SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of INOmax. For information on changes after approval please refer to module 8.

1. Introduction

Persistent pulmonary hypertension of the new-born (PPHN) is a disorder of the transition from foetal to extra-uterine life, a clinical syndrome characterised by persistence of elevated pulmonary vascular resistance producing right-to-left shunting of deoxygenated blood across the still patent foramen ovale and/or ductus arteriosus as well as systemic hypoxemia. PPHN may be idiopathic or associated with parenchyma lung disease as respiratory distress syndrome of prematurity, meconium aspiration syndrome, pneumonia and sepsis or congenital diaphragmatic hernia and pulmonary hypoplasia. Although anatomical changes such as pulmonary vascular smooth muscle hypertrophy may occur and contribute to increased pulmonary resistance, pulmonary vasoconstriction and altered vascular reactivity are central to the pathophysiology of this syndrome. PPHN is an uncommon life threatening condition (the incidence is less than 0.1% of life births and mortality up to 40%).

Conventional therapy consists in hyperoxygenation through mechanical ventilation with hyperoxic gas mixtures, induced metabolic or respiratory alkalosis, deep sedation and pharmacological paralysis to reduce pulmonary vasoactivity. Intravenous vasodilators as sodium nitroprusside or tolazoline may be useful but can lead to severe systemic hypotension, which in turn aggravate the right-to-left shunt.

In the most severe forms of hypoxic respiratory failure, invasive procedures of extracorporeal membrane oxygenation (ECMO) have proven efficacy in reducing mortality compared to conventional therapies. However, these techniques remain procedures of exception performed by a trained and specialised neonatology team. They require catheterisation and often ligation of the cervical great vessels (jugular vein and carotid artery) need continuous blood anticoagulation and use of blood products. Potential drawbacks are related to the increased risk of brain haemorrhage and infarction. Prognosis into adult life is unknown.

Nitric oxide (NO) is the active component of endothelium-derived relaxing factor. Exogenous inhaled NO acts selectively on the pulmonary circulation. In smooth muscle cells, NO activates soluble guanylate cyclase to form cyclic GMP, which in turn promotes a calcium dependent relaxation. It has little systemic effect because of its short half-life caused by inactivation by binding to haemoglobin, rapid oxidation and the interaction with free radicals.

INOmax 400 ppm contains the active drug substance, nitric oxide, formulated as a series of dilutions in nitrogen. It is delivered to the patient via mechanical ventilation after dilution with an air/oxygen mixture using approved (CE-marked) ventilators and delivery devices.

The indication is for the treatment, in conjunction with ventilatory support and other appropriate agents, of newborns ≥ 34 weeks gestation with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, in order to improve oxygenation and to reduce the need for extracorporeal membrane oxygenation. The maximum recommended dose is 20 ppm decreasing to 5 ppm within 4-24 hours provided arterial oxygenation is adequate at lower dose. The 20 ppm dose should not be exceeded. In the pivotal clinical trials, the starting dose was generally 20 ppm.

For this indication, inhaled NO reduced the need for ECMO without altering mortality and morbidity.

2. Chemical, pharmaceutical and biological aspects

Composition

INOmax is a gas for inhalation. It is presented as a compressed gaseous mixture of nitric oxide (active substance) diluted with nitrogen (dilutent). The diluted gas is administered to the patient via

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mechanical ventilator and using a CE-marked delivery system, in which the gas is diluted to a therapeutic concentration with a medical air/oxygen mixture.

Three strengths were initially proposed: 100ppm, 400ppm and 800ppm (mol/mol). The provision of cylinders of three different concentrations increases the chances of an error in administration; the applicant consequently has restricted INOmax to a single 400-ppm strength.

The container is a colour coded aluminium alloy cylinder of 10-litres capacity, closed by stainless steel positive pressure (residual) valve, which presents an unique outlet to eliminate the risk for connection to the wrong type of equipment. Satisfactory packaging component specifications and detail on colour coding and labelling were provided. To ensure that the gas mixture can be delivered at 1 atmosphere and 15°C, an overfill is used; each cylinder containing thus an amount of product equivalent to 1535 litres.

Active substance

The active substance, nitric oxide, is synthesised in a continuous process by the reaction of nitric acid and sulphur dioxide ($2\ HNO_3 + 3\ SO_2 + 2\ H_2O \rightarrow 2\ NO + 3\ H_2SO_4$), followed by further purification steps to remove by-products and moisture. The purified gas is filled in steel gas cylinders fitted with diaphragm sealed stainless steel valves specified for use with pure NO. Compatibility of the gas with the packaging was established. Satisfactory details on the manufacturing process are provided.

The in-process controls performed during the synthesis are minimal. This approach is acceptable, as there are no intermediates in the synthesis, and since synthesis, purification and packaging are performed as a single continuous process, and the potential impurities and assay are effectively controlled on the active substance and sampling could lead to contamination with air. The manufacturing process was validated on three consecutive batches. Results are within the proposed specification and confirm batch-to-batch consistency.

Nitric oxide is a colourless gas and a stable molecule radical, but with the exception that when mixed with air, it immediately forms a brown cloud of nitrogen dioxide. It is not flammable but supports combustion.

Potential impurities in the bulk gas are nitrogen, nitrous oxide, nitrogen dioxide, carbon dioxide, and sulphur dioxide. Other oxides of nitrogen exist but are not considered due to their instability and concentration dependent equilibria and only minute undetectable amounts are likely to exist in the drug substance.

IR and mass-spectrometry have confirmed the structure of nitric oxide. The impurities and related substances are controlled by IR-based method for the assay of nitric oxide and by GC for nitrogen and argon. All analytical methods used were validated. Specifications on all starting materials, impurities and related substances are provided and are acceptable. Batch analysis results from 5 batches of drug substance are presented. Data comply with specifications.

Concerning stability, the active substance stored in cylinders filled at 20 bar was tested for up to 24 months under different temperatures. Increase of the main oxides of nitrogen can be observed, indicating that degradation is dependent on temperature, time and pressure. Degradation is decreased by dilution. Potential degradation products remain within the proposed specifications. Overall, the data support the re-test period of 6 months under the proposed storage conditions.

Other ingredients

The excipient, nitrogen, is used as diluent since it is inert and free from oxygen. Satisfactory details and validation data were provided on the determination of oxygen and carbon monoxide in nitrogen and assay of nitrogen. Compatibility studies were presented demonstrating compatibility between NO/N2 mixtures and the containers and dispensing devices. The choice and specification of excipient used are justified. Specification complies with Ph.Eur. requirements.

Product development and finished product

The pharmaceutical development revolved around the physical properties of the gas mixtures. In presence of oxygen, NO is easily oxidised to NO_2, which is highly toxic. The challenge is to avoid the formation of NO_2. Dilutions are therefore necessary to avoid immediate oxidation after mixing with oxygen/air before administration and to deliver a therapeutic concentration from pure NO. The NO/N_2

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mixture has the same properties as pure nitrogen, and there is no risk of separation, which only occur below -140ºC. The rationale behind the choice of the strength was explained (see also the section Composition). A 10-litres cylinder is selected, since it contains sufficient product to treat one patient and is easily handle in the hospital environment.

The batches of product used in the various clinical studies were identified, and an acceptable degree of equivalency was established.

Dilution of the pure active substance to the finished product is performed in two steps. Intermediate premix is manufactured by dilution of NO with nitrogen into 46.5 litre aluminium cylinders closed with stainless steel valves and teflon rings. From that premix, 400 ppm-finished products are prepared by further dilution with nitrogen. Cylinders of finished product are manufactured in batches of ten by dilution of the NO/N_2 premix with nitrogen into 10 litre cylinders. The premix is filled by weight and the finished product is filled by mass-flow. In-process controls were performed and they are acceptable.

Validation data on batches of finished product indicate the ability of the process to manufacture homogenous finished product with satisfactory filling volumes and pressures.

The control tests and specification for the intermediate and finished products are adequately drawn up and considered to be acceptable. Assay of nitric oxide in the premix and finished product was by an IR-based method as per the method used to determine gaseous impurities. Control of nitrogen dioxide in the finished product was by UV detection. The absence of test for microbiological quality was justified, this was based on the absence of recoverable organism from the gas; but the requirements of Ph. Eur. category 2 products are applied for limits for the microbial status. The validated analytical methods employed are capable of controlling the drug premixes and products within the proposed specifications. The impurity limits in the product specifications guarantee acceptable levels after dilution for administration.

Batch analyses data, resulting from batches of premixes and batches of finished product, met the proposed specification.

The product is manufactured in the facilities that hold the necessary Manufacturing Authorisation (see Annex II of the Opinion).

Stability of the Product

Intermediate premixes - Cylinders were stored at different temperatures (25ºC and 55ºC). Data up to 24 months was available. The NO assay was the only parameter monitored and was within specification limits. The results showed that temperature was not an influencing factor. However, until further stability data on the intended premixes including data on the content of nitrogen dioxide (which will be a better indicator of stability) is provided, the retest period should be restricted to 12 months**.**

Finished product - Cylinders were stored in uncontrolled, unheated storage outdoors, at the ICH conditions and at the conditions to simulate storage exposed to direct sunlight. Data were generated up to 36 months. The parameters monitored were NO assay and nitrogen dioxide and the same specification is applied at release and check. No significant decreases in assay or increases in nitrogen dioxide were observed. The results support the shelf life of 2 years.

Containers and delivery system

The cylinders used for the intermediate premixes and finished product is manufactured according to Directive 84/526/EEC and each unit is identified by a unique number and colour code. The cylinders are closed by stainless steel positive pressure (residual) valves complying with the requirements specified in the draft CEN technical document *"General guidance on the equipment used for inhaled NO therapy"*. Satisfactory specifications and technical details were provided for the cylinders and valves. The ventilators and delivery devices used in the administration of INOmax should be intended for the delivery of NO, CE-marked (i.e. approved according to the Medical Devices Directive) and also meet the CEN requirements for delivery of nitric oxide. The requirements for the administration device are also covered in the SPC**.**

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Discussion on chemical, pharmaceutical and biological aspects

The bulk drug substance is adequately controlled and characterised. The data provide confirm consistency of manufacture and demonstrate adequate stability profile based on the short retest period proposed. The proposed specification is justified.

The manufacture of the intermediate premix and finished product is also adequately defined and controlled. The development pharmaceutics and validation of manufacture are satisfactory. Batch to batch consistency for the finished product has been demonstrated. The finished product exhibits good stability and no degradation is observed even under the extreme storage conditions. The design specification was met over the proposed shelf life.

Overall the chemical-pharmaceutical dossier is well documented and guarantees the quality of the active substance and finished product, both initially and throughout the shelf life. A number of quality points were not resolved at the time of the CPMP opinion. However, these were considered to be without any impact on the efficacy or safety of the product, and are indicated to be addressed post-approval (see section II.3 of this report).

3. Toxico-pharmacological aspects

The pre-clinical dossier comprises data from published literature and additional studies conducted according to GLP.

The high chemical reactivity of nitric oxide in the presence of oxygen renders preclinical studies related to inhaled nitric oxide difficult to perform and to interpret. Moreover, results must be interpreted in the light of not only of inhaled NO but also of inhaled NO_2 levels. NO_2 levels are not always specified, mainly in the older studies.

Pharmacodynamics

Pharmacological properties related to therapeutic indications - The pharmacology of nitric oxide (NO) relevant to its use in hypoxic respiratory failure is centred upon NO's selective properties of causing vasodilatation of hypertensive pulmonary vasculature. Its mechanism of action through generation of c-GMP in smooth muscle cells has been extensively reviewed. Inhaled NO enters the circulation, but red blood cells prevent systemic vasodilatation.

NO reversed pulmonary vasoconstriction induced by hypoxia, hypoxia and acidosis, by vasoconstrictor drugs, by thromboxane/endoperoxide mimetic or by endothelial injury. These NO effects were tested in isolated rat lungs, and in awake and anaesthetised animals as lambs, sheep, rabbit, rat and dog. NO produced a dose-dependent pulmonary vasodilatation over a large range of concentrations (doses from 5 to 120 ppm have been tested). Threshold and plateau for NO activity varied according to the model tested.

Pulmonary vasoconstriction evoked by activation of the coagulation system (endotoxin, lipopolysaccharide) or by infection (group B streptococcus) was also reversed by NO inhalation at doses up to 150 ppm in piglets. In anaesthetised mechanically ventilated guinea pigs, 300 ppm NO decreased methacholine-induced bronchoconstriction. Histamine-induced bronchoconstriction in dogs was also reversed at doses between 100-200 ppm.

The vasodilator effect of NO was independent of cyclooxygenase as indicated by the lack of modification by indomethacin and was additive to that of terbutaline inhalation. In sheep, NO reduced pulmonary vascular resistance independent of pulmonary blood flow; blood flow to poor and unventilated lung areas decreased.

Secondary pharmacology - Effects of NO on the central nervous (CNS), cardiovascular (CVS), and respiratory and immune systems and effects on platelets and metalloproteins were investigated.

CNS - Inhalation of NO at 50 ppm for 180 minutes decreased performance in a discriminative learning task in rats. It also increased the amplitude and prolonged the auditory evoked potentials (AEP) at both 10 and 50 ppm. These effects were not fully understood. However, the results of a clinical trial (NINOS) indicate that this finding probably does not represent a hazard in clinical use. Patients with an average age of 1.7 days were exposed to NO concentrations ranging from 20 to 80 ppm for a mean

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duration of 21 hours; the peak metHb levels were 2%. A long-term assessment of 18-24 months of neurodevelopment indicated no adverse treatment related effects.

CVS and electrocardiograph evaluation of inhaled NO - In a study for acute cardiovascular evaluation of inhaled nitric oxide, 20 anaesthetised Beagle dogs were exposed to NO concentrations of 0, 80, 160, 320 and 640 ppm for 6 hours. Toxic physiological changes were evident in the 320-640 ppm range and consisted of elevated metHb and signs of tissue hypoxia, increased respiratory rate and minute volume, increased heart rate and late hypotension. Systemic pressures decreased late particularly at NO $\geq$ 320 ppm, which was due to decreased vascular resistance attributed to lowered peripheral tissue oxygen concentrations. The death of one dog at 640 ppm was attributed to these effects.

Electrocardiograph changes were found in dogs exposed to NO, some of them were already present during baseline measurements, as premature ventricular depolarisation (sinus tachycardia was observed in a 3 animals in the 80 or 320 ppm group, junctional rhythm in 2 animals in the 80 and 160 ppm group, R on T phenomena in 1 animal from the 640 ppm group). These findings might indicate that exposure to NO in this model produced ventricular irritability.

In six non-anaesthetised Beagle dogs, administration of NO at concentration up to 320 ppm did not affect cardiac conduction, rate or rhythm. It is therefore unlikely that cardiac dysrythmia would result from NO administration in the previous study and more probable that the cardiac anomalies were due to anaesthesia and/or invasive monitoring.

No pathologies were found in the heart in either the rat or dogs studies.

Immune response - The study in mice at low dose NO (10 ppm) for up to 30 weeks suggest that immune responses measured (humoral response to antibodies, graft versus host reaction) were increased in early NO exposure and depressed later.

Effects on platelets and metalloproteins - A prolonged bleeding time has been reported in rabbits and humans, the mechanism is unknown. This effect warrants investigation of interactions with anti-coagulant platelet inhibitory compounds.

Reaction with metalloproteins was evaluated after exposure to 9 ppm NO in mice. NO binds not only haemoglobin and others heme proteins, it can also modify metalloproteins as transferrin and catalase, the clinical implications of this phenomenon is unknown.

Pharmacokinetics

The data was solely from published literature, except for investigations of methaemoglobin (metHb) concentration as a function of time.

Absorption - In rat the percentage of absorption of NO decreased with increasing concentrations, being 90%, 60% and 20% at 138, 270 and 380 ppm respectively. In man, 80% of NO is absorbed in normal breathing and 90% in deep breathing for concentrations up to 5 ppm.

Biotransformation and excretion - The major metabolic pathway of inhaled NO is that the inhaled gas combines with haemoglobin, forming nitrosyl-haemoglobin (NOHb) from which, nitrites (NO_2) and nitrates (NO_3) are generated. In the presence of oxygen, there is a rapid oxidation of NOHb into metHb and the subsequent reduction by methaemoglobin reductase of metHb into ferrous Hb and nitrate. Nitrites and nitrates are in majority excreted in the urine; a small amount is discharged in the oral cavity via the salivary glands. Nitrite is converted to N_2 gas in the stomach. Nitrate in the intestine is partly reduced to ammonia (NH_3), re-absorbed in the body and converted to urea. Most of the metabolites of inhaled NO are excreted from the body within 48 hours.

Methaemoglobin concentration as a function of time - Pharmacokinetic modelling of methaemoglobin concentration-time was investigated in normal dogs inhaling 80, 160, 320, 640 ppm nitric oxide. Based upon an elimination half-life of 3 hours, the time to reach the steady state of % methaemoglobin concentration, in anaesthetised dogs, would be 12-15 hours.
In normal human volunteers inhaling 32, 64, 128 and 512-ppm nitric oxide, model-predicted steady-state % metHb concentrations and metHb formation rates after NO exposures demonstrated a linear relationship between these parameters and NO exposures. Based upon elimination half-life of 1 hour the time to reach steady state % metHb concentrations would be 4-5 hours. These models are probably

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not relevant to clinical application in newborns, which have very different body weight, blood mass and reduced level of activity of methaemoglobin reductase.

Toxicology

Single dose toxicity - Studies in mice, rats and dogs have been reported. The data was mainly from published literature.

In mice, the exposure to NO ranged from 9 ppm for 16 hours to 320 ppm for 8 hours. The main finding reported was increased metHb; NO_2 contamination was not accounted for. In dogs anaesthetised with i.v. pentobarbitone, exposure to 20000 ppm NO resulted in death within 50 minutes; one of two dogs exposed to 5000 ppm also died. Deaths were attributed to a reduction in arterial oxygen content due to methaemoglobinaemia, low arterial pressure and acidaemia.

Mortality can be deduced only in more recent studies where NO_2 levels were controlled. Lethality depends both on the concentration of NO and on the duration of administration. Deaths are related to tissue hypoxia linked to elevated methaemoglobin levels. In Sprague-Dawley rats, doses of 400-500 ppm caused death within 1-3 hours of exposure and 300 ppm caused death in 26 of 30 animals exposed for six hours. In the literature, no death has been reported for 1-hour exposure to NO at 25-260 ppm in rats.

Repeat dose toxicity - Studies were conducted in the mouse, rat and rabbit. The mouse and rabbit data came solely from published literature.

After repeated exposures to low dose of NO (2 to 10 ppm), structural and ultra-structural changes in lung architecture were described in mice (bronchiolar epithelial hyperplasia, hyperaemia, septal enlargement, congestion and increased relative lung weight) and rabbits (fluid containing vacuoles inside the arteriolar endothelial cells and/or the intercellular junctions, and thickening of the alveolar capillary membrane). The changes are typical of the pulmonary responses to NO_2, which was a likely, but unmeasured, contaminant in the whole-body exposure system used.

Sprague-Dawley rats were exposed via a nose only exposure unit to 0, 80, 200, 400 and 500 ppm nitric oxide with 21% oxygen for 6 hours each day for 1, 3 or 7 consecutive days. NO_2 concentrations were maintained at low levels of 0.4, 2.2, 4.0, 6.5 and 7.5 ppm, respectively. All animals on 400 and 500 ppm NO and 24 of 30 animals on 300 ppm died the first day. Two more animals in the 300-ppm group died on the second day. No mortality was recorded in the 80 and 200-ppm groups. The cause of death was considered to be metHb-induced tissue anoxia. Methaemoglobin values on day 1 and 7 were in the normal range in the 80-ppm group (2.7 and 3.6 % at 1 and 7 days) and exceeded 20 % in a dose dependent manner for the other groups. Decreased in platelet counts and elevated blood nitrogen were recorded pre-mortem in the highest dose group.

There was also an increase in the incidence and severity of interstitial oedema in the treated animals relative to the controls.

In the twenty-eight day exposure with recovery of NO study, rats were exposed to concentrations of 0, 40, 80, 160, 200 and 250 ppm of NO via a nose only inhalation system 6 hours a day for 28 days. NO_2 concentrations were 0.1, 0.5, 1.5, 2.5 and 3.4 ppm, respectively. No death was observed in animals receiving up to 160 ppm NO. Methaemoglobin levels were elevated in rats exposed to 160 ppm or more of NO. Female rats exposed to NO showed a higher level of methaemoglobin than males. Explanation of the expert is that females are lighter than males and that methaemoglobin level is related to body weight. Gross pathological examination revealed brown coloration of the lung in early decedents at 200 and 250 ppm. Histopathological examination of a wide range of organs did not detect changes that could be attributed to NO. Abnormal pulmonary findings made after 1 and 7 days dosing at 200 ppm could not be confirmed.

Furthermore, the potential problems of additive toxicity, which may be associated with high concentrations of oxygen and NO2 in the range 0.25 to 2.0 ppm, have been investigated. There appears to be no treatment related adverse effects.

Mutagenicity - A battery of *in vitro* and *in vivo* mutagenicity studies with NO and a bacterial mutagenicity assay with NO_2 were conducted.

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NO doses up to 5000 ppm were evaluated for mutagenicity in *S. typhimurium* strains and *E. coli* both in the presence or absence of metabolic activation. In some Salmonella strains in the presence of metabolic activation, dose related and reproducible mutagenic activity was present at concentrations of 1580 ppm NO or higher. A mutagenic potential of NO was also demonstrated in the mouse lymphoma assay with or without metabolic activation.

NO was clastogenic in the *in vitro* chromosome aberration study performed in the absence of metabolic activation with NO doses from 1500-1800 ppm, which produced a 50-61 % mitotic inhibition. Whereas, there was no evidence of a clastogenic affect in the *in vivo* chromosome aberration study at 40 ppm NO in 30 % oxygen for two hours (NO_2 exposure was up to 1.5 ppm).

NO_2 was shown to be present in all the studies in which it was monitored. In the limited data submitted, the results were contradictory. NO_2 was mutagenic in *S. Typhimurium* strain TA 1535, but was not mutagenic in strain TA100. The positive result occurred at $\geq$ 10 ppm, and in all NO mutagenicity studies NO_2 levels were $\leq$8 ppm, suggesting that the genotoxic activity in the test system resulted from NO and NO_2 in concert. The proposed mechanism of mutagenicity was oxidative deamination of deoxynucleotide.

Reproduction toxicity - No studies have been conducted. This is acceptable in view of the proposed clinical indication and proposed patient population.

Environmental Risk Assessment - Nitric oxide will enter the environment either during therapeutic use or inadvertently when changing gas cylinders. Based on the estimated likely use, the environmental risk of medical NO use is probably negligible.

However, within neonatal units using NO, chronic low dose exposure of medical staff should be avoided. Gas should be trapped at the end of the circuit and ventilation system effective in case of accidental emptying of a cylinder.

Discussion on toxico-pharmacological aspects

A fundamental problem to all toxicology studies was the rapid conversion of high concentrations of NO to oxides of nitrogen in the presence of oxygen. The toxicological literature did not always describe the method of gas administration in details and it is probable that the value of many studies was compromised by the presence of unknown amounts of oxides of nitrogen.

However, the pharmacological basis for treating neonates with hypoxaemic respiratory failure with NO is established. NO was shown to reverse pulmonary vasoconstriction induced by hypoxia, vasoconstrictor drugs, histamine or by activation of the coagulation system or by infection, *in vitro* and *in vivo* in both anaesthetised and awake animal models.

In a limited investigation in rats, concern was that NO had adverse effect on CNS, which may be beyond its hypoxic effect. It is not known whether the CNS toxicity identified was attributable to NO itself or the oxides of nitrogen. Limited reassurance had been provided by only one clinical study with patients been exposed for a mean duration of 21 hours. The major findings related to CVS were an anticipated increase in metHb and ECG abnormalities. The ECG abnormalities were not observed in another study in dogs; these were most probably due to the fact that dogs may be more susceptible to cardiac irritability than human. The NO effect on the immune system in the mouse consisted of an initial increase in some parameters followed by a subsequent decrease; this may be secondary to stress or to the uncontrolled NO_2 levels. The increased bleeding times observed in animal and human warrant caution for the use of NO with anti-coagulant inhibitory compounds.

In acute and repeat toxicity studies, the main findings were the formation of metHb. Deaths were attributed to metHb induced tissue anoxia. The results that NO might cause lung pathology were however not consistent. The potential of additive toxicity, which may be associated with high concentrations of oxygen and NO2 in the range of 0.25 to 2.0 ppm, have been investigated in the rat model, there appears to be no treatment related adverse effects.

Under the test conditions employed, NO was mutagenic in prokaryotic and eukaryotic organisms. The data on clastogenicity was contradictory, NO was clastogenic in one *in vitro* study but not in an *in vivo* study. In the very limited data submitted concerning NO_2, the mutagenicity results were contradictory. INOmax is indicated in newborns $\geq$ 34 weeks gestation, and the use of a genotoxic agent remains a concern and must be considered on a risk/benefit assessment.

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4. Clinical aspects

Clinical pharmacology

The main pharmacodynamic and pharmacokinetic findings are taken from observation from published literature. The primary pharmacodynamic development programme is limited because many of the early studies did not take into account the ready oxidation of NO to NO_2 in air or oxygen containing mixtures. Different delivery systems, quality controls and assays for inhaled nitric oxide have been used. There is no uniform definition of the dose of inhaled nitric oxide. For these reasons a dose response has not been established.

Pharmacodynamics

The pharmacodynamics of inhaled nitric oxide are well documented in published literature although complicated by the wide range of administration devices.

Nitric oxide is an endogenous mediator produced by many cells of the body. It is the active component of endothelium-derived relaxing factor. Unlike other pulmonary vasodilators, it has little systemic effect because of its short half-life caused by inactivation by binding to haemoglobin, rapid oxidation and the interaction with free radicals. The benefit of inhaled NO may depend upon its distribution. Exogenously administered NO diffuses into ventilated area of the lungs, crosses the alveolar-capillary membrane and reaches the arteriolar pulmonary vasculature where vasodilatation occurs. In smooth muscle cells, NO activates soluble guanylate cyclase to form cyclic GMP, which in turn promotes a calcium dependent relaxation. As for endogenous produced NO, the inhaled gas is readily cleared from the circulation by binding to haemoglobin and other protein compounds, preventing systemic vasoactive or other effects.

NO was shown to reverse pulmonary vasoconstriction induced by hypoxia, acidosis or drugs *in vitro* and *in vivo* in animal models. Observations from literature suggest that the effects of inhaled NO on gas exchange and pulmonary artery pressure are apparent within minutes in the majority of newborns with PPHN (persistent pulmonary hypertension of the newborn). Oxygenation improves as a consequence of increased blood pulmonary flow and better ventilation-perfusion mismatch (vasodilatation occurs only in ventilated area of the lung). In adults with severe respiratory failure, the maximum pulmonary vasodilator response to NO, measured in terms of oxygen saturation, may occur at concentrations as low as 1 ppm when NO is administered by sampling gas from the distal tip of the endotracheal tube.

A study conducted in Europe involved 33 neonates (≤ 7 days old) with PPHN and paediatric patients with acute pulmonary hypertension. Dose responses were measured between 0 and 100 ppm of NO for 10 minutes. Although monitoring and delivery devices were imperfect, there was a rapid improvement of oxygenation even at low dose equal or less than 10 ppm.

Pharmacokinetics

- General:

Pharmacokinetics are characterised by nitric oxide being a highly reactive free radical. Observations from the literature indicate that *in vitro* and *in vivo* nitric oxide oxidises to nitrite and also nitrosylates sulphydryl groups in proteins, such as plasma albumin, or even the rubber of tubing, bags and connectors of the anaesthetic circuit, as well as nitrosylating lipids and haemoglobin.

In solution, NO binds heme- or sulphydryl-containing proteins, to form nitrosyl-compounds later metabolised into nitrites and nitrates excreted via the kidneys, and also to some degree through salivary glands into the oral cavity. Nitrite is converted to nitrate principally by a redox reaction involving the iron of haemoglobin. The amount of free NO depends upon the equilibrium dissociation constants of the nitrosylated forms of NO in haemoglobin.

Degradation of NO has been studied in healthy subjects inhaling 25 ppm of NO for 60 minutes and patients with severe heart failure inhaling 20, 40 and 80 ppm in consecutive 10-minute periods (study CTN-NO-93-008). During inhalation of NO, the plasma levels of nitrate increased progressively in both groups. Methaemoglobin also increased. Plasma and urinary clearance of nitrate in healthy subjects averaged 20 ml/min. The relevance of this study is however limited as nitric oxide was mixed in air in concentrations that were not monitored.

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Short-term exposure of inhaled NO in healthy adult volunteers, at a dose of 0.6 to 2.3 ppm for two hours, showed a slight metHb increase from 0.6% to 1.1%.

Methaemoglobin forms also from nitrogen dioxide and the rate can depend upon the degree of extra-corporeal oxidation of nitric oxide to nitrogen dioxide. It seems that nitrite can also be converted to methaemoglobin and HbNO. Accumulation of methaemoglobin, the oxidised form of nitrosylhaemoglobin (NOHb) may occur. This is potentially harmful because of the limited oxygen binding capacity of methaemoglobin.

Some of the cytotoxicity of nitric oxide is thought to be secondary to the formation of peroxynitrite. The tissue concentrations of this highly toxic compound are difficult to measure. Interacting with nitric oxide can inactivate some toxic free radicals.

The extend of exposure of the cerebrospinal fluid (CSF) and central nervous system to nitric oxide and its metabolites is not clear. The absence of haemoglobin and relative absence of proteins in CSF means that some of the interactions of nitric oxide with blood could reverse to release NO, depending upon the dissociation constants of each reaction.

Overall, the degradation pathways for nitric oxide are complex, as might be expected for such a reactive free radical. The majority for endogenously formed NO is eventually metabolised via subsequent conversion to nitrate and metHb. The effects of nitrosylation of lipids and proteins by exogenous or even endogenous nitric oxide are not well understood.

- Interaction studies:

Effects on haemostasis - Reports from the literature suggest that inhaled NO may prolong bleeding time, having a yet undetermined effect on platelets in animals and humans. These data could not be confirmed in the reported trials on humans.

A four way crossover study was conducted to assess the effect on coagulation factors and platelet function of nitric oxide 80 ppm given as a 30 minutes inhalation to healthy male volunteers with aspirin 600 mg and heparin 5000 IU as positive control. No synergistic effect was reported on bleeding time between heparin and NO. The effect on activated clotting time of nitric oxide was similar to the effect of aspirin.

Effects on surfactant - Reports from the literature suggest that inhaled NO and mainly NO_2 in excess of 5 ppm may interact adversely with surfactant, lipids and proteins. Therefore, exogenous surfactant supplementation may be useful before or during the administration of inhaled NO in hypoxic neonatal lung injury. This option has been incompletely studied; exogenous surfactant use has been generally encouraged before randomisation in NO trials.

Interactions with phosphodiesterase inhibitors - Reports from the literature suggest that he cyclic GMP-specific phosphodiesterases inhibitors may have a synergistic effect with inhaled NO on pulmonary arterial pressure that could be useful in clinical practice, but there are few studies in this area.

Clinical efficacy

Persistent pulmonary hypertension of the newborn (PPHN) is a disorder of the transition from fœtal to extra-uterine life. The clinical syndrome is characterised by persistence of elevated pulmonary vascular resistance resulting in hypoxemia secondary to right-to-left shunting of deoxygenated blood across the still patent foramen ovale and/or ductus arteriosus. In human newborns, PPHN may be idiopathic or associated with parenchyma lung disease as respiratory distress syndrome of prematurity, meconium aspiration syndrome, pneumonia and sepsis or congenital diaphragmatic hernia and pulmonary hypoplasia. Although anatomical changes such as pulmonary vascular smooth muscle hypertrophy may occur and contribute to increased pulmonary resistance, pulmonary vasoconstriction and altered vascular reactivity are central to the pathophysiology of this syndrome. Involvement of NO in the adaptation to normal-extra uterine life has been proved in animals.

In the most severe forms of hypoxic respiratory failure, invasive procedures of extracorporeal membrane oxygenation (ECMO) have proven efficacy in reducing mortality compared to conventional therapies. However, these techniques remain procedures of exception performed by a trained multi-disciplinary team of specialised physicians, surgeons, perfusionists and nurses. They require

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catheterisation, and often ligation of the cervical great vessels (jugular vein and carotid artery) need continuous blood anticoagulation and massive use of blood products. Potential drawbacks are related to the increased risk of brain haemorrhage and infarction. Prognosis into adult life is unknown.

The major and widely accepted ECMO criteria in newborn with severe hypoxemic respiratory failure consist in an oxygenation index (OI) of higher than 40 (the oxygenation index is calculated as the mean airway pressure times the fraction of inspired oxygen times 100 times divided by the partial arterial oxygen pressure). In this condition, mortality reached up to 80% in historical conventionally treated controls. In the recent UK ECMO trial, mortality decreased from 59% in ECMO-treated to 32% in control babies with no concomitant increased morbidity in survivors. Prognosis was independent of initial severity of disease.

The purpose of the studies presented was to evaluate the effectiveness of inhaled NO in a population of newborns of 34 weeks gestation age or more, with a grade of severity transiently reaching the main ECMO-criteria (oxygenation index >40) or a lesser severe stage of the disease (oxygenation index ≥25), suffering severe hypoxemic respiratory failure with or without PPHN, in reducing the need of ECMO without altering mortality and morbidity prognoses.

Four main efficacy clinical trials are presented. Three recruited patients with persistent pulmonary hypertension of the newborn (PPHN) and one neonate with hypoxaemic respiratory failure. The clinical trials were performed according to GCP standards, except for INOSG trial. The design, number of patients and status of these patients are given below in Table 1:

Table 1 - Phase III clinical multicentre randomised trials in hypoxaemic respiratory failure of the newborn.

Study	**Indication(s)**	**Design and treatment duration**	**Patients number**	**Centres number**
INO-01/ INO-02	**PPHN** - *Patients enrolled within 72h of birth*.	Prospective, randomised, double-blinded, placebo-controlled, dose-response. *NO 5, 20, 80 ppm inhalation.* *Until threshold criteria or 14 days.*	155	25 US centres
NINOS	**Hypoxemic respiratory** **Failure** - Neonates ≤14 days *(gestational age ≥34 weeks) and oxygenation index ≥25.*	Prospective, randomised, blinded, placebo-controlled. *NO 20-80 ppm inhalation.* *12-336 hours.*	235	19 US and Canadian centres
CINRGI	**PPHN** - *Term neonates (gestational age ≥34 weeks), enrolled within 96 hours of birth.*	Prospective, randomised, double-blinded, placebo-controlled, dose response. *NO 20-5 ppm inhalation.* *4-96 hours.*	186	18 US centres
INOSG	**PPHN**	Randomised, double-blinded, placebo-controlled. *NO 80 ppm inhalation* *for 20 minutes.*	58	7 US centres
Total			634	

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Only two studies, NINOS and CINRGI, are to be considered as pivotal studies for the evaluation of inhaled NO in neonatal hypoxemic respiratory failure (see Tab. 2). Efficacy outcomes can only be deduced from those two pivotal studies, which will be largely described, as the other two studies presented deficiencies in GCP compliance (INOSG study) or in methodology (INO-01/INO-02 study). In addition, studies resulting from compassionate use experience were also reported (Studies NO-93-009, NO-93-011 (AGA), NO-93-010).

Dose-response studies and main clinical studies

Dose response study

A dose finding study (NO-93-003) was conducted with inhaled nitric oxide in neonatal patients with PPHN and paediatric patients with acute pulmonary hypertension, a conclusion could not be drawn as monitoring and delivery devices were imperfect. From recently published clinical trials, lower concentrations of NO, 5 and 10 ppm, were reported. It seems that a lower dose of 10 ppm has similar efficacy to the 20 ppm.

No formal dose response study was performed. The choice of dose of NO is not well defined.

Main studies (CINRGI and NINOS)

1. Description of the study

Both pivotal studies are placebo-controlled, double blind, multicentre prospective randomised with defined endpoints, involving a large number of patients. The endpoints, patients and treatment characteristics concerning NINOS and CINRGI studies are given below in Table 2.

Table 2: NINOS and CINRGI studies.

Study	NINOS	CINRGI
Patients population	Infants with **hypoxic respiratory failure** ≤14 days, gestational age ≥34 weeks and oxygenation index ≥25 mmHg. Diagnosis by echocardiograph evaluation.	**PPHN** neonates with gestational age ≥ 34 weeks, age ≤96 hours and oxygenation index >25 mmHg. Diagnosis by echocardiograph evaluation.
Exclusion criteria	Cardiopathy. Significant structural congenital heart disease or diaphragmatic hernia.	Cardiopathy. Pulmonary hypoplasia and congenital diaphragmatic hernia group, who undergo ECMO.
Patients number	235 (NO=114; placebo (O2)=121)*	186 (NO=97 ; placebo (N2)=89)
Primary efficacy endpoint(s)	The combined outcome of death and/or ECMO rescue before discharge home or 120 days, whichever came first.	Number of patients receiving ECMO rescue.
Secondary efficacy endpoint(s)	The separated death rate and ECMO rescue rate. Assessment of short-term in oxygenation, incidence of infants meeting ECMO criteria, duration of assisted ventilation, incidence of air leak, incidence of chronic lung disease, length of hospital stays, neuro-development outcome at 18-24 months.	The improvement in oxygenation (as measured by the arterial-alveolar oxygen ratio and gradient, the arterial partial pressure of oxygen and the oxygenation index), the safety profiles (as hypotension, elevated methaemoglobinaemia, deterioration of gas exchange), and morbidities and mortalities (as chronic lung disease, neurological abnormalities, survival to hospital discharge).
Statistical method	The primary endpoint was analysed by intention-to-treat paradigm.	The primary endpoint was analysed by intention-to-treat stratified by underlying disease using Cochran-Mantel Haenszel method.
Treatment protocol	The initial dose of NO was 20 ppm or an equivalent flux of oxygen (low flow of gas), increasing to 80 ppm after 30 minutes if the increase in PaO2 was <20 mmHg. Weaning was attempted every 12 hours (2 hours if on high flow gas).	Continuous administration of study gas starting at 20 ppm for 4 hours, then was stepwise withdrawn. The dose was decreased to 5 ppm if the neonates's condition was stable, the PaO2 ≥60 mmHg and the pH 7.55 or lower. If

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	Maximum time on ≥5 ppm NO was 10 days, maximum time of total treatment 14 days.	these criteria were not met, the administration of study gas was maintained and the neonate re-evaluated every 4 hours, up to 24 hours. After 24 hours, the dose was in all cases decreased to 5 ppm. Treatment was continued at 5 ppm until the FiO2 ≤0.7. Placebo was weaned in the same manner. Those who did not tolerate the decrease to 5 ppm at 24 or 96 hours, treatment was considered as failure and discontinued.
Other monitoring	Blood methaemoglobin concentrations at 1, 3, 6, 12, and every 24 hours. NO, NO_2 and total oxides of nitrogen were recorded every two hours in the inspiratory limb of the ventilator.	Blood methaemoglobin concentrations at baseline, 4, 24, 96 hours. Continuous monitoring of FiO2, NO, NO_2 at the tip of the endotracheal tube.
** 230 effectively received study gas, 4 patients who were randomised to placebo and 1 to NO did not receive it.*		

Blinding is difficult to maintain in such trials. It was assured using two independent teams. A blinded clinical team to take care of the patient and an unblinded respiratory therapist to administer and adjust treatment gas. The dedicated unblinded investigator at each site was responsible for the NO cylinders, maintained the bedside stock, calibrated the delivery and monitoring devices, and monitoring of NO, NO_2 and methaemoglobin concentrations. The colour coded NO tank was covered to inhibit its identity.

Study populations - The treatment group were well matched for demographic and baseline characteristics. A complete echocardiograph evaluation was required in each patient to assess PPHN diagnose and to rule out cardiopathy, but diagnosis of persistent pulmonary hypertension was not required if pulmonary parenchymea disease was present. The average patient was full term, appropriate for gestation age.

In NINOS study, the mean age was 1.7 days at randomisation. Baseline oxygenation index (OI) and partial arterial oxygen pressure suggested severe disease: baseline OI was 46 in controls and 47 in NO group; baseline PaO_2 was 45 mmHg in controls and 46 mmHg in NO group. Echograph evidence of PPHN was found in 78% of the patients. Most of the patients (97 %) needed pressure support. Before randomisation, 72% of controls and 71% of the NO group were given surfactant. The type of therapies was well distributed between groups except for the use of vasopressors and alkalosis that were less used in the inhaled NO group.

In CINRGI study, patients enrolled were assigned to one of five diagnostic categories of pulmonary disease (meconium aspiration syndrome, pneumonia, respiratory distress syndrome, idiopathic PPHN, pulmonary hypoplasia syndromes) and then randomly assigned to treatment. The patients, a majority of males, were treated at a mean post-natal age of 30 hours. The control group had a higher mean arterial pressure (56 versus 52 mmHg; both values are in the normal range), but a lower PaO2 (55 versus 78, p-value 0.007) and a higher oxygenation index (44 versus 35, p-value 0.011). The mean level of pressure support was high and identical between the two groups (more then 90% of the patients were on vasopressors on baseline). At entry, 47% of the placebo group and 35% of the NO patients had received exogenous surfactant; 60% and 49% were on high frequency ventilation, respectively in the placebo and NO treated group.

Treatment protocol - The treatment dose was mostly 20 ppm. Both studies used NO at an initial concentration of 20 ppm. In NINOS trial, it could be increased to 80 ppm and in the CINRGI trial, it was decreased stepwise. The placebo consisted of N2 or O2 gas.

Variations in treatment protocol are responsible for different duration of treatments and therefore different duration of NO exposure. It was rarely longer than seven days and restricted to four days maximum in CINRGI study. Also weaning procedures differed in both studies. The mean time on NO for CINRGI patients was 40 hours (median 28 hours) and 71 hours (median 40 hours) for NINOS patients, with large individual variations between patients.

Protocol deviation was observed in CINRGI study, where 15 patients with entry violation were included in the study. Two patients in the placebo group received NO, one of two in the congenital diaphragmatic hernia group. Removal of these patients did not modify the efficacy outcome analysis.

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ECMO criteria - Neonates with treatment failure were only treated with ECMO if usual criteria of severity were met (oxygenation index>40 on 3 of 5 blood gases drawn 30 minutes apart, or PaO2<40 mm Hg for 2 hours, or PaO2<35 mm Hg for 1 hour, or progressive haemodynamic deterioration with systemic blood pressure less then 35 mm Hg).

2. Efficacy results

Clinical Inhaled Nitric Oxide Research Group Study (CINRGI) - Comparison of conventional therapy and nitric oxide in the management of persistent pulmonary hypertension of the newborn: The main findings are given in Table 3 below.

Table 3 – CINRGI Study	**Placebo**	**Inhaled NO**	**p-value**
Received ECMO (% of treatment group)	51/89 (57%)	30/97 (31%)	<0.001*
Death at 28 days (% of treatment group)	5/89 (6%)	3/97 (3%)	0.481**
Chronic lung disease/ patients with information (%)	11/82 (13%)	3/92 (3%)	0.023**
Discharged on O2 or pulmonary medication (%)	10/89 (11%)	6/97 (6%)	0.296**
Duration of hospital stay days (number of patients)	25 (79)	23 (90)	0.198***
At least one abnormal neurologic result/total with results (%)	19/89 (21%)	17/97 (18%)	0.579**
* *Cochran-Mantel-Haenszel chi squared test adjusting for underlying disease* ** *Fischer's exact p-value* *** *Student's t test*			

Primary outcome: It was evaluated on 186 non-lung hypoplasia patients, in an intent-to-treat analysis. There was a significant reduction in the rate of ECMO in the NO group when the 186 non-lung hypoplasia patients were analysed. The reduction with NO remained highly significant after exclusion of the protocol violators. NO reduced ECMO in each stratum of underlying disease except the pulmonary hypoplasia syndromes. The difference among strata was not statistically significant.

Secondary outcome:

Oxygenation - Both the partial pressure of oxygenation (PaO2), the others parameters of oxygenation arterial- alveolar oxygen ratio (PaO2 /PAO2) and alveolar-oxygen gradient (A-a DO2) and the ventilator index were improved significantly in the NO group in comparison with the controls within 24 hours of treatment gas (ANOVA repeated measures at 30 minutes, 1, 4, 12, 24 hours with a p-value ≤ 0.001).

Mortality and morbidity - There was no difference in mortality between groups either at 28 days or at 6 months follow-up. Fewer infants in the inhaled NO group were discharged with diagnosis of chronic lung disease defined as oxygen support at 28 days (p-value 0.023), but the number of patients discharged on oxygen or pulmonary medication did not differ significantly between groups. Duration of hospital stay and neurological abnormalities (abnormal head ultrasound, abnormal computerised axial tomogram, or neurological examination) at discharge were also very similar. There was at least one abnormal neurological assessment in the controls and in the NO group.

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Short-term complications - There was no difference between groups toward a very large number of potential adverse events and biological haematological and chemical variables. Inhaled NO was well tolerated with no evidence of systemic hypotension, the development of significant methaemoglobinaemia (2 in 108 infants had more than 4% methaemoglobin, i.e. 1.9%) nor elevated NO_2. Analysis of these values using a repeated ANOVA yielded a p = 0.001 for methaemoglobin levels and a p = 0.83 for NO_2 levels, suggesting that the delivered dose of inhaled NO used in this trial does not appreciably increase the infant's exposure to NO_2. No patients had NO_2 concentrations above 5 ppm.

Response to treatment and duration on gas - Treatment success occurred in 32/89 (36%) of the placebo treated patients and in 60/97 (62%) of the inhaled NO patients (p-value 0.0001). Mean time on inhaled study gas was 27 hours for placebo and 40 hours for NO.

Outcome in the lung hypoplasia patients: The most common diagnosis of these patients was congenital diaphragmatic hernia. In these patients, both mortality (35-40%) and need for ECMO (80-85%) is higher than for the others, independently from NO treatment.

Neonatal Inhaled Nitric Oxide Study (NINOS) - Inhaled nitric oxide in full-term and nearly full term infants with hypoxic respiratory failure: The main findings are given in Table 4 below.

Table 4 - NINOS Study	**Placebo**	**Inhaled NO**	**p-value:**
Incidence of death and/or ECMO (% of treatment group)	77/121 (64%)	52/114 (46%)	P=0.006
Death (% of treatment group)	20/121 (17%)	16/114 (14%)	P=0.6
ECMO	66/121 (55%)	44/114 (39%)	P=0.0014
PaO2 [mean±SD]	10 ± 52 mm Hg	58 ± 85 mm Hg	P<0.001
Oxygenation index [mean±SD]	1 ± 21	-14 ± 21	P<0.001
A-aDO_2 [mean±SD]	-7 ± 58 mmHg	-60 ± 85 mmHg	P<0.001

Primary outcomes: The incidence of death and/or ECMO decreased significantly (p-value 0.006, CI not given) from 64% in the placebo group to 46% in the inhaled NO group (relative reduction of 28%). This reduction was mainly the result of a significant reduction of ECMO use in the NO group, and not a consequence of reduced mortality. The number of patients who met ECMO criteria in each group was however not different (59% of the NO patients and 69% of controls).

Secondary outcomes: There were statistically significant haemodynamic changes. Inhaled NO lead to a significant short-term improvement in oxygenation. After 30 minutes of gas, PaO2 increased an average 58 ± 85 mm Hg in the NO group and 10 ± 52 mm Hg in the control group (p-value less than 0.001). In the same period of time, the oxygenation index decreased of -14 ± 21 in the NO group while it increased of 1 ± 21 in the controls. The alveolar-arterial gradient decreased in both groups.

There was no statistical difference in the others secondary outcomes as the duration of mechanical ventilation, the incidence of air leak or chronic lung disease, and the length of hospital stay.

As expected, the mean peak levels of methaemoglobin and NO_2 were significantly higher in the inhaled NO group compared to the controls (p-value<0.001) and were related to the administrated

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dose of NO; 23 % of the NO treated patients showed a peak level of NO_2 higher than 1 ppm at any time, 2 % had a toxic level higher than 5 ppm.

Other results: An increase in PaO2 of at least 20 mm Hg (full response) was seen in 15 % of the placebo patients and 51 % of the NO patients (p-value less than 0.001). The majority of patients who did not show a full response at 20 ppm study gas and who were evaluated at the 80 ppm dose had no response at the high gas flow whether they received placebo or inhaled NO (75 % in both groups). The mean duration of administration of NO was 71 ± 79 hours (from less than one day to maximum 14 days). Examination of subgroups (data presented as supplementary post hoc analysis only) of patients stratified by underlying disease, surfactant use, high frequency ventilation use, baseline oxygenation index, initial response to treatment gas, were unable to show significant differences in the relative risk of death and/or ECMO, when the inhaled NO group was compared to the placebo group.

Neurodevelopment outcome: Follow-up at 18 ± 6 months was available for 173 (88 control and 85 NO infants) of 199 survivors or 87% of them (1 late death was recorded in the NO group, 13 controls and 12 inhaled NO patients were lost to follow-up). There were no significant difference between the two groups in reported medical problems and 78% of both groups were classified as neurologically normal and 11% of both groups had cerebral palsy. The incidence of reported seizures was 14.9% for the control group and 4.7% for the NO group ($p<0.039$). There were no statistically significant differences between the groups in mental and motor development, hearing and visual assessments.

Clinical studies in special populations

No clinical studies have been performed in special populations, e.g. in congenital diaphragmatic hernia.

Other studies

The INO-01/INO-02 study failed to reach his objective that was to demonstrate a reduction of mortality and morbidity with inhaled nitric oxide at a dose 5-80 in PPHN of the term newborn. The trial was underpowered, interrupted prematurely because of low enrolment rate as the use of high frequency ventilation and surfactant was excluded by the protocol.

The death rate and the incidence of neurological sequelae were not different between the NO and placebo group. ECMO rescue was used in 29% of the NO group and 39% of the placebo group. There were no statistically significant changes in secondary endpoints. High levels of methaemoglobin (>5%) and NO_2 (>2%) were only seen in the 80 ppm NO sub-group.

The INOSG study planned to evaluate the effect of 20 minutes inhaled high dose (80 ppm) of NO in PPHN of term newborns. Issues related to GCP make efficacy conclusion unreliable.

Compassionate use experience (Studies NO-93-009, NO-93-010 and NO-93-011 (AGA)) - These studies were performed on clinician's initiative, are retrospective studies conducted at an earlier stage of the therapy in Europe, Australia and the USA. Although they suggest that inhaled NO may be effective on improving oxygenation, no deduction in term of efficacy can be driven. Adverse events were limited to some cases of reversible elevated levels of methaemoglobinaemia.

Discussion on clinical efficacy

The pharmacodynamics of inhaled NO are well documented in published literature, although complicated by the wide range of administration devices. Pharmacokinetics are characterised by NO being a highly reactive free radical. It oxidises readily and also nitrosylates sulphydryl groups in proteins or even the rubber of tubing, bags and connectors of the anaesthetic circuit, as well as nitrosylating lipids and haemoglobin. Some of the cytotoxicity is thought to be secondary to peroxynitrite. Some NO may dissociate from nitrosylated compounds and effect the central nervous system as cerebrospinal fluid has no haemoglobin and few proteins to bind NO.

Efficacy outcomes can be deduced from two pivotal studies. The NINOS study involves neonates with hypoxaemic respiratory failure. The primary efficacy endpoint was the combined death rate and/or ECMO rescue rate, which was 64% for placebo and 46% for NO group. The ECMO rescue rate alone was 55% for placebo and 39% for NO. Neurological complications were similar, although the seizure rate was lower with NO. In the second study, CINRGI study, there were 186 neonates with persistent pulmonary hypertension. The primary efficacy variable was ECMO rescue, which was 57% for

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placebo and 31% for NO. Both studies showed an important reduction in ECMO, but it has not been shown that NO decreases mortality. Secondary variables show an early increase in oxygenation, which, in the absence of methaemoglobinaemia, increases tissue oxygen delivery.

The reduction in mortality, as reported in the recent UK ECMO trial, is in contrast to NO therapy, where no statistical significant benefit on survival has been shown. On the other hand, nitric oxide use might decrease ECMO-related complications/sequelae: ECMO arterial access is usually by cannulation of the right common carotid artery which is ligated at decannulation; this has been associated with right cerebral hemispheric ischaemic or haemorrhagic abnormalities and adverse neuro-developmental outcome. Moreover, in the trial populations inhaled NO did not appear to delay the appropriate dispatch of traditional oxygenation therapies (Applicant responses to the CPMP list of Questions).

The choice of NO dose is not well defined. Both pivotal studies used NO at an initial concentration of 20 ppm. In the first study it could be increased to 80 ppm, but this provided no extra benefit. In the second study the concentration was decreased stepwise. Duration was rarely longer than seven days and restricted to four days maximum in the second trial. The optimal concentration of inhaled NO is not known. Recent publications suggested that a lower maximum dose of 10 ppm might have similar efficacy to the 20 ppm dose.

Clinical safety

Patient exposure

In the NINOS study the mean duration of exposure to the study gas in the placebo group was 21 hours (n=117), and the mean duration of exposure to NO was 71 hours (n=113). The initial dose of NO was 20 ppm, but this was increased in some patients to 80 ppm. Only one patient received NO >5 ppm for more than 10 days. This infant showed some response to 20 ppm at randomisation but no response to a higher dose. For this reason the use of the high dose, with subsequent difficulty in weaning, does not appear justified.

In the CINRGI study, the mean duration of exposure to the study gas in the placebo group was 27 hours (n=87), and the mean duration of exposure to NO was 40 hours (n=93). The total time the infant could receive any dose of inhaled NO was 96 hours.

Adverse events and serious adverse event/deaths

In the NlNOS trial the incidence of adverse events of the NO group (n=114):control group (n=121) were: deaths 16:21; intracranial haemorrhage 18:19; periventricular leukomalacia 6:3; brain infarction 7:7; seizures requiring anti-convulsants 16:24; pulmonary haemorrhage 4:6; gastrointestinal haemorrhage 1:1.

In the CINRGI trial the incidence of adverse events for non-lung hypoplasia of the NO group (n=93): control group (n=87) were: deaths 3:5; cardiovascular adverse events 31:31, nervous system 20:27; respiratory system 32:34; suspected sepsis 59:54, positive blood cultures 10:9.

Adverse events that occurred in at least 5% of patients receiving INOmax in the CINRGI study, and that were more common on INOmax than on placebo. These events may or may not be associated with the use of INOmax.

Adverse Event	Placebo (n=89)	INOmax (n=97)
Hypotension	9 (10%)	13 (13%)
Bacteraemia and/or Local Infection	5 (6%)	13 (13%)
Rebound Hypoxemia on Withdrawal	9 (10%)	12 (12%)
Atelectasis	8 (9%)	9 (9%)
Haematuria	5 (6%)	8 (8%)
Hyperglycaemia	6 (7%)	8 (8%)
Stridor	3 (3%)	5 (5%)
Cellulitis	0 (0%)	5 (5%)

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The severity of illness of the neonates in the main efficacy studies complicates safety assessment but it is reassuring that NO was not associated with an increase in the incidence of death, intracranial haemorrhage or pulmonary haemorrhage.

Withdrawal reactions

The use of inhaled NO is now well documented to cause a withdrawal reaction whereby the patient develops increased hypoxemia and pulmonary hypertension if therapy is stopped abruptly. Severe reactions of life threatening pulmonary vasoconstriction have been described with sudden withdrawal of inhaled NO. There were three dangerous withdrawal reactions in earlier trials by the applicant in adult respiratory distress syndrome. Withdrawal can also occur in neonates who are classified as non-responders.

Safety in relation to toxicity of nitric oxide

Nitric oxide is toxic for several reasons. It is highly reactive free radical, it forms the toxic oxidation product peroxynitrite in tissues, it readily oxidises to nitrogen dioxide, and it can form methaemoglobin. The National Institute of Occupational Safety and Health (USA) recommends a time-waited average exposure of 5 ppm of nitrogen dioxide and 25 ppm for nitric oxide. Toxic pulmonary effects of nitrogen dioxide have been observed at 5 ppm or below and concentrations of 0.5 ppm may increase human airway hyper-reactivity. The concentration of nitrogen dioxide administered is influenced by the duration of contact of nitric oxide with oxygen, and the concentration of the oxygen, before the gas mixture reaches the patient's tissues.

Dose-dependent Toxicity - The toxicity of nitric oxide depends on the inhaled concentration of nitric oxide itself and its oxidation products. The inhaled dose is critical, yet early studies did not use strict criteria to define the dose administered to the airway. No simple pharmacodynamic studies were conducted measuring the concentration of NO delivered to the airway and the effect of differences in administration devices, ventilators, ventilator settings, inspired oxygen concentration and interactions with the materials to which the gas is exposed before reaching the patient's airway. The gas concentration should be measured in a specific point in the circuit where gas is analysed for nitric oxide and nitrogen dioxide concentrations, this is reflected in the SPC. The inspired gas at the tip of the endotracheal tube might be appropriate.

Mutagenicity - In AGA study NO-93-006 12 healthy volunteers inhaled 40 ppm in 30% oxygen for 2 hours and there were no chromosome abnormalities detected in peripheral blood lymphocytes.

Laboratory and other findings

Methaemoglobinaemia - In the NINOS study the initial concentration of NO was 20 ppm and could be increased to 80 ppm. The peak methaemoglobin concentration at any time was 2.4% (SD 1.8%) in the NO group.

In the CINRGI study a predefined limit of methaemoglobin of 4% was set. This level was reached by only two patients in the 20-ppm NO group and none of the controls. The peak in mean methaemoglobin concentrations occurred at 4 hours and was 1.3% (SD 0.8) in the NO group and 0.8 (SD 0.6) in the control group.

The formation of methaemoglobin in plasma depends upon the concentration of inhaled NO. Many early studies used NO concentrations, which were not well defined, the delay between gas mixing and administration to the subject allowing oxidation of the NO in air to NO_2. From the two key trials and other more recent data, the incidence of methaemoglobin from NO concentrations of 20 ppm, or below, appears small. Methaemoglobin is of particular importance in the indication requested as the aim is to reverse tissue hypoxia and methaemoglobin has the potential to decrease oxygen delivery to tissues. Neonates may have reduced methaemoglobin reductase activity and are therefore at greater risk of developing methaemoglobinaemia. Concentrations over 5% of methaemoglobin are unusual with inhaled NO concentrations of 20 ppm or below, but can occur. For these reasons the monitoring of methaemoglobin as recommended in the SPC is advisable.

Nitrogen dioxide - NO_2 forms readily when NO is exposed to air or oxygen containing gases. Studies from published data have shown that NO_2 can promote airway inflammation and have an adverse effect on surfactant. Concentrations below 0.5 ppm have been reported to be associated with increased airway hyper-reactivity, the effect on the susceptibility to infection is not known.

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In the NINOS study the peak mean NO_2 concentration at any time was 0.8 ppm (SD 1.2) in the NO group. In the CINRGI study the peak in mean NO_2 concentrations occurred at 1 hour and was 0.19 ppm (SD 0.43) in the NO group and 0.01 ppm (SD 0.3) in the control group.

Advice on the monitoring formation of nitrogen dioxide is given in the SPC.

Peroxynitrite and other oxides of nitrogen - These compounds can be formed as degradation products of NO. Peroxynitrite is of particular concern and it is a free radical that is also generated during the inflammatory process. There is insufficient information to determine if peroxynitrite contributes to inhaled NO toxicity.

Interactions with Haemostasis - In the main efficacy trials presented above, there was no significant increase in intracranial or pulmonary haemorrhage.

Discussion on clinical safety

Toxicity of nitric oxide on clinical grounds appears essentially related to the formation of methaemoglobin and NO_2. Both are dose related. At concentrations of NO above 20 ppm the possible exposure to significant concentrations of NO_2 and methaemoglobin becomes a significant risk.

In the NlNOS trial the incidence of adverse events of the NO group (n=114):control group (n=121) were: deaths 16:21; intracranial haemorrhage 18:19; periventricular leukomalacia 6:3; brain infarction 7:7; seizures requiring anti-convulsants 16:24; pulmonary haemorrhage 4:6; gastrointestinal haemorrhage 1:1. In the CINRGI trial the incidence of adverse events for non-lung hypoplasia of the NO group (n=93): control group (n=87) were: deaths 3:5; cardiovascular adverse events 31:31, nervous system 20:27; respiratory system 32:34; suspected sepsis 59:54, positive blood cultures 10:9.

Although the severity of illness of the neonates in the main efficacy studies makes safety assessment complex, it is reassuring that NO was not associated with an increase in the incidence of death, intracranial haemorrhage or pulmonary haemorrhage. However, hazards as potential central nervous system toxicity, pulmonary toxicity, late malignancies may not be ruled out. The administration of NO should therefore be limited to short period of time, as advised in the SPC.

In addition, NO and NO_2 do have detrimental effects on surfactant. The upper limit in the posology section of the SPC should be limited to 20 ppm NO. The upper limits of exposure to NO_2 and methaemoglobin appear to be too high. The data appear to justify lower limits of 2.5% for methaemoglobin and 0.5 ppm for NO_2, which are reflected in the SPC.

The higher concentrations also appear to increase the difficulty of weaning, even in non-responders.

The complexity of the equipment is of concern. NO must correctly mix with other gases in the ventilator circuit (CE approved device). The concentrations of NO, NO, O2 must be recorded continuously near the tip of the endotracheal tube with an appropriate CE approved device and the blood levels of methaemoglobin regularly checked (within 4 hours of treatment and every 12-24 hours thereafter) as advised in the SPC

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

The CPMP has raised a series of questions, which will be addressed post-approval.

Preclinical pharmacology and toxicology

Overall, the primary pharmacodynamic data provided adequate evidence that the effect of nitric oxide relevant to its intended use in hypoxic respiratory failure is centred upon NO's properties of causing vasodilatation of hypertensive pulmonary vasculature.

Pharmacological effects not related to mode of action that may be of concern were over central nervous system toxicity (one study that need confirmation or invalidation) and possible anticoagulant

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effects. It is not known whether the CNS toxicity identified was attributable to NO itself or the oxides of nitrogen. Limited reassurance had been provided by only one clinical study with patients been exposed for a mean duration of 21 hours. The possible anticoagulant effects could not be confirmed in clinical study (ICR study no 013402). The NO effect on the immune system may be related to uncontrolled NO_2 levels.

From the pharmacokinetic point of view, the percentage NO absorption decreased with increasing concentration in the rat. The major metabolic pathway of NO is combination with haemoglobin to form NOHb with subsequent formation of nitrites and nitrates and elimination. NOHb is also rapidly oxidised to form metHb, which is subsequently reduced to ferrous Hb.

The toxicology programme revealed elevated levels of metHb that induce tissue hypoxia responsible for the reported deaths which dominant acute and chronic toxicity. Oxidative damage by secondary generated oxides of nitrogen remains a poorly controlled variable of nitric oxide inhalation, both in animals and in infants: NO is a free radical that readily forms toxic oxides of nitrogen the presence of oxygen. Modalities of administration of the inhaled NO are therefore susceptible to modify the toxicity profile of the drug to a very large extent.

The concern remains over genotoxicity of nitric oxide itself and the potential genotoxicity of nitrogen dioxide. The use of a genotoxic agent in neonates must be considered on a risk/benefit assessment.

Efficacy

There seems no doubt that inhaled NO can increase oxygenation and decrease pulmonary artery pressure in the group of patients defined in the indication of the SPC. Both pivotal studies showed a significant decreased incidence of the combined outcome death and/or ECMO or ECMO alone in the inhaled NO group compared to the control group. The main evidence comes from the use of a dose of 20 ppm.

There is no convincing evidence that inhaled NO improves survival nor that the treatment with inhaled NO lowers the incidence of pulmonary and neurological morbidity. The Applicant has committed to create a registry of all patients from the main efficacy studies and perform a follow-up examination at 5 years of age. This visit will include an age-appropriate neuro-development examination.

It is possible that lower doses could have an equal or better risk benefit ratio than the 20 ppm dose. Above 20 ppm there is no proven added benefit and the risk of methaemoglobin, NO_2 toxicity and difficulties in weaning become appreciable. Efficacy results cannot be related to the length of exposure to NO and the optimal duration of treatment remains unknown. Minimal exposure to NO and rapid weaning are advised in the SPC.

The use of inhaled NO should reduce the use of ECMO and therefore reduce the incidence of side effects associated with this invasive procedure. In cases where inhaled NO therapy was not successful there could be a delay in the use of other treatments, such as ECMO. For this reason guidance is now included in the SPC on what action to take when inhaled NO therapy is not successful.

Safety

Safety measures provide reassurance that NO does not increase the incidence of chronic lung disease or intra-cranial haemorrhage. However, NO and NO_2 do have detrimental effects on surfactant. Weaning is a major problem, even in patients who have no initial response and is more common with higher concentrations.

There is little in the clinical data to provide reassurance about the preclinical concern of genotoxicity and these patients are being exposed very early in life.

A major clinical objection is that NO concentrations should not exceed 20 ppm. The incidence of NO_2 and methaemoglobinaemia related side effects increases steeply above this limit, with no evidence of added benefit. It is possible that lower actual concentrations of 5 or 10 ppm may have a better risk/benefit ratio. A lower limit of tolerance for the concentration of met-haemoglobin and nitrogen dioxide is recommended.

The risk / benefit ratio of doses higher than 20 ppm has not been documented. Particularly, the impact of moderately elevated levels of NO_2 on an already injured and still immature lung is unknown.

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With reference to the complexity of the equipment, the trials showed benefit in highly specialised centres with adequate investigator training and a dedicated investigator monitoring the NO supply and making the measurements of NO, NO_2 and met-haemoglobin. Key points on training in the use of the device are given in the SPC.

Benefit/risk assessment

The overall benefit/risk assessment is considered to be positive considering that:

- *Efficacy and safety with a lower dose* - The applicant has altered the SPC to limit the upper dose to 20 ppm and limited the exposure to methaemoglobin and nitrogen dioxide as requested. Recent publications suggest that efficacy may plateau in the approximate region of 2-20 ppm. If this is the case, then a concentration of 10 ppm may be as effective as a concentration of 20 ppm. Reducing the maximum dose to which the population is exposed should decrease the risk of genotoxicity and may be associated with a more favourable risk benefit ratio. Halving the loading dose to 10 ppm would improve the theoretic risk, but decrease total exposure by only 10% over 96-hour treatment duration. Initiation of therapy at a maximum of 20 ppm, followed by a quick reduction of the dose to 5 ppm, as tolerated, starting as soon as possible and within 4-24 hours has been proposed.
- *Use, administration and monitoring*- The administration and monitoring equipment is complex. Prescribers would be trained in the use, administration and monitoring of inhaled nitric oxide.
- *Therapy of persisting pulmonary hypertension and positioning of NO* - The positioning of inhaled NO in the therapy of persistent pulmonary hypertension has not been standardised because of the lack of data. Therapies that provide optimum lung inflation (e.g. surfactant, high frequency ventilation, and positive end expiratory pressure) should be applied before and while using NO.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus decision that the benefit/risk profile of INOmax, in conjunction with ventilatory support and other appropriate agents, for the treatment of newborns≥ 34 weeks gestation with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, in order to improve oxygenation and to reduce the need for extracorporeal membrane oxygenation was favourable and therefore recommended the granting of the marketing authorisation.

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