reaching M6 and were randomised (n, %) – the denominator of the proportion is the number of participants enrolled
- Participants having completed all study visits (n, %) – the denominator of the proportion is the number of participants randomized
- Withdrawals (n, %) – the denominator of the proportion is the number of participants enrolled

### 3.1.2. Description of pre-enrolments

- Participants assessed for eligibility in total, per country and per centre (n)
- Participants assessed for eligibility and non-enrolled in total, per country and per centre (n, %) – the denominator of the proportion is the number of participants assessed for eligibility

### 3.1.3. Description of enrolment

- Participants enrolled in total, per country and per centre (n, %) – the denominator of the proportion is the number of participants assessed for eligibility
- Enrolment graph (i.e., the number of participants enrolled from first until last enrolment)
- Monthly enrolment graph (i.e., the number of participants enrolled per month)

### 3.1.4. Description of follow-up

- Expected visits corresponding to the number of participants enrolled (n)
- Visits actually conducted over the number of expected visits (n, %) – the denominator of the proportion is the number of expected visits corresponding to the number of participants enrolled

### 3.1.5. Early trial discontinuation

- Participant withdrawals (after enrolment) (n, %) – the denominator of the proportion is the number of participants enrolled
- Reasons for withdrawal (after enrolment) (n, %) – the denominator of the proportion is the number of withdrawals after enrolment
- Last visit before discontinuation (n, %) – the denominator of the proportion is the number of withdrawals after enrolment
- Time between enrolment date and date of early discontinuation during the run-in period (in months) for those who withdrew
- Time between randomisation date and date of early trial discontinuation (in months) for those who withdrew

### 3.1.6. Description of the population

- Participants to include in the ITT/PP analysis (n, %) – the denominator of the proportion is the number of participants enrolled
- Participants to exclude from the ITT/PP analysis (n, %) – the denominator of the proportion is the number of participants enrolled
- Reasons for exclusion from the ITT/PP analysis (n, %) – the denominator of the proportion is the number of exclusions from the analysis

## 3.2. DESCRIPTION OF DEVIATIONS TO THE PROTOCOL

### 3.2.1. Eligibility criteria assessment

- Participants with unmet eligibility criteria (n, %) – the denominator of the proportion is the number of participants enrolled
- Unmet eligibility criterion per participant

Verification of eligibility criteria is performed by the treating physician and nurses of participating centres prior to participant enrolment using data collected in the medical file. All eligibility criteria (inclusion and exclusion) are listed in Table 1 below.

**Table 1. Eligibility criteria assessed by the treating physician and nurses prior to participant enrolment**

| Inclusion criteria | Exclusion criteria |
|---|---|
| 1. ≥18 years of age | 1. Acute or chronic HCV infection |
| 2. Previously cured or spontaneously cleared HCV infection (i.e., positive HCV RNA test and/or positive anti-HCV antibody in the past with currently negative HCV RNA | 2. Participation in another study offering an HCV testing and/or an intervention targeting behaviours associated with risk of acquiring |
| 3. Self-reported MSM | 3. Receiving HCV treatment |
| 4. Attending care at an human immunodeficiency virus (HIV) treatment centre (for participants with HIV) or a Centre for Sexual Health (CSH) or Centres gratuits d'information, de dépistage et de diagnostic (CeGIDD) (for participants without HIV) | 4. Being under legal guardianship (for participants in France) |
| 5. Sufficient understanding of Dutch or English (for participants in the Netherlands) or French (for participants in France) | 5. Not able or incapable to provide informed consent |
| 6. Accept to be contacted by telephone | 6. Suspected non-compliance with study procedures |

| | |
|---|---|
| 7. Have health coverage within the national healthcare system (for participants in France)<br>8. Have access to internet and an e-mail messaging service | 7. Individuals who are investigators or otherwise dependent persons are also not included in the study |

## 3.3. PARTICIPANT CHARACTERISTICS

The following characteristics are described:

- Epidemiological characteristics at enrolment (M0)
- Clinical characteristics at enrolment (M0) and during follow-up (M6 to M24)
- Behavioural characteristics at enrolment (M0) and during follow-up (M6 to M24)

### 3.3.1. Epidemiological characteristics at enrolment (M0)

- Socio-demographic characteristics
  - Age (in years and in age groups)
  - Gender identity
  - Ethnicity
  - Educational level

### 3.3.2. Clinical characteristics at enrolment (M0) and during follow-up (M6 to M24)

- HCV-related clinical characteristics
  - Previous HCV infections (in counts)
  - Previous HCV treatment (yes/no)
  - HCV genotype
  - Time since first anti-HCV positive test (in years)
- HIV or pre-exposure prophylaxis (PrEP)-related clinical characteristics
  - HIV-status (positive or negative)
  - PrEP-use (yes/no)
  - Type of PrEP regimen (daily or event-driven)
- STI testing and testing outcomes (positive or negative), including chlamydia, gonorrhoea, genital herpes and/or syphilis (self-reported)

#### 3.3.3. Behavioural characteristics at enrolment (M0) and during follow-up (M6 to M24)

- Sexual relationships and sexual behaviour
- Alcohol and drug use
- Influence of COVID-19 measures on sexual behaviour
- Influence of mpox on sexual behaviour

#### 3.3.4. Intervention-related endpoints during follow-up (M12 to M24)

- Testing intervention-related endpoints
  - Including number of free HCV tests used (n, %) – the denominator of the proportion is the number HCV tests distributed.
  - Number of HCV positive test results (n, %) – the denominator of the proportion is the number of HCV tests distributed.
- Behavioural intervention-related endpoints, including website statistics (e.g. frequency of use, time spent on the intervention and the proportion of participants completing all modules of the intervention)
- Type of goals set in the behavioural intervention
- Usability and acceptability of testing and behavioural interventions

### 3.4. PRIMARY ENDPOINT

#### 3.4.1. Main analysis

- **Definition**

  The proportion of participants achieving an HCV-MOSAIC risk score ≥2.0 compared between the run-in (i.e., M0 to M6) and intervention periods (i.e., M12 to M24).
  ORs are calculated along with their 95%CI in the ITT and PP population as described in paragraph 2.1.1.

### 3.5. SECONDARY ENDPOINTS

#### 3.5.1. Incidence rate of HCV reinfections

- **Definition**

  See paragraph 2.1.2.

Number of cases divided by the total person-years of follow-up at risk for HCV reinfection is calculated.

- **Handling of missing data**
  Analysis is performed on all available data.

### 3.5.2. Incidence rate of STI (chlamydia, gonorrhoea, genital herpes and/or syphilis

- **Definition**
  See paragraph 2.1.2.
  Number of chlamydia, gonorrhoea, genital herpes and/or syphilis infections divided by the total person-years of follow-up is calculated.
- **Handling of missing data**
  Analysis is performed in available data.

### 3.5.3. Changes in HCV-related risk behaviour

- **Definition**
  See paragraph 2.1.2.
  Number of sex partners and condom less anal sex acts with casual partners are summarized using means with standard deviations or medians and interquartile range, depending on the distribution of the variable of interest. Counts and percentages for the individual risk behavioural items included in the HCV-MOSAIC risk score are also calculated. Differences in mean continuous outcomes with 95%CI are calculated using linear or Poisson regression coefficients (depending on distribution of outcome measure). Differences in odds of categorical outcomes (i.e., ORs) with 95% CI are calculated using logistic regression. A random-intercept for participant is added to each of the models described in this section.

### 3.5.4. Changes in sexual wellbeing

- **Definition**
  Sexual wellbeing is assessed using three questions, which refer to pleasure and fulfilment from someone's sex life, using a 7-point Likert scale. Responses to these questions will be averaged, thereby summarizing into one continuous score.
  Means and standard deviations or medians and interquartile range during run-in and interventions periods are described, depending on the distribution of the variable of

interest. Differences in mean continuous outcomes with 95%CI are calculated using linear or Poisson regression coefficients).

#### 3.5.5. Differences in HCV-MOSAIC risk scores between arms

- **Definition**

  The proportion of participants achieving an HCV-MOSAIC risk score ≥2.0 at the end of follow-up, compared between intervention arms.

  ORs comparing arms and 95%CI are calculated.

## 3.6. APPENDICES

### 3.6.1. Description of enrolment and follow-up

- Listing of the participants with early trial discontinuation
- Listing of participants excluded from the analysis
- Listing of participants deviating from the protocol

## 4. REFERENCES

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