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Development of non-injectable naloxone for pre-hospital reversal of opioid overdoses:

A Norwegian project and a review of international status

Master´s thesis in Master of Science in Pharmacy

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Abstract

Background and aims: Per year, overdoses kill 69.000 users of illicit and prescription opioids in epidemic pattern worldwide. Among them, 250 people in Norway. Naloxone is an effective antidote for opioid overdose reversal, but approved pharmaceuticals have been limited to invasive administrations. Lay people access to naloxone is initiated to facilitate bystander rescue, but limitations associated with invasive administration constitute a desire for non-injectable formulations. The thesis deals with two separate issues: A) Contribution to recruitment, screening and conduction of a pivotal clinical trial aiming to support marketing authorization for an intranasal naloxone spray. B) Contribution to a systematic review paper on non-peer reviewed patent registrations of non-injectable naloxone formulations.

Method: A) Central elements of good clinical practice were dealt with through developing documents needed for recruitment and inclusion to the clinical trial. B) Patents on non-injectable naloxone formulations were identified through the WIPO PatentScope database. Information on pharmacokinetics and formulations (including stability data) were extracted and analysed. Peer-reviewed literature was reviewed based on a PubMed search using the Boolean search query "(nasal OR intranasal OR nose OR buccal OR sublingual) AND naloxone AND pharmacokinetics".

Results: A) An Information letter with an integrated informed consent form, blood sample storage records, an information flyer and a case report form were developed and used during recruitment and at screening in October and November 2015. 17 subjects were screened, whereof 11 were eligible. 6 subjects were re-screened and 9 new subjects were screened at March 2016, whereof 12 subjects were included. B) 522 WIPO patents and 56 PubMed records were identified, whereof 3 patents and 5 papers were eligible. Pharmacokinetic data for intranasal and sublingual routes were identified and collated. Sublingual bioavailability was F=1%. For concentrated intranasal formulations, bioavailability relative to intravenous and intramuscular were in the range of F=21-42% and F_{IM}=26-57%, and for non-concentrated intranasal naloxone F=11% and F_{IM}=10%, respectively. Intranasal bioavailability is associated positively with dose and negatively with volume.

In summary: A) Taking part in the preparation of a clinical trial on pharmaceuticals will enhance the understanding of good clinical practice, general research and medical ethics principles. B) It is possible to obtain valuable scientific knowledge in the field of development of non-injectable naloxone outside the peer-reviewed literature through a systematic review of registered patents.

Abbreviations

AE	Adverse event(s)
ALAT	Alanine aminotransferase
AR	Adverse reaction
ASAT	Aspartate aminotransferase
$AUC_{0-\infty}$	Area under the curve from time zero to infinity
AUC_{0-72h}	Area under the curve from time zero to 72 hours
AUC_{0-last}	Area under the curve from time zero to last measurement
BBB	Blood-brain barrier
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CHMP	Committee for Medicinal Products of Human use
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	Maximum concentration (in serum or plasma)
CNS	Central nervous system
CONSORT	Consolidated Standards for Reporting Trials
CRF	Case report form
CRO	Clinical research organization
CTU	Clinical trial unit
DnE	AS Den norske Eterfabrik
DOR	δ-opioid receptors
EDTA	Ethylenediaminetetraacetic acid
Cl	Clearance
EBM	Evidence-based medicine
ECG	Electrocardiography
EFTA	European Free Trade Association
EMA	European Medicines Agency
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ENT	Ear, nose and throat
EU	European Union
F	Absolute bioavailability (relative to IV)
F_{IM}	Bioavailability relative to IM
FDA	U.S. Food and Drug Administration
γ-GT	Gamma-glutamyl transferase
GCP	Good Clinical Practise
GPCR	G-protein coupled receptor
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IB	Investigator´s brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use
IDU	Injecting drug users
IEC	Independent ethics committee
IM	Intramuscular
IMP	Investigational medicinal product
IN	Intranasal
IP	Intellectual property
IRB	independent/institutional review board

ISF	Investigator site file
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
KOR	κ-opioid receptors
LOQ	Limit of quantification
MA	Marketing authorization
MAD	Mucosal atomizer device
MCC	Mucociliary clearance
MOR	μ-opioid receptors
ND	Not detected
NDA	New Drug Application
NIDA	National Institute on Drug Abuse
NIH	U.S. National Institute of Health
NOMA	Norwegian Medicines Agency (Statens legemiddelverk, SLV)
NTNU	Norwegian University of Science and Technology (Norges Teknisk-Naturvitenskapelige Universitet)
OD	Overdose
OUS	Oslo University Hospital (Oslo Universitetssykehus)
PCT	Patent Cooperation Treaty
PD	Pharmacodynamic
PI	Principal investigator
PK	Pharmacokinetic
PRISMA	Preferred Reporting Items of Systematic Reviews and Meta-Analysis
PVP	Polyvinylpyrrolidone (Povidone)
PWID	Persons who inject drugs
QUOROM	QUality Of Reporting Of Meta-analyses
RCT	Randomized clinical trial
REC	Regional Committees for Medical and Health Research Ethics (Regionale Komiteer for Medisinsk og Helsefaglig Forskningsetikk, REK)
RP-HPLC	Reversed phase high performance liquid chromatography
SERAF	Norwegian Centre for Addiction Research (Senter for Rus- og Avhengighetsforskning)
SIRUS	The Norwegian institute for alcohol and drug research (Statens Institutt for Rusmiddelforskning)
SL	Sublingual
SmPC	Summary of product characteristics
SQ	Subcutaneous
$t_{1/2}$	Elimination half-life
THN	Take-home naloxone
TMF	Trial master file
t_{max}	Time to maximum concentration (in serum)
UN	United Nations
UNESCO	United Nations Educational, Scientific and Cultural Organization
V_D	Volume of distribution
WHO	World Health Organization
WIPO	World Intellectual Property Organizatio

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1 Introduction

Opioid overdoses (OD) represent a major health problem, killing users of illicit drugs, but also users of legally prescription opioids worldwide. World Health Organization (WHO) estimates 69.000 deaths caused by opioids each year. (1)

Seen in European perspective there is between 6.300 - 8.000 drug induced deaths each year. There have been more than 140.000 drug OD deaths in Europe since the European Monitoring Centre for Drug Addiction (EMCDDA) started to register drug OD deaths twenty years ago. A majority of these deaths are caused by opioids, mainly heroin. (2)

The situation in Norway is no exception. Registration of deaths in Norway caused by narcotics started in 1977. It is necessary to distinguish the terms *deaths caused by narcotic use* and *narcotic related deaths*, where the latter also include accompanying death causes, e.g. infections, violence and accidents. ODs in Norway caused by narcotic use have increased gradually until it accelerated around 1990 accompanying the increased misuse of heroin injections. A peak of 400 deaths in 2001 was the largest number in one year. In recent years OD deaths seems to have stabilized at approximately 250 fatalities per annum. (3) The Norwegian mortality rate is considered to be paradoxically high taken into account the relatively low prevalence of drug users among the total Norwegian population. (3, 4) This is probably connected to the fact that a large proportion of drug users in Norway inject their drugs, but it should also be seen in context of concomitant misuse of alcohol or benzodiazepines. It has also been pointed out that Norwegian death rates can be attributed to a pattern of use similar to the Norwegian drinking culture, characterized by high consumption over a relatively short time period. When it comes to narcotic related deaths, the Norwegian death rates are similar to comparable European countries. This is explained with a lower impact of infections, violence and accidents among the Norwegian narcotic related deaths (3).

The Norwegian Institute for Alcohol and Drug Research (SIRUS) has estimated the number of high-risk opioid users in Norway for the period 2010-2012 to be approximately 7.700. High-risk opioid users represent a heterogeneous group that also includes persons addicted to legally prescription opioids. The same report estimated that the number of injecting drug users (IDUs) was 8.400 in 2012, or 2.5 per 1.000 capita. High-risk opioid users and IDUs are thus overlapping groups (4).

Many OD deaths can probably be avoided if proper aid is provided within the crucial time frame before opioid induced respiratory depression causes the heart to stop and death eventually occurs. Resuscitation from opioid OD is mainly about reversing the respiratory depression, and the antidote naloxone is a vital part of this treatment. (2)

The challenge is to enable access to naloxone and ensure early administration of the antidote. One of the approaches toward this is introduction of Take-Home Naloxone (THN) programs (see 2.3 Take-home naloxone), where naloxone is distributed to users, agency staff, peers and carers, often in combination with relevant education and training.

Although some variability regarding clinical practise, the approved routes of administering naloxone have for decades been intravenously (IV), intramuscularly (IM) or subcutaneously (SQ). (1)

Persons who inject drugs (PWID) represent a patient group with high prevalence of blood-born diseases. (5, 6) Needle-free pharmaceuticals are proposed as a solution to reduce the risk for blood contamination by paramedics, together with far more attainable lay-person rescue. (7) Some of the THN programs as well as some ambulance services use various methods of administering intranasal (IN) naloxone already, but these are "off-label" formulations without marketing authorization (MA) from legal drug authorities. Knowledge of both efficacy and pharmacokinetic (PK) properties of IN and other non-injectable naloxone formulations is only to a limited extent established. The extensive use of off-label naloxone formulation in acute OD resuscitation constitutes a need for evidence-based treatment regimen and approved pharmaceutical products. (8, 9)

This master thesis seeks to deal with two separated issues: A) Planning, recruitment, screening and facilitation of a clinical trial meant to support a MA of an IN naloxone product. B) Contribution to a systematic review of patent applications for non-injectable naloxone formulations, including a joint first authorship of a systematic review paper. These two issues are methodically separated as Part A and Part B. The results- and the discussion sections are hence also divided into Part A and Part B, respectively.

1.1 Part A - The clinical trial, OPI 15-002

This study, named OPI 15-002 / SMR-3089 (EudraCT no: 2005-0023355-10), is aiming to support a MA for a new IN medicinal product for human use. Previous clinical studies on the same formulation has shown the following results:

Table 1 PK results from earlier studies of the formulation

Study	Sample size n	IN Dose mg	C_{max} ng/ml (SD)	t_{max} min (SD)	F % (SD)	F_{IM} % (SD)
OPI 12-001	5	2.0	4.19 (2.19)	16.0 (8.2)	56 (20)	-
OPI 13-001	12	0.8	1.45 (0.60)	18.1 (10.2)	61 (19)	-
		1.6	2.56 (1.54)	17.9 (6.6)	60 (30)	-
OPI 14-001	12	0.8	1.58 (0.70)	36 (13)	-	71.5 (23)

The objectives of OPI 15-002 were as follows:

- Primary objective:
    - Investigate the systemic exposure and PK profile after one IN naloxone dose of 1.4 mg compared to 0.8 mg IM and 0.4 mg IV.

- Secondary objectives:
    - Investigation of dose proportionality following one and two doses of 1.4 mg IN naloxone
    - Determination of absolute- and relative bioavailability of IN naloxone.
    - Evaluate bioequivalence criteria for 1.4 mg IN naloxone in relation to both 0.8 mg IM naloxone and 0.4 mg IV naloxone.
    - Investigation of safety and tolerability for the intranasal formulation of 14 mg/ml.

In context of the contribution to OPI 15-002/SMR-3089, ethical aspects of Good Clinical Practise (GCP) will be addressed and discussed.

1.2 Part B - Review of patent applications of non-injectable naloxone

Part B of this master thesis represents the contribution of a shared first authorship on a review article together with PhD student Rebecca McDonald, Addiction Department, Kings College, London (head: professor John Strang).

The aim was to present evidence from public non-peer reviewed patent registrations on non-injectable naloxone formulations, intending compensate for missing peer-reviewed literature on the field of non-injectable naloxone development.

The aims of the review were threefold:

1. To trace the concept and product development by route of administration.
2. To describe the non-injectable naloxone formulations for which human in vivo data are available.
3. To describe and compare human PK data reported in the patent documents.

In addition to what was included into the review article, in this thesis the patent applications were also examined for formulation stability data, to elucidate different excipients- and formulation aspects in relation to stability and degradation of naloxone in the respective formulations.

Based on the above-described parts of the thesis, it is proposed that:

1. Taking part in the preparation of a clinical trial on pharmaceuticals will enhance the understanding of good clinical practice, general research and medical ethics principles.

2. It is possible to obtain valuable scientific knowledge in the field of development of non-injectable naloxone outside the peer-reviewed literature through a systematic review of registered patents.

2 Theoretical background

2.1 Opioids

The terms *opioid* and *opiate* are terms used alternately on the same group of pharmaceutical analgesic compounds based on the opium poppy *(Papaver somniferum)*. Both opioids and opiates are used in medicine as sedatives and pain reliefs. Opiates are natural alkaloids derived from the opium poppy and does not include synthetic opioids, exemplified by *morphine*, *codeine* and *thebaine*. Opioids also include semi-synthetic and fully-synthetic analogues to the opium alkaloids, like for instance *methadone*, *buprenorphine* and *heroin*, and is therefore a wider term. (10) In this thesis the term opioid(s) will be used unless other is specified.

Opioids affect the body primary in the central nervous system (CNS), i.e. the brain and the spinal chord, but also in the gastrointestinal tract. The physiological response depends on the type of opioid, but the typical effects are pain reduction, sedation, constricted pupils, euphoria, drowsiness, nausea and respiratory depression. (2)

2.1.1 Opioid receptors

Opioid receptors are G-protein coupled receptors (GPCR) found in a wide spectre of CNS tissue including the pain-modulation pathways consisting of the medulla, locus coeruleusa and the central gray area. Also the midbrain, the limbic and the cortical structures contains opioid receptors. (11) Opioid receptors are also expressed in peripheral systems like gut-, vascular-, lung- and cardiac cells. (12)

Opioid receptors can be divided into three main groups; *μ-opioid receptors* (MOR), *δ-opioid receptors* (DOR) and *κ-opioid receptors* (KOR). These three groups have in common that they constitute analgesic effect when stimulated. The most widespread opioid receptor in the body is the MOR. Activation of MOR can also cause respiratory depression and constipation. A lot of opioids cause effect on MOR, with heroine as an example of an opioid with strong agonistic effect on this receptor. (2, 13)

There are furthermore two subclasses of MOR; μ_1- and μ_2-receptors. Almost all analgesic effects of opioids are ascribed to their binding to μ_1-receptors, while side effects such as respiratory depression, reduced gastrointestinal motility, bradycardia, euphoria and physical dependence are mostly related to μ_2-receptor activation. (10)

2.1.2 Agonism and antagonism

Substances constituting a response when interacting with a receptor are called *agonists*, and substances preventing such response, by binding to the same receptors, are referred to as *antagonists*. An antagonist having affinity for the same binding site on the same receptor as an agonist, competes on binding to the receptor, and this is referred to as *competitive antagonism*. The power balance is depending on the binding affinity and intrinsic activity of both the agonist and the antagonist. Examples of opioid agonists are *morphine*, *fentanyl*, *methadone* and *heroin*, while opioid antagonists such as *naloxone* and *naltrexone* block the opioid receptors and prevent the physiological effects of the opioid agonists. (2)

Agonistic stimulation of opioid receptors inhibits the cyclic adenosine monophosphate (cAMP) pathway. (11) cAMP is a derivate of adenosine triphosphate (ATP) involved in signal transduction in a wide series of biological processes. (14) Opioids also modulate calcium and potassium ion-channels, leading to hyperpolarization and inhibited neural activity. In addition to this, recent research suggests that also other transduction pathways are depressed by opioid stimulation (11)

2.1.2 Opioid addiction

Opioid addiction is a powerful physiological response to opioid exposure over a relatively "long" period. The brain adjusts to the exposure, resulting in a more or less normal function when the opioid receptors are stimulated and abnormal when not. (15) Stimulation of opioid receptors spontaneously inhibits the cAMP pathway and hereby the cAMP levels, but with time the cAMP levels will gradually recover to normal levels, and in presence of an opioid antagonist (e.g. naloxone) the cAMP levels will rise far above the baseline levels. (14)

Clinically, it is necessary to distinguish the terms *tolerance* and *dependence*. Tolerance is described as the need to intake higher and higher dosages to achieve the same effects, as a result of opioid receptors becoming gradually less responsive due to constant stimulation. (15) It has also been shown that long-term exposure of morphine has resulted in elevated cAMP levels, and such deviant regulation of cAMP is suggested to explain tolerance. (11)

Dependence is described as the presence of withdrawal symptoms if the receptors are not stimulated (by opioid agonist), and is typically leading to repeated exposure and further inducement of tolerance. (15)

2.1.3 Opioids causing respiratory depression

When opioids activate MORs, the release of noradrenalin from the neurons is reduced, leading to decreased respiration and lowered blood pressure, as well as drowsiness. (15) A depressant effect on the respiratory centre of the brain decreases the ability of inspiration (i.e. breathing inwards), while the ability of expiration (i.e. breathing outwards) remains unaltered. The respiratory frequency can become irregular and slow, followed by hypercapnia (elevated CO_2 levels) and hypoxaemia (low O_2 level). If vital organs do not receive enough oxygen, there is a risk for organ failure, coma or even death. (2)

Concomitant drug use, for instance intake of benzodiazepines, alcohol or other sedatives contributes to an elevated risk for respiratory depression. Doses of opioids that normally would be tolerated for one specific individual, might prove fatal in combination to other concomitant drugs, with respiratory depression and OD as results. (2)

2.2 Naloxone

2.2.1 Chemical properties

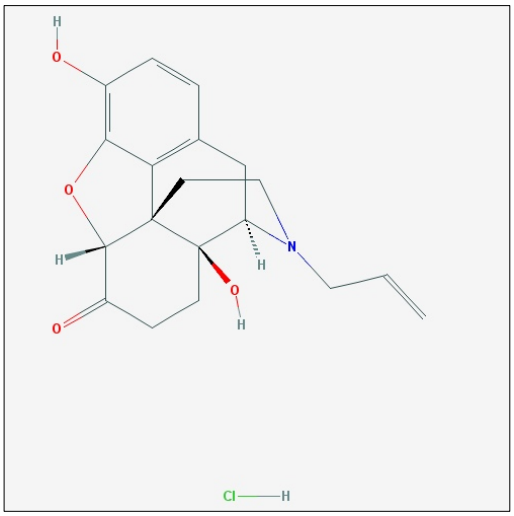

Figure 1 Structure formula of naloxone hydrochloride

The International Union of Pure and Applied Chemistry (IUPAC) describes naloxone chemically as (4R,4aS,7aR,12bS)-4a,9-dihydroxy-3-prop-2-enyl-2,4,5,6,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7-one. (16)

The molecular formula of naloxone base is $C_{19}H_{21}NO_4$. The molecular weights for naloxone base, naloxone hydrochloride and naloxone hydrochloride dihydrate are 327.38 g/mol, 363.84 g/mol and 399.87 g/mol, respectively (17, 18) Naloxone hydrochloride has a pKa=7.94 (19)

2.2.2 Naloxone as a part of opioid overdose treatment

Naloxone, a derivate of thebaine, is a competitive opioid antagonist well known for its ability to reverse opioid OD. (18) Naloxone was developed in the early 1960s by dr. Fishman and dr. Lewenstein. (20) In 1971, the U.S. Food and Drug Administration (FDA) approved the first injectable naloxone product. (2) Several generic alternatives have later appeared.

Naloxone is used worldwide, and is listed on the WHO Model list of Essential Medicines under the category *Antidotes and other substances used in poisonings*. (21)

In most countries naloxone is a prescription drug, and the access is limited to the supply from professional healthcare personnel. The approved routes of naloxone administration have for decades been limited to parenteral routes, comprising IV, IM, or SQ administration. (1)

IV is the standard administration route according to Summary of Product Characteristics (SmPC) for parenteral injectable naloxone with MA approved by Norwegian Medicines Agency (NOMA). IM injection is recommended if IV administration is not possible. (22, 23) In other countries, also SQ administration is accepted. (24)

2.2.3 Mechanism of action

Naloxone works as an antidote by competitive antagonism on opioid receptors. Naloxone binds strongly to MOR, but also to some degree to KOR and DOR. (13)

Naloxone can reverse the effects of opioids, including the respiratory depression as a result of an opioid OD. The primary goal for naloxone treatment is to re-establish spontaneous ventilation, without inducing acute withdrawal symptoms. (25)

2.2.4 Pharmacokinetic properties of naloxone

When administered orally, naloxone is absorbed well by the gastrointestinal tract, but is highly degraded due to extensive hepatic first-pass metabolism. (22, 23) The systemic bioavailability after oral administration is therefore low. (26)

Naloxone easily distributes to tissues and body fluids, including the brain. The distribution volume (V_D) is approximately 2 lkg^{-1}. (22) When administered intravenously naloxone has a serum half-life of 4,7 minutes in the distribution phase. The proportion bound to proteins is in the range of 32-45%. (13)

The metabolism mechanism is primarily hepatic phase 2-glucuronide conjugations. The major metabolite is naloxone-3-glucuronide, which is eliminated through renal extraction. The plasma half-life ($t_{1/2}$) for parenteral administered naloxone is 1-1,5 hours (3 hours in babies). The total clearance (Cl) is hereby 22 $ml*kg*min^{-1}$. (13, 22, 23)

The PK parameters for other administration routes than IV are only to a limited degree disclosed in literature. Dowling et al. (27) reported absolute bioavailability (F) of 35% and a median time to maximum concentration (t_{max}) of 12 minutes for IM administration. They also reported F=4% and t_{max} of 6-9 minutes for IN administration of the same parenteral fluid (5 ml of 0.4 mg/ml) attached to a mucosal atomizer device (MAD). (27)

Evzio®, the first naloxone auto-injector for IM and SQ administration was approved by FDA, 3th April 2014. (28) Evzio´s SmPC reports a t_{max} of 15 minutes, maximum plasma concentration (C_{max}) of 1.24 ng/ml and a $t_{1/2}$=1.28 hours after single administration of Evzio 0.4 mg injection (1 mg/ml). Evzio also reports data from a single 0.4 mg IM administration using a standard syringe, which achieved a t_{max} of 20 minutes, C_{max}=1.07 ng/ml and $t_{1/2}$=1.36 hours. (29)

The first and so far only non-injectable naloxone formulation is the Narcan® IN spray approved 18th November 2015. The SmPC of Narcan® nasal spray discloses PK parameters for 4 mg dosage (one spray, one nostril) and 8 mg (one spray, each nostril), with t_{max} of 0.50 and 0.33 hours, C_{max} of 4.83 and 9.70 ng/ml, and an area under curve from time zero to last measurement ($AUC_{last)}$ of 7.87 and 15.3 ng*h/ml, respectively. The corresponding dose-normalized bioavailability values relative to IM administration (F_{IM}) were 46.7% (4 mg) and

43.9% (8 mg). The reference treatment described was 0.4 mg IM injection with t_{max}=0.38 hours, C_{max}=0.88 ng/ml and AUC_{last}=1.72 ng*h/ml. (30)

2.2.5 Side effects and adverse events

Naloxone has an encouraging safety profile. The SmPC for Naloxon B. Braun 0.4 mg/ml parenteral solution claims that single IV doses of 10 mg naloxone hydrochloride are well tolerated without any side effects or change of laboratorial parameters. In absence of other agonistic or antagonistic effects on opioid receptors, naloxone has practically no pharmacologic effect on man. (22) In the absence of exogenous opioids, the effects of naloxone are few, giving an advantageous safety profile. (10)

For people addicted to opioids, opioid withdrawal symptoms constitutes a different picture. Buajordet et al. (31) conducted a prospective observational study in Oslo in the ambulatory emergency service that aimed to determine the characteristics and frequencies of adverse events (AE) related to out-of-hospital administration of naloxone. They included 1.192 acute opioid OD episodes and assessed AE after parenteral administration of naloxone. An initial IM dose of 0.4-0.8 mg (body weight depended) was given together with 0.4 mg IV. Depending on the response, the IV dose could be repeated up to a maximum of 1.6 mg (a total dose of 2.4 mg). There were 726 reported AEs among 538 patients. The most frequently observed AEs were confusion, headache, nausea, vomiting, aggressiveness and tachycardia. Only 0.3% of the opioid OD patients given naloxone experienced AEs leading to hospitalization, supporting the image of naloxone as a relatively safe compound. The study concluded that serious complications due to naloxone were rare. (31)

There are case reports though where AEs such as severe hypertension, atrial tachycardia, ventricular fibrillation, general convulsion, asystole, pulmonary edema and violent behaviour are seen in context of naloxone treatment. (32, 33)

It can be questioned whether AEs such as the aforementioned should be seen in direct relation to naloxone, to the opioid withdrawal as a result of the naloxone treatment, by the opioid intoxication itself, concomitant drug use or underlying medical conditions. (33)

2.2.6 Prehospital challenges

The number of emergency calls related to OD in Oslo and Akershus is 1.300-1.500 per year (3). Patients suffering an opioid OD are typically found in poor condition at places not ideal for medical treatment, and are typically treated on site as prehospital patients. (34)

Depending on which opioid that has caused the patient´s OD, there is a risk of recurrent toxicity and re-entering a stage of respiratory depression after initial effect from naloxone. If the $t_{1/2}$ of the opioid is longer than what it is for naloxone, the opioid might re-occupy the receptors and re-induce OD. (35) The situation will sometimes demand re-administration of naloxone to prevent re-intoxication.

IV administration provides that the entire dose enters the systemic blood circulation. (36) The IV route gives a quick onset of action, but this advantage is often ousted by the time taken to establish IV access on people having poor veins. (34) Parenteral administration limits the administration of naloxone to only trained personnel. However, in prehospital settings worldwide, there seem to be a drift away from IV as the preferred route. (37, 38) A survey in England 2005 among ambulance and police services, revealed that a majority of the services would chose the IM administration route (49%). 16% would chose IV, 1% chose SQ and 23% preferred a combination of routes, with IV and IM as the most preferred combination. (39)

Ambulance staff carry out their work under circumstances involving blood exposure. Leiss et al. (40) studied various risk factors for blood contamination among American paramedics and the incident rates for infection by different administration routes. They reported the incidence rates for needle stick injuries among US paramedics to be 1.3 pr. 10.000 calls (95% CI: 0.5-2.0) or 0.8 pr. 10.000 patients (95% CI 0.3-1.3). In the same report they claimed there were more than 10.000 reported needle sticks pr. year among paramedics in the United States (40).

Another problem with parenteral based naloxone is the risk for contamination with bacterial infections and blood borne viruses for the medical staff or any bystander that administer the antidote. Typically hepatitis C (HCV) and B virus (HBV) are highly prevalent, but also human immunodeficiency virus (HIV). (5, 41) The prevalence of HCV among PWIDs is estimated to 43% in the European Union (EU)/European Free Trade Association (EFTA) region (27 EU member states plus Norway, Iceland, Liechtenstein and Switzerland), and 60 % globally. (6, 42) Sharing of paraphernalia such as syringes, needles, cottons and cooking devices makes illegal drug use a main reason behind new incidents of HCV infection. (42, 43)

An epidemiological systematic review estimated that one in five of PWID is infected with HIV globally. (44)

Rapid injection with high initial dose (i.e. IV administration) causing rapid opioid withdrawal is pointed out as a plausible explanation for agitating behaviour. (31) There are several reports of agitation and violent behaviour after naloxone administration when reviving patients with acute opioid OD. (33, 45, 46) The presence of infectious needles can therefor represent an elevated hazard for the paramedics working in the field. Agitation is suggested explained both as an acute opioid withdrawal symptom and/or as a result of unmasking underlying personality disorders. (33) Another suggested explanation is that opioids can suppress effects from concomitant drugs, which might come to surface during naloxone treatment. Blood samples from unconscious patients with evidence of heroin OD in Copenhagen area support the impression that concomitant drug abuse is usual among heroin addicts. Benzodiazepines, amphetamines and cocaine are typical examples of drugs combined with heroin/opioids. (47) The exemplified concomitant drugs are all associated with aggressive behaviour. (48-50)

Slower injection is suggested as a possible solution to prevent agitation. (33) Slowly IV injection after initial IM injection is also recommended administration form due to guideline for paramedics treating opioid OD in pre-hospital environment in Oslo. (51) SQ Administration of naloxone in pre-hospital settings with insufficient IV access, have also been proposed as a preferable solution by some ambulance services. (37)

In recent years there has been an increasingly interest for development of non-injectable naloxone formulations to address the abovementioned problems, and in United States, the National Institute on Drug Abuse (NIDA) has given financial support to development of such pharmaceuticals. (52)

2.3 Take-Home Naloxone

Restoring the patient´s breathing and provide basic life support and resuscitation is essential for survival of an opioid OD. Administration of naloxone is a central part of the acute treatment regimen, (1) and several countries have started programs to ensure early layperson access to naloxone. (53)

Prescription drug OD are most likely to occur in private homes. (54) OD victims using illicit drugs are more likely to be found in public places than victims of overdoses caused by

prescription drugs, and most heroin ODs happens in presence of others. (55) This constitutes an opportunity for early intervention (i.e. before an ambulance arrives), and this awareness has contributed as a gate-opener for a new way of ensuring access of naloxone, where bystanders and peers are supposed to help administer naloxone to opioid OD victims.

THN programs are community-based programs meant to prevent opioid ODs and reduce the number of deaths by providing education and distribution of naloxone to drug users and people likely to witness an OD. There are three different target populations for THN distribution. These are *users*, *agency staff* with high probability of user interaction, and *carers*. The latter include family members, peers and other close contacts of users. (2) The proposal of naloxone distribution to drug users was first time mooted in 1992. (56)

In the United States, such programs have existed since 1996 when an harm reduction organization called the Chicago Recovery Alliance first started to hand out naloxone to drug users in Cook County, Illinois. (53, 57)

Today THN programs are implemented in more than fifteen countries worldwide. (58) Currently, there are seven European nations that have implemented any form of THN programs. Those nations are Estonia, Germany, Italy, Spain, Denmark, Norway and the United Kingdom. (2) The programs differ in size, format and the type of naloxone formulation used. In 2011, Scotland was the first country in the world to offer a public funded THN program, which also aims to evaluate pre-post comparison of the program. (59) Scotland and Wales have implemented nationwide distribution, whereas others have programmes dedicated to smaller geographic areas, like for instance Norway who provides THN to IDUs in Bergen and Oslo. Some programmes, for instance the one in Norway, does not distribute naloxone for injection. Due to the lack of approved pharmaceutical products, the project uses an improvised IN kit that is temporary approved by the Norwegian Medicinal Agency (NOMA). This non-approved IN kit consists of a 1mg/ml naloxone syringe attached to a MAD. This naloxone solution is developed for parenteral injections. (3, 4) By October 2014, 456 kits had been distributed. (2) The Danish THN programs also provides an improvised IN kit, but includes a needle for IM administration as back-up in case IN administration fails. (2)

The first report in peer-reviewed litereature of survival of OD as a direct result of THN provision was published in 2001, and it referred to two projects, one in Berlin and the other in

the island of Jersey. Overall there were 34 cases where THN was given, all with a positive survival outcome. There were no AEs reported, other than expected withdrawal symptoms. (60) Cook County, Illinois, reported the reversal of an upward trend of opioid ODs after the introduction of the THN program. (57) A study conducted in London estimated that approximately two-thirds of bystander-witnessed heroin ODs with a fatal outcome, could have been avoided if naloxone had been accessible to the bystander(s). (61) A recent published systematic review paper aiming to find the impact on overdose-mortality caused by THN distribution as well as its safety profile, concluded that THN reduces overdose-mortality with a low rate of AE. (58)

The Norwegian Parliament, Stortinget, has proclaimed a "zero-goal" in connection to efforts to reduce the number of OD deaths in Norway. The Ministry of Health and Care Services is sponsoring a project led by The Norwegian Centre for Addiction Research (SERAF) where THN is distributed free of charge to drug users in Oslo and Bergen. SERAF will evaluate the project and is espected to advise on possible extension or expansion of the project by the end of 2016. (3, 4)

2.3.1 Scepticism to THN

Counterarguments against THN programs have been raised. There are reports of bystanders not being able to successfully attach the MAD unit to the syringe containing naloxone. (62)

It has been questioned whether easier access to an antidote would result in ignorance of the hazards of drug use, and hence lead to increased or riskier use. A structured interview survey among IDUs and participants in methadone programs concluded that the risk for more hazardous opioid use caused by introduction of THN was unlikely. (61) Still, bystanders have pointed at a fear of agitation as a result of the acute opioid withdrawal. Also the fear for needle-stick injuries accompanied with infection by blood-borne diseases have been underlined and pointed out as arguments for choosing non-injectable naloxone formulation in THN programs. An Australian survey aimed to identify which administration routes that were preferred among IDUs, and it concluded that the IN route was most preferred. (63)

Also, questions regarding legal aspects have been raised. One question is whether it is legally acceptable that a bystander administers a drug to whom the antidote was prescribed to. Conversely, is it acceptable if a drug user whom the naloxone was prescribed to, uses his/her own naloxone supply to rescue a peer? (64) In many jurisdictions it is considered

controversial to prescribe a prescription drug to a recipient that is not examined or not even known to the prescriber. However, in at least fifteen US states, THN programs were made possible thanks to the introduction of the Good Samaritan laws, granting legal immunity to bystanders assisting an OD victim. (65)

The implementation of improvised IN naloxone kits into opioid OD treatment in community settings, has been criticized for being prematurely. (66) Despite the lack of evidence-based treatment regimens nor authorized pharmaceuticals, an increasing use of IN naloxone has been going on in the ambulance service since early 2000s. (34, 67-71) The missing continuity and slow evolvement of new non-injectable naloxone products, together with the continued distribution off-label formulations, has been criticised for being unethically. (8, 9)

2.4 Non-injectable naloxone

The PK parameters for non-injectable naloxone formulations are only known to a limited extent. (72)

The criteria to support a New Drug Application (NDA) for a non-injectable naloxone product were presented by FDA in 2012. (73) Three candidate routes for non-injectable administration were identified through a recent systematic review that applied the latter criteria to the peer-reviewed literature. The three possible administration routes identified were the sublingual (SL), buccal and IN administration routes. (72)

2.4.1 Sublingual naloxone

The above-mentioned review of candidate non-injectable routes, left the SL route with least credibility compared to the buccal- and IN route, due to the possibility of obstructed access to SL mucosa caused by a closed mouth or vomit and high inter-subject variability. (72) The inter-subject variability of SL naloxone was shown to be high in an effect study from 1990. (74)

FDA granted fast track admission for a NDA of a new SL naloxone product in 2015. (75)

2.4.2 Buccal naloxone

The first clinical trials on a new buccal delivery tablet developed through collaboration between the Addiction Department and the Institute of Pharmaceutical Science at King´s College, London, are currently under conduction. (72)

2.4.3 Intranasal naloxone

The IN route has been pointed out as a promising non-injectable administration route that could address the above-mentioned problems related to invasive administration. (34, 76)

In 1984, a complete absolute bioavailability, F=101%, was shown for IN naloxone in rats, pointing at the IN route as an interesting route for future non-injectable naloxone administration. (77)

The first human in vivo clinical trial (1992) of IN naloxone was a test method developed to identify physically-dependant opiate users by the use of IN naloxone. All subjects having opiate-positive urine samples had significantly increased opiate withdrawal symptoms after IN naloxone administration. The researchers also suggested that the IN route is as effective as parenteral administration. (76)

The IN administration of 2 mg/5 ml by Dowling et al. (27) (see 2.2.4 Pharmacokinetic properties of naloxone) achieved only F=4% and F_{IM}=36%.

Two Australian randomized clinical trials (RCT) have been published on IN administration of naloxone. Both studies compared 2 mg IN and 2 mg IM. Kelly et al. (68) administered 5 ml of a 0.4 mg/ml IN naloxone solution, where they successfully reversed the opioid OD in 74% of the cases. Kerr et al. (69) administered 2 ml of a 1mg/ml solution, which reversed the opioid OD in 89% of the cases. In both studies the IN unit was administered through a syringe attached to a MAD.

A WHO meta-analysis of the above-described two RCTs found no significant difference between the IN and IM administrations of naloxone. (1)

Sabzghabaae et al. (71) conducted a RCT where they compared the pharmacodynamic (PD) effects of IN to IV administration of naloxone. The trial was conducted on one hundred overdosed patients divided into equally sized groups (n=50). Both IN and IV groups received

0.4 mg naloxone, where the IN group received 1 ml containing 0.2 mg naloxone into each nostril. The IN group demonstrated longer time to adequate response, but had a significantly higher consciousness level after administration compared to the IV group ($p<0,001$). One of the findings was a different level of agitation between IN versus IV administration routes. There were twelve cases of agitation following the IV treatment and none in the IN group. The researchers implied the delayed clinical exposure due to nasal absorption as a possible explanation for this observation, and saw this as a possible advantage for the IN route. They noted, however, that this observation could also be explained by a possible higher number of patients with drug addiction in the IV group. (71)

The benefits achieved from IN naloxone administration have been questioned. Zuckerman and his colleges put a critical spotlight on the increasing focus on IN administration of naloxone, implying that studies of THN programs undermine reports of individual cases of unsuccessful administrations and adverse outcomes. They also reported a case where IN naloxone failed to reverse an OD for a 26 years old male who had masticated two 25 μg fentanyl patches. In the particular case, 1 mg non-concentrated naloxone was sprayed into each nostril via a MAD. (78)

2.5 Intranasal drug delivery route

2.5.1 Nasal physiology

Olfaction is the main physiological functions for the nasal cavity. Particle filtration, humidification and heating the incoming air are other important functions for protection of the lower airways. (79, 80)

Air entering through the nasal vestibule enters the nasal cavity. The nasal cavity is a two-compartment cavity separated by a "wall" called the medium septum. Each of the two compartments has three different openings, also called the upper-, middle- and lower meatus which is leading further into the cavity. These narrow openings are separated by three horizontally turbinates also called the inferior-, middle- and superior nasal concha. (80) The narrow passageways created by the turbinates constitute a mucosal surface area of 150-180 cm^2 covered with 2-4 mm thick layer of mucosa. (79)

One of the main functions of the nasal mucosa is *mucociliary clearance* (MCC). Cilia are motile appendages attached to the surface of epithelial cells. Three types of epithelial cells are located in the human nasal cavity, *squamous*, *respiratory* and *olfactory* epithelial cells. The anterior part of the nasal vestibule is covered with squamous, but no cilia. The respiratory epithelia covering the major surface of the human nasal cavity appears after approximately one centimetre, and is essential for MCC. The olfactory epithelial cells are located in the posterior nasal cavity. (81)

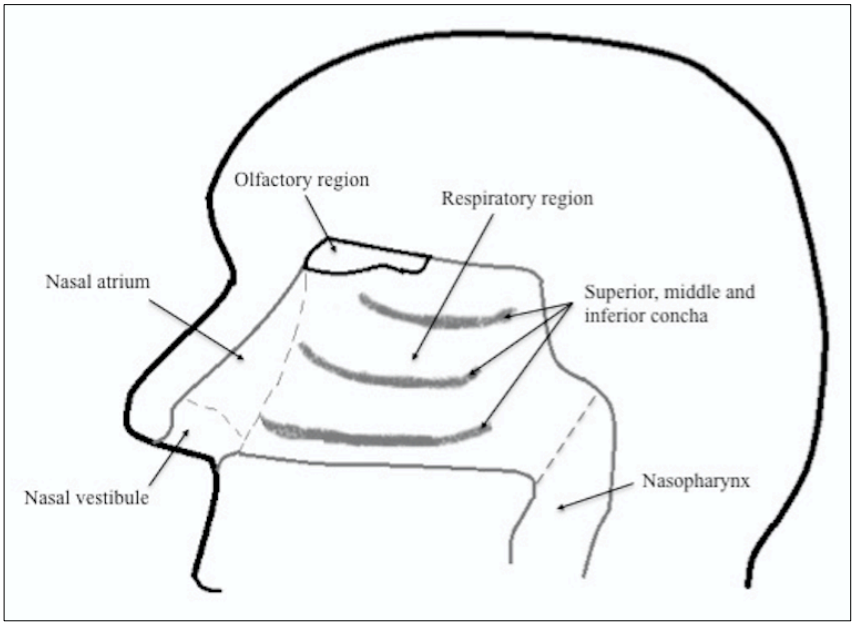

Figure 2 The nasal cavity

MCC is a mechanism of importance for the protection of the bronchi, as particles and pathogens adhere to the nasal mucus layer instead of following the air-flow to the lungs. The mucus with the attached particles is transported by ciliary motion, often referred to as a "conveyer belt", via the nasopharynx and further down the gastrointestinal tract. (82)

2.5.2 Intranasal route for drug administration

The IN route has been recognized as a route for drug administration for decades, especially for decongestants and other drugs with local topical effects. There is also an increasing attention towards the IN route´s systemic delivery properties. The nose is easily accessible, has an appropriate mucosal area for absorption and has an extensive systemic blood supply. (82) Systemic absorption via IN administration bypasses the hepatic first-pass metabolism,

which can degenerate drugs absorbed via the gastro-intestinal tract. (83) As mentioned in the section *2.2.4 Pharmacokinetic properties of naloxone*, this is relevant to naloxone, which is subject to extensive degradation by hepatic first-pass metabolism. Other nasal advantages are fast absorption, rapid onset, avoidance of gastrointestinal irregularities such as gastric stasis and vomiting. (80)

Factors affecting the systemic absorption of IN administrated drugs are:

- Drug concentration
- Drug vehicle – delivery system
- Time in contact with mucosal tissue (residence time)
- Venous drainage of mucosal tissue
- Chemical properties like; pH, tonicity, ionization, molecular size and lipophilic properties

Committee on Drugs, 1997: 143-52. (84)

Certain physiochemical properties need to be met if a drug shall reach the systemic blood flow through the IN route. Molecular size (i.e. molecule weight) has a great impact on the absorption. In general, molecules designed for systemic delivery through the IN route should have a molecular weight <1000 Da for achieving good systemic bioavailability. In presence of *absorption promoters* this can be further stretched to approximately <6000 Da. Larger molecules represent a risk for damaging the nasal cavity. (83)

Another factor with influence on the absorption, and therefore also the systemic bioavailability, is whether drug is ionized or un-ionized. Lipophilic compounds are in general more likely to be absorbed over the nasal mucosa than hydrophilic compounds. The environmental pH can affect the compound´s degree of ionization, and hence the lipophilic properties. The pH on the surface of mucosal cells in the nasal cavity is about pH 7.39, and approximately pH 5.5-6.5 in the mucosal layer. An additional aspect is the fact that the pharmaceutical formulation itself can modify the environmental pH, and hereby indirectly affect the absorption of the drug. (83)

There are different techniques to improve the systemic bioavailability of a formulation intended for IN use. One way is to increase the nasal *residence time* for the compound. By adding bio-adhesives or excipients that increases the viscosity of the formulation, one can reduce MCC, and hereby enhance nasal residence time. Examples of such excipients hydroxypropyl methylcellulose (also known as hypromellose) and polyacrylic acid (Carbopol). Another way to improve systemic bioavailability is to add excipients enhancing the nasal absorption. These works by increasing the rate of the compound´s passage through

the mucosal layer in the nasal cavity, or by changing the structure of the epithelial cells. There are several examples of such absorption enhancers, for instance surfactants, bile salts and cyclodextrins. (83) Polyvinylpyrrolidone (PVP), also known as Povidone K30/K90, has the ability to thicken formulations and stick to mucosal membranes, and hereby act both as a viscosity enhancer and a bio-adhesive agent. (85) A third way of improving systemic bioavailability is by altering the physiochemical properties of the compound by modifying its structure. One can use different salt forms of the active compound, or make changes to the *auxophore*, which is the part of the molecule that is not involved in binding to the target. (83) The term *bioisosterism* is used on replacement of single atoms or specific groups of the auxophore and by this way alter the physiochemical properties of the drug molecule and hence improve the PK properties. Ideally, this can be done without decreasing the binding affinity between the binding target and the *pharmacophore*, i.e. the part of the drug molecule that binds to the target. (86)

Drugs designed to serve effect in the brain need to cross the blood-brain barrier (BBB), which limits the entry of drugs into the CNS. The BBB hereby serve as protection against unwanted substances into brain tissue. The nasal mucosa is the only location in the body providing direct connection between the atmosphere and CNS through olfaction cells, constituting the so-called *nose-to-brain theory*. (80) There are substantial evidence for this route being relevant in animals, and some evidence in humans. (87, 88)

Standard volume for approved metered dose-pump nasal solutions is in the range between 25-200 µl. (89) To avoid run-off from the nose and further down the pharyngeal cavity, a maximum volume per nostril of 150 µl is recommended. (79, 90)

2.6 Evidence-based medicine

There are several definitions on evidence-based medicine (EBM). A simple definition from 1996 says:

> Evidence based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients.

Sackett, 1996: 71-2 (91)

For this definition to make sense one must first look at what is meant by the term *evidence*, a term even philosophers disagree on how to define. In addition, various languages will translate the term differently, giving it different meaning as a result of the translation it self, with terms like *proof*, *fact* and *knowledge* as examples of translations. Some philosophers include the aspect of *belief* into the definition, by defining evidence as *grounds of belief*. EBM context suggests a broad approach to the term *evidence*, saying that *"any empirical observation or report of a symptom or mental state constitutes potential evidence, whether systematically collected or not"*. This would include reports from patients, clinical observations of individuals, clinical trial results and more. (92)

There are three epistemological principles of EBM: 1) Chase the truth, in the meaning of finding the best available evidence identified through systematic summaries, rather than limited samples of evidence. 2) Recognise to what degree the evidence is trustworthy. 3) Evidence is necessary, but not sufficient to make good clinical decisions. Benefits, burdens, risks and costs are, together with the patient´s values and preferences, considerations that must be taken into account. (92)

2.7 Clinical research

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) defines a clinical trial as:

> Any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamics effects of an investigational product, and/or to identify any adverse reactions to an investigational product, and/or to study absorption, distribution, metabolism and excretion of an investigational product with the object of ascertaining its safety and/or efficacy. The terms clinical trial and clinical study are synonymous.

ICH, 2002: page 6 (93)

2.7.1 Historic retrospect on the evolution of clinical trials

The historic perspective on development of clinical trials can be traced back to 500 BC and the biblical descriptions in the "Book of Daniel" telling the story of King Nebuchadnezzar who ordered his soldiers to eat only meat and drink wine, except for a group of rebels who was allowed to eat only vegetables and water and them apparently becoming better nourished.

In 1974, a ship surgeon named James Lind conducted the first known controlled clinical trial. This is referred to as the Scurvy Trial, because of his parallel approaches on treating scurvy among sailors. He discovered the beneficial effects of eating oranges and lemons. (94)

The concept of *randomization* was launched in 1923, but the first RCT was first conducted in 1946 when streptomycin was tested on pulmonary tuberculosis. (95)

The Nuremberg tribunal from 1947 that judged the crimes committed in World War Two, formed ten ethical standards about experiments on humans to which physicians must commit, called The Nuremberg Code. The Declaration of Helsinki from 1964 adopted these principles, and has greatly influenced further adoption of ethical standards into different countries regarding research on humans. The Declaration of Helsinki has later been modified plural times. (96)

Unfortunately, unethical experiments did not stop there. In 1966, Henry K. Beecher published a report documenting that subjects had been recruited into high-risk intervention studies without knowing. (97, 98) As a result to this, independent ethics committees (IEC) have been created worldwide. In 1977 the Council of Europe signed the Oviedo convention (Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application Biology and Medicine). The convention discusses the rights of the subjects attending clinical researches as well as the researchers´ obligations. The Oviedo convention sets requirements on the quality of the research with respect to scientific value, quality and qualifications of the personnel conducting the research. (99)

In 1949, a non-governmental organization called Council for International Organizations of Medical Sciences (CIOMS) was established by WHO and United Nations Educational, Scientific and Cultural Organization (UNESCO). (100) CIOMS focuses on bioethics, and is issuing key specific guidelines for application of ethical principles. One central guideline is the *International Ethical Guidelines for Biomedical Research Involving Human Subjects*,

often referred to as the "green book". CIOMS consists of more than 60 member organizations, and is an associate partner of UNESCO and in official relations to WHO. (101)

2.7.2 Good Clinical Practise

ICH provides guidelines for GCP, the ICH-GCP guidelines, that unify standards for EU, Japan and the United States and thus making acceptance of clinical data mutual. (93)

1. Clinical trials should be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).
2. Before a trial is initiated, foreseeable risks and inconveniences should be weighted against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if anticipated benefits justify the risks.
3. The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.
4. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
5. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
6. A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favourable opinion.
7. The medical care given to, and medical decisions made on behalf of, subject should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
8. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
9. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
10. All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.
11. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
12. Investigational products should be manufactured, handled and stored in accordance with applicable good manufacturing practice (GMP). The should be used in accordance with the approved protocol.
13. Systems with procedures that assure the quality of every aspect of the trial should be implemented.

ICH, 2002: p.11-12 (93)

The person conducting a clinical trial is referred to as an *investigator*. If the there is a team of investigators, the main responsible person is referred to as *principal investigator* (PI). (93) The PI is a person with impartiality from the sponsor. The PI has the responsibility to make sure that the investigation is conducted in accordance to GCP. (102)

The *sponsor* manages and finances the study. (93) This can be an individual, an university, a non-profit organization, a pharmaceutical company or even a government. A common approach in front of a clinical trial is that the sponsor provides a collection of relevant information for the trial called *investigator´s brochure* (IB). Information on physical,

biological and chemical properties, known pharmacological data, including preclinical studies, safety and toxicity is included into the IB. (102)

The *protocol* is a comprehensive document describing in details how the clinical trial is to be conducted. The background and rationale for the clinical trial and its aims and endpoints are explained. The study design is fully described with its methodology, hence its number of subjects and methods for statistical analysis is disclosed. (93, 102) The protocol describes the subject selection process with its inclusion- and exclusion criteria and describes the study visit procedures in detail. It also contains information on how the subject´s safety, welfare and confidentiality are provided. (102)

Information related to the individual subject is recorded into a *case report form* (CRF). The CRF is a printed, optical or electronic form to where all protocol relevant information about the subject is to be recorded. (93) The subject´s baseline data and recorded vital signs, haematology, clinical observations, AE, as well as comments from the subject and the investigator(s) is recorded into this form. The structure of the document should reflect the different stages of the trial, and cover the recording of markers relevant to the endpoints. The CRFs are parts of the regulatory documentation from where the data are analysed statistically. (102)

To ensure that the clinical trial is conducted as described in the protocol and with accordance to GCP principles, the study must be monitored. This most important function of the monitoring is to ensure the subject´s rights, safety and well-being. (93) This includes monitoring effects and AE, and make sure that this information is recorded into the CRF. Sometimes a sponsor uses a contracted organization to monitor or even conduct the clinical trial. Such an organization is referred to as a *clinical research organization.* (CRO) (102) Formally documentation of the duties and functions of the CRO is required. (103)

At the beginning of a clinical trial, a *trial master file* (TMF) must be established. This is a collection of documentation relevant for the conduction of the trial. The TMF allows evaluation of the clinical trials integrity and compliance to GCP, and serves as a basis for inspection by monitor/CRO and authorities. If the study is conducted at plural sites, or if the sponsor and investigator are located at different places, then an *investigator site file* (ISF) is established at the specific study site. GCP inspectors regard the ISF(s) and TMF as the entire TMF, collectively. (103)

2.7.3 Ethical considerations

According to the ancient Hippocratic oath, the prime duty of a physician is to *"avoid harming the patient"*. (94)

Four basic principles of biomedicine ethics, *respect for autonomy*, *beneficence*, *non-maleficence* and *justice*, were described in 1983. (104) Informed consent is an important aspect of autonomy, and being capability to communicate with the subject is a prerequisite. The principles of beneficence and non-maleficence interferes with each other, since whenever a healthcare worker (or researcher) is intervening on a patient (or subject), there is a risk for doing harm. Importantly, for this ratio (c.f. risk-benefit ratio, described later) to be found favourable, knowledge based on medical research must be provided. The principle of justice may be divided into *legally justice*, *rights* and *distributive justice*, where the latter points out that the distribution of resources must be fair. (105)

There are several guidelines and handbooks for design and conduction of clinical research, based on the fundaments from the abovementioned Nuremberg code, The Declaration of Helsinki, CIOMS and more. One example is provided by the U.S. National Institute of Health (NIH), which operates with seven main requirements that all must be fulfilled for a clinical trial to be considered ethical. (106) The composition and division of topics may slightly differ in various countries´ ethical guidelines, but contextually they are harmonized, and analogous to NIH´s. NIH´s seven requirements are:

- Social value
- Scientific validity
- Fair subject selection
- Favorable risk-benefit ratio
- Independent review
- Informed consent
- Respect for enrolled subject

NIH, 2016 (106)

The terms *social value* refers to the clinical trial´s ability to improve the health or well-being on a society level. (106)

Subjects offering their valuable time and even taking the risk of letting researchers "use" their body, must be assured that their contribution is leading to results having *scientific validity*, i.e. use of valid methods leading to statistically verifiable results answering the scientific question. The results from the study should produce new useful knowledge. (106)

Fair subject selection refers to a subject cohort being relevant and able to answer the scientific question, but selected in a way that minimizes risk exposure. (106) A phase 1 clinical trial typically recruits healthy volunteers that might get a financial compensation for their contribution and participation, while the following phases are normally conducted among the target population. (102)

Subjects in a clinical trial should be exposed to minimal risk and maximal benefit. The more risk associated with the study, the more benefit should be achievable. The risks and benefits should hence be balanced in a *favorable risk-benefit ratio*. A high social benefit (social value) can compensate for a lack of individual benefits for the study participants, but in those cases the risks must be low for the study being ethically justified. Another aspect to this is the burden a survey puts on the participants, for instance the use of time. Researchers should not occupy more time or expose the subjects to more risk than absolute necessary to answer their research question. The financial compensation to the subject should not be considered in the context of risk-benefit ratio. (102, 106)

To ensure that ethical considerations are taken care of, it is required to get an *independent review* by a group of competent people with no connection to the research. (106) Different countries have different institutional solutions for this, often referred to as independent/institutional review board (IRB) or IEC. (102) The IRBs/IECs monitors studies, and help researchers fulfil ethical requirements, but they also provide certainty to both subjects and the society in general about the research being safe and ethically. (102, 106)

An *Informed consent form* is signed by the subjects who have agreed to participate after being informed about the details of the clinical trial. The process of giving informed consent can be divided in three: 1) *disclosure*, *understanding* and *voluntariness*. A precondition for this is that the researcher gives a full disclosure of what participation means, including what risks are involved and what kind of responsibility the subject has through participation. For the subject to make a good decision whether he or she wants to participate, he or she must understand the purpose, risks, benefits and alternatives of the study. All participation into clinical trials must be based on voluntariness. (106) Tight boundaries between researchers and participants can interfere with the ability of autonomous voluntary consent. (102) Voluntary consent is also the one point out of ten, being emphasized in the Nuremberg code from 1947. (96)

Researchers must show *respect for subjects* involved. This means to keep any information about the subjects confidential and allow them to leave the study at any time they want. It also means to check the well-being of each individuals and remove subjects who get exposed to increased risk along the way, and inform subjects about possible changes in risks or other information relevant for the subjects. The results should be shared with the subjects, and by this way include them in the partnership of the study. (106)

2.7.4 Clinical trial phases

The traditional linear development progress for an investigational medicinal product (IMP) is starting as a lead compound found through an irrational or more or less rational approach in the early *drug discovery phase*. In the drug discovery phase target validation is essential, finding *proof of principle* on that the drug exerts effects on the relevant target, be it a receptor, an enzyme, a ion channel or similar. Traditional drug discovery phase often includes optimization of the compound, improving its physiochemical properties due to stability, PK properties and more. If the compound is considered eligible, it moves into the *drug development phase*. Pre-clinical methodology including animal testing on basal pharmacology, toxicology (including carcinogenicity, genotoxicity, and reproductive toxicology) is then conducted. If the compound still is found likely to become a successful drug, it proceeds to clinical trials in humans. (107)

The first experiments in humans are called *phase 1* clinical trials. Endpoints of phase 1 clinical trial are varying of the nature of the IMP, and whether the IMP follows a traditional linear phase progression. The primary goal of a traditional phase 1 clinical trial is to determine *tolerability* and *safety* of the IMP in humans. PK properties are monitored, and to some extent also PD activity. Usually, healthy volunteers are recruited, but it is not ethically acceptable to expose healthy volunteers to drug candidates with a more unfavourable risk-benefit ratio. In such cases volunteers from the patient population can be used if that is considered both ethically and scientifically relevant seen in context of other possible treatment regimen for the individual subjects. (102)

Phase 1 clinical trials are often *open label* studies, meaning the subject is aware of what treatment that is given at any time. The number of participants is low compared to the subsequent phases, often 20-80. Sometimes the number of subjects can be even lower, depending on the design of the trial. PK properties can be determined by sampling of blood,

urine, stool or other physiological parameters. The subject will be observed for any physiological changes like pain, fever, discomfort etc. Vital signs like for instance heart beat frequency, blood pressure, respirational frequency etc., are monitored. Behavioural matters may also be of interest. (102)

The transition and distinction between different phases is not always clear and rigid. In general, the objectives of *phase 2* clinical trials are safety, efficacy and mechanism of action. Phase 2 trials are also called *therapeutic exploratory trials*, and has larger sample sizes than phase 1 trials. The subjects are recruited from the target population. The included subjects are randomized into case- and control groups. The controls will have either placebo or current standard treatment, an ethical consideration depending mainly on whether there is an already existing treatment method. Randomization and the blinding provide statistically valid comparative data on the efficacy and safety of the IMP. Sometimes phase 2 trials are divided into two sub-categories, *phase 2a* and *phase 2b*. Phase 2a clinical trials are mainly attributed to *proof-of-concept* and efficacy, while phase 2b clinical trials are dose-range finding studies. Efficacy is measured by using specific endpoints that correlates with the interaction between the disease and the IMP. Definitive endpoints may be mortality and survival, for instance in case of a clinical trial conducted on a new cancer drug. Surrogate endpoints are markers that indirectly correlate to efficacy, for instance blood pressure in cases of antihypertensive treatment. From phase 2 clinical trials it is often possible to determine effective dosage regimen. (102)

Phase 3 clinical trials aim to confirm efficacy in a larger targeted population than in phase 2, and are often referred to as *therapeutic confirmatory trials*. They are typically conducted as multisite trials over 3-5 years, involving hospitals with different demographic location. This makes it possible to conduct studies with larger sample sizes, often in the range of hundreds or thousands, which provides results that take into account ethnic and demographic variability. Large sample sizes increase the chance of detecting rare AEs. Phase 3 clinical trials are sometimes called *pivotal trials*, reflecting the nature of such studies´ tendency to make- or break the success of the IMP. (102)

Phase 4 clinical trials are observational post-marketing approval studies conducted to see if there are long-time effects or side effects revealed in real-life situation. The patient population will typically be more heterogeneous than in the earlier phase 1-3 clinical trials. (102)

Not all IMPs are tested through traditional linear phase 1-4. For generic drugs, new formulations/entities, and for studies of new administration routes, it may be adequate to conduct phase 1 bioequivalence studies (see 2.7.5 Bioequivalence) that bridge data from authorized pharmaceuticals. (108)

2.7.5 Bioequivalence

According to European Medicines Agency (EMA), a clinical trial aiming to support a MA for a product with a new administration route, must demonstrate bioequivalent to an authorized reference product and treatment regimen. This is thoroughly described in "Guideline on the investigation of bioequivalence" from Committee for Medicinal Products for Human use (CHMP). The approach is similar for generics, new dosage forms and strengths, as for development of entities with new routes of administration, where bridging from existing data from authorized reference products is used to determine bioequivalence in pivotal clinical studies. Crossover study design is the study design of choice, and the sample size must be ≥12. (108)

In order to determine whether the IMP is bioequivalent to the reference treatment, one need to report the PK parameters area under the curve from start to infinity ($AUC_{0-\infty}$), AUC_{0-last}, C_{max} and t_{max}. The area under the curve reflects the clinical exposure of the IMP in the study subject´s blood plasma (or serum). The absorption rate is of importance for the values of C_{max} and t_{max}. The elimination rate constant (λ_z) and termination half-life ($t_{1/2}$) can optionally be reported. (108)

The sampling period must cover the concentration time curve long enough to cover ≥80% of the $AUC_{0-\infty}$. In fact, one needs to determine the λ_z to make a reliable estimate of $AUC_{0-\infty}$. In order to estimate λ_z at least three to four log-linear concentration samples of the terminal phase is needed. It is not required to use a sample period longer than 72 hours for immediate release formulations. For drugs having especially long half-lives, it is considered acceptable to use truncated AUC values at 72 hours (AUC_{0-72h}). Single-dose studies need to show IMP/reference product ratios for both C_{max} and AUC_{0-last} within 80-125% with 90 % confidence interval. (108)

Healthy volunteers are considered adequate subjects for detection of formulation differences in bioequivalence studies. As a general rule, subjects should be between 18 and 55 years old and have normal body mass index (BMI). A medical examination of the subject including clinical laboratory tests and extensive review of medical history should be conducted as a part of the screening for assessment of eligibility. Precautions and special medical investigations may be required depending on the therapeutic class and safety profile of the drug. (108)

2.7.6 Crossover study design

In a crossover clinical trial each included subjects receive all treatments, and the observed effect from one treatment is compared to the effect of the other treatment(s) within the same subject. The subjects are not randomized into different treatment groups, only into which order they receive the different treatments. (109)

The hierarchy of evidence puts crossover design (n-of-1) clinical trials at the very top, over systematic reviews of plural RCTs (meta-analyses) (92) Because the study subjects serves as their own controls, crossover study design provides high statistical power and precision. The reason for this is less variability *within* subjects than *between* subjects. This reduces the sample size requirements compared to independent group design studies. There are two reasons for this: 1) It is necessary to include less subjects to get the same precision on the difference between the interventions (half as many participants if based on two treatment arms). 2) The reduced variance of the estimated difference between the treatments lowers the requirements of the sample size. (109)

The recruitment may therefore be easier for a crossover study compared to a case-control study, since the number of subjects would be lower. In addition to the reduced number itself, the participant may be more willing to join due to more predictability, i.e. knowledge on what treatment they will receive. (109)

There are though some problems to crossover design. Subjects dropping out of the study will influence the results in higher degree than subjects leaving from an independent group design study. *Carry-over effects* from one treatment may also affect on the results from the following treatments. To avoid remaining treatment drug in the body from the first treatment, it is necessary to determine a sufficient washout-period between the treatments. If the first treatment cures or changes the baseline condition in the patient, this could influence on the

validity of the results from the next treatment. The baseline condition must therefor be stabile. (109)

2.7.7 Regulatory considerations - the application process

Before a clinical trial on human subjects is initiated, the sponsor has the responsibility getting the clinical trial approved by both the drug regulatory authority and the IRB/IEC in the respective country. In Norway, NOMA is the drug regulatory authority. (110) The Norwegian IEC is called Regional Committees for Medical and Health Research Ethics (REC). REC has four regional offices, named REC South East, REC West, REC Central and REC North. (111)

After an application for a start-up of a clinical trial is handed over to NOMA, the agency has a 60 days deadline to assess whether the application is approved or not. If NOMA has any questions that need to be answered before approval, they will address their questions within 30-35 days. Then the researchers must answer these questions within day 45 in order to get NOMA to state an answer at day 60. If NOMA does not have any questions, then the application can get approved at day 30-35. If NOMA needs an expert group opinion, the maximum time to get an answer extends to 90 days. (110, 112)

REC will consider the relevance and design of the clinical trial. They will assess whether the trial has a justified risk-benefit ratio, weighted in relation to the benefits for both the individual trial subject and the future patient population (c.f. 2.7.4 Ethical considerations). The suitability of the investigator(s), the facilities involved and documents such as the protocol, IB, informed consent form (including the recruitment and information process in relation to obtaining consent) will also be considered. The size of compensations for both subjects and investigators will be considered. Also, clauses in any contracts between sponsor and investigator, as well as insurance issues are assessed by REC. (112)

The application to REC can be sent in parallel to the NOMA application. The processing time for an application at REC is maximum 60 days, analogue to the NOMA application. REC may ask the researchers to supplement the application once. If so, there will be a “clock-stop”, and the time spent on providing this supplement is added to 60 days. If REC needs to consult an expert group to answer the application, the processing time can be prolonged to 90 days. (110, 112)

2.8 Frameworks for reporting results from studies

2.8.1 CONSORT

Reporting of clinical research should be *clear*, *complete* and *transparent*. In 1996, a guidance called Consolidated Standards of Reporting Trials (CONSORT) was established. The objective of CONSORT is to improve the quality of reporting of clinical trials. This includes a minimum set of recommendations, i.e. a flow-diagram and a checklist on how a trial should be designed, analysed and interpreted. The CONSORT statement has later been revised plural times. (113) Researchers following CONSORT are encouraged to use the CONSORT endorsement, and templates for CONSORT checklist and flow-diagram are available. (114)

In a commentary on the CONSORT 2010 Statement, a dermatologist named Hywel C. Williams wrote:*"Finally, as a practicing clinician, it is so much easier to read trials that follow CONSORT in order to see exactly what was done, by whom and when."* (115)

2.8.2 PRISMA

The importance of systematic reviews and meta-analyses in healthcare has been increasing the recent years, helping clinicians to keep updated on their respective field, but also as a base for development of guidelines for clinical practice. (116) A study published in 1987 examined the scientific quality of 50 review articles in four leading journals, and found that none met all scientific criteria (e.g. quality assessment of studies included into the review). (117) Little had improved in 1996 when a study measuring the quality of reporting in meta-analyses, expressed a growing concern for the standard, and that methodological issues still remained unsolved. (118)

An international group called QUality Of Reporting Of Meta-analyses (QUOROM) was developed in 1999. QUOROM focused on increasing the quality and addressing the sub-optimal reporting in meta-analyses of RCTs. The group updated their guidelines in 1999 to address the concept of systematic reviews, and changed their name to Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA). (116)

The idea of PRISMA is to ensure quality in the reporting of systematic reviews and meta-analyses, and a minimum set of requirements are available on their website. Two central requirements are the need for an a priori search protocol and the including of a flow chart of

the study selection process. PRISMA also helps peer-reviewers and editors to critically appraise systematic reviews and meta-analysis. (116)

2.9 Patent applications

2.9.1 World Intellectual Property Organization

World Intellectual Property Organization (WIPO) is one of the sixteen self-funded specialized agencies of the United Nations (UN) together with the WHO, The World Bank, UNESCO and more. (119)

WIPO´s main task is to ensure respect for the intellectual properties (IP) worldwide. WIPO aims to enable creativity, innovation and protection of IPs. WIPO also manages several international agreements regarding IP. (119, 120)

The Berne Convention was created in 1886. This was the first agreement regarding protection against illegal copying of products in the world. This convention serves as a fundament for WIPO till present day. WIPO was established in 1967, but the organization was first up and running in 1970. It was incorporated into UN in 1974. WIPO´s headquarter is located in Geneva, Switzerland. WIPO consists of 188 member states and 250 organizations serving as observers. (119, 120)

The Patent Cooperation Treaty (PCT) is an international treaty that deals with international patent properties. PCT is administered by WIPO, which provides access to international patent applications in full text on the day of publication through the WIPO PatentScope database. (119, 120)

3 Materials and methods

This master is methodically separated into two parts, A and B. The original plan was to entirely relate the master thesis to the conduction of what is here describes as Part A, the conduction of a clinical trial for a new IN medicinal product. Since this study was postponed for 6 months due to a suspected quality issue of formulation/device, it was necessary to include other aspects into the thesis. Part B represents the contribution of a joint first authorship of a systematic review paper on patent applications for non-injectable naloxone formulation, a collaboration project with the Addiction Department at King´s College, London.

NOTE: The methodology of Part A regarding study design and conduction of the clinical study is nevertheless described, because it was considered necessary to explain the nature and rationale of the study.

3.1 Part A - Materials

To accommodate the previously described need for approved non-injectable medicinal products and evidence-based treatment regimen, this study aimed to support an application for MA of a new IN naloxone product. In addition, this study should provide new knowledge of PK parameters for the IM route.

The IMP of this study is a 14 mg/ml IN naloxone spray, developed by professor Ola Dale at Norwegian University of Science and Technology (NTNU), who also is the PI of the study. The IMP is contains naloxone hydrochloride dihydrate, equivalent to naloxone hydrochloride in the ratio 11:10 (cf. molecular weight, section 2.2.1) The composition is revealed in Table 2.

Table 2 Composition of the IMP

Naloxone hydrochloride "DnE" 14 mg/ml	
Naloxone hydrochloride dihydrate	1,54 g
Povidone K30	0,1 g
Glycerol	1,2 g
Disodium edetate	0,05 g
Benzalkonium chloride solution	0,04 g
Citric acid monohydrate	0,2 g
Sodium citrate dihydrate	0,28 g
Sodium hydroxide/ Hydrochloric acid	qs ad pH 4,3±0,2
Water for injections	ad 100 ml

The spray device used is Aptar Unitdose nasal spray system.

The comparator product is Nalokson B.Braun 0.4mg/ml. This is an injectable NOMA authorized product with the MA-number 06-4660. It is formulated with naloxone hydrochloride dihydrate, but the concentration is corresponding naloxone hydrochloride 0.4 mg/ml. It is stabilized to osmolality level 270-310 mOsmo/kg with pH 3.1-4.5, adjusted by sodium chloride and diluted hydrochloric acid. (22)

Sponsor of the study is Den norske Eterfabrikk (DnE), and the study is monitored by the CRO, Smerud Medical Research. At the time of writing, this two-centre study is under conduction at the clinical trial unit (CTU) at Oslo University Hospital (OUS) and at the CTU at NTNU/St. Olavs Hospital, Trondheim University Hospital. The undersigned master student has completed a GCP course organized by Unit for Applied Clinical Research, NTNU.

The clinical trial was named *"Bioavailability of nasal naloxone compared to injected naloxone"*. The study has two parallel identification codes, OPI 15-002 and SMR-3089. OPI 15-002 is the internal code used within NTNU, while SMR-3089 is the code registered at the CRO. The study was registered with EudraCT no: 2005-0023355-10. The study will hereby be referred to as OPI 15-002.

3.2 Part A - Methods - Contribution to the clinical trial, OPI 15-002

3.2.1 Study design

OPI 15-002 is a randomized, open-label 4-way cross over study.

The study has a sample size of twenty-two subjects, where twelve is recruited to the NTNU/St. Olavs Hospital site. The remaining ten subjects will participate at the study site at OUS.

The included subjects will be randomized into a four-period, four-treatment crossover design, where each treatment representing a visit day at the CTU:

- Treatment A: 1.4 mg IN naloxone
- Treatment B: 2 x 1.4 mg (2.8 mg) IN naloxone
- Treatment C: 0.4 mg IV naloxone
- Treatment D: 0.8 mg IM naloxone

The first intervention visit will be conducted <60 days after the screening (visit 1). There will be a washout period of at least 72 hours between the administrations of the naloxone doses to eliminate carry-over effects and interference on the serum naloxone concentrations from one visit to another.

The order of the four interventions is randomized in a 1:1:1:1 ratio based on the randomization of the possible orders: ACDB, BDCA, CBAD and DABC. There will be no blinding.

Subjects will receive a compensation of 1.000 NOK per intervention day. If they chose to leave the study before completing all visits, they will be compensated for the intervention days completed.

Fifteen venous blood samples will be collected to determine PK and systemic exposure as a result of the naloxone administration at each of the four intervention visits (visit 2-5). The first blood sample will be collected about 10 minutes prior to naloxone and the following 14 blood samples will be collected in a given time regimen up until 360 minutes after naloxone administration. Each blood sample has a volume of approximately 6 ml. The blood samples will centrifuged for 10 minutes at 2200 rcf before pipetted into two cryo-tubes, constituting the A and B samples, and immediately frozen at -20°C before moved to a -80°C freezer within the end of the day.

At each intervention recordings of vitals signs will be performed, hence blood pressure, heart rate, respiration rate and oxygen saturation. Safety blood samples for haematology and biochemistry will be collected after the last PK-blood sample (360 min), along with recordings of symptoms nausea, vomiting, headache, dizziness and nasal irritation.

A follow-up visit (Visit 6) will be performed 3-30 days after the last intervention visit (Visit 5). This visit will address any AE. A follow-up rhinoscopy performed by an ENT specialist, similar to the assessment conducted prior to the first IN administration will be conducted after the last IN administration and at latest the same day as the follow-up visit.

A flow-chart of the study visits is revealed in Table 3, page 38.

Table 3 Study flow chart

Visit no.	**Visit 1 (screening)**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
Time	*-60 days*	*Day 1*	*≥72 h after naloxone administration Visit 2*	*≥72 h after naloxone administration Visit 3*	*≥72 h after naloxone administration Visit 4*	*3-30 days after Visit 5*
Informed consent	x					
Inclusion/exclusion	x	x				
Medical history	x					
Concomitant medication	x	x	x	x	x	
Randomization		x				
Physical examination	x					(x)
Assessment of nasal mucosa (rhinoscopy)	x *					x **
Vital signs	x	x	x	x	x	
ECG	x					
Safety blood samples (haemotology/biochemistry)	x	x	x	x	x	
PK blood samples		x	x	x	x	
Administration of naloxone		x	x	x	x	
Assessment of adverse reactions		x	x	x	x	
Assessment of local irritation in the nose		x	x	x	x	
Assessment of adverse events		x	x	x	x	x

**=Assessment of nasal mucosa prior to the first IN administration of naloxone*
***=Assessment of nasal mucosa after the last IN administration of naloxone and at latest the same day as visit 6.*

The further described methodology refers to activity at NTNU/St. Olavs Hospital, Trondheim University Hospital.

3.2.2 Sketching the information letter and informed consent form

During March 2015 a preliminary sketched information letter and informed consent form was developed based on a draft to a protocol for the clinical trial. The design of the information letter/informed consent form was based upon a template called *Mal informasjonsskriv legemiddelutprøving*, obtained from REC. Also the previous clinical trials on the same project (OPI 13-001, OPI 14-001 and OPI 15-001) used analogue structure on their respective information letters/informed consent forms. The information letter with the included informed consent form, was subsequently collaboratively revised and adapted to the changes in the protocol that later occurred, and was ultimately completed by the CRO during late September 2015.

3.2.3 Developing forms for recording storage information of blood samples

The applications to NOMA and REC South East were submitted in parallel. While waiting for both NOMA and REC to process the application for the planned clinical trial, the recruitment and other practical aspects to the conduction of the study was prepared.

Forms were developed for recording storage information of blood samples and logistics between the CTU, a satellite room with an -80°C freezer and for shipment to an external lab (Vitas AS) where the analyses of the blood samples were to be done.

3.2.4 Recruitment of subjects – development of an info flyer

The planning of recruitment began prior to receiving the approvals from REC and NOMA, although the recruitment it self did not start before approval was granted.

Simultaneously to the planning of recruitment, the undersigned was asked to present the research project to the pharmacy master students at the Faculty of Medicine, NTNU. A short presentation was held with emphasis on the epidemic aspects of the OD situation and rationale for the project, including brief information on already conducted studies on the project. This was also an opportunity to disseminate information about the upcoming study, and perhaps prepare for recruiting process.

Afterwards a brief information flyer about the project was sent by e-mail to all master students and medical students at the Faculty of Medicine, NTNU, distributed through the same senior executive officer at the faculty who had requested the above-mentioned presentation.

3.2.5 Setting up the case report form, CRF

A start-up meeting for the clinical trial was conducted 1th October 2015, attended by the sponsor, the CRO, staff from the CTU, as well as the PI and the undersigned master student. It was already clarified from previous dialogue that the CRO had the responsibility of creating a web-based CRF. At the time of the meeting the web-CRF was not finished. One of the main tasks pointed out in this start-up meeting was the need for a paper edition of the web-CRF given status as source data documentation for the screening visit (visit 1), the intervention visits (visits 2-5) and the follow-up visit (visit 6). The preparation of a paper-CRF was delegated to the undersigned master student, and was a dynamic process conducted in

concordance and dialogue with the CRO, since both the paper-CRF and the web-CRF had to be designed in accordance to the study protocol and still fit various practicalities at the CTU.

Another concrete task was to create binders for each individual subject. These binders were to contain the signed informed consent forms and all subject relevant documents, including the aforementioned paper-CRFs, electrocardiography (ECG) printouts and other laboratory printouts. Since the CRF were subject to a series of changes and updates, it was a time consuming task to keep the binders up to date and ready for start-up of the screening visits on a short notice. The 30th day response form NOMA led to an adjustment of the protocol and hence the CRF. NOMA advised performing of a visual inspection of the nasal mucous membrane before and after nasal administration. This was resolved by including a rhinoscopic assessment conducted by an ear-nose-throat (ENT) specialist at the screening or separate to the first IN administration, and at the follow-up visit or separate visit between the last IN administration and the follow-up visit.

3.2.6 Screening

The application for clinical trial was approved by NOMA at 16th October 2015. The list of potential subjects that had reported interest for the project was then used to invite subjects to screening. Questions from people showing interest were answered in thorough manner, either by e-mail or telephone. Then followed the organizing of screening days, including scheduling screening appointments for each subject.

In order to participate in the study the subjects would have to meet all the following inclusion- and exclusion criteria:

- Inclusion criteria
    1. Provision of a signed written informed consent
    2. Healthy men and women aged 18-40 years
    3. ECG without any pathological abnormalities
    4. Have a BMI range of 18,5-26,0 kg/m^2
    5. Female subjects with child bearing potential must use high efficacy contraception. For the purpose of this study acceptable contraception is defined as sterilization, oral contraceptives, patch, implants, vaginal ring, hormonal IUD or copper IUD through out the study until last visit
    6. Laboratory values within reference values for the following haematology and biochemistry tests:
        a. Haemoglobin (Ref. values; female: 11,7-15,3g/dl, male: 13,4-17,0g/dl)
        b. Creatinine (Ref. values; female: 45-90μmol/l, male: 60-105 μmol/l)
        c. ASAT (Ref. values; female: 15-35 U/l, male: 10-70 U/l)
        d. ALAT (Ref. values; female: 10-45 U/l, male: 10-70 U/l)
        e. γ-GT (Ref. values; female: 10-45 U/l, male: 10-80 U/l)

- Exclusion criteria
    1. Subjects using medication on a regular basis, including regular use of nasal spray of any form
    2. History of prior drug allergy
    3. Subjects having local nasal disease or nasal surgery for the last two months
    4. Pregnant and breast-feeding women. A serum HCG below 3 U/l must be demonstrated in females of child-bearing potential at screening visit
    5. Current drug or alcohol abuse, which in the opinion of the Investigator should preclude participation in the study
    6. Have received another new medical chemical entity (defined as a compound which have not been approved for marketing) or has participated in any other clinical study that included drug treatment within 3 months of the administration of investigational product in this study
    7. Hypersensitivity to naloxone or any of its excipients.
    8. Investigator considers subject unlikely to comply with study procedures, restrictions and/or other requirements.

Seventeen potential participants were screened at 28th October and 4th November 2015. The screening (visit 1) included an approximately 30 minutes long conversation between the subject, the undersigned master student and a medical screening doctor (investigator) where the rationale, background and aims of the study were explained. The voluntariness and the subject´s free will to leave at any time were emphasized. Eligible subjects were then asked if she/he wanted to participate to the study. The informed consent form was first signed by the subject, followed by the signature of the undersigned master student. A complete review of

the subject´s past medical history, diseases and concomitant medication was undertaken by the medical screening doctor, and documented by filling in the paper-CRF.

The subject´s height and weight was measured and used to calculate the BMI. A vital sign evaluation was performed, including measurement of blood pressure, heart rate, respiration rate and oxygen saturation. Blood samples for clinical chemistry were collected. A twelve lead ECG was performed, and evaluated by a cardiologist. Female subjects were tested for pregnancy by a blood sample of human chorionic gonadotropin (HCG) level.

Individual appointments at the ENT specialist at the outpatient clinic at St. Olavs Hospital were arranged. All eligible subjects had their first visit to the ENT specialist completed shortly after the screening visit, and prior to first IN treatment, in accordance to the protocol.

3.2.7 Re-screening and screening of new subjects

Because the postponement of the clinical trial exceeded the validity of the screening (60 days), it was necessary to perform a re-screening visit of the subjects included from the initial screening. This was approved by REC 2nd March 2016, which also in the same resolution extended the validity of the ECG and assessment of nasal mucosa (rhinoscopy) from the initial screening to 26 weeks.

The re-screening of subjects included from the initial screening was performed in the period 16-30th March 2016.

Screening of nine new subjects replacing excluded subjects and subjects withdrawn by own will, was performed in the period between 10th March and 15th April 2016.

3.2.8 Postponement of the clinical trial – new tasks needed.

The planned start-up of the clinical trial was put on hold shortly after the two screening days in October and November. It was discovered random unsatisfactory deviations of the amounts of naloxone delivered by some of the spray devices.

The same spray device (Aptar) is used in other pharmaceutical products, including the IN anti-migraine product, Imigran (sumatriptan) and the cancer breakthrough painkiller, Instanyl (fentanyl). Common for both Imigran and Instanyl is the lack of viscosity increasing excipients. (121, 122) At this point there was a suspicion that it could be the IMP´s viscosity

or other formulation aspects that was causing the deviations. The sponsor started an investigation to find out the reason(s) behind, and address these deviations. The sponsor wanted to clarify this issue before continuing the study, and decided to postpone the start up of the clinical trial.

Consequently, the basis for a complete master thesis was lost, and a new related direction had to be found.

3.3 Methods Part B - Review of non-injectable naloxone formulations

At the time of the postponement of the clinical trial (Part A), John Strang, Head of the Addiction Department at King´s College, London, his PhD student Rebecca McDonald and professor Ola Dale was planning to cooperate on a review of available literature, including the writing of a review article based on a systematic search and analysis of patent applications regarding non-injectable naloxone formulations for opioid OD reversal, as well as a review of peer-reviewed literature identified through PubMed search. There was a need for pharmaceutical competence, and the undersigned was therefore invited to take part in this project that also included a joint first authorship of the paper. See reference (123).

An initial exchange of information on relevant patent applications between the two research groups was done. The inclusion criteria for the review were determined to be patents regarding non-injectable naloxone that contained human in vivo PK data.

Later, an a priori search protocol in accordance with the PRISMA requirements was established. This included both a search for relevant patent applications and a systematic search for peer-reviewed literature, as further explained in a three-stage approach described here:

Stage 1: The WIPO PatentScope database (https://patentscope.wipo.int/search/en/search.jsf) was searched for non-injectable naloxone formulations. The WIPO PatentScope was searched for English-language ("Language: EN") patents registered with any international patent office ("Office(s): all") containing the search term "naloxone" within their first page. According to aim 2 (see section 1.2 Part B - Review of patent applications of non-injectable naloxone), only patents for non-injectable naloxone containing human PK data were included for further analysis.

Stage 2: Human PK data were extracted and summarized from relevant patent records. The PK values for C_{max}, $AUC_{0-\infty}$ and AUC_{0-last} were generated into dose-adjusted per mg values, to allow for comparability between the formulations.

The calculation of per mg-adjusted values of and C_{max} and AUC was done as follows:

$$C_{max}\text{per mg (ng/ml)} = \frac{C_{max}\ (ng/ml)}{Dose\ (mg)}$$

$$AUC\ \text{per mg (ng} * \text{h/ml)} = \frac{AUC\ (ng * h/ml)}{Dose\ (mg)}$$

In cases where variation was reported as coefficient of variation (CV%) this was converted to standard deviation (SD) for consistent comparison, by the use of the following formula:

$$SD = \frac{CV\%\ x\ Mean}{100}$$

If information about F or F_{IM} were not disclosed, these values were computed manually as follows:

$$F = \left(\frac{AUC_{0-\infty\ IN}}{AUC_{0-\infty\ IV}}\right) \times \left(\frac{Dose_{IV}}{Dose_{IN}}\right)$$

$$F_{IM} = \left(\frac{AUC_{0-\infty\ IN}}{AUC_{0-\infty\ IM}}\right) \times \left(\frac{Dose_{IM}}{Dose_{IN}}\right)$$

The PK parameters, including those manually calculated, were included into a table for easier comparison.

For the IN treatments, t_{max} as well as per mg-adjusted values of $AUC_{0-\infty}$ and C_{max} were plotted against volume administered per nostril to see if the size of any of these parameters were associated with volume. Also, to see how dose impacts the $AUC_{0-\infty}$, C_{max} and t_{max} values, these values were plotted against dose.

Stage 3: PubMed was searched for human PK data of injection-free naloxone delivery in order to supplement and crosscheck the identified patent data obtained in Stages 1 and 2. Based on the aforementioned systematic review (72) which pointed at nasal, buccal and SL route as potential non-injectable routes, the Boolean search query "(nasal OR intranasal OR nose OR buccal OR sublingual) AND naloxone AND pharmacokinetics" was used.

It was known before the WIPO PatentScope search was conducted that University of Kentucky and professor Daniel Wermeling, known for the developing of an IN naloxone product, could be involved in other patents of interest. An additional search was therefore conducted within the WIPO PatentScope database using the keyword “Wermeling”. A similar search was conducted using the Boolean search query “naloxone AND Kentucky”.

The abovementioned stages were conducted by Rebecca McDonald and Øyvind D. Glende (undersigned), under supervision of professor John Strang and professor Ola Dale. First, a common agreement about the methods was established. McDonald conducted the WIPO PatentScope and PubMed searches, followed by a selection process collectively conducted by Glende and McDonald. Glende retrieved the PK and formulation information from relevant patents, collated the information into tables and performed exploratory analysis of the PK parameters.

3.3.1 Retrieving stability data from patent applications

In addition to what was relevant for the review article, the included patent applications from the WIPO PatentScope search were also reviewed for stability testing data. It was of interest to see if the patent applications contained information on formulation aspects, hence testing and choice of different excipients with regard to stability and degradation. In same manner it was interesting to see which pH and tonicity levels used by the applicants, and the rationale behind the choice of such levels.

This data was only retrieved from the patents already included into the aforementioned patent review.

4 Results

4.1 Part A - The clinical trial, OPI 15-002

4.1.1 Information letter, including informed consent form

The final edition of the information letter was completed in cooperation with the CRO based on the draft made during the spring 2015.

The information letter has an introduction part describing the background and rationale for the study, as well as clarifying the study design. The introduction also provides information on possible benefits (none) and disadvantages and a clarification on how health information, including the blood samples, will be handled and safeguarded. The authorizations from both REC and NOMA are accounted for in this section. A specific paragraph, emphasizing that participation is voluntary and that subjects are free to leave the study at any time without giving any explanation, is included in this section.

The information letter is further divided into a Chapter A and B, where Chapter A is introduced with a compilation of the inclusion/exclusion criteria. Chapter A also describes the background, rationale, aims, study design and safety in details. Information of insurance, compensation and contact information is also described in chapter A of the information letter. Chapter B is addressing topics such as safeguarding of privacy, bio-banking and information on how the study is financed.

The informed consent form is integrated into the final section of the information letter. *See Appendix A.*

4.1.2 Forms for recording storage information of blood samples

In accordance to GCP, forms for recording storage information of blood samples and logistics between the CTU, the satellite room with an -80°C freezer and shipment information to the external analysis lab (Vitas AS), safeguards overview and control over where the respective blood samples are located at any time.

It was developed separate forms for A-samples and B-samples. *See Appendix B and C.*

4.1.3 Recruitment of subjects - the information flyer

After the presentation for the pharmacy master students and the brief flyer was distributed, there was a good response from students wanting to know more about the project, and even at this point signalizing their willingness to participate. More surprisingly was the response from people showing interest from outside the Faculty of Medicine, NTNU. Some said they had heard about the nasal naloxone project in media, and caught interest when hearing from students whom had received the flyer. All questions from people showing interest were answered properly, mostly by e-mail, but also by telephone.

It was also at this point necessary to emphasize to all potential subjects that the study depended on approval from both REC and NOMA before formal recruitment (i.e. screening) could start. The list of people showing interest to participate later became the fundament for recruitment of subjects to the upcoming screening.

After the approval from NOMA and REC was received, the sponsor introduced a flyer developed by the CRO, on the grounds that the flyer developed by the undersigned might be too suggestive. *See Appendix D and E.*

4.1.4 Paper-CRF

The developed paper-CRF reflects the requirements according to the study protocol. The natural sequence of events during the study visits is reflected by the order of the recordings in the paper-CRF, which at the same time mirrors the web-CRF. *See Appendix F.*

The individual subject´s paper-CRFs are inserted into separate binders. The paper-CRF is clearly divided with separator sheets into six different sections consisting of the screening (Visit 1), the four PK sessions (Visit 2-5) and the follow-up visit (Visit 6).

The paper-CRF serves as collection of all source data from each individual subject. In addition to the sections specific for each visit (including screening and follow-up visit), the paper-CRF contains registration forms for AR, AE, a specific form for registration of local irritation in the nose, a medical history log and a concomitant medication log. Finally, the paper-CRF contains a form for the PI´s signature, attesting that the paper-CRF is completed and that all data is entered accurately and correctly under the responsibility of the PI.

4.1.5 Results from the screening

The initial screening was performed at the CTU at St. Olavs hospital, at 28th October and 4th November 2015.

Seventeen subjects were screened during the initial screening, whereof ten female and seven male subjects. The age of the subjects ranged between 20 to 39 years, with a mean age of 25.4 years, SD=6.1. Height and weight were captured for fourteen of the screened subjects (7 males, 7 females) with measurements in the range of 162.9-191.8 cm (mean=175.3 cm, SD=9.1) and 52.4-85.1 kg (mean=69.3 kg, SD=11.6). The corresponding BMI values were in the range of 17.7-29.2 kg/m^2 (mean=22.5 kg/m^2, SD=2,8).

Six subjects, all females, were excluded based on the screening. Five subjects did not meet the inclusion criteria. Two subjects had a BMI outside the reference value of 18,5–26,0 kg/m^2, (29,2 and 17,7 kg/m^2, respectively), and three subjects did not use high efficacy contraception. Finally, one subject was excluded based on the assessment of the nasal mucosa (rhinoscopy) that revealed allergic rhinitis, moderate secretion and swelling, plus a single nasal polyp.

Box-plots of BMI among screened subjects compared to included subjects, shows that BMI did not exclude male subjects, and that BMI was slightly lower for female subjects (Figure 3).

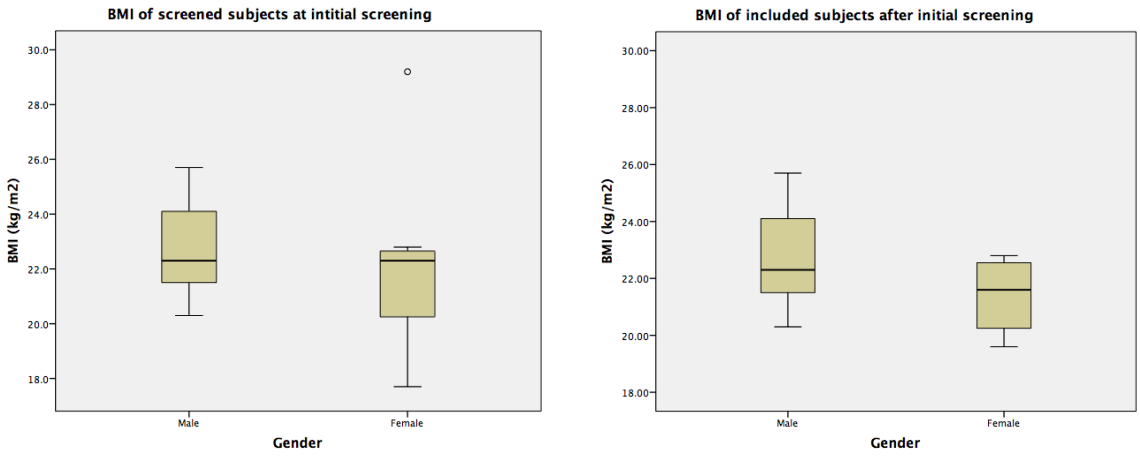

Figure 3 Box-plots of BMI among screened (left) and included subjects of the initial screening (right)

Eleven subjects met the inclusion- and exclusion criteria, and were eligible for participation to the study.

4.1.6 Re-screening and screening of new subjects

The organization of screening appointment for the days in October and November 2015 and the re-screening and subsequent screening was conducted through dialogue (mostly by e-mail) with each individual subject. Some general information was sent out in common to all relevant subjects, but all incoming questions were answered on individual basis. Maintaining a low threshold for subjects to make contact and ask questions was considered important. An exemplified e-mail sent out in common prior to re-screening can be seen in *Appendix G.*

Among the eleven subjects included at the initial screening by autumn 2015, six (4 males, 2 females) subjects met for re-screening in March 2016. One of the initially included subjects had at this point exceeded the upper limit of age, and was therefor not re-screened. Four subjects rejected further participation. No participants were asked the reason for rejection, but several subjects reported up-coming exams as reason. Among the six re-screened subjects, two females were excluded due to start-up of concomitant medication (long-period antibiotics and antihistamine treatment, respectively).

Nine new subjects (5 males, 4 females) were screened (including ECG and nasal mucosa assessment) to complete twelve included subjects at the study site, whereof one male was excluded due to elevated levels of ASAT/ALAT test.

The age for the twelve included subjects after re-screening and screening were in the range of 21-29 years (mean=24.5 years, SD=2.3). Height and weight were in the range of 166.1-191.3 cm (mean=179.1 cm, SD=8.6) and 59.9-90.3 kg (mean=71.8 kg, SD=9.3). This gives a corresponding BMI range of 20.9-24.7 kg/m^2 (mean=22.3 kg/m^2, SD 1.1). Figure 4 and 5 (page 51) shows box-plots of BMI among male and female subjects and a CONSORT flow diagram of inclusion/exclusion process.

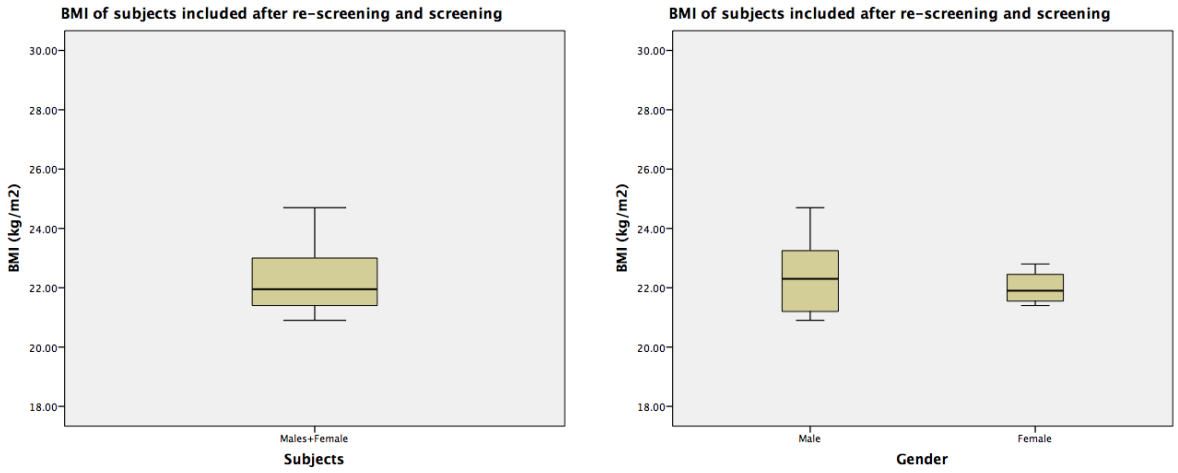

Figure 4 Box-plot of BMI among included subjects regardless of gender (left) and by gender (right)

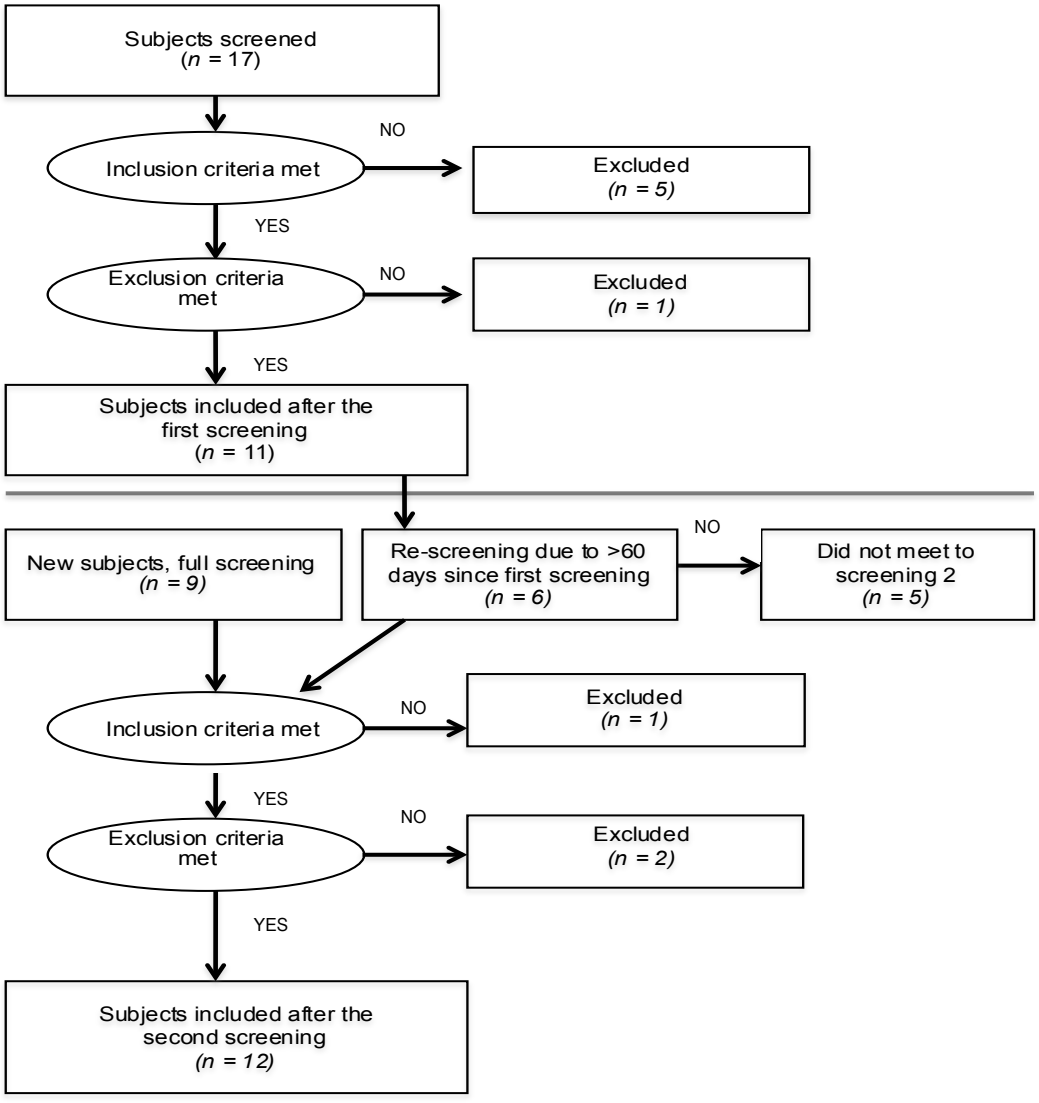

Figure 5 CONSORT flow diagram of inclusion and exclusion of subjects

4.2 Part B - Review of non-injectable naloxone formulations

This review is also presented as a manuscript for a systematic review article submitted for publication 11th May 2016. The name of the review article is "Patent applications for non-injectable naloxone for opioid OD reversal: search and retrieve analysis of World Patent records". See reference (123).

The results presented and described in the article are rewritten and rendered in section 4.2.1 through 4.2.4.

4.2.1 Stage 1 - selection of patent applications

522 records were identified from the WIPO Patentscope database search through the First page search with the search term "naloxone". A cross-check for known applications were conducted, and it was found that the 522 records did not capture the Lighlake patent(s) which cover the approved Narcan® nasal spray, because the search term "naloxone" was not present in the first page of the patent. 5 additional matching patents were added manually based on a front-page with the search term "Lightlake".

480 patents were excluded based on its title. Of the remaining 47 records, 10 were removed based on their abstracts. The remaining 37 patents were downloaded, and full-text reviewed for in vivo data (including attachments and supporting documents). 14 patents contained in vivo PK data, whereof 10 were excluded for the following reasons: 5 patents reported animal data and 6 patents were duplicates (earlier or later versions of a patent which differed only by patent claims and/or the same patent was applied for in different countries). The three patents eligible for inclusion are presented in Table 4.

Table 4 Patents included

Patentnumber	Year of publishing	Name	Reference
WO/2012/156317	2012	Euro-Celtique	(124)
WO/2015/095644	2015	AntiOp	(125)
WO/2015/136373	2015	Lightlake	(126)

The three patents all comprise the inventions of formulations for IN naloxone spray administration, but one applicant (Euro-Celtique) also presented PK data for a SL formulation. The selection process is shown as a PRISMA flow-chart in Figure 6, page 53.

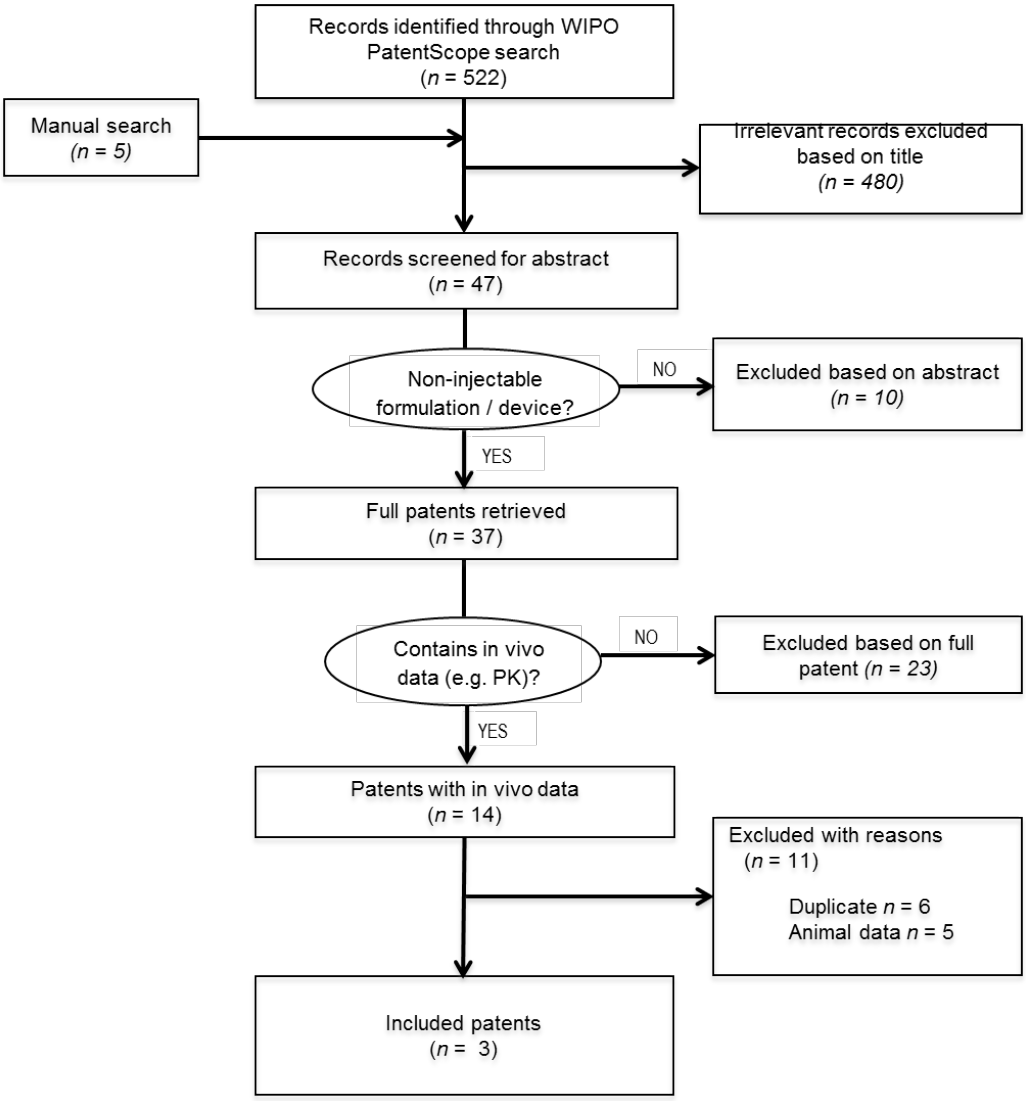

Figure 6 PRISMA diagram of the patent selection (123), with permission

The additional search for relevant patent applications from professor Daniel Wermeling and University of Kentucky generated no additional patent applications to include.

4.2.2 Stage 2 - comparison of formulations

The Euro-Celtique (WO/2012/156317) patent revealed no other formulation details except that the two IN formulations used, contained naloxone hydrochloride at the concentrations 20mg/ml and 40 mg/ml, and that the SL formulation contained naloxone hydrochloride at concentration 16 mg/ml diluted in 0,9% sodium chloride solution adjusted to pH 5.6.

Information on the formulations presented in the patents by AntiOp (WO/2015/095644) and Lightlake (WO/2015/136373) is shown in Table 5.

Table 5 Formulations from patents with excipients displayed per ml (123), with permission

Function	Component	Formulation					
		AntiOp 10mg/ml	Lightlake 10mg/ml	Lightlake 20mg/ml	Lightlake 40mg/ml	Euro-Celtique 20 mg/ml	Euro-Celtique 40mg/ml
Active ingredient	Naloxone HCl	n/a	10mg	n/a	n/a	20mg	40mg
	Naloxone HCl dihydrate	10mg *	n/a	22mg *	44mg *	n/a	n/a
Buffer	Citric acid anhydrous	4.8mg	n/a	n/a	n/a	NR	NR
Preservative	Benzyl alcohol	5.0mg	n/a	n/a	n/a	NR	NR
	Disodium EDTA dihydrate	3.7mg	n/a	n/a	n/a	NR	NR
	Disodium ededate	n/a	n/a	2.0mg	2.0mg	NR	NR
	Benzalkonium chloride	n/a	0.1mg	0.1mg	0.1mg	NR	NR
Isotonicity adustment	Sodium chloride	qs. 365-425 mOsm	7.4mg	7.4mg	7.4mg	NR	NR
pH adjustment	HCl	qs pH 4.25±0.1	qs target pH	qs pH 4.5	qs pH 4.5	NR	NR
	NaOH	qs pH 4.25±0.1	n/a	n/a	n/a	NR	NR
Carrier/solvent	Purified water	qs 1ml	qs 1ml	qs 1ml	qs 1ml	NR	NR

Annotations: * 1.1mg of Naloxone HCl dihydrate are dose-equivalent to 1mg Naloxone HCl (ratio 11:10); NR = not reported.

There are similarities, but also differences between the formulations of AntiOp and Lightlake. Neither AntiOp nor Lightlake used viscosity enhancers to increase the residence time in the nasal mucosa or absorption enhancers in their formulations. AntiOp reported stability tests on different formulations with and without such excipients, but chose to exclude them due to observed increased degradation. Both AntiOp and Lightlake used edetic acid (disodium EDTA dihydrate or disodium edetate, respectively) as preservative. Other similarities were the use of sodium chloride for osmotic adjustment and hydrochloric acid to adjust pH, although AntiOp in addition used sodium hydroxide for pH adjustment.

When it comes to differences, the AntiOp formulation contained a citrate buffer, while Lightlake´s did not. Another difference was the choice of preservatives. AntiOp used benzyl alcohol while Lightlake used benzalkonium chloride for preservation of their formulations.

The pH was slightly more acidic for the AntiOp formulation (pH 4.25) compared to the Lightlake formulation (pH 4.5).

Which naloxone form used by AntiOp is ambiguously reported in the patent application. In the part of the patent describing formulation aspects AntiOp describes a formulation containing 10 mg/ml naloxone hydrochloride dihydrate, whereas the PK section describes a formulation of 10 mg/ml naloxone hydrochloride. 10 mg/ml naloxone hydrochloride dihydrate is equivalent to 9.1 mg/ml naloxone hydrochloride. (17, 18)

4.2.3 Stage 2 - comparison of pharmacokinetics

All the three included patent applicants used crossover study design, although the sample sizes differed from 7 to 35 subjects per treatment arm.

AntiOp described two crossover studies, hereby referred to as Trial 1 and Trial 2. Trial 1 had six treatment arms, whereof three treatment arms covered the reference administration routes IV (0.4 mg), IM (1 mg) and SQ (1 mg) administration routes. Two IN treatment arms (1 mg and 2 mg) covered the 10 mg/ml IMP given as 0.1 ml into one or two nostrils respectively, and a final IN treatment arm using non-concentrated 1 mg/ml solution with a MAD attached to a syringe, analogue to the off-label formulations used in various THN programs and ambulance services.

AntiOp´s Trial 2 was a three-way crossover study. It had a treatment arm for 0.4 mg IM administration, a 2 mg IN administration (1 spray of 0.1 ml into each nostrils) and a 2+2 mg IN administration (2 sprays of 0.1 ml into each nostril with 5 minutes interval).

Lightlake presented results from two different crossover studies, hereby referred to as Study 1 and Study 2. Study 1 was a three-way crossover study in which they tested a 10 mg/ml IN formulation given as 2 mg (1 spray of 0.1 ml into each nostrils) and 4 mg (2 sprays of 0.1 ml into each nostrils) against 0.4 mg IM administration.

In Study 2, Lightlake was testing two concentration of the IN formulation, i.e. 20 mg/ml and 40 mg/ml. In this five-way crossover both concentrations were administered as 0.1 ml into one or two nostrils, corresponding a dose-range of 2-8 mg. Study 2 also included a 0.4 mg IM treatment arm.

Euro-Celtique conducted a four-way crossover study that included the two IN doses 8 mg (20 mg/ml) and 16 mg (40 mg/ml) given as 0.2 ml into each nostril. Euro-Celtique included a 1mg IV injection, but also a 16 mg/1 ml liquid SL saline solution administered and kept under the tongue for 5 minutes. In the main document of the Euro-Celtique patent, the reported IN

PK results are dose-adjusted to 1.2 and 1.6 mg. The summarised original PK data for the actual doses were available in table format as an appendix.

The Euro-Celtique patent reported bioavailability as F, whereas the more recent Lightlake and AntiOp patents provided F_{IM} values, in accordance to NDA criteria received by FDA in 2012. (73)

Intranasal route:

F: Euro-Celtique reported F values of 32% and 27% for their 20 mg/ml and 40 mg/ml respectively. We were not able to replicate those values by manually calculation of F based on neither the dose-adjusted $AUC_{0\text{-last}}$ values from the description of the patent nor the original $AUC_{0\text{-last}}$ and $AUC_{0\text{-}\infty}$ values provided in the appendix. AntiOp did not report F values, but since they included an IV arm into their Trial 1, we were able to manually compute F=36% (0.1 ml one nostril only) and F=42% (0.1 ml per nostril) for the 10 mg/ml formulation. Computed F value for non-concentrated off-label formulation was only 11%. We were not able to estimate F values for Lightlake´s studies, since they did not include IV treatment arms.

F_{IM}: The highest F_{IM} value (57 %) was achieved in the Study 1 by Lightlake, when 0.1 ml of the 10 mg/ml formulation was administered into both nostrils. Interestingly, the F_{IM} was lower (48%) when the administration volume per nostril was doubled to 0.2 ml. The F_{IM} values for the 20 mg/ml were 54% (0.1 ml, one nostril) and 55% (0.1 ml, each nostrils). The 40 mg/ml achieved 49% (0.1 ml, one nostril) and 45% (0.1 ml, each nostrils). AntiOp´s reported F_{IM} values for the 10 mg/ml formulation were 34% (0.1 ml, one nostril), 31-39% (0.1 ml, each nostrils), and 26 % (0.1 ml, each nostril + re-administration after 5 minutes, i.e. total volume of 0.2 ml per nostril). The non-concentrated off-label formulation (1 mg/ml) achieved a F_{IM} of 10%.

Per mg adjusted AUC and C_{max}: The Lightlake 20 mg/ml formulation achieved the highest C_{max} value (1.66 ng/ml) and $AUC_{0\text{-}\infty}$ value (2.48 ng*h/ml) when 0.1 ml was administered into each nostril. Based on the original PK data from the appendix, $AUC_{0\text{-}\infty}$ value of Euro-Celtique´s 20 mg/ml was even higher (2.76 ng*h/ml) than Lightlake´s, but the respective C_{max} value for the same treatment arm was found to be much higher by per mg adjusting the original data than by the reported data. The lowest C_{max} (0.27 ng/ml) and $AUC_{0\text{-}\infty}$ (0.45 ng*h/ml) were achieved by the non-concentrated off-label formulation (1 mg/ml) from AntiOp´s Trial 1.

t_{max}: The t_{max} values for the IN formulations ranged from 0.27 hours (AntiOp, 1 mg/ml, 1 ml into each nostril) to 0.5 hours (AntiOp 10 mg/ml, 0.1 ml into one nostril, Lightlake 40 mg/ml, 0.1 ml into one nostril).

$t_{1/2}$: The longest IN $t_{1/2}$ values was reported by Euro-Celtique with 9.5 hours (20 mg/ml) and 9.1 hours (40 mg/ml), but these data were only available through the original data appendix which for $t_{1/2}$ only included 4 subjects. The $t_{1/2}$ values for the Lightlake and AntiOp treatment arms fell in the range 1.2-2.1 hours.

Sublingual route:

The mean parameters achieved by the 16 mg/ml SL treatment arm included in the Euro-Celtique patent was F=1%, per mg adjusted $AUC_{0-\infty}$= 0.06 ng/ml, per mg adjusted C_{max}= 0.09 ng/ml, t_{max}= 0.67 hours (median) and $t_{1/2}$= 1.13 hours.

The summarized PK parameters are shown in Table 6, page 58.

Table 6 PK parameters from patent applications (123), with permission

Route	Study	n	Conc. (mg/ml)	Nostrils #	Dose (mg)/ volume (ml)	F%	F_{IM}%	t_{max} (h)	$t_{1/2}$ (h)	Observed values			Dose-adjusted values (permg)		
										C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng*h/ml)	AUC_{0-last} (ng*h/ml)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng*h/ml)	AUC_{0-last} (ng*h/ml)
IV	AntiOp Trial 1	13	0.4		0.4/1.0			*0.03±0.1*	*1.28±0.2*	*3.87±2.7*	*1.67±0.5*		*9.68*[a]	*4.18*[a]	
	Euro-Celtique	11	1		1.0/1.0			0.85±1.6	0.89±0.1[e]	17.9±29.9	12.6±12.4[e]	10.5±7.2	17.9[a]	12.6[a]	10.5[a]
IM	AntiOp Trial 1	13	NA		1.0/NA	106[a, d]		*0.33±0.5*	*1.41±0.3*	*2.54±1.0*	*4.43±1.2*		*2.54*[a]	*4.43*[a]	
	AntiOp Trial 2	34	0.4		0.4/1.0			*0.17 (0.1, 1.0)*	1.38±0.3	1.05±0.4	1.67±0.4		2.63[a]	4.18[a]	
	Lightlake 1	14	0.4		0.4/1.0			0.34±0.1	*1.21±0.2*	0.77±0.2	1.42±0.3	1.38±0.3	1.91[a]	3.55[a]	3.45[a]
	Lightlake 2	28	0.4		0.4/1.0			*0.42 (0.1, 2.0)*	1.19[b]	0.91±0.3	1.83±0.4	1.79±0.4	2.26±0.7	4.57±1.1	4.48[a]
SQ	AntiOp Trial 1	13	NA		1.0/NA	99[a, d]	94[a, d]	*0.17±0.3*	*1.59±0.6*	*2.72±0.8*	*4.15±1.1*		*2.72*[a]	*4.15*[a]	
IN	AntiOp Trial 1*	13	10	2	2.0/0.2	42[a, d]	39[a, d]	*0.42±0.3*	*1.53±0.2*	*1.95±1.1*	*3.47±0.8*		*0.98*[a]	*1.74*[a]	
	AntiOp Trial 1*	13	10	1	1.0/0.1	36[a, d]	34[a, d]	*0.50±0.2*	*1.41±0.3*	*0.84±0.5*	*1.52±0.5*		*0.84*[a]	*1.52*[a]	
	AntiOp Trial 1	7	1	2	2.0/2.0	11[a, d]	10[a, d]	*0.27±0.1*	*1.64±0.3*	*0.53±0.2*	*0.90±0.2*		*0.27*[a]	*0.45*[a]	
	AntiOp Trial 2*	33	10	2	2.0/0.2		31[a, d]	*0.33 (0.3, 0.8)*	1.37±0.3	1.78±1.0	2.63±1.3		0.89[a]	1.32[a]	
	AntiOp Trial 2*	35	10	2+2[c]	4.0/0.4		26[a, d]	*0.42 (0.2, 1.0)*	1.41±0.3	3.06±1.6	4.42±2.2		0.77[a]	1.11[a]	
	Lightlake 1	14	10	2	2.0/0.2		57	0.33±0.1	1.19±0.1	2.32±1.0	3.44±1.0	3.41±1.0	1.16[a]	1.72[a]	1.71
	Lightlake 1	14	10	2	4.0/0.4		48	0.31±0.1	1.22±0.1	4.55±2.9	5.68±1.6	5.63±1.6	1.14[a]	1.42[a]	1.41
	Lightlake 2	28	20	1	2.0/0.1		54	*0.33 (0.3, 1.0)*	1.70[b]	3.11±1.1	4.86±1.5	4.81±1.5	1.56±0.6	2.43±0.7	2.41
	Lightlake 2	28	20	2	4.0/0.2		55	*0.33 (0.1, 0.5)*	2.09[b]	6.63±2.3	9.91±2.7	9.82±2.7	1.66±0.6	2.48±0.7	2.46
	Lightlake 2	28	40	1	4.0/0.1		49	*0.50 (0.2, 1.0)*	2.00[b]	5.34±2.4	8.87±3.3	8.78±3.3	1.34±0.6	2.22±0.8	2.20
	Lightlake 2	28	40	2	8.0/0.2		45	*0.33 (0.2, 1.0)*	1.91[b]	10.3±4.0	16.1±3.8	15.9±3.8	1.29±0.5	2.01±0.5	1.99
	Euro-Celtique	11	20	2	8.0/0.4	22)[a, d]		0.34±0.2	9.48±3.9[f]	12.8±4.5	22.0±4.2[f]	20.1±4.9	1.60[a]	2.76[a]	2.51[a]
	Euro-Celtique	12	40	2	16.0/0.4	(21)[a, d]		0.39±0.2	9.09±2.7[f]	18.3±7.5	42.8±10.6[f]	32.8±10.2	1.14[a]	2.67[a]	2.05[a]
SL	Euro-Celtique	11	16		16.0/1.0	(1)[a, d]		3.91±10.6	1.13±0.2[f]	0.90±0.4	1.50±0.4[f]	2.67±1.8	0.06[a]	0.09[a]	0.17[a]

Annotations: Values for t_{max}, C_{max}, AUC, $t_{1/2}$ denote mean ±SD, except for values in italics. Values in italics denote median ±SD or median (min, max). Inconsistent information between the patent and the PK data whether the formulation contained10mg/ml Naloxone HCl dihydrate or 10mg/ml Naloxone HCl. Dose-adjusted values (per mg) in table are based on Naloxone HCl. [a] calculated values; [b] harmonized mean; [c] re-administration after 5 minutes; [d] calculated F and F_{IM} values based on $AUC_{0-\infty}$; [e] sample size = 3; [f] sample size = 4; NA = not available; IV = Intravenous; IM = Intramuscular; SQ = Subcutaneous; IN = Intranasal; SL = Sublingual.

Figure 7 displays plots of per mg dose-adjusted $AUC_{0-\infty}$ and C_{max} values and t_{max} values against volume (left side), and $AUC_{0-\infty}$, C_{max} and t_{max} values against dose (right side). The graphs indicate a positive linear correlation between dose and $AUC_{0-\infty}$ and C_{max}, and a negative correlation between volume and $AUC_{0-\infty}$ and C_{max}.. There is no clearly apparent associations for t_{max}.

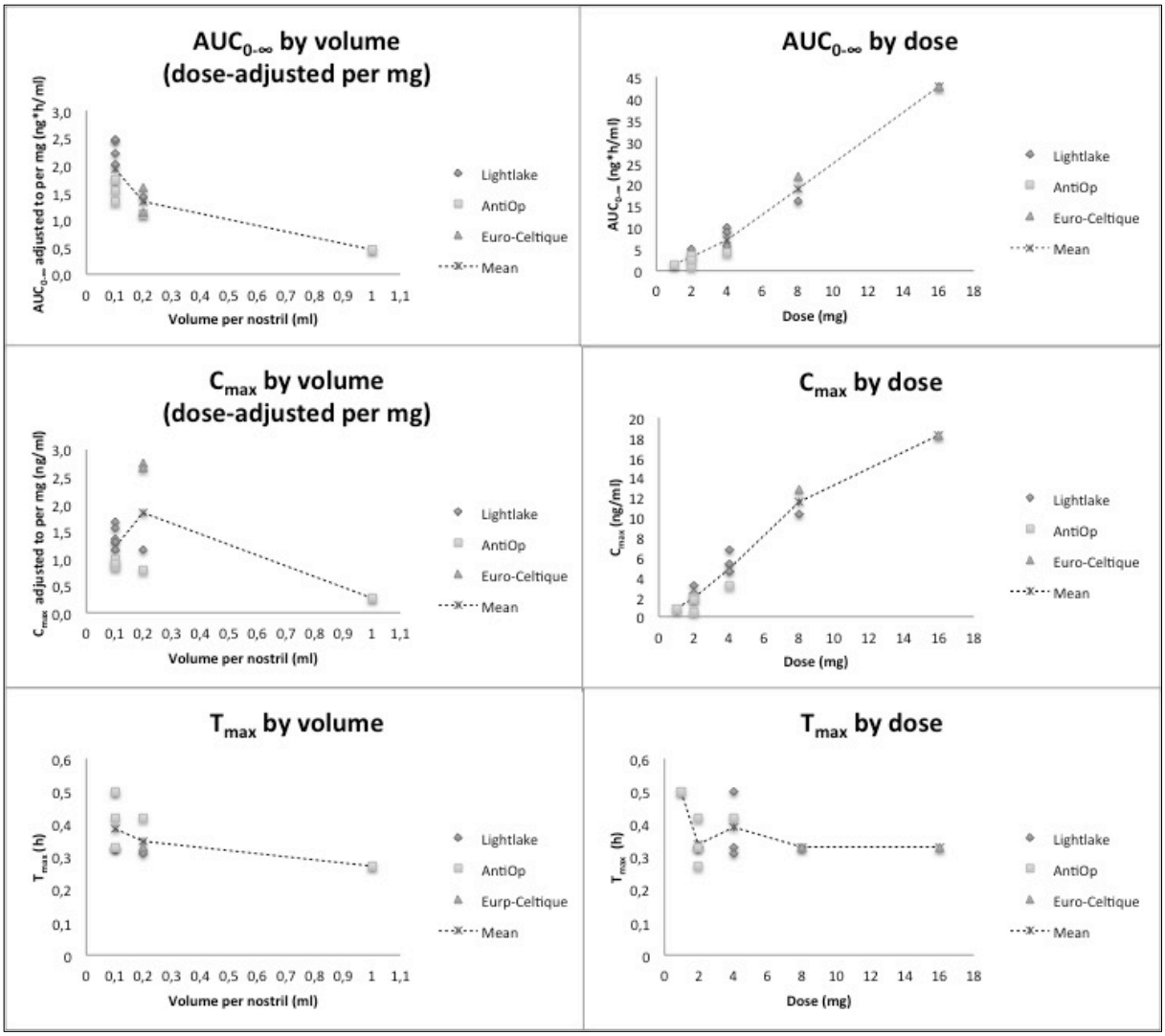

Figure 7 $AUC_{0-\infty}$, C_{max} and t_{max} plotted by volume and dose (123), with permission

4.2.4 Stage 3 - Results from PubMed search

The PubMed search for supplementing and/or crosschecking peer reviewed papers for naloxone PK matched with the three routes suggested administration routes nasal, buccal and sublingual administration, generated 56 matches. 46 papers were excluded based on abstract

due to no data from human naloxone studies. The ten remaining papers were examined on full text basis, whereof four were excluded based on not containing PK data and one because it was a review article. A flow chart of the PubMed selection is displayed in Figure 8.

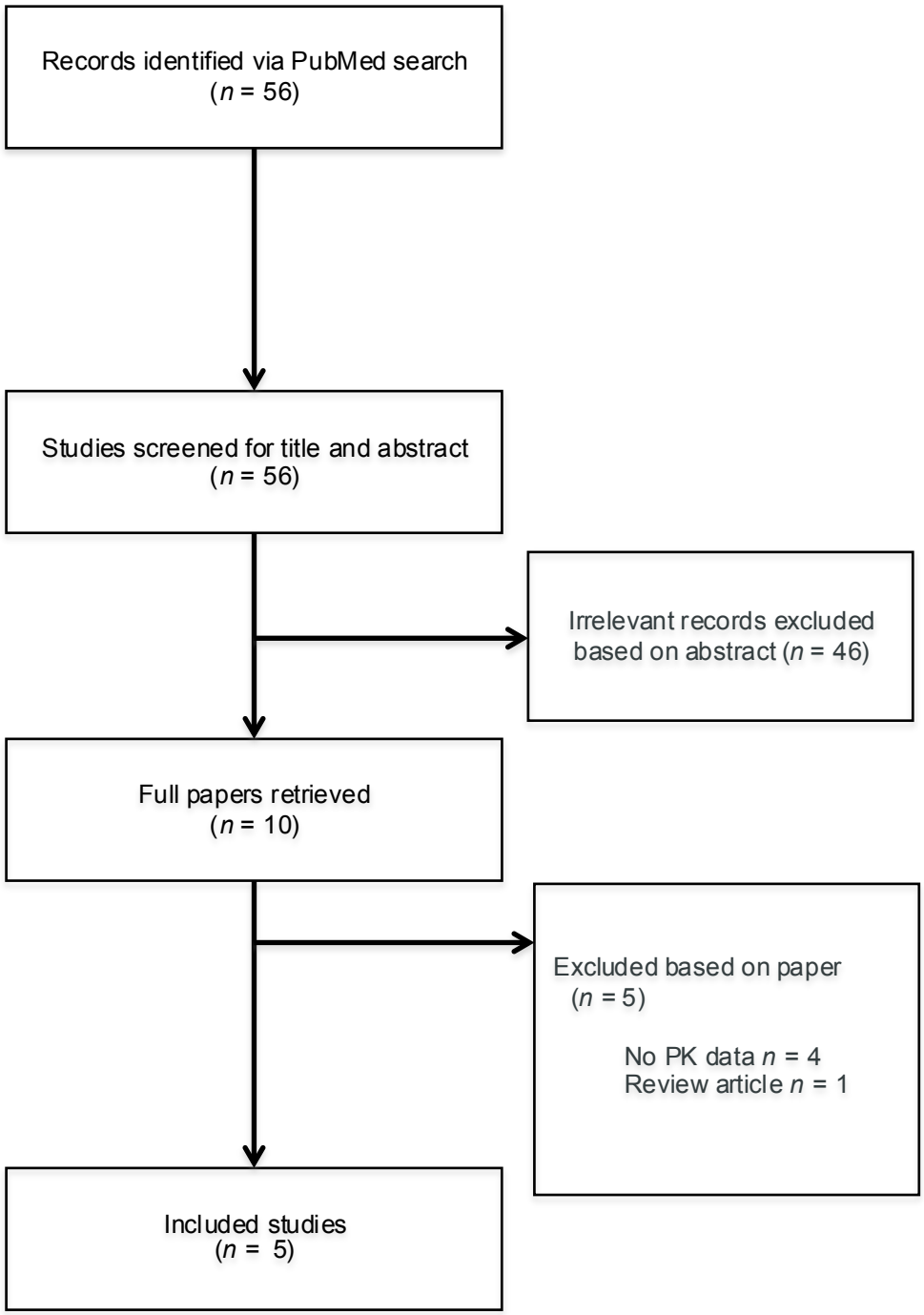

Figure 8 PRISMA diagram of PubMed search (123), with permission

Of the five eligible papers, three contained PK data on SL naloxone and two on IN. No identified paper contained information on buccal PK. The list of included papers is shown in Table 7.

Table 7 Eligible papers from PubMed search

Authors:	Year:	Title:	Route:
Dowling et al.	2008	Population pharmacokinetics of intravenous, intramuscular, and intranasal naloxone in human volunteers.	Intranasal
Middleton et al.	2011	The pharmacodynamic and pharmacokinetic profile of intranasal crushed buprenorphine and buprenorphine/naloxone tablets in opioid abusers.	Intranasal
Harris et al.	2004	Pharmacokinetics and subjective effects of sublingual buprenorphine, alone or in combination with naloxone: lack of dose proportionality.	Sublingual
Fischer et al.	2015	Pharmaceutical and pharmacokinetic characterization of a novel sublingual buprenorphine/naloxone tablet formulation in healthy volunteers.	Sublingual
Nasser et al.	2015	Pharmacokinetics of Sublingual Buprenorphine and Naloxone in Subjects with Mild to Severe Hepatic Impairment (Child-Pugh Classes A, B, and C), in Hepatitis C Virus-Seropositive Subjects, and in Healthy Volunteers.	Sublingual

The paper from Dowling et al. (27) (see also 2.2.4 Pharmacokinetic properties of naloxone and 2.4.3 Intranasal naloxone) describes an open-label crossover study with five treatment arms; 0.8 mg IV, 2.0 mg IV, 0.8 mg IM, 0.8 mg IN and 2.0 mg IN, to assess PK parameters for naloxone in six healthy volunteer subjects. The investigational product given in all treatment arms was a 0.4 mg/ml solution. The IN administration achieved only F=4% and F_{IM}=36% and a t_{max}=6-9 minutes. The authors suggest that the low bioavailability may be attributed the high volume administered (5 ml for the 2.0 mg IN treatment arm). One of six subjects refused to receive the 2.0 mg IN treatment due to the high administration volume. (27)

Middleton et al. (127) describe a randomized double-blinded, placebo-controlled crossover study design to compare PK/PD profiles of IN administration of crushed SL tablets of buprenorphine and buprenorphine/naloxone. The study had six treatment arms; placebo IN, 2 mg buprenorphine IN, 8 mg buprenorphine IN, 2/0.5 mg buprenorphine/naloxone IN, 8/2 mg buprenorphine/naloxone IN and 0.8/0.2 buprenorphine/naloxone IV. The subjects were ten recreational prescription drug users. The IN 2/0.5 mg route achieved F= 24%, t_{max}=18 min, C_{max}=0.39 ng/ml and $AUC_{0\text{-}72h}$=0.4 ng*h/ml. The results of the IN 8/2 mg route was F=30%, t_{max}=20 min, C_{max}=1.60 and $AUC_{0\text{-}72h}$=2.02.

The SL route showed poor bioavailability. Harris et al. (128) conducted a study on non-dependant opioid user where they assess dose-effect proportionality of buprenorphine alone and in combination with naloxone, but found this comparison impossible since many of the naloxone plasma concentration levels were below the limit of quantification (0.05 ng/ml).

Fischer et al. (129) conducted crossover studies where naloxone was administered sublingually as 1.4 mg and 2 mg doses together with buprenorphine. The naloxone C_{max} values were <0.4 ng/ml and t_{max} of 0.8 hours for both dosages.

Nasser et al. (130) tested the impact of hepatic impairment and HCV infection on buprenorphine and naloxone PK. The study revealed a 3-14-fold increase of $AUC_{0\text{-last}}$ and 3-11-times higher C_{max} among the subjects with moderate to severe hepatic impairment.

4.3 Results from the stability data screen

Two of the three included patent applications presented data from stability tests, namely AntiOP and Lightlake.

4.3.1 Anti-OP stability tests

This applicant reported a total of four incremental stability tests.

The first assay tested stability of pH and osmolarity for formulations of naloxone hydrochloride 20 mg/mL in citrate buffer at pH 3.0, 4.0 and 5.0, respectively. The samples were stored at 60°C or exposed to light for 15 days. The samples were analysed for pH and osmolarity at day 0-15 and for impurities (types not specified) at day 15 with reversed phase high performance liquid chromatography (RP-HPLC) method. The results at day 15 showed that the pH remained relatively stable throughout the test period for all pH values. pH 5.0 showed most degradation with a relative retention time (RRT) of 0.52 causing the largest peak area. The applicant points out that a lower pH appears to protect the naloxone formulation from degradation. The formulations changed their appearance from clear, colourless solution to very slightly tint of yellow, but still clear solution for all three pH values (pH 3.0, 4.0 or 5.0) when stored at 60°C for 15 days.

The second test evaluated a series of excipients likely to include in an IN naloxone hydrochloride formulation with regard to degradation, pH, osmolality and purity. Thirteen combinations of excipients including buffers, preservatives, oxidants and viscosity enhancers, were tested with naloxone 20 mg/mL at pH 5.0 (some combinations were also tested at pH 4.0 and 4.5). The formulations were stored at 60°C for four weeks.

According to the applicant, this second test supported the observation from the first test showing that a decreased pH minimized the oxidative degradation. The presence of a common nasal product preservative, benzalkonium chloride, was found to further increase the degradation. Ascorbic acid and propyl paraben were also found to increase the degradation of naloxone (degradation products not specified). HPLC analysis indicated that the preservative methyl paraben, propylene glycol and glycerine had negative impact on the formulations, especially because of increased naloxone degradation and increased level of impurities.

According to the applicant, it was their pre-understanding that an IN naloxone formulation should contain permeability- and viscosity enhancers, such as exemplified sorbitol, hypromellose, polypropylene glycol, polyethylene glycol and glycerine, this to increase the residence time in the nasal cavity. However, the exemplified excipients were found to increase degradation. The applicant summarizes that such excipients might work individually, but the tested combinations of these were judged to be unfavourable for an IN naloxone formulation, and thus were omitted.

Based on the above, four formulations were chosen for further analysis. These formulations contained naloxone 20 mg/ml. Oxygen rich storage condition at 60°C was designed and accelerated 12 weeks stability testing with respect to changes in pH and osmolality, impurities and degradation of naloxone were conducted. This revealed that a formulation containing parabens (methyl- and propyl paraben) had elevated degradation and was therefor excluded. The three other formulations contained benzyl alcohol as a preservative agent. One of these formulations was also excluded based on increased degradation. Unlike the other three formulations, this formulation comprised sodium citrate, glycerine and propylene glycol. Based on this test, the two most promising formulations were selected for another 4 week accelerated stability study.

The two selected formulations were tested in stoppered vials with nitrogen- and oxygen overlays and stored at 60°C for 4 weeks. One of the formulations showed a markedly

increased degradation when oxygen was used as overlay. This was the most complex of the two formulations, comprising hypromellose and sorbitol, as well as the excipients in common with the other (citric acid, EDTA and benzyl alcohol). There was no essential alteration of pH and osmolarity. The formulation showing the best stability properties comprised naloxone 20 mg/mL, citric acid (25mM), EDTA (10mM) and benzyl alcohol (0,5 %).

Two batches of this formulation were produced in nasal spray device, and stored for 12 months at:

- 25°C / 60 % humidity
- 40°C / 75 % humidity

Both batches were stored in upward and downward positions, to see if degradation was influenced by contact with the stopper (downward position). The naloxone-related degradation products (10-α-hydroxynaloxone, oxymorphone, noroxymorphone, 10-β-hydroxynaloxone, 7,8-didehydronaloxone, 2,2´-bisnaloxone and 3-O-allynlnaloxone) were determined as either not detected (ND) or below limit of quantification (<LOQ), with no significant differences between upward or downward positioning. (125)

4.3.2 Lightlake stability tests

This applicant conducted two different stability tests. The first test was conducted with a formulation comprising naloxone 10 mg/mL, sodium chloride, disodium edetate, hydrochloric acid, benzalkonium chloride and purified water. The pH was not disclosed in the patent application. Two batches were stored at 25°C / 60 % humidity and tested at 0, 3, 6, 9 and 12 months, where batch 1 was nude and batch 2 was mounted in a Pfeiffer BiDose device. The applicant concluded that both batches showed that the composition was storage-stable.

The second test was conducted with two concentrations; 20 mg/mL and 40 mg/mL. The excipients were the same as in the first experiment. The pH was adjusted to 4,5 (3,5-5,5). The formulations were stored in three different environments;

- Room temperature/light conditions
- Room temperature/dark conditions
- 25°C / 60 % humidity (protected from light).

The assemblies were tested for pH and impurities at 0, 2 and 10 months. At 10 months a clear, yellow appearance was observed for the samples stored at room temperature/light conditions,

and these samples also had the highest degradation and impurity levels. The appearance was clear and colourless for the assemblies analysed at 0 months and those stored in 2 and 10 months at 25°C/60 % humidity, protected from light. (126)

5 Discussion

5.1 Part A - The clinical trial, OPI 15-002

Treatment with non-injectable naloxone is a hot topic in field of subject, and in particular IN administration has been given attention by experts as well as government authorities worldwide.

Although IN naloxone undoubtedly is saving lives of opioid OD victims through THN distribution programs and rescue by ambulance personnel, it is an important principle that also vulnerable, marginalized patient populations (e.g. drug users) have the same rights to be treated with approved pharmaceutical products in accordance to the principles of EBM. This should be seen in relation to justice, one of the general principles of medical ethics (see. 2.7.3 Ethical considerations), hence fair distribution of available resource, including innovation and development of dedicated pharmaceuticals.

Even though this master thesis´ time frame was not able to include the completion of OPI 15-002 and draw results answering aims and endpoints of the study, it is possible to extract valuable information regarding the preparation and the recruitment process.

5.1.1 GCP aspects

This study was conducted on healthy volunteers. In addition to this being a regulatory requirement for phase 1 studies of this kind, there are ethical aspects of the terms “healthy” and “volunteers” which deserve to be discussed.

The health aspect is perhaps not as obvious as the principle of voluntariness, but can be discussed in context of the third principle of ICH-GCP saying; *“The rights, safety and well-being of the trial subject are the most important considerations and should prevail over interests of science and society”*. The principle is emphasizing the importance of minimizing risk exposure to the study subjects. In general, it is easier to ensure safety and well-being for healthy subjects compared to subjects with more various health conditions. This also explains the rationale for strict inclusion-/exclusion criteria that may seem irrelevant to both the target patient population and academic interests. Recording of vital signs at screening and during intervention visits are examples on how the same principle is being safeguarded, but also assessment of nasal mucosa before and after IN treatment.

The excluding of female subjects not using high-efficacy contraception elucidate the same ethical aspect, namely the protection of a potential unborn foetus and its mother.

Also the follow-up of subjects being excluded based on medical findings is relevant to ethics. This can be exemplified with the subject being excluded due to elevated ASAT/ALAT values. This subject was offered an additional ASAT/ALAT test at the CTU, and was forwarded to its general practitioner for further assessment. Although unknown to the research group, this may potentially have revealed an underlying health condition for the subject that hypothetically needs treatment or life-style adjustments.

The forms for storage recording of blood samples and the CRF are examples of tools for safeguarding the tenth ICH-GCP principle as a contribution to accurate reporting, interpretation and verification.

In terms of a favourable risk-benefit ratio, this study did not bring individual benefits to the subjects, but the risks are also low. The safety of naloxone at this dose-range is considered high in healthy subjects where opioid withdrawal symptoms are not a likely observation. Still, a favourable individual risk-benefit ratio is impossible to achieve when the benefit is zero. According to ethical requirements (see 2.7.3 Ethical considerations) a high social value can compensate for the lack of individual benefits if the risk is low. It seems reasonable to suggest that a potentially authorized evidence based nasal naloxone spray should be considered having high social value, and hence fulfil the ethical requirement.

The autonomy of the subjects needs to be facilitated through awareness of communication at all stages of the process. In addition to thorough and impartial dissemination, this was exemplified by letting the subject be the first to sign the informed consent form. If the informed consent form first was signed by a researcher, the subject may feel more obligated to sign, and the autonomy and voluntariness would be threatened. The information letter and its integrated informed consent form, as well as the introducing talk-through at the screening visit, contributes to *disclosure*, *understanding* and *voluntariness*, important elements of informed consent. It was also considered of importance to communicating a low threshold for asking questions and respectfully answer every single question, thoroughly. Some subjects also expressed their acknowledgements for good information.

5.1.2 Gender specific issues

The aims of the study and its study design did not necessitate equal distribution of gender, but there were gender specific inclusion/exclusion criteria, i.e. females had to not be pregnant or breast feeding, use high-efficacy contraceptives and demonstrate a serum-HCG level <3 U/l. A majority of the subjects screened at the initial screening visit were females (10/17). After a few screenings it became clear that it was necessary to elucidate inclusion criterion no. 5, saying that female subjects with child-bearing potential must use high-efficacy contraception. Three subjects were excluded at the screening visit based on this criterion solely. The inclusion- and exclusion criteria were listed as a compilation text in the information letter, but the detailed per protocol information on what is defined as “high-efficacy contraception" was not revealed in the information letter. This may likely have gotten subjects to wrongly believe that they were eligible. The comments from the excluded females were that they had interpreted this point as irrelevant to them because of either not presently being sexually active or that they used condoms, which per protocol is not considered high-efficacy contraception. The realization of this information weakness and its potential for embarrassment for the female subjects as well as the ethical aspects of not wasting the subject´s valuable time, made us clarify this issue per e-mail before screening for the remaining screening visits. A more thorough talk-trough of the inclusion criteria at an earlier stage of the screening visit was also implemented, and noteworthy, no female subjects were excluded by this criterion at neither the rest of the initial screening in the autumn 2015 or at the following screening/re-screening during spring 2016.

5.1.3 Recruitment among students - a representative cohort?

It can be questioned whether recruitment among students at the Faculty of Medicine represents a too narrow cohort selection of subjects. A subject age in the range of 21-29 years may be considered a very tight range. An age range at this size is probably a direct result of recruitment targeted against students, even though being student was obviously not a criterion. In fact, several of the screened subjects were not students.

The box-plots of BMI among included subjects (Figure 4) are pointing at narrowness of the cohort. This figure shows that BMI variation is far within the limits of the inclusion criterion (no. 4) saying that subjects must have a BMI range of 18.5-26.0 kg/m^2. Especially among the female subjects, the BMI range was tight. This is also interesting in relation to an internal

debate prior to the study start-up pointing at this inclusion criterion being strict, and with limited relevance to the target population. Importantly, the inclusion criteria is not chosen to represent the target population, but to ensure the important ethical consideration of safeguarding that volunteer subjects are safe during the study, as also highlighted in section *5.1.1 GCP aspects*.

On the other hand, it can be counter-argued that students at such faculty has a generally better understanding of medical terms, regulatory requirements regarding clinical research and conceptual understanding of informed consent, possibly leading to better concordance and adherence to the requirements of participation.

In relevance to the ethical requirements of clinical trials regarding volunteer informed consent (see 2.7.3 Ethical considerations), one could also question whether students at the same faculty would feel committed to participate based on affiliation or not. Based on the latter, it was emphasized clearly both verbally and in the information letter that participants were free to leave the clinical trial at any time, without explaining why.

These topics were debated within the research group, but it was concluded that the relatively large amount of students at the faculty should be able to bring diversity among the eventual included subjects. Also, the PI does not have any bindings or direct relation to students at the faculty.

NOTE: several of the screened and hence included participants were not students at the Faculty of Medicine, but had heard of the study through friends and chose to take contact.

5.1.4 Advertising - was the flyer too suggestive?

The flyer that was distributed to the students at the Faculty of Medicine prior to the approval from REC and NOMA was considered to be too suggestive by the sponsor, and was eventually replaced by a less detailed version created by the CRO. However, this version was first used for recruitment during spring 2016, because the number of potential subjects showing interest was adequately large before this decision was made.

The version created by the CRO is briefer, more neutral and perhaps less appealing than the version created by the undersigned. The CRO version does not reveal much background information and rationale for the study. It was discussed and considered okay to include such information when creating the original version, based on the fact that the topic was highly

elucidated in media at the time and it appeared reasonable to connect this topicality to the upcoming study.

Before the distribution of the flyer, it was a discussion within the research group, together with the CRO, whether it was okay to distribute information on the upcoming study before approval from REC and NOMA. It was obvious to all involved that a reservation of approval before recruitment, had to be underlined. Still, it may be argued that such information distribution falls under the category of advertising, and hence only should be conducted after approval.

5.1.5 Motivation for participation - was it easy money?

An interesting question is what was the motivation for the subjects to participate. This question was not raised to the participants. However, some comments still reached us, saying that the nature of the study and the ultimate aim of saving people from opioid ODs was appealing. Some subjects told us that they recognized participation as an opportunity to learn more about clinical trials, and this should be seen in context of the cohort containing students from the Faculty of Medicine.

Others commented that the financial compensation was easily achieved. This leads to an interesting question regarding the ethical consideration about compensation. The subject compensation was meant to compensate for the time used by the participants, and was not associated with the risk/benefit ratio. In the internal discussion on this topic, one argument was that students might be tempted to join by wrong motivations if the compensation was too good. A counter-argument to this was that if the compensation was too low, then the plausibility for recruiting others than students would be close to non-existing. A previous study on the same project (OPI 14-001) paid 1.500 NOK per research day. OPI 14-001 occupied as many hours per day as OPI 15-002. One major difference was that OPI 14-001 included a controlled sedative IV infusion with the highly potent anaesthesia opioid *remifentanil*. Arguments were raised saying that remifentanil infusion spoke for a higher economical compensation than an otherwise similar PK study that did not involve remifentanil, and it was suggested to pay 750 NOK per research day for this study. Another study on the same project (OPI 15-001) paid 750 NOK per research day. OPI 15-001 also included remifentanil infusion, but the time consume was shorter (approximately 3 hours). However, the ethical principle saying that the level of compensation should be independent

from the risk-benefit ratio assessment (cf. 2.7.3 Ethical considerations) should be weighted in such discussions. This spoke for a compensation in the same size as OPI 14-001. An overall consideration of the use of subject´s time without their willingness to take more risk than otherwise, made a compensation of 1.000 NOK per research day for OPI 15-002 seem reasonable.

In relevance to the motivation and the size of the financial compensation, one should take into account that some of the initially included subjects who rejected re-screening stated their upcoming exams as a reason for not attending re-screening, even though none of them were asked to state a reason for leaving the study. This indicates that the compensation it self were not a too weighty motivation.

5.1.6 Strengths and limitations - Part A

Part A of this master thesis with its appendixes covers the recruitment process of a clinical trial in details, and it includes ethical discussions relevant to such processes. It shows how other tasks in relevance to GCP could be safeguarded while waiting for regulatory and IRB approval, such as designing the information letter with the informed consent form, as well as facilitation of traceability by designing the blood sample storage sheets and the paper-CRF.

This master thesis gives an example of a detailed and modern CRF, capable of capturing and safeguarding important source data from a multiple visit PK crossover study.

A detailed overview of a screening process is revealed. The importance of meticulous communication and detailed explanation of concepts that may be misinterpreted, although seem obvious to medical personnel, is highlighted and exemplified.

The lack of results answering the research question, as a consequence of the postponement, is a weakness for Part A of this master thesis.

5.2 Part B - Review of non-injectable naloxone formulations

The review paper (123) examines development activity of non-injectable naloxone illustrated by the use of the PRISMA framework. All the included patents were from 2012 or newer. A systematic review of peer-reviewed literature identified through PubMed search is also disclosed.

It is high activity in the field of development of non-injectable naloxone. That is understandable, seen in relation to the problematic issues in the field together with high death rates deaths caused by opioid ODs. The field is likely broader than what this review captures, i.e. others may not yet have registered their inventions as patents, or their patents simply not being included to this review due to no presented PK data.

Information on PK for non-injectable routes is only to a limited extent available in peer-reviewed literature. Enhanced focus on non-injectable administration routes, as well the widespread use of off-label products, yields for more pharmacological knowledge among these routes. It was therefore considered valuable to retrieve information from non-peer-reviewed patent applications. The scientific value of such data can rightly be questioned, not least because of the comparison of data reported in different ways (e.g. mean vs median, SD vs min,max) and the reporting of unlike parameters (e.g. $AUC_{0\text{-last}}$ vs $AUC_{0\text{-}\infty}$). Also different length and interval of blood sampling regimens questions the grounds for comparison. Still, the lack of peer-reviewed literature, together with awareness of these limitations, justifies this study.

5.2.1 Formulation aspects and PK parameters

The review paper (123) discusses and compares the PK profiles of the included patent applications. Noteworthy, interesting formulations aspects may likely have been omitted from the review due to the inclusion criterion saying that only those patent applications containing human in vivo PK data should be included.

The two applicants AntiOp and Lightlake disclose information about their formulation´s excipients and chemical properties, while Euro-Celtique does not. There are striking similarities between the AntiOp and Lightlake formulations. They do not contain viscosity increasing agents nor absorption enhancers. Both formulations must therefore assumingly be quite aqueous, and that is an interesting element that increases the justification of comparison

between these two formulations. By and large, this also legitimizes the comparison against the improvised non-concentrated off-label MAD-attached solution, which AntiOp had included in their Trial 1 (pilot), thus replicating the treatment used in THN programs and ambulance programs.

Despite the disclosed similarities between Lightlake and AntiOps formulations, the AntiOp formulation achieved slightly lower per mg adjusted values of C_{max} (0.77-0.98 ng/ml) and $AUC_{0-\infty}$ (1.11-1.74 ng*h/ml) compared to Lightlake (C_{max}=1.14-1.66 ng/ml and $AUC_{0-\infty}$ =1.42-2.48 ng*h/ml), as displayed in the graphs in Figure 5 and Table 2. This may be explained by the discrepancy of information whether the AntiOp formulation contained 10 mg/ml naloxone hydrochloride or 10 mg/ml naloxone hydrochloride dihydrate. AntiOp describes their exemplified IN formulation containing 10 mg/ml naloxone hydrochloride dihydrate, whereas in the PK section they listed 10 mg/ml naloxone hydrochloride. If the formulation contained 10 mg/ml naloxone hydrochloride dihydrate, this would correspond to 9.1 mg/ml naloxone hydrochloride. The calculations of per mg values for PK comparison assumed that AntiOp contained 10 mg/ml naloxone hydrochloride. It is therefore possible that the reported per mg values of AntiOp are under-estimated. With this uncertainty revealed, it was not considered necessary to include plots of per mg-adjusted values of a corresponding "speculative" AntiOp 9.1 mg/ml naloxone hydrochloride formulation.

Another possible explanation for AntiOps slightly lower per mg adjusted C_{max} and $AUC_{0-\infty}$ values may be the fact that AntiOp´s IMP formulation is slightly more acidic with a pH 4,25 compared to Lightlake´s pH 4.5. The pKa value of naloxone hydrochloride (and naloxone hydrochloride dihydrate) is 7,94. A lower pH should then imply more dissociated ionized naloxone and less lipophilic properties, and hereby less absorbed naloxone. This together with the uncertainty of which naloxone salt form actually used in the AntiOp formulation, could explain why AntiOps per mg-adjusted C_{max} and $AUC_{0-\infty}$ values were slightly lower than the ones of Lightlake.

Unlike the formulations described in Part B, the IMP described in Part A contains excipients increasing viscosity and bio-adhesiveness, i.e. Povidone K30 and Glycerol. In theory, such excipients should improve systemic bioavailability. Also the disclosed F (56-61%) and F_{IM} (71.5%) from earlier studies of the IMP (Table 1) indicate that this may be correct. (NOTE: the F_{IM} value was assessed under influence of remifentanil.)

Further publication of human in vivo PK data for non-injectable naloxone is desired, and the upcoming results from OPI 15-002 will hopefully contribute to that. Studies examining the addition of excipients such as absorption enhancers and viscosity increasing agents to IN naloxone formulations, and their impact on PK parameters and bioavailability would be of greatest interest.

5.2.2 Intranasal administration - Volume matters

Interesting association between volume administered and both $AUC_{0-\infty}$ and C_{max} is shown. Unfortunately, it was not possible to determine a valid cut-off value, that points out a maximum volume for IN administration of naloxone. A systematic study on administration volumes aiming to determine the optimal range of nasal administration volume would be of greatest interest for the particular field of research. Still, it seems clear that 1 ml, as used in the improvised off-label syringes with MAD, is a far too high volume with the achievement of only F=11% and F_{IM}=10%, compared to the more concentrated ones achieving F in the range 20-42% and F_{IM} in the range 26-57%. These low values are by and large confirming the findings by Dowling et al. (27) of a F=4 % when administering an even larger volume (5 ml) of 0.4 mg/ml of an improvised solution attached to a MAD, as revealed through the PubMed search. (123)

From the above it seems clear that administration volume matters, and the observation is further strengthened by the fact that the Lightlake´s formulation of 10 mg/ml achieving the highest F_{IM}, was reduced from 57% to 48% when the volume was doubled from 0.1 ml per nostril to 0.2 ml per nostril. Therefore, the present use of current off-label treatment with non-concentrated naloxone formulations should be replaced by authorized non-injectable pharmaceuticals adhering to the principles of EBM. The need for evident medical knowledge to intervene balanced within the ethical principles of beneficence and non-maleficence, supports the latter proposal.

5.2.3 Sublingual administration - a dead end?

The SL route appears to be examined in a lesser extent as a possible non-injectable administration route for naloxone. Although earlier identified as a candidate route (72), the position of the SL route seems weakened based on this systematic review. The bioavailability revealed through the Euro-Celtique patent is more or less analogue to the per-oral administration route. (123)

The PubMed search highlighted an interesting additional aspect revealing that persons with hepatic impairment, a condition that is common among the target population, had manifold naloxone levels in their blood plasma. This may very well indicate that the uptake of naloxone is in fact not due to SL absorption, but rather as GI absorption facilitated by reduced hepatic first-pass metabolism.

Still, it should be emphasized that optimized and appropriate SL formulations may improve the SL routes performances.

5.2.4 Discussion of stability testing data

The conducted WIPO PatentScope search was not designed specifically to identify stability data, and stability data was not included into the review paper. An appropriate search protocol built to identify formulation aspects, including stability data, would likely capture plural relevant patent applications, due to not being limited to those patents containing human in vivo PK data, which was one of our inclusion criteria for this systematic review.

Although the AntiOp patent describes better observed degradation properties for a formulation lacking commonly used excipients in IN formulations (i.e. absorption promotors and viscosity increasing agents), these excipients should not be depreciated based on this patent solely. The selection of formulation was based on comparison of "cocktails" of excipients, and not systematic examination of one by one excipient. Excipients such as the benzalkonium chloride (preservative) and glycerine (preservative, co-solvent and viscosity enhancer) may have been identified as unsuitable on wrong basis.

A systematic stability study of different excipients in a naloxone formulation would be of great interest. It should be noted that such studies may in fact exist, although not identified through this review, because of a targeted focus on patents containing human in vivo PK data.

An observation common to both Lightlake and AntiOp was the appearance of a yellow tinted solution after storage under various conditions. None of the applicants suggests an explanation for this, but AntiOp disclosed this observation being independent of pH (range 3.0-5.0) for their 10 mg/ml formulation. Lightlake´s observed the same for their 20 mg/ml and 40 mg/ml formulations, which suggests this phenomenon being independent of concentration range, at least to some degree. The Lightlake samples turning yellow was either stored in light or dark condition at room temperature. This suggests that light exposure is not the reason. The

samples stored at controlled temperature/humidity conditions (25°C/60% RF, protected from light) remained clear and colourless. This indicates that temperature and/or humidity does matter. It was not disclosed in the patents whether the yellow tint was caused by degradation of naloxone or other excipients in the formulations.

In addition, previous stability testing assays of different concentration levels of the formulation that later would become the IMP described in Part A, also experienced yellow colouring after 8 months when stored in 75% RH at 30°C and 40°C, but not at 4°C. The intensity of the colour was here depending on the concentration.

An appropriately designed systematic review addressing stability data within patent applications (regardless PK data content), as well as systematic stability tests, may bring relevant information to surface.

Although not described through this review, another thinkable way of "avoiding" IN stability issues is the use of nasal powder formulation. This may be a trace for further research on IN naloxone, and PK studies on designed powder formulations would be of greatest interest.

5.2.5 Strengths and limitations - Part B

The review paper is the first systematic review of concentrated non-injectable naloxone formulations of peer-reviewed literature in PubMed and published data from patent applications registered in the WIPO PatentScope database. Both academic views and pharmaceutical progress perspectives are therefore captured by the review article. (123)

There are noteworthy limitations regarding the study. Firstly, one can ask if the First page search within the WIPO PatentScope was a strong enough approach to cover all relevant patent applications, due to the fact that it did not capture the Lightlake patent, since Lightlake did not include the term "naloxone" within the front page of their patent. If we were to conduct a full-text search, the WIPO PatentScope would have identified >19.000 hits, which would by far exceeded our capacity. We must therefor recognize the possibility that relevant patents may not been captured by this review. Secondly, it is likely that the results from both the peer-reviewed literature and at least the patent registrations would be subject of publication bias, i.e. presenting non-significant results. A third limitation is based on the fact that we did not have access to subject raw data, which made it necessary to do our analysis based on summary of data provided by the applicants. This, together with different sample

sizes, made it difficult to set up consistent and identical comparison methods. The summary data from the applicants are not presented in same formats. Different measures of central tendency and spread were used. Lightlake, AntiOp Trial 2 and Euro-Celtique expressed their results in mean (except t_{max} and $t_{1/2}$), whereas AntiOp listed their Trial 1 results as median.

For Euro-Celtique, we were not able to reproduce their reported bioavailability values in the descriptive part by manual calculation from the original data. A possible explanation to this is outlier-removal, but the subtleties of such selection and calculation remains unknown.

AntiOp did not report $AUC_{0\text{-last}}$ values, so the manually calculations of absolute- and relative bioavailability were performed by using the reported $AUC_{0\text{-}\infty}$ values. The accuracy of that modelling is questionable, depending on the unreported differences between the $AUC_{0\text{-last}}$ and $AUC_{0\text{-}\infty}$ values. The length of the sampling periods constituting the $AUC_{0\text{-last}}$ values differed between 8 and 36 hours. This would likely affect the modelling of $AUC_{0\text{-}\infty}$ values and hence the depending measures (i.e. absolute- and relative bioavailability).

5.3 Bridging Part A and Part B

There are connecting elements in Part A and B of this thesis.

Cross over study design in healthy volunteers was used in both OPI 15-002, described in Part A, and all the studies in the patent applications, described in Part B. This is in accordance with "Guideline on the investigation of bioequivalence" by CHMP" (see 2.8 Bioequivalence). The sample size of OPI 15-002 was 22 subjects (4- way cross-over), which is within the range of sample sizes of the studies describes in the patents (n=7-35).

The fact that OPI 15-002 is not linked to patents also underscores the point in the Part B review that there may be developmental work in this field that was invisible in both the patent world and the peer-review world, thus supporting this shortcoming of finding all studies. However, protocol elements of OPI 15-002 can be found in the database clinicaltrials.gov.

An important difference between the studies described in the patent review was that OPI 15-002 used 0.8 mg IM administration as reference treatment, whereas the studies described in the patents used 0.4 mg IM treatment, except Euro-Celtique that did not include an IM treatment arm. According to consultant in anaesthesia at OUS, Arne Skulberg (personal

communication, May 2016)) the standard initial treatment of opioid OD in the Oslo Ambulance Service is 0.8 mg IM. OPI 15-002´s use of 0.8 mg IM is therefore representative to the Norwegian practice. However, it should be noted that OPI 15-002 also included a 0.4 mg standard reference IV group.

Previous practice with IV administration resulted in cases of opioid withdrawal and drug seeking behaviour. (31) It does seem reasonable to assume that administrations resulting in high C_{max} may induce opioid withdrawal symptoms such as agitation. Therefor it seems relevant to raise the question whether this could be a problem for some of the IMPs from the patents, but not least for the FDA approved Narcan® nasal spray (see 2.4.3 Intranasal naloxone). The latter spray produced 5.5 to 11-fold higher IN C_{max} and 4.5 to 8.9-fold higher AUC_{last} compared to reference treatment (0.4 mg IM). It should be noted that the dose of the IMP in OPI 15-002 is chosen to produce higher serum concentrations than for the 0.8 mg IM reference treatment, but without risking a considerable overshoot of C_{max} compared to reference.

The two parts of the thesis, Part A and Part B, compliments one another. Part A deals with important aspects of GCP, which is essential in drug development clinical trials, moreover the general ethical principles of the conductance of clinical studies such as for instance by the informed consent procedure which is a paramount of the Helsinki declaration. In addition, general medical ethical issues such as beneficence, non-maleficence and justice are highlighted in the context of this research aiming to develop scientific knowledge and lead an approved pharmaceutical product for a vulnerable target population. Finally, the transparency frameworks of CONSORT and PRISMA are used for guidance in Part A and Part B, respectively.

5.3.1 In summary

1. Taking part in the preparation of a clinical trial on pharmaceuticals will enhance the understanding of good clinical practice, general research and medical ethics principles.

2. It is possible to obtain valuable scientific knowledge to the field of development of non-injectable naloxone, also outside the peer-reviewed literature, namely through a systematic review of registered patents, although with certain limitations.

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Appendix A: Information letter and informed consent form

Forespørsel om deltakelse i legemiddelutprøving

"Biotilgjenglighet av nasal nalokson sammenlignet med injisert nalokson"
EudraCT nr: 2015-002355-10

Bakgrunn og hensikt

Dette er en forespørsel til deg om å delta i et forskningsprosjekt hvor vi skal sammenligne legemiddelet nalokson gitt som **nesespray** med nalokson gitt som **injeksjon**. Friske kvinner og menn mellom 18 og 40 år kan delta. Nye medisiner, også nye måter å gi et kjent medikament på, må testes i friske frivillige før de kan brukes i behandling av syke. Målsettingen er å få markedsføringstillatelse for produktet.

Nalokson er motgift mot heroin og lignende stoffer, som samlet kalles *opioider*. Nalokson gis som livreddende behandling for å gjenopprette pusting ved overdoser. I dag gis nalokson med sprøyter, enten *intravenøst* (i en blodåre) eller *intramuskulært* (i en muskel).

Nesepray kan gis av folk uten helsefaglig kompetanse, for eksempel andre rusbrukere eller pårørende. Da kan livgivende behandling, nesespray og førstehjelp bli gitt overdosepasienter selv før ambulanse har kommet til stedet. Det kan være problematisk å sette injeksjoner på personer som har tatt overdose, dels fordi de kan ligge kronglete til, dels fordi det kan være vanskelig å finne egnede blodårer å sette injeksjon i. Ved å gi nalokson som nesespray reduseres fare for blodsmitte.

Hva innebærer studien?

Dette er en overkryssingsstudie hvor du fordelt på fire forsøksdager vil få nalokson i alt fire ganger; to ganger som nesespray, én gang som intramuskulær injeksjon og én gang som intravenøs injeksjon. Rekkefølgen på de fire forsøksdagene vil bli tilfeldig (randomisert).

Det skal være 22 deltakere i denne studien, hver med i alt seks besøk. Det første besøket vil være for informasjon og samtykke. Her vil vi ha samtale om helse, ta blodprøver og EKG samt at det vil bli gjort en undersøkelse av nesen din. Deretter følger fire forsøksdager som hver varer i ca. 7 timer. Mellom hver av forsøksdagene må det gå minst 72 timer. Forsøkene vil derfor strekke seg over en periode på minimum 2 uker. Innen fire uker etter siste forsøksdag, vil vi ha en oppfølgingssamtale med deg.

Mulige fordeler, ulemper og alvorlige bivirkninger

Du har ingen fordeler av å delta i studien, men din deltakelse kan komme andre til nytte i framtiden. Vi vil kompensere deg for den tiden du bruker, ev. reise og mat med kr 1000 for hvert forsøk, 4000 kr totalt. Sykehuset vil be om skattekort for utbetalingen, og du vil kunne bli trukket i skatt.

Nalokson har liten effekt på mennesker som ikke først har fått et opioid. Tidligere forskning har ikke klart å vise skadelige effekter av forgiftning i friske frivillige selv i doser 10 ganger større enn de som gis i denne studien, men det finnes rapporter om uønskede hendelser i spesielle, postoperative pasienter. Du vil være under kontinuerlig overvåkning av helsepersonell under forsøkene. Studien foregår ved Forskningsposten hvor vi har tilgang på ekstra personell dersom det skulle bli nødvendig. Du kan når som helst avbryte forsøket, og du vil kunne ta kontakt med oss mellom forsøkene hvis du ønsker det.

Hva skjer med prøvene og informasjonen om deg?

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien.

Noen av blodprøvene vil fryses og sendes til Vitas AS for analyse. De får kun tilsendt blodprøvene, og ingen informasjon om deg.
Blodprøvene vil oppbevares i en spesifikk biobank opprettet kun for dette prosjektet etter de er analysert. Hensikten er å kunne reanalysere blodprøver om det skulle bli nødvendig.
Prøvene vil lagres på studiesenteret etter analysen.
Innen ett år etter at studien er publisert vil blodprøvene destrueres, men senest 31.12.2019.

Vi vil ikke få tilgang til din pasientjournal på sykehuset. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Listen som kan koble ditt navn til koden vil bli oppbevart hos prosjektleder, og bare personell med ansvar for studien har tilgang til denne. Opplysninger slettes 15 år etter at sluttrapport fra prosjektet foreligger. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Godkjenninger

Studien er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk og Statens legemiddelverk.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke deg fra studien uten at det får noen konsekvenser for deg. Du undertegner samtykkeerklæringen dersom du ønsker å delta.

Ytterligere informasjon og samtykkeerklæring

Ytterligere informasjon om studien finnes i Kapittel A – *utdypende forklaring om hva studien innebærer*.

Ytterligere informasjon om biobank, personvern, økonomi og forsikring finnes i Kapittel B – *personvern, biobank, økonomi og forsikring*.

Samtykkeerklæring følger etter Kapittel B – *Signeres av den som samtykker til å delta i studien. Personen som har informert om studien, kan bekrefte at informasjonen er gitt*.

Kapittel A – utdypende forklaring om hva studien innebærer

Kriterier for deltakelse

Friske menn og kvinner i alderen 18-40 år, med BMI mellom 18,5-26 kan delta i studien.

Tilstander som gjør at du ikke kan delta i studien:

- Kvinner som er gravide eller ammer
- Kvinner som ikke bruker sikker prevensjon
- Bruk av faste medisiner eller naturlegemidler (bortsett fra prevensjon)
- Lokal nesesykdom eller operasjon i nesen de siste to måneder
- Dersom du noen gang har opplevd allergiske reaksjoner på medisiner
- Unormale blodprøver og/eller EKG

Forkjølelse eller annen forbigående sykdom gjør at forsøksdagene utsettes til 7 dager etter at du har blitt frisk.

Enkeltvis inntak av medisin (inkludert reseptfri medisin, nesespray, naturmedisin, urtemedisin o.l.) gjør at forsøksdagene må utsettes etter siste inntak. Hvor lang tid utsettelsen blir avhenger av halveringstiden på medisinen. Derfor må du ta kontakt med oss dersom du tar noen medisiner utover prevensjon i studieperioden, slik at vi kan planlegge for å utsette besøkene.

Bakgrunnsinformasjon

Opioider er en samlebetegnelse for en bestemt gruppe stoffer som virker dempende på nervesystemet. I medisinsk sammenheng brukes opioider først og fremst som sterke smertestillende legemidler. Noen av de mest brukte er *morfin*, *kodein*, *tramadol* m.fl. Opioider kan også gi rus og regelmessig bruk fører til avhengighet. Heroin er et eksempel på et veldig kraftig opioid som brukes av mange rusmisbrukere. Dersom dosene blir høye kan dette føre til overdose. Overdose resulterer i redusert eller opphørt pust, små pupiller og bevisstløshet. Dette kan føre til hjertestans.

Opioidoverdose er et alvorlig problem blant rusmisbrukere. I Norge dør omtrent 230 personer av slike overdoser hvert år, flere enn de som dør i trafikken. De som injiserer heroin eller andre opioider har den høyeste risikoen for å dø av overdose. Døden inntrer som følge av pustestans.

For å redde liv er det nødvendig med umiddelbar behandling med en motgift, og nalokson er motgiften som oftest blir benyttet. Nalokson blokkerer bestemte reseptorer i nervesystemet. På den måten får ikke opioidet (f.eks. heroin) bundet seg til reseptoren og utøvd effekt.

Nalokson opphever pustestansen som kommer av overdosen. Nalokson gis i dag som intravenøs og intramuskulær injeksjon, hvor av den første krever god teknikk og den andre tar lengre tid før medisinen virker. Standard behandlingsopplegg i Norge i dag er å gi 0,4 - 0,8 mg intramuskulært og/eller 0,4 mg intravenøst. Den første for langvarig effekt, den andre for rask respons. Sistnevnte er et viktig poeng siden pasienten raskt må begynne å puste igjen, men man må også ta høyde for at rusmidlet som oftest har lengre virkningstid enn motgiften.

Nalokson som nesespray har blitt foreslått som behandling av overdoser, det kan gis raskt av nesten alle, uten mye opplæring eller risiko for sprøytestikk. . Det er i dag forsøk med utplassering av nalokson nesespray til rusbrukere, men dagens spray er ikke optimal og har kun midlertidig godkjennelse. Potensielt kan nasalt nalokson alene oppfylle de samme kravene som kombinasjonen av intravenøst og intramuskulært, ved at effekten kommer raskt, men også har like god varighet. Det er ønskelig å kunne administrere medikamentet nasalt fordi det fjerner risikoen for at personellet stikker seg på sprøytespisser, og blir utsatt for blodsmitte fra en risikogruppe med tanke på blodbårne sykdommer. Dessuten er det ofte

vanskelig å sette intravenøse sprøyter på mennesker som injiserer rusmidler.

Det finnes få gode studier av nasalt nalokson. I de fleste studiene har man brukt nesespray med for lav konsentrasjon av nalokson. Det har derfor vært nødvendig å gi for store volum, noe som har resultert i at mye renner ned i magesekken, og ikke blir tatt opp i blodet. Det er ønskelig at volumet som gis er så lite som mulig og utstyret må være enkelt å bruke... I våre studier har vi vist at ca 60% av dosen som gis blir tatt opp i kroppen.

Oppsummert er det slik at nasalt nalokson kan få en viktig rolle innenfor akuttmedisinen, men for at dette skal bli en realitet trengs bedre formuleringer. Med dette menes hvordan medisinen er laget, hvilke hjelpestoffer den inneholder, og hvilken type utstyr som trengs for å gi medisinen.

Mål

Dette er en av flere studier som søker å bidra til bedre behandling av overdoser ved å introdusere motgift som kan gis som en nesespray. Målet med denne studien er å vise at nalokson som nesespray gir tilsvarende blodkonsentrasjoner av nalokson som dagens behandling.

Dette er en overkryssingsstudie hvor 22 friske frivillige vil delta. Overkrysningsstudie betyr at hver deltager får alle de fire ulike måtene å gi nalokson på, både som intramuskulær og intravenøs injeksjon, og som nesespray i to ulike doser. Vi sammenlikner hvert individ med seg selv, i motsetning til andre studieformer hvor forskjellige grupper deltagere får ulike alternativer.

Undersøkelser, blodprøver som deltakerne blir utsatt for

Den første dagen vil det være frammøte for informasjon og samtykke, og dersom du etter å ha fått informasjon og ønsker å delta vil vi gjøre en helseundersøkelse. Vi vil stille deg spørsmål om den generelle helsetilstanden din. Vi har taushetsplikt, og det er viktig at du er åpen og ærlig. Du vil bli intervjuet med hensyn på kriteriene for deltakelse i studien og det blir foretatt noen undersøkelser. Vi vil ta et EKG (hjerteprøve) og blodprøver, hvor vi måler hemoglobin og lever- og nyreprøver. Slimhinnen i nesen din vil bli undersøkt av en øre-nese-hals lege. Du vil også bli spurt om du tidligere har hatt neseblødninger eller andre symptomer fra nesen. Kvinner får også tatt en graviditetstest. Deretter følger fire forskningsdager som varer i 7 timer hver. I løpet av fire uker etter at de er gjennomført vil vi ha en oppfølgingssamtale med deg. Studien vil totalt strekke seg over minst 2 uker.

Slik vil en forskningsdag se ut

I løpet av de fire forskningsdagene vil du få nalokson på tre ulike måter; *nasalt*, *intramuskulært* og *intravenøst*. Du vil kun få én av formuleringene pr gang, og rekkefølgen vil være tilfeldig. Det er viktig at du sier fra om du har vært syk, tatt medisiner eller andre endringer som oppstår mellom studiedagene. Dette er en legemiddelstudie hvor vi også ser på potensielle bivirkninger av nalokson nesespray. Du må si ifra om du får overraskende helseplager, må til lege/ sykehus eller opplever andre endringer i din helsetilstand.

De fire ulike doseringene du vil få er:

- **Intranasalt:** 0,1 ml nesespray a 14 mg/ml = **1,4 mg** nalokson
- **Intranasalt:** 2 x 0,1 ml nesespray a 14 mg/ml = **2,8 mg** nalokson
- **Intramuskulært:** 2 ml injeksjon i skulder a 0,4 mg/ml = **0,8 mg** nalokson
- **Intravenøst:** 1 ml injeksjon i vene a 0,4 mg/ml = **0,4 mg** nalokson

Du vil bli lagt i en seng eller i en egnet stol. Her vil du kobles til EKG, og vi vil sette inn en et venekateter (venflon) i den ene armen din. Dette for å forenkle blodprøvetaking.

Det vil bli tatt blodprøve av deg 10 min før og 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 240 og 360 minutter etter at du har fått nalokson. Dette høres kanskje mye ut, men mengden blod pr blodprøve er kun ca. 6 ml, noe som gir et samlet blodprøvevolum på 150 ml = 1,5 dl. Til sammenligning tapper blodbanken 4,5 dl dersom man er blodgiver. Dersom du ønsker det vil detbli gitt anledning til å forlate forskningsposten mellom de siste målingene,. Prøvene vil så fryses før de sendes til laboratoriet hvor nalokson nivået vil bli bestemt.

Det må gå minst 72 timer mellom hver av de fire forskningsdagene, det vil si at det tar minst 2 uker fra oppstart første forskningsdag og til gjennomført fjerde forskningsdag.

Innen 4 uker etter siste forskningsdag vil vi ha en oppfølgingssamtale med deg.

Dersom det går mer en 60 dager fra den første helseundersøkelsen før vi kan gjennomføre den første forskningsdagen, vil deler av helseundersøkelsen gjøres på nytt, blant annet vil det bli tatt en ny blodprøve.

Dersom dosen av nalokson er betydelig lavere enn forventet vil vi kunne be deg om å komme til et ekstra besøk for å gi deg denne dosen på nytt. Du vil da bli bedt om å være på Forskningsposten i 2 timer for observasjon før du kan gå hjem. Videre vil du bli bedt om å komme til et nytt besøk tidligst 3 dager senere.

Sprøytestikk

Denne studien innebærer at du vil bli stukket med nåler. På den første besøksdagen vil vi ta vanlige blodprøver fra armen. På forskningsdagene vil du få lagt inn en venekanyle (venflon) som det vil tas blodprøver fra. På to av fire forskningsdager vil du få en sprøyte med nalokson, den ene dagen som intravenøs injeksjon (stikk i blodåre), andre dagen som intramuskulær injeksjon (stikk i skuldermuskel). Sistnevnte kan være litt ømt noen timer etterpå.

Oppfølgingsbehandling

Det vil være en oppfølgingssamtale innen fire uker etter siste behandling. Der vil vi intervjue deg med fokus på mulige bivirkninger, hvordan du har opplevd forsøket og vi vil gjennomføre medisinske undersøkelser om det er behov for det.
I perioden mellom fjerde forskningsdag og oppfølgingssamtalen vil igjen en øre-nese-hals lege undersøke slimhinnen i nesen din.

Sikkerhet

Nalokson er et velprøvd legemiddel som normalt tolereres veldig godt. Vår erfaring fra tidligere studier viser ingen bivirkninger eller komplikasjoner, men noen kjenner en smak i svelget noen minutter etter å ha fått nesespray.

For å lese mer om Nalokson kan du lese fullstendig preparatomtale her:
http://slv.no/_layouts/Preparatomtaler/Spc/06-4660.pdf

Studien foregår på Forskningsposten.. Dette er spesielle lokaler som er utstyrt med den utrustning og personell som trengs for sikker gjennomføring av medisinske forsøk.

Om farmasøytisk spesialpreparat uten markedsføringstillatelse kan inngå i utprøvingen

Nalokson er et godkjent preparat til intravenøst og intramuskulært bruk. Den nasale formuleringen som her har ikke markedsføringstillatelse. Formuleringen er gjort av internasjonale samarbeidspartnere med spesialkompetanse innen farmasøytisk formulering, og som i tillegg har høy akademisk kompetanse. Denne studien er et samarbeidsprosjekt med et norsk, farmasøytisk firma. Målsettingen er å søke om markedsføringstillatelse blant annet med bakgrunn av denne studien.

Mulige fordeler/bivirkninger/ubehag

Du vil ikke ha noen fordeler med å delta i studien. Nytten vil komme andre til gode i fremtiden dersom nasalt nalokson kommer til bruk i behandling av overdoser, både for pasientene og ambulansepersonell.

Nalokson er et svært sikkert legemiddel. Tidligere forskning har ikke klart å vise bivirkninger/toksisitet av nalokson (i friske frivillige, selv i 10 ganger større doser enn de som gis i dette prosjektet). Det finnes rapporter om uønskede hendelser, for eksempel lungeødem i postoperative pasienter. Slike har funnet sted hos pasienter med hjertesykdom eller som har brukt potensielt hjerteskadelige medikamenter. Den aktuelle nesesprayen har vært testet i tre studier med til sammen 30 deltagere uten å vise noen alvorlige bivirkninger utover lette smaksopplevelser hos de som har testet det.

Studiedeltakernes ansvar

Når du deltar i studien er vi avhengige av at alle opplysninger som blir gitt er korrekte. Prosjektleder kan ta deg ut av studien, på medisinsk grunnlag eller etter eget initiativ. Som studiedeltager vil du bli opplyst så raskt som mulig dersom ny informasjon blir tilgjengelig som kan påvirke din villighet til å delta i studien. Du vil også få informasjon om mulige beslutninger/situasjoner som gjør at din deltagelse i studien kan bli avsluttet tidligere enn planlagt.

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke deg fra studien uten at det får noen konsekvenser for deg. Du undertegner samtykkeerklæringen dersom du ønsker å delta.

Kompensasjon for studiedeltakeren dersom det skjer studierelaterte skader (forsikring)

Du vil være forsikret i henhold til Pasientskadeerstatningsordningen og Legemiddelansvarsforeningen.

Kompensasjon til dekking av utgifter

Vi kompenserer deg for den tiden du bruker med kr 1000 for hvert forsøk, 4000 kr totalt. Dette beløpet skal beskattes.

Tidsskjema

Planlagt gjennomføring er høsten 2015 og våren 2016.

Kontaktperson

Navn:
Institusjon:
Telefon:
E-post:

Kapittel B – Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som registreres om deg er alder, kjønn, høyde, vekt din informasjon om eventuelle tidligere sykdommer, legemiddelbruk, allergier, EKG og blodprøvesvar.

Representanter fra sponsor, Statens legemiddelverk og kontrollmyndigheter i inn- og utland kan få utlevert studieopplysninger. Formålet er å kontrollere at studieopplysningene er korrekte. Alle som får innsyn i informasjon om deg har taushetsplikt.

Innsynsrett og oppbevaring av materiale

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller blitt brukt i vitenskapelige publikasjoner.

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien.

Blodprøvene vil fryses og sendes til Vitas AS for analyse. De får kun tilsendt blodprøvene, og ingen informasjon om deg. Blodprøvene vil oppbevares i en spesifikk biobank opprettet kun for dette prosjektet etter de er analysert. Hensikten er å kunne reanalysere blodprøver om det skulle bli nødvendig. Prøvene vil lagres på NTNU etter analysen. Innen ett år etter at studien er publisert vil blodprøvene destrueres, men senest 31.12.2019.

Vi vil ikke få tilgang til din pasientjournal på sykehuset eller andre helseinstitusjoner. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Listen som kan koble ditt navn til koden vil bli oppbevart på hos prosjektleder, og bare personell med ansvar for studien har tilgang til denne. Opplysninger slettes 15 år etter at sluttrapport fra prosjektet foreligger. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Finansiering

Studien er støttet av forskningsmidler fra Helse Midt-Norge. Den Norske Eterfabrikk er sponsor og finansierer brorparten av studiens direkte utgifter. Vi som gjennomfører studien mottar ikke honorar fra sponsor.

Forsikring

Du er forsikret i henhold til Pasientskadeerstatningsordningen og i Legemiddelansvarsforsikringen.

Godkjenninger

Studien er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk, Helseregion Midt-Norge og Statens legemiddelverk.

Informasjon om utfallet av studien

Som deltaker har du rett å få informasjon om resultatet i studien. Du kan gi beskjed om du ønsker dette.

Samtykke for deltakelse i studien

Jeg er villig til å delta i studien.

Dato:_____

_____
Navn (blokkbokstaver)

_____
Signatur

Bekreftelse på at informasjon er gitt deltakeren i studien

Jeg bekrefter å ha gitt informasjon om studien.

Dato:_____ Rolle i studien: _____

_____
Navn (blokkbokstaver)

_____
Signatur

Appendix B: Blood sample storage record form, A samples

SAMPLE STORAGE & ANALYTICAL LOG

Naloxone A-samples

Date: _____ Subject nr:_____ Subject initials: _____ Visit number (2-5) :_____ Adm. Route (IV, IM, IN or 2xIN):_____

Study: OPI 15-002, EudraCT no.: 2015-0023355-10 *Bioavailability of nasal naloxone compared to injected naloxone*	**Sponsor:**	AS Den norske Eterfabrikk Box 23 Høybråten, 1005 Oslo
Doc. Arch.: Investigator site file	**Principal Investigator:**	Ola Dale, Professor ISB, NTNU, Box 8905, MTFS, 7491 Trondheim

Sample number	Box nr:	Location:	Deviations/ Comments:	Time storage at -80 freezer at Clinical Research Facility (hh:mm)	Signature:	Samples forwarded Date:	Logistical method / company:	Signature:
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Appendix C: Blood sample storage record form, B samples

SAMPLE STORAGE & ANALYTICAL LOG

Naloxone B-samples

Date: _____ Subject nr:_____ Subject initials:_____ Visit number (2-5) :_____ Adm. Route (IV, IM, IN or 2xIN):_____

Study: OPI 15-002, EudraCT no.: 2015-0023355-10 *Bioavailability of nasal naloxone compared to injected naloxone*	**Sponsor:**	AS Den norske Eterfabrikk Box 23 Høybråten, 1005 Oslo
Doc Arch.: Investigator site file	**Principal Investigator:**	Ola Dale, Professor ISB, NTNU, Box 8905, MTFS, 7491 Trondheim

Sample number:	BOX nr:	Location:	Deviations/ Comments:	Time transported to -80 freezer at room 13-003-017 (hh:mm)	Signature:	Samples forwarded Date:	Logistical method / company:	Signature:
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Appendix D: Information flyer (developed by undersigned)

Hei farmasistudent!

Vil du være med å lage livreddende medisin mot heroin/opioid-overdoser?

Vi har utviklet en nesespray for behandling av heroinoverdoser. Virkestoffet er nalokson, og dette gis pr i dag som injeksjoner. Problemet med injeksjon er at det ofte kan være vanskelig å gi ute i felten, dels fordi personer som har tatt overdose kan ligge trøblete til, dels fordi det er vrient å injisere på disse pasientene. I tillegg er det en viss smittefare knyttet til dette for ambulansepersonell. Nalokson som nesespray er en del av myndighetenes nye strategi for å redusere antallet overdosedødsfall.

Jeg er masterstudent i farmasi ved NTNU, og sammen med anestesilege Arne Skulberg og forskerlinjestudent Ida Tylleskär gjennomfører vi studier av nasalt nalokson til bruk ved behandling av opioidoverdoser. Professor Ola Dale ved ISB leder prosjektet. Legemiddelprodusenten Den norske Eterfabrikk er studiens sponsor. Dette er med andre ord norsk farmasøytisk innovasjon.

For å få godkjent den nye nesesprayen, trenger vi å bevise at produktet er like bra eller bedre enn dagens behandling. Én sentral faktor i dette arbeidet er å vise at blodkonsentrasjonene man oppnår med nesesprayen er sammenlignbare med standardbehandling. Det er vi godt i gang med, og vi har allerede gjennomført flere kliniske studier, blant annet under simulert heroinoverdose vha. opiodiet remifentanil.

Den studien vi nå skal i gang med er en ren farmakokinetisk studie hvor vi ønsker å sammenligne biotilgjengeligheten til den nye nesesprayen med dagens intravenøse og intramuskulære administrasjonsmåter. Vi er derfor på utkikk etter 24 friske frivillige studiedeltakere (12 her i Trondheim, 12 i Oslo) som i høst kan hjelpe oss et stykke vei videre. Har **du** lyst til å hjelpe oss?

Det dreier seg om 4 besøk, hvor du vil få følgende behandling i randomisert rekkefølge:

- 0,4 mg IV (intravenøst)
- 0,8 mg IM (intramuskulært)
- 1,4 mg IN (intranasalt)
- 2,8 mg IN ------”-------

Deltakelse er frivillig, og du kan når som helst, uten å oppgi årsak, trekke deg fra studien.

Du kan se oss på Schrødingers Katt her:
https://tv.nrk.no/serie/schrodingers-katt/DMPV73001914/25-09-2014#t=20m18s

Arne Skulberg vant den nasjonale Forsker Grand Prix 2014 gjennom dette prosjektet:
https://tv.nrk.no/serie/kunnskapskanalen/MDFP15003214/10-01-2015

Er dette farlig?
Nei, deltakernes sikkerhet er første prioritet. Nalokson er et svært trygt legemiddel, og det har i praksis ingen farmakologiske effekter når man ikke er påvirket av opioider. De dosene vi opererer med her er langt under toksiske. Noen kan kjenne en metallsmak i munnen kort tid etter nesesprayinntak.

Forsøkene vil finne sted under svært trygge omgivelser på Forskningsposten i AHL-bygget på St. Olavs hospital.

Hvem kan delta?
Alle friske kvinner og menn i alderen 18-40 år.

Kvinner må bruke "sikker prevensjon" (f.eks. p-piller) for å kunne delta, men siden dette er en legemiddelstudie kan du ikke bruke andre faste medisiner.

Når?
Oppstarten avhenger av at vi får klarsignal fra SLV og REK. Studien vil foregå på dagtid i oktober/november 2015. Du vil måtte sette av 4 hele dager til deltakelsen. Du vil ikke måtte være fysisk til stede hele dagen, men du må være tilstede når vi skal ta blodprøver.

Kompensasjon?
Ja, du vil motta 1000 kr pr studiebesøk, altså til sammen 4000 kr.

Hørtes dette spennende ut?

Ta kontakt med meg, så skal du få mer informasjon om prosjektet!

Slik kan du nå meg;
Mail: oyvidan@stud.ntnu.no , Mobil: 950 83 316

Jeg svarer mer enn gjerne på spørsmål!

Mvh
Øyvind D. Glende
Farmasistudent - NTNU

Appendix E: Information flyer (developed by the CRO)

Vi søker friske frivillige for deltagelse i klinisk studie.

Ved Forskningsposten, <St.Olavs hospital/ Rikshospitalet> skal det gjennomføres en klinisk studie hvor det skal testes ut å gi legemiddelet nalokson via nesen.
Nalokson brukes i dag som behandling ved overdose av opioider og gis da som en injeksjon med sprøyte. Det er nå utviklet en nesespray med nalokson. I denne studie skal vi sammenligne hvor mye nalokson friske personer har i blodet etter at de har fått nalokson via en injeksjon og ved nesespray.

For deltagelse i studien må du:

- Være mellom 18 og 40 år
- Ha normalt EKG
- Ha BMI mellom 18.5-26 kg/m^2
- Benytte sikker prevensjon i studieperioden

Du kan ikke være med i studien hvis du:

- Bruker andre medisiner regelmessig
- Har påvist allergi mot medisiner

Det vil være 6 besøk i forbindelse med studien hvor hvert besøk kan ta opp til 7 timer. Det vil være minst 3 døgn mellom hvert besøk.
Du vil få kompensasjon ved deltagelse i studien.

Studien er godkjent av Regional-etisk Komite, Region Midt-Norge og Statens Legemiddelverk.

Hvis du er interessert i å få vite mer om prosjektet, vennligst kontakt studiesykepleier…… på telefon……

Appendix F: Paper-CRF

Medical journal - OPI 15-002 /SMR 3089

Bioavailability of nasal naloxone compared to injected naloxone

Protocol Identification Number:

OPI 15-002 v. 3.0, date 01.10.2015

EudraCT Number: 2015-002355-10

Subject number: _____

Subject initials: _____

Study no: OPI 15-002

Table of Contents

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Visit 1 – Screening (Day -60-0)

Date: _____ (yyyy/mm/dd)

Subject's Identification

Initials: _____

Date of subject's informed consent: _____ (yyyy/mm/dd)

Date of birth: _____ (yyyy/mm/dd) Age: _____

Sex: Male ☐
Female ☐

Weight _____ (kg)

Height _____ (cm)

Calculated BMI _____ (kg/m^2)

Inclusion criteria

1. Provision of Informed Consent — Yes ☐ No ☐

2. Healthy men and women aged 18-40 years — Yes ☐ No ☐

3. ECG without any pathological abnormalities — Yes ☐ No ☐

3. Have a BMI range of 18.5-26.0 kg/ m2 — Yes ☐ No ☐

5. Female subject with child bearing potential must use high efficacy contraception. For the purpose of the study acceptable contraception is defined as sterilization, oral contraceptives, patch, implants, vaginal ring, hormonal IUD or copper IUD throughout the study until the last visit.

Yes ☐ No ☐ NA ☐

6. Laboratory values within reference values for the following haematology and biochemistry tests: Yes ☐ No ☐
   a. Haemoglobin
   b. Creatinine
   c. ASAT
   d. ALAT
   e. Gamma GT

Exclusion criteria

In order to participate in the study subjects must not meet any of the following exclusion criteria:

1. Subjects using medication on a regular basis, including regular use of nasal spray of any form. Yes ☐ No ☐

2. History of prior drug allergy Yes ☐ No ☐

3. Subjects having local nasal disease or nasal surgery for the last 2 months Yes ☐ No ☐

4. Pregnant or breast feeding women. A serum HCG below 3 U/L must be demonstrated in females of child-bearing potential at Screening Visit. Yes ☐ No ☐ NA ☐

5. Current drug or alcohol abuse, which in the opinion of the Investigator should preclude participation in the study. Yes ☐ No ☐

6. Have received another new medical chemical entity (defined as a compound which have not been approved for marketing) or has participated in any other clinical study that included drug treatment within 3 months of the administration of investigational product in this study. Yes ☐ No ☐

7. Hypersensitivity to naloxone or any of its excipients. Yes ☐ No ☐

8. Investigator considers subject unlikely to comply with study procedures, restrictions and/ or other requirements. Yes ☐ No ☐

Is the subject eligible for the study? **Yes ☐ No ☐**
If No, please complete the Study termination form

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Check questions

Does the subject have any current medical condition or illness, or relevant condition in the past which may render the subject at unacceptable risk or confound the study assessments? Yes ☐ No ☐

If Yes, please record these in the Medical History log

Is the subject currently using any concomitant medications? Yes ☐ No ☐

If Yes, please confirm that subject is eligible and complete the Concomitant Medication form

Did the standard physical examination reveal any additional conditions which have previously not been recorded in the CRF? Yes ☐ No ☐

If Yes, please confirm that subject is eligible and complete Medical History log

History of Medical Conditions

Disease/system	No	Yes	If yes, specify details:
Cardiac	☐	☐	_____
Vascular	☐	☐	_____
Respiratory	☐	☐	_____
Nasal disease	☐	☐	_____
Gastro intestinal	☐	☐	_____
Hepatic	☐	☐	_____
Kidney	☐	☐	_____
Hematological	☐	☐	_____
Neurological	☐	☐	_____
Psychiatric disorder	☐	☐	_____
Muscular/skeletal	☐	☐	_____
Endocrine	☐	☐	_____
Other:	☐	☐	_____

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Comments on medical conditions:

_____

_____

_____

_____

Habits

Smoking habits:
- Non- smoker ☐
- Ex-smoker ☐
- Current smoker ☐

Exercise habits:
- Never ☐
- Occasionally ☐
- Regularly ☐

Alcohol habits:
- Never ☐
- Occasionally ☐
- Regularly ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Vital sign screening

Has a vital sign evaluation been performed Yes ☐ No ☐

Oxygen saturation _____ (%)

Blood pressure after being seated for 5 minutes

Systolic _____ (mmHg)
Diastolic _____ (mmHg)

Heart rate: _____ (bpm)

Respiration rate: _____ (resp./min)

ECG performed and printout presented to cardiologist? Yes ☐ No ☐

ECG printout is attached to this file? Yes ☐ No ☐

ECG interpretation form signed by cardiologist and attached to file Yes ☐ No ☐

Is there any abnormal findings on the ECG? Yes ☐ No ☐

If Yes, please confirm that subject is eligible or go to Early termination page in eCRF

Subject forwarded to ENT specialist for rhinoscopy Yes ☐ No ☐

Rhinoscopy form attached to file Yes ☐ No ☐

Laboratory Analysis

Were blood samples taken for haematology and clinical chemistry Yes ☐ No ☐

Date: _____ (yyyy/mm/dd) Time: _____ (hh.mm)

Lab-ID: _____

Laboratory value printout attached to file? Yes ☐ No ☐

Laboratory values within reference values for the following haematology and biochemistry tests: Yes ☐ No ☐

a. Haemoglobin
b. Creatinine
c. ASAT
d. ALAT
e. Gamma GT
f. HCG

Comments on laboratory analysis

_____

_____

_____

Medications

Use of any medications on regular basis, including nasal spray device of any kind Yes ☐ No ☐

If Yes, complete "Pre-trial and concomitant medication log" (page 51).

ECG interpretation form (to be completed by cardiologist)

ECG is performed and determined without any pathological abnormalities? Yes No

☐ ☐

Attestation of cardiologist:

Date:_____(yyyy/mm/dd) _____

Signature cardiologist

Rhinoscopy form (to be completed by ENT specialist doctor), Screening

Rhinoscopy is performed and determined without any pathological abnormalities? Yes ☐ No ☐

1. Mucosa: Colour and Swelling — Abnormal / Normal
2. Secretion: Amount and colour — Abnormal / Normal
3. The presence of polyps — Yes / No
4. Concha interior for swelling — Yes / No

If Abnormal / Yes – please specify:_____

_____

The following will be assessed by patient history:

1. History of nasal blockage — Yes / No — Date start:_____ Date stop:_____
2. Epistaxis — Yes / No — Date start:_____ Date stop:_____
3. History of nasal discharge — Yes / No — Date start:_____ Date stop:_____
4. History of anosmia / hyposmia — Yes / No — Date start:_____ Date stop:_____

If Yes, please provide start and stop date

Attestation of ENT specialist:

Date:_____(yyyy/mm/dd)

Signature ENT specialist doctor: _____

The assessment is performed at Screening or at separate visit between screening and first administration of nasal spray? Yes ☐ No ☐

Date:_____(yyyy/mm/dd)

Signature (sub-) investigator:_____

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Other comments by study personnel:

_____

_____

Declaration

I certify that all data for visit 1 have been filled out completely and correctly.

Yes No
☐ ☐

If no, comment:

_____

_____

_____

_____

_____ Date: _____ (yyyy/mm/dd)
Signature of Study Personnel

I certify that all data for visit 1 have been checked for correctness and completeness.
All study requirements have been fulfilled according to the protocol.

Yes No
☐ ☐

If no, comment:

_____

_____

_____

_____

_____ Date: _____ (yyyy/mm/dd)
Signature of (sub-) investigator (MD)

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Visit 2 Inclusion, randomisation and pharmacokinetic session 1

Date: _____ (yyyy/mm/dd)

Confirmation of Inclusion criteria

	Yes	No
Have there been any changes to the previously confirmed inclusion criteria that would render the subject ineligible for the study?	☐	☐

1. Provision of signed written informed consent
2. Healthy men and woman aged 18-40 years
3. ECG without any pathological abnormalities
4. Have a BMI range of 18,5-26 kg/m^2
5. Female subject with child bearing potential must use high efficacy contraception. For the purpose of this study acceptable contraception is defined as oral contraception, patch, implants, vaginal ring, hormonal IUD, copper IUD, sterilization through out the study until last visit
6. Laboratory values within reference values for the following haematology and biochemistry tests:
    a. Haemoglobin
    b. Creatinine
    c. ASAT
    d. ALAT
    e. Gamma GT

Confirmation of Exclusion criteria

	Yes	No
Have there been any changes to the previously denied exclusion criteria that would render the subjects ineligible for the study?	☐	☐

1. Subjects using medication on a regular basis, including regular use of nasal spray form of any kind
2. History of prior drug allergy
3. Subjects having a local nasal disease or nasal surgery for the last 2 months
4. Pregnant or breast feeding women. A serum HCG below 3 U/L must be demonsrated in females of child-bearing potential at Screening Visit
5. Current drug or alcohol abuse which in the opinion of the Investigator should preclude participation in the study
6. Have received another new medical chemical entity (defined as a compound which has not been approved for marketing) or have participated in any other clinical study that included drug treatment within 3 months of the administration of investigational product in this study
7. Hypersensitivity to naloxone or any of its excipients
8. Investigator considers subject unlikely to comply with study procedures, restrictions and or other requirements

Check question	Yes	No
Does the subject have any additional current medical condition or illness, or relevant condition in the past which may render the subject at unacceptable risk or confound the study assessments, not recorded at Screening?	☐	☐

	Yes	No
Have the subject used any concomitant medication during the last 7 days?	☐	☐

If Yes, Please confirm that at least 5 x max $t_{1/2}$ have passed Since last dose of the concomitant medication was taken.

5 x _____(max $t_{1/2}$) = _____hours.

	Yes	No
Have 5 max $t_{1/2}$ passed since last dose of concomitant medication?	☐	☒

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Randomization

Please confirm that patient meets all the inclusion criteria
and no exclusion criteria, and can be randomized? Yes ☐ No ☐

Randomization number:_____

Treatment for visit 2: _____

Treatment for visit 3: _____

Treatment for visit 4: _____

Treatment for visit 5: _____

Date: _____ (yyyy/mm/dd) _____

Signature of (sub)- investigator

Treatment during visit 2 (double check with randomisation list):

IN 1,4 mg ☐
IN 2,8 mg ☐
IM 0,8 mg ☐
IV 0,4 mg ☐

(2 x 1,4 mg within one nostril with 3 minutes interval)

Administrated in left arm/nostril ☐
right arm/nostril ☐

Treatment administered by: _____

Batch number on ampoule/device: _____

Weight of nasal spray / filled syringe* before administration_____(g) (5 decimals)

Weight of nasal spray / filled syringe* after administration _____(g) (5 decimals)

* Braum omnifix 2,5 ml syringe, weighed with needle attached

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Vital Signs

Scheduled time Rel. to naloxone. (min)	**Time** (hh.mm)	**Heart rate**	**Resp. rate** (bpm)	**Oxygen saturation** (%)	**Blood pressure** (mmHg)	
					Systolic	Diastolic
- 10						
15						
30						
45						
60						
90						
120						
240						
360						

Is any of the recordings clinically significant? Yes No ☐ ☐

If Yes, confirm that the subject is eligible and complete the Adverse Event log (visit independent log) and Medical History log (visit independent log)

Subject is found eligible: _____

Signature of (sub-) investigator

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

PK Blood samples

Scheduled time Relative to adm.	**Actual time** (hh.mm)	**Labelling** OPI-15-002 AAA_XX_YZZ* Date for sampling	**Comments on blood sampling**
- 10		____ _ ____ _ 201	
Naloxone given		No sample	
2		____ _ ____ _ 202	
5		____ _ ____ _ 203	
10		____ _ ____ _ 204	
15		____ _ ____ _ 205	
20		____ _ ____ _ 206	
25		____ _ ____ _ 207	
30		____ _ ____ _ 208	
35		____ _ ____ _ 209	
45		____ _ ____ _ 210	
60		____ _ ____ _ 211	
90		____ _ ____ _ 212	
120		____ _ ____ _ 213	
240		____ _ ____ _ 214	
360		____ _ ____ _ 215	

* AAA is subject initials
XX is subject identification number
Y is visit number

Venous samples drawn from
left arm ☐
right arm ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Safety blood samples

Were safety blood sample for hematology and clinical chemistry taken after the last PK sample at 360 min? Yes ☐ No ☐

Date of safety blood sample:_____(yyyy/mm/dd)

Specify time of safety blood sample taken:_____(hh:mm)

Safety blood found within references values and print is attached to subject file? Yes ☐ No ☐

If No, Comment:
_____
_____
_____
_____

Local irritation in the nose

VAS scale of pain regarding local irritation in the nose, is completed by the subject Yes ☐ No ☐

Completed VAS scale attached to this document Yes ☐ No ☐

Rhinorrhoea? Yes ☐ No ☐

Comments:_____

Itching? Yes ☐ No ☐

Comments:_____

Loss of smell sensation? Yes ☐ No ☐

Comments:_____

Expected Adverse Reactions

Epistaxis	☐ Mild symptoms, intervention not indicated
	☐ Moderate symptoms, medical intervention indicated (nasal packing, cauterization, topical)
	☐ Transfusion radiologic, endoscopic, or operative intervention indicated (haemostasis of bleeding site)
	☐ Life-threatening consequences, urgent intervention indicated
	☐ Death
Nausea	☐ Loss of appetite without alteration in eating habits
	☐ Oral intake decreased without significant weight loss, dehydration or malnutrition
	☐ Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospalization indicated
Vomiting	☐ Grading 1= 1-2 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 2= 3-5 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 3= >=6 episodes (separated by 5 minutes) in 24hrs. tube feeding, TPN or hospitalization
	☐ Grading 4= Life threatning consequences; urgent intervention
Headache	☐ Grading 1 = Mild Pain
	☐ Grading 2 = Moderate pain, limiting instrumental ADL
	☐ Grading 3 = Severe pain, limiting self-care, ADL
Dizziness	☐ Grading 1 = Mild unsteadiness or sensation of movement
	☐ Grading 2 = Moderate unsteadiness or sensation of movement limiting instrumental ADL
	☐ Grading 3 = Severe unsteadiness or sensation of movement, limiting self-care ADL

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Has the subject experienced any adverse events, or have there been any changes to pre-existing adverse events since the last visit? Yes No

(Describe below) ☐☐

_____

_____

Is any of this considered an adverse event? Yes No ☐☐

If yes: Adverse Event form is filled out and included in Participant folder? Yes No ☐☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Declaration

I certify that all data for visit 2 have been filled out completely and correctly. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of Study Personnel

I certify that all data for visit 2 have been checked for correctness and completeness. All study requirements have been fulfilled according to the protocol. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of (sub-) investigator

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Visit 3 - Pharmacokinetic session 2

Date: _____ (yyyy/mm/dd)

Check questions

Does the subject have any additional current medical condition or illness, or relevant condition in the past which may render the subject at unacceptable risk or confound the study assessments, not recorded at Screening (Visit 1)? Yes ☒ No ☐

Have the subject used any concomitant medication during the latest 7 days? Yes ☐ No ☐

If Yes, name of medication:_____

Please confirm that at least 5 x max $t_{1/2}$ have passed since last administration:

5 x _____(max $t_{1/2}$) = _____hours

Is it more than 5 x max $t_{1/2}$ since last administration? Yes ☐ No ☒

Randomisation / Treatment

Randomization number:_____

Treatment during visit 3 (double check with randomisation list):

IN 1,4 mg ☐
IN 2,8 mg ☐
IM 0,8 mg ☐
IV 0,4 mg ☐

(2 x 1,4 mg within one nostril with 3 minutes interval)

Administrated in left arm/nostril ☐
right arm/nostril ☐

Treatment administered by _____

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Batch number on ampoule/device _____

Weight of nasal spray / filled syringe* before administration_____(g) (5 decimals)

Weight of nasal spray / filled syringe* after administration _____(g) (5 decimals)

* Braum omnifix 2,5 ml syringe, weighed with needle attached

Vital signs

Scheduled time Rel. to naloxone. (min)	**Time** (hh.mm)	**Heart rate**	**Resp. rate** (bpm)	**Oxygen saturation** (%)	**Blood pressure** (mmHg)	
					Systolic	Diastolic
- 10						
15						
30						
45						
60						
90						
120						
240						
360						

Is any of the recordings clinically significant? Yes ☐ No ☐

If Yes, confirm that the subject is eligible and complete the Adverse Event log (visit independent log) and Medical History log (visit independent log)

Subject is found eligible: _____

Signature of (sub-) investigator

PK Blood samples

Scheduled time Relative to adm.	**Actual time** (hh.mm)	**Labelling** OPI-15-002 AAA_XX_YZZ* Date for sampling	**Comments on blood sampling**
- 10		____ _ ____ _ 301	
Naloxone given		No sample	
2		____ _ ____ _ 302	
5		____ _ ____ _ 303	
10		____ _ ____ _ 304	
15		____ _ ____ _ 305	
20		____ _ ____ _ 306	
25		____ _ ____ _ 307	
30		____ _ ____ _ 308	
35		____ _ ____ _ 309	
45		____ _ ____ _ 310	
60		____ _ ____ _ 311	
90		____ _ ____ _ 312	
120		____ _ ____ _ 313	
240		____ _ ____ _ 314	
360		____ _ ____ _ 315	

* AAA is subject initials
XX is subject identification number
Y is visit number

Venous samples drawn from
left arm ☐
right arm ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Safety blood samples

Were safety blood sample for hematology and clinical chemistry taken after the last PK sample at 360 min? Yes ☐ No ☐

Date of safety blood sample:_____(yyyy/mm/dd)

Specify time of safety blood sample taken:_____(hh:mm)

Safety blood found within references values and print is attached to subject file? Yes ☐ No ☐

If No, Comment:

_____

_____

_____

_____

Local irritation in the nose

VAS scale of pain regarding local irritation in the nose, is completed by the subject Yes ☐ No ☐

Completed VAS scale attached to this document Yes ☐ No ☐

Rhinorrhoea? Yes ☐ No ☐

Comments:_____

Itching? Yes ☐ No ☐

Comments:_____

Loss of smell sensation? Yes ☐ No ☐

Comments:_____

Expected Adverse Reactions

Epistaxis	☐ Mild symptoms, intervention not indicated
	☐ Moderate symptoms, medical intervention indicated (nasal packing, cauterization, topical)
	☐ Transfusion radiologic, endoscopic, or operative intervention indicated (haemostasis of bleeding site)
	☐ Life-threatening consequences, urgent intervention indicated
	☐ Death
Nausea	☐ Loss of appetite without alteration in eating habits
	☐ Oral intake decreased without significant weight loss, dehydration or malnutrition
	☐ Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospalization indicated
Vomiting	☐ Grading 1= 1-2 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 2= 3-5 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 3= >=6 episodes (separated by 5 minutes) in 24hrs. tube feeding, TPN or hospitalization
	☐ Grading 4= Life threatning consequences; urgent intervention
Headache	☐ Grading 1 = Mild Pain
	☐ Grading 2 = Moderate pain, limiting instrumental ADL
	☐ Grading 3 = Severe pain, limiting self-care, ADL
Dizziness	☐ Grading 1 = Mild unsteadiness or sensation of movement
	☐ Grading 2 = Moderate unsteadiness or sensation of movement limiting instrumental ADL
	☐ Grading 3 = Severe unsteadiness or sensation of movement, limiting self-care ADL

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Is the subject experienced any adverse events, or have there been any changes to pre-existing adverse events since the last visit? Yes No
(Describe below) ☐ ☐

_____

_____

Is any of this considered an adverse event? Yes No ☐ ☐

If yes: Adverse Event form is filled out and included in Participant folder? Yes No ☐ ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Declaration

I certify that all data for visit 3 have been filled out completely and correctly. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of Study Personnel

I certify that all data for visit 3 have been checked for correctness and completeness. All study requirements have been fulfilled according to the protocol. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of (sub-) investigator

Visit 4 - Pharmacokinetic session 3

Date: _____ (yyyy/mm/dd)

Check questions

Does the subject have any additional current medical condition or illness, or relevant condition in the past which may render the subject at unacceptable risk or confound the study assessments, not recorded at Screening (Visit 1)? Yes ☐ No ☐

Have the subject used any concomitant medication during the latest 7 days?

Yes ☐ No ☐

If Yes, name of medication:_____

Please confirm that at least 5 x max $t_{1/2}$ have passed since last administration:

5 x _____(max $t_{1/2}$) = _____hours

Is it more than 5 x $t_{1/2}$ since last administration? Yes ☐ No ☐

Randomization

Randomization number:_____

Treatment during visit 4 (double check with randomisation list):

IN 1,4 mg ☐
IN 2,8 mg ☐
IM 0,8 mg ☐
IV 0,4 mg ☐

(2 x 1,4 mg within one nostril with 3 minutes interval)

Administrated in left arm/nostril ☐
right arm/nostril ☐

Treatment administered by _____

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Batch number on ampoule/device _____

Weight of nasal spray / filled syringe* before administration_____(g) (5 decimals)

Weight of nasal spray / filled syringe* after administration _____(g) (5 decimals)

* Braum omnifix 2,5 ml syringe, weighed with needle attached

Vital Signs

Scheduled time Rel. to naloxone. (min)	**Time** (hh.mm)	**Heart rate**	**Resp. rate** (bpm)	**Oxygen saturation** (%)	**Blood pressure** (mmHg)	
					Systolic	Diastolic
- 10						
15						
30						
45						
60						
90						
120						
240						
360						

Is any of the recordings clinically significant? Yes ☐ No ☐

If Yes, confirm that the subject is eligible and complete the Adverse Event log (visit independent log) and Medical History log (visit independent log)

Subject is found eligible: _____

Signature of (sub-) investigator

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

PK Blood samples

Scheduled time Relative to adm.	**Actual time** (hh.mm)	**Labelling** OPI-15-002 AAA_XX_YZZ* Date for sampling	**Comments on blood sampling**
- 10		____ _ ____ _ 401	
Naloxone given		No sample	
2		____ _ ____ _ 402	
5		____ _ ____ _ 403	
10		____ _ ____ _ 404	
15		____ _ ____ _ 405	
20		____ _ ____ _ 406	
25		____ _ ____ _ 407	
30		____ _ ____ _ 408	
35		____ _ ____ _ 409	
45		____ _ ____ _ 410	
60		____ _ ____ _ 411	
90		____ _ ____ _ 412	
120		____ _ ____ _ 413	
240		____ _ ____ _ 414	
360		____ _ ____ _ 415	

* AAA is subject initials
XX is subject identification number
Y is visit number

Venous samples drawn from
left arm ☐
right arm ☐

Safety blood samples

Were safety blood sample for hematology and clinical chemistry taken after the last PK sample at 360 min? Yes ☐ No ☐

Date of safety blood sample:_____(yyyy/mm/dd)

Specify time of safety blood sample taken:_____(hh:mm)

Safety blood found within references values and print is attached to subject file? Yes ☐ No ☐

If No, Comment:

_____

_____

_____

_____

Local irritation in the nose

VAS scale of pain regarding local irritation in the nose, is completed by the subject Yes ☐ No ☐

Completed VAS scale attached to this document Yes ☐ No ☐

Rhinorrhoea? Yes ☐ No ☐

Comments:_____

Itching? Yes ☐ No ☐

Comments:_____

Loss of smell sensation? Yes ☐ No ☐

Comments:_____

Expected Adverse Reactions

Epistaxis	☐ Mild symptoms, intervention not indicated
	☐ Moderate symptoms, medical intervention indicated (nasal packing, cauterization, topical)
	☐ Transfusion radiologic, endoscopic, or operative intervention indicated (haemostasis of bleeding site)
	☐ Life-threatening consequences, urgent intervention indicated
	☐ Death
Nausea	☐ Loss of appetite without alteration in eating habits
	☐ Oral intake decreased without significant weight loss, dehydration or malnutrition
	☐ Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospalization indicated
Vomiting	☐ Grading 1= 1-2 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 2= 3-5 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 3= >=6 episodes (separated by 5 minutes) in 24hrs. tube feeding, TPN or hospitalization
	☐ Grading 4= Life threatning consequences; urgent intervention
Headache	☐ Grading 1 = Mild Pain
	☐ Grading 2 = Moderate pain, limiting instrumental ADL
	☐ Grading 3 = Severe pain, limiting self-care, ADL
Dizziness	☐ Grading 1 = Mild unsteadiness or sensation of movement
	☐ Grading 2 = Moderate unsteadiness or sensation of movement limiting instrumental ADL
	☐ Grading 3 = Severe unsteadiness or sensation of movement, limiting self-care ADL

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Has the subject experienced any adverse events, or have there been any changes to pre-existing adverse events since the last visit? Yes No

(Describe below) ☐ ☐

_____

_____

Is any of this considered an adverse event*? Yes No ☐ ☐

If yes: Adverse Event form is filled out and included in Participant folder. Yes No ☐ ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Declaration

I certify that all data for visit 4 have been filled out completely and correctly. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of Study Personnel

I certify that all data for visit 4 have been checked for correctness and completeness. All study requirements have been fulfilled according to the protocol. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of (sub-) investigator

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Visit 5 – Pharmacokinetic session 4

Date: _____ (yyyy/mm/dd)

Check questions

Does the subject have any additional current medical condition or illness, or relevant condition in the past which may render the subject at unacceptable risk or confound the study assessments, not recorded at Screening (Visit 1)? Yes ☒ No ☐

Have the subject used any concomitant medication during the latest 7 days? Yes ☐ No ☐

If Yes, name of medication:_____

Please confirm that at least 5 x max $t_{1/2}$ have passed since last administration:

5 x _____(max $t_{1/2}$) = _____hours

Is it more than 5 x $t_{1/2}$ since last administration? Yes ☐ No ☒

Randomization

Randomization number:_____

Treatment during visit 5 (double check with randomisation list):

IN 1,4 mg ☐
IN 2,8 mg ☐
IM 0,8 mg ☐
IV 0,4 mg ☐

(2 x 1,4 mg within one nostril with 3 minutes interval)

Administrated in left arm/nostril ☐
right arm/nostril ☐

Treatment administered by _____

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Batch number on ampoule/device _____

Weight of nasal spray / filled syringe* before administration_____(g) (5 decimals)

Weight of nasal spray / filled syringe* after administration _____(g) (5 decimals)

* Braum omnifix 2,5 ml syringe, weighed with needle attached

Vital Signs

Scheduled time Rel. to naloxone. (min)	**Time** (hh.mm)	**Heart rate**	**Resp. rate** (bpm)	**Oxygen saturation** (%)	**Blood pressure** (mmHg)	
					Systolic	Diastolic
- 10						
15						
30						
45						
60						
90						
120						
240						
360						

Is any of the recordings clinically significant? Yes ☐ No ☐

If Yes, confirm that the subject is eligible and complete the Adverse Event log (visit independent log) and Medical History log (visit independent log)

Subject is found eligible: _____

Signature of (sub-) investigator

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

PK Blood samples

Scheduled time Relative to adm.	**Actual time** (hh.mm)	**Labelling** OPI-15-002 AAA_XX_YZZ* Date for sampling	**Comments on blood sampling**
- 10		____ _ ____ _ 501	
Naloxone given		No sample	
2		____ _ ____ _ 502	
5		____ _ ____ _ 503	
10		____ _ ____ _ 504	
15		____ _ ____ _ 505	
20		____ _ ____ _ 506	
25		____ _ ____ _ 507	
30		____ _ ____ _ 508	
35		____ _ ____ _ 509	
45		____ _ ____ _ 510	
60		____ _ ____ _ 511	
90		____ _ ____ _ 512	
120		____ _ ____ _ 513	
240		____ _ ____ _ 514	
360		____ _ ____ _ 515	

* AAA is subject initials
XX is subject identification number
Y is visit number

Venous samples drawn from
left arm ☐
right arm ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Safety blood samples

Were safety blood sample for hematology and clinical chemistry taken after the last PK sample at 360 min? Yes ☐ No ☐

Date of safety blood sample:_____(yyyy/mm/dd)

Specify time of safety blood sample taken:_____(hh:mm)

Safety blood found within references values and print is attached to subject file? Yes ☐ No ☐

If No, Comment:
_____
_____
_____
_____

Local irritation in the nose

VAS scale of pain regarding local irritation in the nose, is completed by the subject Yes ☐ No ☐

Completed VAS scale attached to this document Yes ☐ No ☐

Rhinorrhoea? Yes ☐ No ☐

Comments:_____

Itching? Yes ☐ No ☐

Comments:_____

Loss of smell sensation? Yes ☐ No ☐

Comments:_____

Expected Adverse Reactions

Epistaxis	☐ Mild symptoms, intervention not indicated
	☐ Moderate symptoms, medical intervention indicated (nasal packing, cauterization, topical)
	☐ Transfusion radiologic, endoscopic, or operative intervention indicated (haemostasis of bleeding site)
	☐ Life-threatening consequences, urgent intervention indicated
	☐ Death
Nausea	☐ Loss of appetite without alteration in eating habits
	☐ Oral intake decreased without significant weight loss, dehydration or malnutrition
	☐ Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospalization indicated
Vomiting	☐ Grading 1= 1-2 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 2= 3-5 episodes (separated by 5 minutes) in 24 hrs.
	☐ Grading 3= >=6 episodes (separated by 5 minutes) in 24hrs. tube feeding, TPN or hospitalization
	☐ Grading 4= Life threatning consequences; urgent intervention
Headache	☐ Grading 1 = Mild Pain
	☐ Grading 2 = Moderate pain, limiting instrumental ADL
	☐ Grading 3 = Severe pain, limiting self-care, ADL
Dizziness	☐ Grading 1 = Mild unsteadiness or sensation of movement
	☐ Grading 2 = Moderate unsteadiness or sensation of movement limiting instrumental ADL
	☐ Grading 3 = Severe unsteadiness or sensation of movement, limiting self-care ADL

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Has the subject experienced any adverse events, or have there been any changes to pre-existing adverse events since the last visit? Yes No
(Describe below) ☐ ☐

_____

_____

Is any of this considered an adverse event*? Yes No ☐ ☐

If yes: Adverse Event form is filled out and included in Participant folder. Yes No ☐ ☐

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Declaration

I certify that all data for visit 5 have been filled out completely and correctly. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of Study Personnel

I certify that all data for visit 5 have been checked for correctness and completeness. All study requirements have been fulfilled according to the protocol. Yes ☐ No ☐

If no, comment:

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of (sub-) investigator

Rhinoscopy interpretation (to be completed by ENT specialist doctor)

Rhinoscopy is performed and determined without any pathological abnormalities? Yes No ☐ ☐

1	Mucosa: Colour and Swelling	Abnormal / Normal
2	Secretion: Amount and colour	Abnormal / Normal
3	The presence of polyps	Yes / No
4	Concha interior for swelling	Yes / No

If Abnormal / Yes – please specify:

_____
_____
_____

Attestation of ENT specialist:

Date:_____(yyyy/mm/dd)

Signature ENT specialist doctor:_____

The assessment is performed at Visit 6 or at separate visit between last administration of nasal spray and Visit 6? Yes No ☐ ☐

Date:_____(yyyy/mm/dd)

Signature (sub-) investigator:_____

Visit 6 – Follow up

Date: _____ (yyyy/mm/dd)

Check questions

Has there been any change in concomitant medication since last visit?

Yes No ☐☐

If Yes, comment :_____

_____

_____

Did the standard physical examination reveal any additional conditions which have previously not been recorded in the CRF?

Yes No ☐☐

If Yes, comment:_____

_____

_____

Has the subject experienced any adverse events, or have been any changes to pre-existing adverse events since last visit?

Yes No ☐☐

If Yes, comment:_____

_____

_____

Declaration

I certify that all data for visit 6 has been filled out completely and correctly.

Yes ☐ No ☐

If no, comment:

_____

_____

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of Study Personnel

I certify that all data for visit 6 have been checked for correctness and completeness. All study requirements have been fulfilled according to the protocol.

Yes ☐ No ☐

If no, comment:

_____

_____

_____

_____

Date: _____ (yyyy/mm/dd) _____
Signature of (sub-) investigator

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Study Termination

Did the subject complete the study? Yes No

☐☐

Date completion: _____(yyyy/mm/dd)

Date of withdrawal: _____ (yyyy/mm/dd)

Withdrawn from Treatment ☐
or Study ☐

Primary reason for withdrawal:

Did not meet the selection criteria
Withdrawal of informed consent ☐
Adverse event* ☐
Lost to follow up ☐
Investigators discretion ☐
Other** ☐

If Other, specify: _____

Date of last study medication taken:_____(yyyy/mm/dd)

Adverse Reaction log, 3089

Subject no:_____

AE term	Intensity	Frequency	Date start	Date stop	Sign.
Nausea					
Vomiting					
Headache					
Dizziness					
Local irritation in the nose					

Adverse Events log, 3089

Subject no:_____

AE term	Severity	Rel. to IMP	Date start	Date stop	Action taken	Sign.

Severity: Mild, moderate, severe
Relationship to study drug: Unrelated, unlikely, possible, probable, not assessable
Action taken: None, Study drug discontinued permanently, Remedial therapy, Hospitalization required or prolonged, Other
If other, specify:

_____

Local irritation in the nose, 3089

Subject no.:_____

AE term	Intensity	Frequency	Date start	Date stop	Sign.
Pain					
Nasal congestion					
Rhinorrhoea					
Epistaxis					
Itching					
Loss of smell sensation					

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Medical History log

No.	All current diagnosis, symptoms and findings. Relevant past diagnosis	Date of onset (yyyy/mm/dd)	Has the condition worsened during the study?		Is condition continuing after study end?		Stop date of medication (yyyy/mm/dd)	Signature
			Yes	No	Yes	No		
1								
2								
3								
4								
5								
6								
7								

Study no: OPI 15-002

Subject initials: _____
Subject number: _____
Page filled in by (initials):_____

Pre-trial and concomitant Medication log

No	Medication (generic name)	Dose	Unit	Regi-men	Route	Indication	Start date (yyyy/ mm/dd)	Drug to be continued after study		Stop date (yyyy/ mm/dd)	Reason for adm.
								Yes	No		
1											
2											
3											
4											
5											
6											
7											

Unit: appl, caps, dram, gtts, grain, gram, I/U, inj, meq, mEq, mg, mg/kg, mL, ng, puff, ounce, tabs, units, spray, other (specify)

Regimen: daily, twice daily, 3 times daily, 4 times daily, every other week, once weekly, twice weekly, 3 times weekly, 4 times weekly, monthly, twice per month, as needed, single dose, not applicable, other (specify)

Route: Intraocular, nasally, orally, dietary, topically, subcutaneously, transdermal, intraspinal, intravenous, perineural, urethal, rectally, inhaled, vaginally, sublingually, capsules, ear, intraperitoneally, nebulized, percutaneous, intradermally, other (specify)

Reason for administration: Medical history, adverse event, other (specify)

Principal Investigator's signature

All data in this medical journal and case report form has been entered under my responsibility, and to the best of my knowledge is accurate and complete.

Date: _____ (yyyy/mm/dd)

_____
Principal Investigator's signature

Appendix G: E-mail sent out prior to re-screening

Hei

Endelig ser det ut til at vi kommer i gang med klinisk utprøvning for nasalt nalokson.

Vi håper at du fremdeles ønsker å delta i studien, og sender derfor nå ut litt informasjon om datoer for screening og forskningsdager.

I og med at det har gått en stund siden du var screening, er det nødvendig å gjøre deler av screeningen på nytt. Planlagte datoer for screening er satt til **onsdag 16. mars og fredag 18. mars**. Fint om du kan gi en tilbakemelding på om du kan én eller begge datoer.

Sender dessuten med en foreløpig oversikt over hvilke dager vi har satt av på Forskningsposten til forskningsdager i perioden **29. mars - 31. juni** (NB! Excel-arket har én side pr måned.) Vi må ut fra kapasitet på Forskningsposten og deltakernes muligheter, sammenstille en plan, og det er derfor kjempefint om du kan angi hvilke av disse dagene du har anledning på.

Hver deltaker gjennomfører 4 forskningsdager, med minimum tre døgn mellom hver forskningsdag.

Ta gjerne kontakt dersom spørsmål!

Med vennlig hilsen

Øyvind Glende
oyvidan@stud.ntnu.no , mobil 95083316

og

Ola dale
ola.dale@ntnu.no , mobil 91199255