# Statistical analysis plan (SAP)

***Section 1: Administrative information***

**1a Trial**

Comparison intramuscular versus intranasal naloxone for reversal of opioid toxicity – the IMvIN study

**1b Trial registration numbers**

P23-055
EUCT: 2023-505338-93-00
ISRCTN: 21068708

**2 SAP version**

Version 1.0 Date: 01-11-2023

**3. Protocol version**

This document has been compiled in accordance with the study protocol version 2.0, which was published on August 1, 2023.

**4.a. SAP revisions – revision history, with justification and timing**

| Protocol version | Updated SAP version no | Section number changed | Description and reason for change | Date changed |
|---|---|---|---|---|
| NA | NA | NA | NA | NA |

**5. Names, affiliation, and roles of SAP contributors**

M. van Lemmen, investigator and author SAP

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**6. Roles and Responsibility – signatures**

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c. Signature Principal investigator

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***Section 2: Introduction***

**7. Background and rationale**

Opioid-induced respiratory depression (OIRD) is a serious complication associated with opioid treatment or opioid abuse. Administering the opioid receptor antagonist naloxone is the current treatment.. Nevertheless, there is still a significant amount of unresolved information regarding the most effective method of medication administration to guarantee the rapid and complete reversal of opioid-induced respiratory depression (OIRD). In this study we will compare the effectivity of two naloxone formulations in reversing fentanyl-induced respiratory depression in both healthy volunteers and chronic opioid users.

## 8. Objectives

*Research hypothesis*

The null hypothesis posits that there is no difference in the number of administrations of intramuscular naloxone 5 mg (ZIMHI system) and intranasal naloxone 4mg (Narcan) needed to achieve full reversal of fentanyl-induced apnea in healthy volunteers.

*Study objectives*

The primary objective is to determine the number of administrations of intramuscular naloxone (ZIMHI system) and intranasal naloxone (Narcan) on complete reversal of fentanyl-induced respiratory depression in healthy volunteers.

Secondary objectives are:
(1) Identify the number of administrations necessary for of either intramuscular naloxone (using the ZIMHI system) and intranasal naloxone (Narcan) on complete reversal of fentanyl-induced respiratory depression in chronic opioid users;

(2) To compare the time in minutes to full reversal of ventilation (defined as achieving at least 80% of baseline ventilation) following administration of intramuscular naloxone (ZIMHI) versus intranasal naloxone (Narcan);

(3) To compare the pharmacokinetic parameters of naloxone, including Cmax, Tmax, area under the curve (AUC), and half-life ($t_1/_2$), following intramuscular (IM, 5 mg) and intranasal (IN, 4 mg) administration.

Exploratory objective

(1) To evaluate the incidence and severity of muscle rigidity following fentanyl or naloxone administration, as assessed by muscle stiffness (N/m).

*Section 3: Trial Methods*

## 9. Trial design

The trial has a randomized open label design. Each subject will visit the laboratory twice, with a 7-10 day interval between visits. (the order of IM and IN administration will be randomized).

### 10. Randomisation

Randomization list (1:1) will be generated in R by an independent member of the Anesthesiology department The treatment is not blinded.

### 11. Sample size

There are no data on either IN or IM naloxone dose requirements for full reversal of ventilation. We did perform a sample size calculation in GraphPad PRISM and obtained a power > 90% assuming 12 subjects with a median of 1.5 administrations for the IM formulation and 2.5 administration for the IN administration (i.e., a median delta of 1). The chronic opioid user population is added for comparative reasons and should be considered a pilot for future studies. The number of chronic opioid users is arbitrary as this population is included as a pilot set for hypothesis generation and sample determination for future studies.

### 12. Framework

The IMvIN trial protocol states the objective is "The number IM and IN administrations needed to restore ventilation to baseline levels in healthy volunteers". Therefore, both the primary and secondary outcomes are tested for equivalence.

## 13. Statistical Interim analyses and stopping guidance

*13.a. Information on Interim analyses specifying what interim analyses will be carried out and listing of time points*

Interim analyses are not planned

*13.b. Any planned adjustment of the significance level due to interim analysis*

NA

*13c: Details of guidelines for stopping a trial early*

NA

### 14. Timing of final analysis

The primary outcome, the first secondary outcome, the second secondary outcome, and the exploratory outcome will be reported first. After this report, a second report will be made with the pharmacokinetic analysis (third secondary outcome).

**15. Timing of outcome assessments**

The schedule of study procedures is described in paragraph 10.3 of the study protocol. In short the study consists of two visits separated by at least 7 days to ensure no carryover takes place. The primary outcome is the number of administrations needed of IM vs IN to reverse opioid induced respiratory depression in healthy volunteers.

***Section 4: Statistical Principles***

**16. Level of statistical significance**

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level.

**17. Description of any planned adjustment for multiplicity, and if so, including how the type 1 error is to be controlled**

Holm's sequential Bonferroni procedure will be used to adjust for multiple testing.

**18. Confidence intervals (CI) to be reported**

All confidence intervals will be 95% and two sided.

**19. Adherence and protocol deviations**

*19.a. definition of adherence to the intervention and how this is assessed including extent of exposure.*

Compliance is not assessed as both drugs are administered under supervision of study personnel.

19.b. Description of how adherence to the intervention will be presented

NA

*19.c. Definition of protocol deviation for the trial*

The following are pre-defined major protocol violations with a direct bearing on the primary outcome:

1) Any active medical condition, organ disease or concurrent medication or treatment that may either compromise subject safety or interfere with study endpoints
2) Currently receiving medication-assisted treatment for the treatment of opioid-use disorder

*19.d. Description of which protocol deviations will be summarised*

. Protocol deviation are classified prior to database lock. The number (and percentage) of patients with major and minor protocol deviations will be summarized by treatment group with details of type of deviation provided. No formal statistical testing will be undertaken.

20 Analysis populations

The per-protocol analysis includes only the complete cases. Statistical analysis will be conducted on the data from healthy volunteers. The opioid user group is exploratory, and therefore no statistical analysis will be performed on their data.

***Section 5: Trial Population***

**21. Screening Data**

The following summaries will be presented for all screened subjects:
Enrolment: the number of days recruiting, the number of subjects screened, the number of subjects recruited, the number of screened subjects not recruited, and the reason for non-recruitment. This summary will be provided.

22. Eligibility

The number of patients who are ineligible for randomization, if any, will be reported, along with the reasons for their ineligibility.

23. Recruitment

The "CONSORT" diagram comprising the number of people screened, eligible, consented, randomised, receiving their allocated treatment, withdrawal/lost to follow-up.

**24 Withdrawal/Follow-up – level of withdrawal**

*24.a. Level of withdrawal e.g. from intervention and/or from follow up*

The extent of consent withdrawal will be tabulated (classified as "consent to continue follow-up and data collection" "consent to continue data collection only", "complete – no further follow-up or data collection".

*24.b. Timing of withdrawal/lost to follow up data*

This will be presented in CONSORT diagram format rather than as a table, with withdrawal causes and quantities and/or exclusion from analysis.

*24.c. Reasons and details of how withdrawal/lost to follow up data will be presented*

The numbers (with reasons) of losses to follow-up over the course of the trial will be summarized by treatment arm.

**25 Baseline patient characteristics**

*25a: List of baseline characteristics to be summarized*

Participant age, sex at birth, opioid use (oral morphine equivalents), both overall and separately for the healthy volunteers and chronic opioid users.

*25b: Details of how baseline characteristics will be descriptively summarized*

Categorical data will be summarized by numbers and percentages. Continuous data will be summarized by mean, SD and range if data are normally distributed and median, IQR and range if data are non-normally distributed. Minimum and maximum values will also be presented for continuous data. statistical tests will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

***Section 6 Analysis***

**26. Outcome definitions**

*26.a. Specification of outcomes and timings.*

Primary endpoint is the number of administrations of intramuscular versus the number of administrations in intranasal naloxone in the population of healthy volunteers.

*26.b. Specific measurement and units*

The number of administrations, the number of doses required of intramuscular or intranasal naloxone.

*26c: Any calculation or transformation used to derive the outcome*

NA

27. Analysis methods

The primary endpoint is the number of administrations of IM vs IN naloxone in healthy volunteers and will be compared using non-parametric test (Wilcoxon signed rank test) in R with $p < 0.05$ considered statistically significant.

**Secondary endpoints:**

(1) the number of administrations of IM vs IN naloxone in chronic opioid users will be compared using non-parametric test (Wilcoxon signed rank test) in R.

(2) The time in minutes to full reversal of ventilation (as defined as achieving at least 80% of baseline ventilation) following administration of IM versus IN naloxone will be compared using non-parametric test (Wilcoxon signed rank test) in R.

(3) The pharmacokinetics parameters (including Cmax, Tmax, area under the curve (AUC), and half-life ($t_{1/2}$)) of naloxone (IM or IN) and fentanyl will be analyzed by descriptive analysis.

(3) Effect of fentanyl and naloxone (IM or IN) on muscle tone will be analyzed by descriptive analysis.

Finally, we will perform a PK/PD analysis of the interaction of naloxone and fentanyl using the administration mode as covariate. The analysis shall focus on (1) naloxone bioavailability, (2) delay in response to naloxone, and (3) change in minute ventilation. The PK/PD model shall incorporate $CO_2$ kinetics as well as a model of the respiratory controller. See Ref. Hellinga et al. 2023.

Citations

1. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/