Product: Cysteamine bitartrate IV (NM002/Nylexa®)

Chemical Name: 2-aminoethanethiol/(2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1) (salt)

Investigator's Brochure

Edition Number: Version 1.1

Release Date: 22 September 2021

Replaces Previous Edition Number: Version 1.0 dated 03 September 2021

Confidentiality Statement

This Investigator's Brochure is a confidential document for the sole information and use of recipients and investigators associated with the performance and review of studies relevant to the development of the NovaBiotics compound, cysteamine bitartrate IV (Nylexa®)

Signature Page

Sponsor: N/A as submitted by REMAP-CAP team

DATE:

Primary Contact:

Coordinating Investigator

DATE:

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. SUMMARY

NovaBiotics Ltd is developing NM002/cysteamine bitartrate IV (NM002/Nyelxa®) as a novel therapy for the treatment of community acquired pneumonia (CAP). Cysteamine bitartrate (IV) will be investigated as a separate domain specific appendix (DSA) in the international platform study - Randomised Embedded, Multi-factorial, Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) - in critically ill patients hospitalised with community acquired pneumonia (CAP).

The active pharmaceutical ingredient (API) or drug substance of NM002/Nyelxa® is cysteamine bitartrate. Cysteamine is a derivative of the amino acid cysteine (**Section 3.0**). Cysteamine (as bitartrate and hydrochloride salts) has been licensed in Europe and the US for the management of nephropathic cystinosis in adults and children 6 years and older (currently marketed as Cystagon® and Procysbi®) and approved for human clinical trials in a range of other conditions for more than 25 years.

Cysteamine is an aminothiol derivative of co-enzyme A and aminothiols have been developed and approved in oral, topical and intravenous (IV) form for a range of indications (summarised in **Table 13)**.

Cysteamine is already in late-stage clinical development by NovaBiotics as an intervention in the complex respiratory infections that drive the acute pulmonary exacerbations of cystic fibrosis (CF). This Investigator Brochure includes a detailed summary of NovaBiotics's entire development programme to date and an overview of the nonclinical and clinical findings relevant to the application of cysteamine bitartrate in the treatment of patients hospitalized with CAP.

NovaBiotics's preclinical data (Section 4.0) demonstrates that cysteamine has unique, dual antimicrobial properties: Antiviral and Antibacterial (anti-biofilm, anti-virulence and antibiotic-potentiating), is anti-inflammatory and mucoactive.

The results from the 2 clinical trials (Section 5.0) conducted by NovaBiotics of oral cysteamine bitartrate (Lynovex®) in patients experiencing pulmonary exacerbations of cystic fibrosis (EudraCT 2014-000284-40 and EudraCT 2015-004986-99, the CARE-CF 1 trial) show that cysteamine is well tolerated by CF patients, demonstrates anti-inflammatory effects and resulted in statistically significant benefit in the resolution of respiratory infection as measured by various outcomes (Devereux *et al* 2020).

The safety & toxicological potential of cysteamine bitartrate (Cystagon, 150 mg hard gel capsules) has been characterised in a number of preclinical tests performed ahead of previous marketing authorisations for its use as a therapeutic intervention for cystinosis (**Section 4.4**). Other aminothiols given as IV preparations are licensed for use.

There are over 30 years of clinical use history and data from cysteamine's application in cystinosis, safety and pharmacokinetic outcomes from previous clinical studies in healthy volunteers and cystinosis patients and also post-marketing data of cysteamine bitartrate in cystinosis patients are already described (**Section 5.4**).

2. INTRODUCTION

2.1. Background to cysteamine bitartrate in the treatment of patients hospitalized with CAP

Community Acquired Pneumonia (CAP) is a global health challenge: a significant cause of hospitalisation and illness world-wide. Respiratory tract infections are the leading cause of deaths from infectious disease globally and are the leading cause of deaths in developing nations. In the UK alone 220,000 patients are diagnosed every year with pneumonia and the urgent requirement for an effective, sustainable solution to treat CAP patients is ongoing; this need heightened when new respiratory epidemics (influenza, coronavirus) occur. Even with the deployment of vaccines for respiratory viruses such as influenza, COVID-19 and a range of bacterial pathogens, hospitalisations/ICU care requirements and mortality from resulting pneumonias will remain significant.

NovaBiotics Ltd have been investigating oral cysteamine bitartrate (Lynovex®/NM001) as an interventional treatment in infectious pulmonary exacerbations of cystic fibrosis (CF) for more than 16 years and have conducted two phase-2 clinical trials to date. Orphan drug designation was granted to NovaBiotics (EU and USA, in 2011 and 2014 respectively) for cysteamine bitartrate for the treatment of cystic fibrosis (CF) and fast track designation was granted in the US in 2018.

Preclinical and clinical studies conducted by NovaBiotics have shown that cysteamine bitartrate has numerous properties of direct relevance to the treatment of CAP:

- Immunomodulatory (anti-inflammatory)
- Antimicrobial (antibacterial and antiviral)
- Antibiotic potentiating
- Anti-virulence (including preventing biofilm formation)
- Mucolytic

Cysteamine bitartrate in IV form (NM002/Nylexa®) has been developed to enable administration to those patients critically ill with pneumonia (and in ICU, possibly ventilated and on organ support) for whom oral or inhaled formulations are not feasible or certainly not ideal. The Cysteamine/Nylexa® domain of the REMAP-CAP study will investigate the efficacy of cysteamine bitartrate in the treatment of patients hospitalised with CAP.

Evidence from NovaBiotics' studies with cysteamine bitartrate suggest that it could prevent the progression of respiratory disease to acute respiratory distress syndrome (ARDS) and the need to ventilate patients, through both its antimicrobial and immunomodulatory properties. Cysteamine could however, also have benefit in later/end stage disease and ARDS largely through its immunomodulatory actions in targeting the hyperinflammatory response and potentially preventing lung fibrosis.

Cysteamine bitartrate has immunomodulatory properties that could be of benefit in resolving inflammation/hyperinflammation. Inhibition of the glycine cleavage system in

patient cells by cysteamine causes a temporary restriction in *de novo* pyrimidine synthesis which potentiates broad-spectrum host-directed type I interferon antiviral activity (Lucas-Hourani *et* al., 2013), and is known to inhibit Th1 driven inflammation (Dimitrova *et* al., 2002). The restriction of de novo pyrimidine synthesis in host cells is a recognised antiviral drug development strategy with wide applicability across multiple viral infections and not effected by emergence of viral variants or serotypes. Cysteamine has reported antiviral activity against influenza (Yamashita *et* al., 2017) as well as coronaviruses. Furthermore, in CAP patients with secondary bacterial infection and patients with drug resistant or otherwise recalcitrant bacterial infections, cysteamine could be of benefit as it potentiates the effects of antibiotics and improves their efficacy. The potential utility of cysteamine bitartrate in treating drug resistant pneumonias where conventional antibiotics may no longer work is significant.

With multiple properties of direct relevance to the resolution of CAP, including CAP as a consequence of COVID-19, cysteamine bitartrate could be a rapidly deployable treatment of significant benefit to those patients that develop severe and even critical disease.

2.2. Therapeutic Class

Cysteamine is an aminothiol – produced as a breakdown product of co-enzyme A. It is an endogenous innate immune-defence effector molecule, and it has the below reported properties:

- Anti-inflammatory
- Antimicrobial
    - Antiviral
    - Antibacterial
        - anti-biofilm
        - anti-virulence
        - antibiotic-potentiating
- Mucolytic

2.3. Preclinical Evaluation of the Medicinal Product

NovaBiotics has performed a range of nonclinical (*in vitro*, *in vivo* and *ex vivo*) studies (Section 4.0) to determine the anti-inflammatory, antimicrobial and mucolytic functionality of cysteamine bitartrate.

2.4. Clinical Evaluation of the Medicinal Product

2.4.1. Summary of existing clinical data

Cysteamine and aminothiols have been extensively investigated for human use and aminothiols have already been approved for IV use (see **Table 13** for a summary of aminothiols and their clinical indications). Oral cysteamine bitartrate is a licensed medicinal

product for the treatment of cystinosis. and NovaBiotics have conducted two clinical studies investigating cysteamine bitartrates' potential as an intervention adjunct to standard of care in infectious pulmonary exacerbations of cystic fibrosis (see **Section 2.4.2**).

2.4.2. Available Clinical Data for Cysteamine in acute respiratory infections

To date NovaBiotics has included over a hundred CF patients in two clinical trials (NCT02212431/EudraCT 2014-000284-40 and NCT03000348/ EudraCT 2015-004986-99, (Devereux *et al* 2020) investigating oral cysteamine bitartrate (NM001/Lynovex®) as an adjunct to standard of care therapy to resolve the respiratory infections that underlie pulmonary exacerbations in CF.
The key findings from the trials were that Cysteamine bitartrate (in oral form):

1. was well tolerated when taken for up to 5 weeks
2. accumulated in the lung at 4 times the levels seen in plasma
3. demonstrated anti-inflammatory effects in short term low dose exposure with the optimal dose regimen of 450mg twice daily: cysteamine reduced white blood cell count (WBC) by $2.46x10^9$ /l (95% CI 0.11, 4.80), p=0.041 at day 14 compared with the standard of care and placebo. All treatment regimens containing cysteamine reduced CRP when compared collectively to standard of care (p=0.049) at day 14, and 450 mg cysteamine twice a day reduced geometric mean CRP 2.57 nmol/l (95% CI 0.15, 0.99), p=0.049. Elevated CRP is considered to have prognostic value for poor outcome in COVID-19 (Luo *et al*., 2020; Chen *et al*., 2020) and is also associated with severe cases of CAP (Almirall *et al*., 2004).
4. improved ventilatory function (in patients in the treatment arm relative to placebo) over a 14-day period.
5. improved patients' symptoms such as 'chest tightness' and 'feeling feverish' as recorded in the patient reported outcome measures (PROMS) using the Cystic Fibrosis Respiratory Symptom Diary-Chronic Respiratory Infection Symptom score (CFRSD-CRISS).

The improvements in patient reported outcome measures, combined with the physiological outcomes (of reduced CRP and WBC) suggest an improvement in the resolution of pulmonary inflammation which might be triggered by the dual anti-infective, and immunomodulatory properties of cysteamine, and could be of benefit in CAP and COVID-19.

3. PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1. Drug Substance

3.1.1. Chemical Name and Structure

The systematic chemical name for the API is 2-aminoethanethiol and for the IMP in bitartrate salt form ((2R,3R)-2,3-dihydroxybutanedioate (1:1) (salt).

Cysteamine bitartrate formula: $C_2H_7NS \bullet C_4H_6O_6$
Cysteamine bitartrate molecular weight: 227 amu

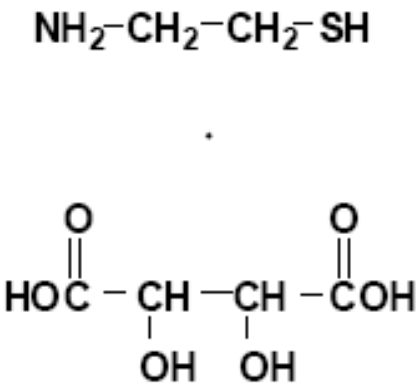


Figure 1. Chemical structure of cysteamine bitartrate

3.1.2. General Properties

Cysteamine, in both free base and bitartrate form is a white amorphous powder, highly soluble in aqueous solutions. The pH of the free base at ambient temperature is approximately 12 and the pH of the bitartrate form is approximately 6.5. The pKa1 and pKa2 values for cysteamine are 8.19 and 10.61, respectively, indicating that this compound will primarily exist in the cation form at neutral pH when buffered by its bitartrate salt or other factors.

3.2. Medicinal Product

The drug product Nylexa® will be provided as a 200 mg/mL solution of cysteamine bitartrate in 2.5 mL of water containing 500mg of for intravenous administration.

Cysteamine bitartrate will be administered intravenously every 8 hours (three times daily) as a bolus infusion via a central or peripheral line, given over 10 minutes. The dose is 5mg/Kg based on measured or estimated body weight with a total dose not exceeding 500mg (one vial). The calculated dose per mg/Kg is diluted in 50 or 100mls of 0.9% sodium chloride.

3.2.1. Description, Storage and Handling

Description

IV vials contain 200mg/mL of cysteamine bitartrate (500mg/vial) in a clear 2mL vial (VC002-13C) with a fill volume of 2.5mL. Cysteamine bitartrate IV vials (see **Figure 1**) are provided frozen and should be immediately stored in a freezer between -15°C to -25°C.

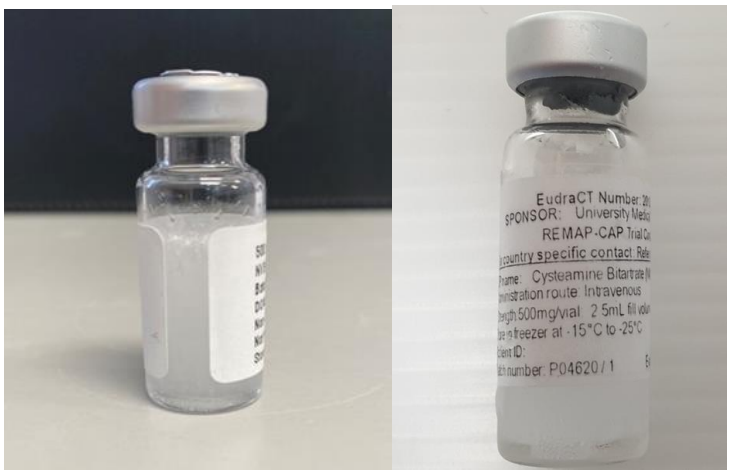


Figure 2. Representative frozen cysteamine bitartrate vials with 2.5mL fill volume.

Boxes (box size of 20cm (L) x 7cm (W) x 7cm (H)) containing 30 vials of frozen cysteamine bitartrate (sufficient for 10 days of treatment (3 vials per day)). The box and insert are white. See **Figure 3.**

Figure 3. Representative IMP box with IV cysteamine bitartrate vials

Storage and handling

Cysteamine bitartrate is temperature sensitive; vials must be stored in the freezer at -15°C to -25°C and can be thawed by one of the methods given below.

Guidance for thawing

The cysteamine bitartrate vials are stored frozen (-15°C to -25°C) and require to be thawed by one of the methods given below in **Figure 4**.

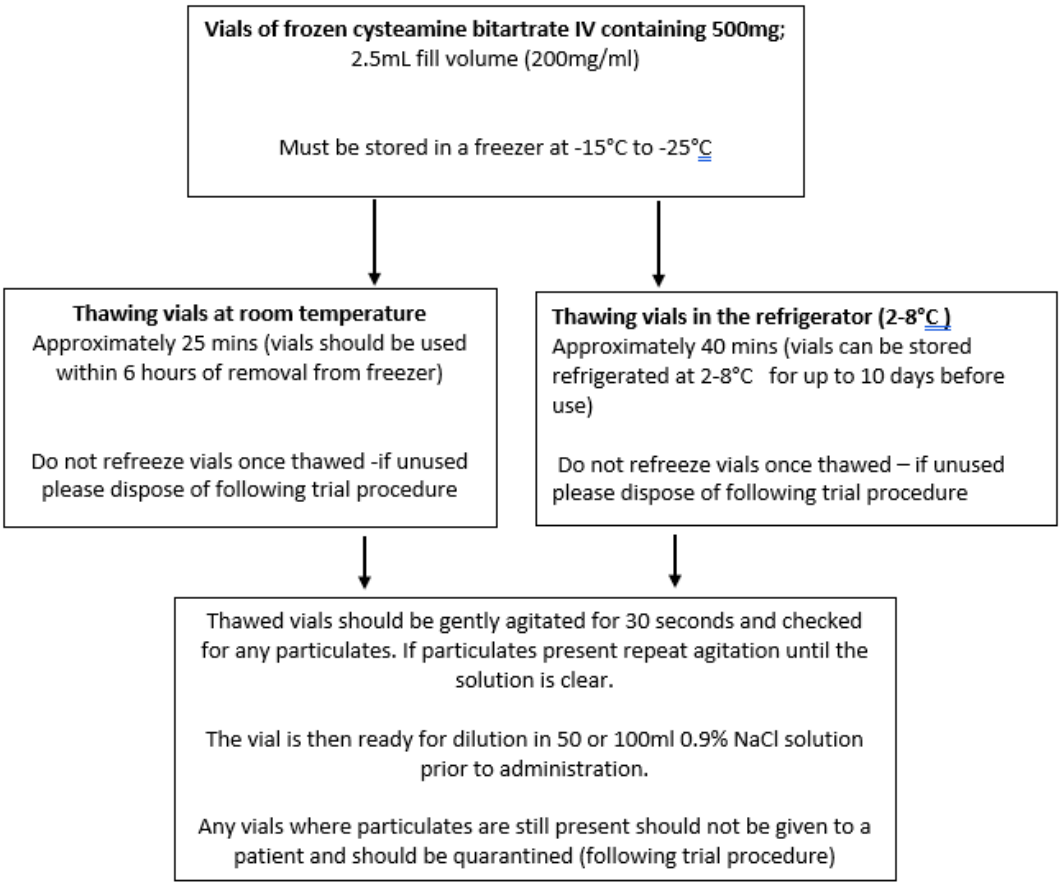


Figure 4. Methods for thawing frozen cysteamine bitartrate IMP vials

Preparation of Cysteamine bitartrate for IV administration

Cysteamine Bitartrate (200mg/mL) is a concentrated solution and should be diluted prior to administration intravenously (5mg/Kg of estimated/actual body weight, see **Table 1**, dose banding table below) with each single administered dose not to exceed 500mg. Cysteamine bitartrate should be diluted in 50 mL or 100 mL 0.9% NaCl solution prior to administration and given as below.

Table 1. Dose banding table for cysteamine bitartrate

Estimated or measured body weight (kg) Based on the upper weight in each 5kg band	Calculated dose in mgs (to give 5mg/Kg of body weight)	Required volume (mL) of 200mg/mL solution – rounded to nearest decimal point
35-40kg	200mg	1.0mL
41-45kg	225mg	1.1mL
46-50kg	250mg	1.3mL
51-55kg	275mg	1.4mL
56-60kg	300mg	1.5mL
61-65kg	325mg	1.6mL
66-70kg	350mg	1.8mL
71-75kg	375mg	1.9mL
76-80kg	400mg	2.0mL
81-85kg	425mg	2.1mL
86-90kg	450mg	2.3mL
91kg and over	475mg-500mg*	*Calculate the dose up to the extractable volume of the vial

*The total fill volume of each vial is 2.5 mL, however the total extractable volume varies between vials so for patients weighing 91kg and over use the maximum extractable volume from the vial to calculate the dose.

The infusion may be made in an aseptic environment or at the bedside, depending on local regulations. If made at the bedside, administer infusion immediately after preparation and discard remaining vials following local guidance. If preparing at the bedside, face shields should be worn in addition to standard PPE. There is no data supporting the safety of handling or preparation of cysteamine bitartrate by people who are pregnant or trying to conceive. Cysteamine bitartrate should be handled and prepared in line with local guidelines.

Calculate the volume (mL) of the thawed cysteamine bitartrate required (based on patient's estimated/actual body weight) of 200 mg/mL cysteamine bitartrate drug product (see **Table 1**, dose banding table above for guidance).

1. Cysteamine bitartrate is very sensitive to air, therefore care should be taken not to introduce air bubbles while drawing the solution from the vial into the syringe and on transferring into the infusion bag. Withdraw the required volume of cysteamine bitartrate using a needle and 1 mL or 5 mL syringe and add to a 50 mL or 100 mL 0.9% NaCl solution infusion bag (Baxter is the preferred giving set but is not essential to use).
2. The remaining contents of the vial should be disposed of in pharmaceutical waste.
3. Invert the infusion bag 10 times to ensure homogenous solution.
4. Inspect the bag. Only bags which are clear and free of visible particles can be infused.
5. It is recommended to administer cysteamine bitartrate as soon as it is diluted in the 50mL or 100mL 0.9% NaCl solution. However, if made in an aseptic environment and there is a situation where cysteamine bitartrate cannot be administered immediately, the 50mL or 100mL 0.9% NaCl bags with diluted cysteamine bitartrate can be stored in refrigerator at 2-8°C for a maximum of 24 hours prior to administration.

6. Attach the bag to the administration set and prime the line with the cysteamine bitartrate infusion then administer infusion intravenously over set at a flow rate to administer the entire bag within 10- minutes. Please note cysteamine bitartrate should not be given via the same line as beta lactams (see Potential drug interactions).
7. Flush line at the same rate as the infusion with 20mL of 0.9% sodium chloride.

Dosing

Cysteamine will be administered every 8 hours at a dose of 5 mg/kg of estimated or measured body weight, with the administered dose not to exceed 500 mg. The dose will be diluted in 50 or 100 mL of 0.9% saline and administered as an intravenous infusion over 10 minutes via a central or peripheral venous catheter.

If clinically significant hypotension occurs during infusion, the infusion rate should be slowed and, if necessary, ceased.

Monitor patients for

- Suspected or proven allergic or hypersensitivity reaction sufficient to require interruption of infusion or treatment or both. These may include one or more of the following clinical findings - urticaria, pruritus, facial flushing, wheezing, dyspnoea, and hypotension
- *Hypocalcaemia that is symptomatic or requires treatment or both
- Total neutrophil count less than 2.0×10^9 /L
- For any patients on glyceryl trinitrate – patients should be monitored for possible hypotension, which can be serious and may be indicated by headaches.

*Amifostine is an aminothiol drug that has been associated with hypocalcaemia. As cysteamine is an aminothiol compound, hypocalcaemia may be a potential side effect.

Potential Drug Interactions

Beta-lactam antibiotics are not contraindicated, but co-administration of cysteamine with beta-lactams should be avoided (as direct mixing in a line can affect the chemistry of the drugs and potentially reduce beta-lactam potency). Cysteamine is an antibiotic potentiator and is designed for co-administration with antibiotics but:

Through different lines if given concomitantly

- Through different ports of the same line sequentially
- If only a single line is available/accessible, cysteamine and beta-lactams should be administered at different times (a few minutes apart)

Glyceryltrinitrate (GTN): Simultaneous administration with cysteamine may increase the vasodilatory and platelet aggregation-inhibiting effect of glyceryl trinitrate. If such combined treatment is considered necessary, the patient should be monitored for possible hypotension, which can be serious and may be indicated by headaches.

Duration of therapy

The duration of cysteamine administration is 10 days, i.e. 30 doses. Cysteamine may be ceased at the time of ICU discharge in patients who are discharged from ICU before completion of the 10-day course. Omission of three or more consecutive doses is a protocol deviation. After 10 days, decisions regarding cysteamine therapy are at the discretion of the treating clinician. If clinically significant hypotension occurs during infusion, the infusion rate should be slowed and, if necessary, ceased.

Rationale for Dose Selection of 5mg/kg of IV cysteamine bitartrate

A 5mg/kg body weight IV dosing regimen of three times a day was selected for the first clinical study in CAP/COVID-19 – and the rationale for selecting this was based on the following:

1. The clinical trial data of oral cysteamine in infectious pulmonary exacerbations of cystic fibrosis (described above) showed that administration of up to 450 mg of oral cysteamine given four times a day for up to 5 weeks, was tolerated in patients. A twice daily dose of 450 mg of oral cysteamine for 14 days (approximately 7.5 mg/kg body weight) brought about therapeutic benefit (as measured by patient reported outcome scores (PROM and CRISS) and resulted in a statistically significant benefit in the resolution of respiratory infection in patients. This was associated with both a reduction in white blood cell counts and C-Reactive protein (CRP). 450mg bd dosing achieved a plasma C_{max} 2.86 mg/mL and of note, cysteamine concentrated in lung secretions at a sputum:plasma ratio of 4.2:1 in these patients. Devereux *et al* 2016 and Devereux *et al* 2020).
2. Cysteamine is licensed for use in cystinosis patients at up to 2 g/day for life, 1 g every 12 hours (total daily dose of 33.3 mg/kg based on a 60 kg patient). https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/203389s010lbl.pdf
3. *In vitro* experiments carried out with doses of cysteamine within the therapeutic range and below the anticipated C_{max} in plasma using 5 mg/kg, resulted in a reduction in viral load with 229E (coronavirus) and significant reductions in IL-6 release at concentrations (NovaBiotics, unpublished data). As mentioned above, concentration of cysteamine should lead to 4.2 times this level being reached in the lungs). Similarly, therapeutic concentrations do show minor reductions in CPE induced by SARS-CoV-2 in Vero cells at 72 hours post-infection.
4. In addition, a preclinical study conducted by NovaBiotics demonstrated in a murine (thigh infection) model of infection with drug resistant *Pseudomonas aeruginosa,* that intravenous administration of 1.25 mg/kg - 12.5 mg/kg was well tolerated and effective in reversing antibiotic resistance against ciprofloxacin.
5. Cysteamine has been given IV to one young male cystinosis patient (Gahl *et al*., 1995) at a 10 mg/kg dose. The plasma C_{max} of cysteamine measured was 71 µM (5.478 mg/L) – which can be compared with the less bioavailable oral administration of 7.5 mg/kg cysteamine given in the NovaBiotics clinical trial leading to a 2.86 mg/L cysteamine level in the plasma (as detailed above).

6. Aminothiols have a short half-life (cysteamine has a half-life of 3.7 hours) hence the PK/PD rationale for an IV administration given 3 times a day. NovaBiotics' previous research including a literature search, suggests that the C_{max} for IV will be higher than for oral dosing (less bioavailability) as it avoids first pass metabolism and the T_{max} will be earlier.
7. Other licensed IV aminothiols such as n-acetylcysteine and amifostine are administered at much higher doses (over 100 mg/kg and 910 mg/m^2 respectively) administered over longer infusion times than proposed for IV cysteamine bitartrate in the REMAP-CAP Study.

3.2.2. Similarity to known products

Cysteamine and aminothiols have been extensively investigated for human use and aminothiols have already been approved for IV use (see **Table 13** for a summary of aminothiols and their clinical indications). Oral cysteamine bitartrate is a licensed medicinal product (both in Europe and the US) for the treatment of cystinosis in adults and children with more than 30 years of clinical safety data collected.

The two licensed oral formulations of cysteamine bitartrate for the treatment of cystinosis in adults and children are:
https://www.drugs.com/pro/cystagon
https://www.drugs.com/pro/procysbi-delayed-release

Currently the IMP cysteamine bitartrate is not licensed in IV form, IV cysteamine bitartrate has been given two young cystinosis patients who were unable to take oral cysteamine (Gahl *et al* 1995 and Bendel-Stenzel *et al* 2008) and in some acetaminophen overdose patients at very high doses (See below).Other aminothiols are licensed for use in IV form (given by infusion and at higher doses generally than intended for cysteamine bitartrate for CAP (see **Table 13**):

Amifostine (Ethyol) as a cytoprotective adjuvant therapy given during radiotherapy and chemotherapy treatment for cancer:
https://www.drugs.com/pro/ethyol

N-acetylcysteine (NAC) is licensed as a treatment for paracetamol overdosing:
https://bnf.nice.org.uk/drug/acetylcysteine.html

In acetaminophen overdose NAC can limit the damage done by the toxic metabolite N-acetyl-p-benzoquinone (NAPQI) by boosting GSH stores which forms a non-toxic adduct with NAPQI (Figure 3). The recommended dosing regimen for using NAC to treat acetaminophen overdose is high, reaching 300 mg/kg in 21 hours. Other thiol-based drugs have been investigated for the same indication, including the delivery of high doses of IV cysteamine in a few studies (Douglas *et al* 1976 and Prescott *et al* 1976).

Potential interaction with glyceryl trinitrate (GTN)

GTN and NAC work together because GTN generates nitric oxide (NO) for vasodilation, and some of this may form S-nitroso-n-acetylcysteine with NAC which is a more stable NO donor. Cysteamine would work in the same way as S-nitrosocysteamine can form from cysteamine and NO at physiological pH (Fraser-Pitt *et al*., 2018).
Simultaneous administration of these drugs may increase the vasodilatory and platelet aggregation-inhibiting effect of glyceryl trinitrate. If such combined treatment is considered necessary, the patient should be monitored for possible hypotension, which can be serious and may be indicated by headaches.

4. NON-CLINICAL STUDIES

4.1. Introduction

For the purposes of this IB NovaBiotics has confined the information provided herein to the non-clinical studies relevant to the investigation of the pharmacology of the compound pertinent to the potential treatment of CAP. The unique multi-action properties of cysteamine bitartrate have been demonstrated in *in vitro* and *in vivo* studies, as well as an *ex vivo* sputum study of CF patients.

4.2. Non-clinical Pharmacology

4.2.1. Preclinical Anti-inflammatory Activity

NovaBiotics has demonstrated the anti-inflammatory activity of cysteamine in *in vitro* experiments summarised in **Table 2** below.

Cysteamine dose-dependently inhibits the activity of human recombinant neutrophil elastase and over-activation of this enzyme can cause tissue destruction at inflamed sites. Through inhibition of glycine decarboxylase activity cysteamine can also increase the type I interferon response and reduce IL-6 responses to viral challenge, a key mediator of the dysregulated hypercytokinaemic response in some patients to viral infection.

Table 2. Preclinical *in vitro* evidence of anti-inflammatory activity of cysteamine

Evaluation	Objective	Summary of results
Preclinical in vitro experiments	To determine the effect of cysteamine on the enzymatic activity of recombinant human neutrophil elastase activity	Dose-dependent inhibition of recombinant human neutrophil elastase activity by both cysteamine bitartrate and free base. The oxidised disulfide, cystamine, had no effect. NovaBiotics unpublished data
Pre-clinical in vitro ELISA experiments on airway epithelial cell responses to viral infection	To determine the effect on innate immune responses to cell challenge with human coronavirus 229E	Cysteamine increased the interferon beta response to this virus (which actively interferes with this response during infection) at therapeutic doses. It also reduced the IL-6 response to viral challenge. NovaBiotics unpublished data
Quantitative PCR	To determine the effect of cysteamine on the interferon response to challenge with human coronavirus 229E	Type I interferon transcript responses were upregulated by cysteamine treatment in A549 cells infected with HCoV 229E, including increases in IFNB1 and IL-10RB genes. NovaBiotics unpublished data

4.2.2. Preclinical Anti-viral activity

Cysteamine has known antiviral activity and NovaBiotics have elucidated a novel cysteamine mediated host-directed antiviral mechanism which has broad applicability against viral infection (which should not be impacted by any viral mutations and the mechanism of action indicates activity will extend beyond COVID and possibly other coronaviruses/respiratory viruses for example influenza). NovaBiotics has demonstrated reduced viral load in treated cell lines and protection from infection with human coronavirus 229E. Cysteamine's antiviral activity is summarised in **Table 3.**

Table 3. Summary of cysteamine's anti-viral activity

Evaluation	Objective	Summary of results
Pre-clinical *In vitro* experiments to determine if cysteamine disrupts the metabolism of glycine	To confirm the ability of cysteamine to inhibit eukaryotic glycine decarboxylase (part of the glycine cleavage system) determined to be important in the antiviral responses to influenza and MERS-CoV (Zhou *et al*., 2019)	Cysteamine blocks glycine utilization at therapeutic doses NovaBiotics unpublished data
Pre-clinical *in vitro* experiments to determine if cysteamine could reduce	To determine if cysteamine reduced the cytopathology (CPE) induced in vero cells	Cysteamine provided complete protection of cell monolayer at 128 mg/L up to 96 h post-

SARS-CoV-2 infection in cell culture model	by infection with SARS-CoV-2 and whether cysteamine reduced the viral load (as determined by quantitative PCR)	infection, significant reduction (~10%) in CPE could also be demonstrated at therapeutic doses as low as 0.25 mg/L at 72 h post infection compared with control. IC50 21.36 at 72 h post infection Cysteamine significantly reduced absolute viral copy number at 48 h post infection with as little as 1 mg/L cysteamine prior to any observable CPE. From 72 h pot-infection, significant reductions in viral load remain for treatments of 64 mg/L and higher as determined by quantitative PCR. NovaBiotics unpublished data
Pre-clinical *In vitro* experiments to determine if cysteamine reduces the viral load of human coronavirus	Determine the effect of single and multi-doses of cysteamine on viral load of infected airway epithelial cells	Cysteamine reduces the viral load of human coronavirus 229E (measured by TCID50 or pfu/ml) in A549 cells at therapeutic doses. NovaBiotics unpublished data
Pre-clinical *in vitro* experiments to determine if cysteamine reduces the level of viral RNA	Quantitative real time PCR evaluation of viral mRNA in infected airway epithelial cells following treatment with cysteamine	Cysteamine reduced the viral load of 229E as determined by quantitative PCR. NovaBiotics unpublished data
Pre-clinical *in vitro* assessment of airway epithelial cell integrity after challenge with human coronavirus 229E and treatment with cysteamine	To determine if treatment with cysteamine reduced cytopathology (CPE) and improved epithelial monolayer integrity in infected epithelial cells	Cysteamine treated cells had greater monolayer confluence, less CPE, and less cell debris than untreated infected cells as determined by both qualitative and quantitative microscopy. NovaBiotics unpublished data
Literature search	Evidence for antiviral activity	Existing literature shows cysteamine mediated antiviral activity versus HIV (Ho *et al.,* 1995; Bergamini *et al*., 1996), and influenza (Yamashita *et al*., 2017), though the mechanism is unclear.

4.2.3. Preclinical Antibacterial Activity

Cysteamine has a range of antibacterial properties (summarized below in **Table 4** *in vitro* and *in vivo* pharmacology studies pertinent to the treatment of acute respiratory infections including CF) pertinent to CAP.

Table 4. Summary of *in vitro* and *in vivo* antibacterial studies of cysteamine pertinent to treatment of acute respiratory infections

Evaluation	Objective	Summary of results
Non-clinical pharmacology	*In vivo* murine studies of IV cysteamine bitartrate as an antibiotic potentiator in treatment of P. aeruginosa infection	Cysteamine's tolerability/safety and efficacy as an intravenous antibiotic potentiator was demonstrated in a ciprofloxacin-resistant P. aeruginosa murine thigh infection model. Cysteamine bitartrate administered intravenously at 1.25 mg/Kg and 12.5 mg/kg 5 minutes post ciprofloxacin dosing resulted in a statistically significant restoration of antibacterial efficacy against an established thigh infection as measured by a reduction in cfu/ml and cfu/g of P. aeruginosa tissue burden versus ciprofloxacin alone. Cysteamine plus ciprofloxacin brought about a resolution of infection comparable to the positive (colistin) control at lower overall doses than orally administered cysteamine (expected with oral cysteamine's pk/pd properties and bioavailability). Fraser-Pitt *et al* 2018
Non-clinical pharmacology	Determine adjunct antimicrobial activity of 5% API formulation *in vivo* with murine acute lung infection model with *P. aeruginosa* ATCC 27853 with and without inhaled tobramycin co-therapy	5% inhaled cysteamine alone treatment caused a non-significant 0.53 log reduction in cfu/g of *P. aeruginosa* burden in lung. Tobramycin at 0.188 mg (total) had no significant effect on burden. The combination of tobramycin at this and 5 and 10% formulations of cysteamine caused highly significant reductions in microbial burden of 5.97 and 4.94 log reductions (cfu/g) respectively (Fraser-Pitt *et al*., 2018)
Non-clinical pharmacology	To assess the anti-biofilm properties of cysteamine against CF relevant bacterial pathogens and therefore potential effectiveness of cysteamine in treating microbial biofilm infections associated with CF.	Cysteamine prevents *Pseudomonas* spp and *B. cenocepacia* biofilm formation *in vitro* Cysteamine eradicates established *Pseudomonas* spp biofilms *in vitro* Cysteamine elicits a post-antimicrobial/(PAE) effect and delays the growth recovery of cells from *Pseudomonas* biofilms treated with antibiotic agents Charrier *et al* 2014, Devereux *et al* 2015, 2016, Fraser-Pitt *et al* 2016, 2018.

Non-clinical pharmacology	To assess the antimicrobial activity of cysteamine; as monotherapy and in combination against CF relevant bacterial pathogens	Cysteamine is directly antimicrobial and against a range of CF bacterial pathogens (>100 type strain and CF clinical isolates tested) Cysteamine retains its antimicrobial potential in artificial sputum medium Cysteamine significantly reduces or eradicates CF patient derived sputum microbial burden when applied alone or in combination with tobramycin (and other antibiotics) *ex vivo* in CF sputum Cysteamine is microbicidal and kills *Pseudomonas* spp within 3 h *in vitro* Cysteamine is active against difficult to treat and emerging CF bacterial pathogens including *Burkholderia* spp and *Mycobacterium abscessus* complex *in vitro* and *ex vivo* in sputum and potentiates the activity of standard of care antibiotics against these pathogens The antimicrobial activity of cysteamine is driven by conversion to cystamine (in the presence of trace amounts of transition metals) and the generation of reactive oxygen species (ROS). The uptake of cystamine into the bacterial cell also generates intracellular ROS and (via forming mixed disulfides) disrupts bacterial metabolism and stress response pathways. Cysteamine synergises with a number of classes of antibiotics (except penicillins - limitations of *in vitro* assay) in its antimicrobial activity against CF bacterial pathogens. It does this as cystamine via disruption of cell stress responses (probably including efflux) and metabolism. Both cysteamine and cystamine potentiate co-trimoxazole (folate synthesis combination). Cysteamine reverses antibiotic resistance/insensitivity in a range of CF bacteria *in vitro* (under standard CLSI conditions) and *ex vivo* (eradicating antibiotic resistant microbes from CF sputum samples) Cysteamine can also potentiate the activity of reactive oxygen and reactive nitrogen species. Cysteamine is an anti-virulence factor, inhibiting pyocyanin and other key virulence effector molecules produced by *Pseudomonas aeruginosa.* Charrier *et al* 2014, Devereux *et al* 2015, 2016, Fraser-Pitt *et al* 2016, 2018 and NovaBiotics unpublished data.

4.2.4. Pre-Clinical Anti-Mucolytic Activity

Cysteamine bitartrate has been shown to accumulate in the lungs (see Clinical studies, Devereux *et al* 2016, Devereux *et al* 2020) where it is anticipated to be rapidly mucolytic and able to loosen thick sputum, a highly advantageous trait in the treatment of CF and other acute respiratory infections. Cysteamine has been shown to breakdown mucus in *in vitro* and *ex vivo* experiments performed by NovaBiotics (detailed below in **Table 5**):

Table 5. Studies on the mucolytic activity of cysteamine

Non-clinical pharmacology	To determine the mucoactive potential of cysteamine *in vitro* and *ex vivo* to assess the potential of cysteamine as a mucolytic agent	Cysteamine significantly reduces the viscoelasticity of isolated porcine mucin *in vitro* Cysteamine disrupts mucins produced at the apical aspect of fully differentiated normal human bronchial epithelial cells *in vitro* and increases the volume of free airway surface fluid that can be recovered apically from these cultures Studies with recombinant mucin demonstrate cysteamine MOA is different from N-acetylcysteine. Cysteamine forms hydrophilic mixed disulfides with susceptible cysteines in the cystine-knot (von Willebrand factor like) domain Studies with human saliva and sputum and Alcian blue demonstrate the reduction of polymeric mucin glycoprotein by physiological concentrations of cysteamine Rheological analysis demonstrates cysteamine has largest impact on yield stress of sputum, distinct from and superior to DNAse I Cysteamine significantly reduces the viscoelasticity of CF patient derived sputum *ex vivo* Charrier *et al* 2014, Devereux *et al* 2015 and 2016 NovaBiotic's unpublished data
Non-clinical pharmacology	Determine mucolytic efficacy *ex vivo* in CF sputum for 5%, 10%, 15% and 20% loading cysteamine bitartrate API in mannitol/leucine DPI formulations	Confirmation of mucolytic activity in CF sputum superior to or equal to DNAseI where used as controls. Sponsor's unpublished data

4.2.5. Summary

The non-clinical data confirm that cysteamine has pharmacodynamic activity relevant to the acute bacterial and viral respiratory infection setting. *In vitro* and *ex vivo* studies carried out by NovaBiotics (Charrier *et al* 2014 and Devereux *et al* 2015, 2016) have shown cysteamine to be mucolytic in activity – cysteamine breaks the cysteine disulfide bonds in polymeric mucin relevant to NovaBiotics's development of cysteamine in the treatment of CF. Cysteamine also potentiates antibiotics, and potentially host-immune mediators, against relevant bacterial pathogens (Fraser-Pitt *et al*., 2018) and non-published data suggests immunomodulatory activity which could aid in the resolution of viral infection.

4.3. Pharmacokinetics and Product Metabolism in animals

NovaBiotics have not assessed pharmacokinetics or product metabolism in animals.

4.4. Non-clinical Toxicology

Cysteamine bitartrate is a licensed therapy in oral form for the treatment of cystinosis, and other aminothiols are licensed in IV form (**Table 13**). The toxicological studies conducted to support the approval of cysteamine as a cystinosis therapy are outline below.

4.4.1. Single Dose Toxicology

The LD50 in a mouse model was 625 mg/kg of body weight following oral delivery and 250 mg/kg via intraperitoneal delivery. The lethal dose in rats following a single oral dose of cysteamine bitartrate was 660 mg/kg of body weight.

4.4.2. Carcinogenicity

Cysteamine bitartrate was not mutagenic in the Ames test and resulted in a negative response in an *in vitro* sister chromatid exchange assay in human lymphocytes, but a positive response in a similar assay in hamster ovarian cells. Cysteamine bitartrate has not been tested for its carcinogenic potential in long-term animal studies.

4.4.3. Reproductive Toxicity

Reproduction studies showed embryofoetotoxic effects (resorptions and post-implantation losses) in rats at the 100 mg/kg/day dose level and in rabbits receiving cysteamine 50 mg/kg/day. Teratogenic effects have been described in rats when cysteamine is administered over the period of organogenesis at a dose of 100 mg/kg/day.

This is equivalent to 0.6 g/m^2/day in the rat, which is less than half the recommended clinical maintenance dose of cysteamine, i.e., 1.30 g/ m^2/day. A reduction of fertility was observed in rats at 375 mg/kg/day, a dose at which body weight gain was retarded. At this dose, weight gain and survival of the offspring during lactation was also reduced. High doses

of cysteamine impair the ability of lactating mothers to feed their pups. Single doses of the drug inhibit prolactin secretion in animals. Administration of cysteamine in neonate rats induced cataracts.

Studies in male and female rats given oral cysteamine bitartrate doses of 75 mg/kg of body weight per day (450 mg per square meter of body surface area (mg/sq m) per day) showed no evidence of impairment of fertility or reproductive performance (Thomson Micromedex 2005). However, another study in male and female rats given 375 mg/kg per day (2250 mg/sq m per day) revealed a reduction in survival of the offspring. Repeat breeding reproduction studies were conducted in male and female rats (Nikseresht *et al*., 2017).

Cysteamine was found to have no effect on fertility and reproductive performance at an oral dose of 75 mg/kg/day (450 mg/m2/day, 0.4 times the recommended human dose based on body surface area). At an oral dose of 375 mg/kg/day (2,250 mg/m2/day, 1.7 times the recommended human maintenance dose based on body surface area), it reduced the fertility of the adult rats and the survival of their offspring.

4.4.4. Genotoxicity

Genotoxicity studies have been performed: although in published studies using cysteamine, induction of chromosome aberrations in cultured eukaryotic cell lines has been reported, specific studies with cysteamine bitartrate did not show any mutagenic effects in the Ames test or any clastogenic effect in the mouse micronucleus test.

5. EFFECTS IN HUMANS - CLINICAL STUDIES

5.1. Introduction

Cysteamine was initially applied therapeutically in cystinosis in 1976 and cysteamine bitartrate (as Cystagon®) was first approved in 1994 in oral form for the treatment of cystinosis. The current dosing recommendations for the approved indication of treatment of cystinosis are:

> For children up to age 12 years: 1.30 g/m^2/day of the free base divided four times daily.
>
> For patients over age 12 and over 110 lbs weight: 2g/day, divided four times daily.

Clinical studies in healthy volunteers and patients with nephropathic cystinosis, plus post-marketing experience provide >30 years of safety/other clinical data (**Sections 5.3 and 5.4**).

NovaBiotics has carried out two clinical trials of oral cysteamine bitartrate patients experiencing pulmonary exacerbations of cystic fibrosis (EudraCT 2014-000284-40 and EudraCT 2015-004986-99 – CARE-CF 1) for oral cysteamine.

Cysteamine Bitartrate is not licensed in IV form but cysteamine has been administered as an infusion: as a potential treatment of acute paracetamol poisoning (Douglas *et al* 1976 and Prescott *et al* 1976) and to two young cystinosis patients who were unable to take oral cysteamine (Gahl *et al* 1995 and Bendel-Stenzel *et al* 2008).

5.2. Pharmacokinetics & Product Metabolism in Humans

5.2.1. Clinical Pharmacology

There is no available clinical pharmacology data for IV cysteamine bitartrate however there are examples in the literature of cysteamine given IV to two young cystinosis patients (Gahl *et al* 1995 and Bendel-Stenzel *et al* 2008): In the study by Gahl et al., 1995 in a paediatric cystinosis patient, 5 mg/kg cysteamine free base IV was administered (by infusion over 10 minutes in 50 mL normal saline) achieved a Cmax of 2.7 mg/L at 30 min. Cysteamine free base is much more reactive and likely to be removed from the system faster than cysteamine bitartrate salt.

A summary of pharmacokinetic studies for oral cysteamine bitartrate in adult cystic fibrosis patients (performed by NovaBiotics) is presented in **Table 6** below and **Table 7** shows the association between non-clinical pharmacology and pharmacokinetic-dynamics of oral cysteamine:

Table 6. Summary of pharmacokinetic studies for oral cysteamine

Evaluation	Results Summary
Ph2b CARE CF 1 Study, (Devereux *et al* 2020) Pharmacokinetics following a (final) single dose (day 14, final on-treatment day) of oral cysteamine bitartrate in protocol population component of 89 CF patients	The geometric mean plasma levels of cysteamine free base measured post final dose (ng/mL) were 85.65, 45.26, 129.20, 104.11, 153.38, and 10.00 in the cysteamine bitartrate 450 mg QD, 150 mg TID, 450 mg BID, 300 mg TID, 450 mg TID, and placebo groups, respectively. Equivalent plasma levels were achieved from 450 mg BID and 450 mg TID which supports the efficacy signals not increasing from 450 mg BID to 450 mg TID. The geometric mean sputum concentration levels for the cysteamine bitartrate 450 mg QD, BID, and TID doses were 342.2, 233.8, and 497.9 ng/mL of free base, respectively. For the TID regimens of cysteamine bitartrate 150 mg, 300 mg, and 450 mg, mean sputum free base concentrations were 150.0, 283.8, and 497.9 ng/mL, respectively. Additionally, cysteamine concentrations in the sputum were higher than those measured in plasma for all of the different cysteamine dose regimens investigated.
Study (Ph2a) previously performed by NovaBiotics Pharmacokinetics in blood and sputum of a single dose of	Cysteamine concentrations were quantified from serial plasma samples taken after the first dose of 450 mg cysteamine bitartrate. Cysteamine was quantified in sputum samples expectorated after the final dose of 450 mg QDS (four

cysteamine bitartrate in 10 CF patients	times daily) taken for two weeks. For 4 participants these samples were taken 3 hours post-dose and for 2 subjects 6 hours post-dose (after a change in the protocol based on results from the first 4 subjects). For the 4 sputum samples taken 3 hours post-dosing, sputum cysteamine free base concentrations were 210, 55, 114 and 317 µg/mL and for the 2 sputum samples taken six hours post-dosing they were 470 and 175 µg/mL. Examination of each pharmacokinetic profile revealed the presence of a two-compartmental model. Mean half-life of cysteamine in plasma was determined as 222 min, the T_{max} as 72 min and C_{max} of 2.86 µg/mL. Longer half-life versus cystinosis patients and healthy subjects per literature; compartmentalisation. The median (IQR) ratio of sputum to plasma cysteamine concentration in CF patients was 4.2 (0.98-8.84) and explains the compartmentalisation. Sputum cysteamine C_{max} therefore >12 µg/mL That cysteamine reaches higher concentrations/appears to accumulate in sputum than plasma is a potentially advantageous from a pharmacodynamic perspective.

Table 7. Association between non-clinical pharmacology and pharmacokinetic-dynamics of oral cysteamine

Non-clinical pharmacology	To link concentrations of cysteamine achieved in plasma and sputum in CF patients dosed with 450 mg cysteamine bitartrate BID and non-clinical pharmacological activities detailed above	Cysteamine C_{max} concentration achievable in sputum dosing with 450 mg cysteamine bitartrate was ≥12 µg/mL (free base). Concentration of cysteamine required to elicit antimicrobial effects and potentiate the activity if antibiotics against bacteria including Pseudomonas (in sputum) ≤2 µg/mL Concentration of cysteamine required to inhibit neutrophil elastase activity ≤7.7 µg/mL Concentration of cysteamine required to elicit anti-virulence effects ≤4 µg/mL Concentration of cysteamine required to prevent Pseudomonas aeruginosa biofilm formation in response to tobramycin ≤ 2 µg/mL NovaBiotics Data submitted for publication 2021

5.3. Clinical safety and efficacy

5.3.1. Clinical Efficacy

There is no available clinical efficacy data for the intravenous formulation of cysteamine bitartrate to date. NovaBiotics has previously conducted two clinical trials of oral cysteamine bitartrate in stable cystic fibrosis (CF) patients (UK study, EudraCT 2014-000284-40) and in CF patients experiencing an infectious (Gram negative bacteria) pulmonary exacerbation of CF (UK, Italy, US) (NBTCS02; NCT 002212431, IRAS 195249, EudraCT 2015-004986-99, (Devereux *et al* 2020,) A summary of both studies is given in **Table 8** below and **Table 9** shows a summary of anti-inflammatory activity in CARE-CF 1 clinical trial.

Table 8. Summary of clinical experience of cysteamine bitartrate in cystic fibrosis

Oral Cysteamine Bitartrate (NM001/Lynovex®) clinical data in Cystic Fibrosis	
Phase 2b Clinical Trial (CARE-CF-1) Efficacy and Safety of oral cysteamine in adult CF patients being treated for an exacerbation of CF-associated lung disease 89 adult patients with Gram-negative pulmonary exacerbation of their CF disease 14 days of treatment	Minimum once daily dose of 450mg required for clinical benefit but 450mg BID appears to be more clinically meaningful. Sputum microbial load was determined not to be an appropriate efficacy outcome method. Clinically meaningful Patient Reported Outcome Measure (PROM) end-point of CFRSD-CRISS (Cystic Fibrosis Respiratory Symptom Diary-Chronic Respiratory Infection Symptom Score) was the most appropriate end-point with which to assess the impact of cysteamine (plus standard of care treatment) on the resolution of the acute infections in exacerbations of the CF-associated respiratory disease. The CRISS was supported by aspects of the CFQ-R (Cystic Fibrosis Questionnaire Revised). The clinically meaningful improvements observed in the CFRSD-CRISS and the CFQ-R Respiratory Symptoms, Vitality, Health Perceptions, Weight, and Digestive Symptoms domains indicate improvement in patient outcomes. Specifically, in the CFRSD-CRISS, there was an improvement in participants in the cysteamine 450 mg BID group feeling less feverish, having less tightness in the chest, and decreased coughing compared to placebo. These patient-reported improvements were supported by laboratory measures of improvement in FEV_1 percent predicted (chest tightness domain), neutrophil elastase levels, blood leukocyte counts, and CRP in the 450 mg BID group (feverishness) compared to placebo. The efficacy signals of cysteamine appear to be supported by the plasma cysteamine levels. (Devereux *et al* 2020)

Phase 2a Clinical Trial An open label investigation of the tolerability and pharmacokinetics of oral cysteamine in adults with Cystic Fibrosis. 10 adult CF patients with stable disease	Cysteamine was generally well tolerated at up to 450mg 3 times daily (see section 5.3.2 for full safety details). Confirmation that oral cysteamine is absorbed and it does reach the sputum- the concentration in sputum is several-fold higher than that in plasma. Data from this study demonstrated that cysteamine was able to reduce microbial sputum load and reduce sputum viscosity, both of which are highly desirable attributes.

Six-armed study (CARE-CF 1) conducted to determine the effect of cysteamine on participants experiencing an acute pulmonary exacerbation of cystic fibrosis.

Table 9. Summary of anti-inflammatory activity in CARE-CF 1 clinical trial

Objective	Summary of results
Determination of any effect on neutrophil elastase from patient sputum	There was a reduction in median active neutrophil elastase concentration found in the sputum of participants on 450 BID, 300 TID and 900 total daily dose (TDD, though insignificant p>0.05).
Determination of any effect on patient reported outcome measures.	There was a significant improvement (reduction) in CFRSD-CRISS score above that for placebo alone of 9.85 points (p=0.05). This included significant reductions in the feeling feverish subdomain (p=0.016)
Determination of any effect on white blood cell count.	Cysteamine significantly reduced white blood cell count beyond that reduction seen for placebo alone by 2.46 x 10^9 cells/litre (p=0.041) for the 450 BID dose.
Determination of any effect on C-reactive protein.	Cysteamine significantly reduced CRP in all cysteamine treatment groups compared with control (p=0.049) as well as by $\log_{10}$ 0.41 nmol/L in excess of placebo at the 450 BID dose (p=0.0489)

5.3.2. Clinical Safety

There is currently no safety data for the intravenous formulation of cysteamine bitartrate. As detailed above there are examples in the literature of cysteamine given IV (Gahl *et al* 1995 and Bendel-Stenzel *et al* 2008) where the main side effects were nausea and lethargy with some association with hepatotoxicity seen in the young patient detailed in Bendel-Stenzel (2008) publication. There are also examples of IV cysteamine bitartrate given back in 1976 as a treatment for acetaminophen poisoning at very high doses (Douglas et al 1976, Prescott et all 1967).

Safety of the oral formulation was evaluated in participants of the exploratory, phase 2b, multi-centre, double-blind, randomized, parallel-group, placebo-controlled, 6-arm study (CARE-CF-1) by monitoring AEs, serious adverse events (SAEs), vital signs, laboratory parameters (chemistry, haematology, and urinalysis), physical examinations, and concomitant therapy. Additionally, a Data Safety Monitoring Board (DSMB) reviewed the

study at periodic intervals, as specified in the DSMB Charter (Devereux *et al* 2020). An overview of Treatment Emergent Adverse Events (TEAEs) is provided in **Table 10**.

Table 10. Overall summary of treatment-emergent Adverse Events (safety Population) CARE-CF-1

Characteristic	Placebo (N=17)	Cysteamine 450 mg TDD		Cysteamine 900 mg TDD		Cysteamine 1350 mg TDD	Total Cysteamine (N=72)
		450 mg QD (N=11)	150 mg TID (N=15)	450 mg BID (N=15)	300 mg TID (N=16)	450 mg TID (N=15)	
Total Number TEAEs	30	29	40	33	45	36	183
Total Number TESAEs	1	1	2	1	0	1	5
Number (%) of Participants Reporting at Least One:							
TEAE	9 (52.9)	10 (90.9)	11 (73.3)	12 (80.0)	10 (62.5)	13 (86.7)	56 (77.8)
TEAE by Severity[a]							
Mild	7 (41.2)	6 (54.5)	5 (33.3)	6 (40.0)	4 (25.0)	7 (46.7)	28 (38.9)
Moderate	2 (11.8)	4 (36.4)	6 (40.0)	5 (33.3)	5 (31.3)	6 (40.0)	26 (36.1)
Severe	0	0	0	1 (6.7)	1 (6.3)	0	2 (2.8)
TEAE by Relationship[b]							
Not Related	5 (29.4)	6 (54.5)	5 (33.3)	4 (26.7)	2 (12.5)	6 (40.0)	23 (31.9)
Related	4 (23.5)	4 (36.4)	6 (40.0)	8 (53.3)	8 (50.0)	7 (46.7)	33 (45.8)
TEAE Leading to Discontinuation of Study Drug	1 (5.9)	1 (9.1)	1 (6.7)	1 (6.7)	1 (6.3)	1 (6.7)	5 (6.9)
TEAE Requiring Dose Interruption of Study Drug	0	0	0	0	0	0	0
TEAE Causing Death	0	0	0	0	0	0	0
TESAE	1 (5.9)	1 (9.1)	2 (13.3)	1 (6.7)	0	1 (6.7)	5 (6.9)
TESAE by Severity[a]							
Mild	0	0	0	0	0	0	0
Moderate	1 (5.9)	1 (9.1)	2 (13.3)	0	0	1 (6.7)	4 (5.6)
Severe	0	0	0	1 (6.7)	0	0	1 (1.4)
TESAE by Relationship[b]							
Not Related	1 (5.9)	1 (9.1)	1 (6.7)	1 (6.7)	0	1 (6.7)	4 (5.6)
Related	0	0	1 (6.7)	0	0	0	1 (1.4)

[a] Participants reporting more than one adverse event were counted only once using the highest severity.
[b] Participants reporting more than one adverse event were counted only once using the closest relationship to study drug. Not related events included those reported as "Unlikely" or "Not Related" to study drug; related events included those reported as "Possibly Related," "Probably Related," or "Definitely Related" to study drug. **Source:** NovaBiotics Clinical Study Report NBTCS02 for the CARECF-1 study (Devereux *et al* 2020).

More TEAEs were reported in all of the cysteamine treatment groups compared to placebo, and the majority of the TEAEs reported were mild in severity. Two TEAEs were reported as severe (decreased appetite [Study Days 1-8] resolving while study drug continued), and an initially non-serious TEAE of infectious pulmonary exacerbation of CF that started on Study

Day 17, became serious on Study Day 24 due to hospitalization, and resolved on Study Day 31 with hospital discharge. (cysteamine 450 mg BID) The event term was subsequently amended by the Investigator to bronchial hyper-reactivity and was considered by the Investigator to be unrelated to study drug.

The most common TEAEs were nausea, headache and vomiting, which were reported by participants in all 5 of the cysteamine treatment groups. This is compared to a single reported headache and no cases of nausea or vomiting in the placebo group. The incidence of these events within each of the cysteamine groups did not appear to increase with any increasing cysteamine dose regimen, nor with increasing TDD of cysteamine.

For the TEAEs reported with a preferred term other than nausea, headache, or vomiting, the differences between the cysteamine treated groups and placebo were, in general, small. Two participants experienced mild and transient increases in liver function tests (ALT/AST and alkaline phosphatase). Study drug continued and the events improved in the continued presence of the study drug; neither of these 2 reports reached the accepted criteria for drug-induced hepatic impairment. The time course of the increase and reduction in liver enzymes suggests perhaps that they were a response to the infective exacerbation rather than the antibiotics or study drug.

All TEAEs leading to study drug discontinuation resolved by the end of the study, with the exception of the non-serious TEAEs of abdominal pain, nausea, and chest discomfort (all moderate in severity), reported in one participant who withdrew from the study.

One participant in each of the 6 treatment groups, including placebo, experienced a total of 9 TEAEs leading to study drug discontinuation. Of the 5 participants in the cysteamine treatment groups experiencing TEAEs that led to discontinuation, the event was reported as a SUSAR for one participant; was reported as definitely related for another participant; as possibly related in 2 participants; and as unrelated to study drug in 1 participant. The TEAE leading to discontinuation in the placebo group was reported as possibly related to study drug.

No deaths occurred during the study and only one SUSAR and 5 other SAEs were reported. With the exception of the SUSAR, all other SAEs were considered by Investigators to be unrelated to study drug.
The reported SUSAR was of depression and it is noted that the participant had a history of depression and had previously experienced a very similar clinical event, as reported for the SUSAR, and prior to entry to the study.

In conclusion, oral cysteamine in doses up to 450 mg TID were, in general, well-tolerated with no emergent safety concerns arising during this study.

The patient study (Phase 1/2a) 'An open label investigation of the tolerability and pharmacokinetics of oral cysteamine in adults with Cystic Fibrosis' EudraCT 2014-000284-40 AEs/SAEs are summarised in **Table 12**. No adverse events unrelated to those previously

reported post marketing of cysteamine for cystinosis were observed during the phase I/IIa study of cysteamine in CF patients.

5.4. Post Marketing Experience of oral cysteamine bitartrate and of IV aminothiols

The API cysteamine bitartrate is marketed in oral form as Cystagon® and Procysbi® for the unrelated condition of cystinosis and key product and safety information are summarised for reference in **Table 11** in Section 6 and in detail at the below links:

https://www.drugs.com/pro/cystagon.html
https://www.drugs.com/pro/procysbi-delayed-release

There is no marketing experience for the IV form of cysteamine bitartrate – other IV aminothiols that are marketed include: Ethyol (amifostine) and N-acetylcysteine and key product and safety information are summarised for reference in **Table 11** in Section 6 and in detail at the below links:

https://www.drugs.com/pro/ethyol.html
https://bnf.nice.org.uk/drug/acetylcysteine.html

5.5. Conclusions

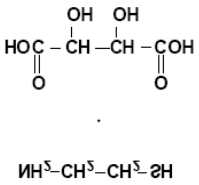
Cysteamine bitartrate has the potential to offer benefits to all CAP patients regardless of the infectious cause and as part of a readiness strategy for future pandemics. Cysteamine bitartrate accumulates in the lung, improves host defence responses to viral infection (including SARS-CoV-2 and influenza) and potentiates antibacterial therapy in the case of primary bacterial pneumonia or superadded bacterial infections secondary to viral pneumonia. Uniquely, cysteamine appears to play a key role in resolving infection and appropriately regulating causative inflammation in addition to its antimicrobial (antiviral, antibacterial) activity.

As outlined in the above **Sections 4 and 5** cysteamine bitartrate is already in late-stage clinical development for the treatment of complex respiratory infections that drive pulmonary exacerbations of cystic fibrosis (CF) lung disease. In the CARE CF 1 phase 2b study conducted (by NovaBiotics) across the UK, Italy and US, oral cysteamine bitartrate as an adjunct to standard of care CF exacerbation antibiotic therapy, resulted in a statistically significant benefit in the resolution of respiratory infection. This was associated with a reduction in white blood cell counts and C-Reactive protein (CRP) in patients. This clinical data and its relevance to CAP is supported by a raft of preclinical data on cysteamine's antimicrobial and anti-inflammatory properties (as previously described).

Cysteamine (in IV form as Nylexa®) has potential as an antimicrobial-immunomodulatory intervention for CAP by not only targeting the cause of the disease (viral and bacterial infections), but also in modulating the immune response to the infection which could bring about significant patient benefit.

6. SUMMARY OF DATA AND GUIDANCE FOR INVESTIGATORS

Table 11. Key product and safety information

Investigational Medicinal Product	**Nylexa®/NM002 (Cysteamine bitartrate) (IV)**
Chemical name and structure of API	Cysteamine bitartrate formula: $C_2H_7NS \bullet C_4H_6O_6$ 
Current Marketed Products	Cystagon® and Procysbi® in the US and the EU Oral preparations for the treatment of nephropathic cystinosis
IMP under investigation	Nylexa®/NM002 (Cysteamine bitartrate) (IV)
Post-marketing Safety Experience of Cystagon® and Procysbi® - oral cysteamine bitartrate	https://www.drugs.com/pro/cystagon.html https://www.drugs.com/pro/procysbi-delayed-release **Indication: Cystinosis** **Given Orally** **Below are summary findings – for full information please use the above links**
Contraindications	Hypersensitivity to cysteamine/penicillamine. Avoid alcohol consumption
Warnings	Skin rash, CNS symptoms, Gastrointestinal Ulceration/bleeding One report of interstitial nephritis with early renal failure – causal relationship not established
Precautions	Gastrointestinal Symptoms of nausea, vomiting, anorexia and abdominal pain Benign Intracranial Hypertension with papilledema – causal relationship not established Ehlers-Danlos-like syndrome Leukopenia and abnormal liver function studies
Pregnancy and breastfeeding warnings	Studies in animals have shown reproductive toxicity, including teratogenesis and fetotoxicity at doses less than the recommended human maintenance dose. Observed teratogenic findings were cleft palate, kyphosis, heart ventricular septal defects, microcephaly, and exencephaly. There are no adequate and well controlled studies in pregnant women. **UK:** Use should be avoided during the first trimesters of pregnancy; use is not recommended unless clearly needed.

	AU, US: This drug should not be used during pregnancy unless the benefit outweighs the risk to the foetus.
Adverse Drug Reactions	• Cardiovascular-hypertension • Gastro-intestinal-Nausea, bad breath, abdominal pain, dyspepsia, constipation, gastroenteritis, duodenitis, gastrointestinal ulceration and bleeding • Central nervous System- somnolence, encephalopathy, headache, seizures, ataxia, confusion, tremor, hyperkinesia, decreasing hearing, dizziness, jitteriness • Psychiatric-nervousness, abnormal thinking, depression, emotional liability, hallucinations, nightmares • Integumentary-urticaria • Urogenital – interstitial nephritis • Clinical Laboratory Findings – abnormal liver function, anaemia, leukopenia
Overdose	2 cases of human overdosage reported to date. Vomiting and dehydration experienced. Full recovery made. In case of overdose the respiratory and cardiovascular systems should be supported. No specific antidote is known. Haemodialysis may be considered as cysteamine is poorly bound to plasma proteins
As there is no safety experience of IV cysteamine bitartrate a summary of Post-Marketing Safety Experience for the IV aminothiol: Ethyol® *(amifostine)* has been given as an indication of potential safety issues that could be seen with IV cysteamine bitartrate – *please note: the intended doses for the IMP use in the clinical trials are significantly less than for Ethyol® so similar side effects may not be seen*	https://www.drugs.com/pro/ethyol.html **Indication: for Reduction of Cumulative Renal Toxicity with Chemotherapy** **By intravenous infusion** **Below are summary findings – for full information please use the above link**
Contraindications	Hypersensitivity to cysteamine/penicillamine
Warnings and Precautions	• Hypotension and Cardiovascular Events • Severe Cutaneous Reactions • Hypersensitivity • Nausea and Vomiting • Hypocalcaemia
Pregnancy and breastfeeding Warnings	**Use is contraindicated in pregnancy:** Risk Summary: Animal studies have revealed evidence of embryotoxicity at doses lower than the equivalent recommended human dose. Comments: -Adequate methods of contraception should be encouraged. -If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential harm to the foetus.

	Animal studies have revealed evidence of embryotoxicity. There are no controlled data in human pregnancy. It is not known whether this drug can cause foetal harm or adversely affect reproductive capacity in humans. **Use is contraindicated in breastfeeding:** Excreted into human milk: Unknown Excreted into animal milk: Data not available Comments: -The effects in the nursing infant are unknown.
Adverse Drug Reactions	Closely monitor patients receiving anti-hypertensive medications or other drugs that could cause or potentiate hypotension.
Overdose	The most likely symptom of overdosage is hypotension, which should be managed by infusion of normal saline and other supportive measures, as clinically indicated.
As there is no safety experience of IV cysteamine bitartrate a summary of Post-Marketing Safety Experience for the IV aminothiol: acetylcysteine has been given as an indication of potential safety issues that could be seen with IV cysteamine bitartrate *please note: the intended doses for use for the IMP in the clinical trials are significantly less than for acetylcysteine so similar side effects may not be seen*	https://bnf.nice.org.uk/drug/acetylcysteine.html **Indication: Paracetamol overdosage** **By intravenous infusion** **Below are summary findings – for full information please use the above link**
Cautions with IV use	asthma (see Side-effects for management of asthma but do not delay acetylcysteine treatment); atopy; may slightly increase INR; may slightly increase prothrombin time
Side Effects	**With parenteral use** acidosis; anaphylactoid reaction; angioedema; anxiety; arrhythmias; cardiac arrest; chest discomfort; cough; cyanosis; eye pain; eye swelling; generalised seizure; hyperhidrosis; hypertension; hypotension; joint disorders; malaise; nausea; pain facial; respiratory disorders; skin reactions; syncope; thrombocytopenia; vasodilation; vision blurred; vomiting **Anaphylactoid reactions (with intravenous use)** can be managed by suspending treatment and initiating appropriate management. Treatment may then be restarted at lower rate.
Clinical trial Safety experience of oral Lynovex®/NM001 (cysteamine bitartrate) in Cystic Fibrosis – see section 5.3.2	NM001 (Lynovex®) has been granted Orphan Drug Designation by the FDA (12-3687) and previous to that, by the Europeans Medicines Agency (EU/3/11/928) for the treatment of CF. Oral

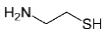
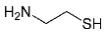
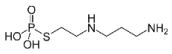
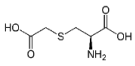
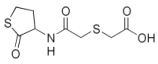
	cysteamine bitartrate was also granted Fast Track Development status (February 2018) in the U.S for the treatment of acute infections pulmonary exacerbations in CF.
Phase 2b Clinical Trial Efficacy and Safety of oral cysteamine in adult CF patients being treated for an exacerbation of CF-associated lung disease 89 adult patients with Gram-negative pulmonary exacerbation of their CF disease	Oral doses of cysteamine up to 450mg BID were generally well tolerated with no emergent safety concerns Most common Adverse Events reported were nausea, headache and vomiting Severe decreased appetite – reported in 2 individuals Bronchial hyper-reactivity (deemed to be unrelated to study drug by investigator) One SUSAR of depression (noted that patient had prior history of depression) Abdominal pain, nausea and chest discomfort (all moderate in severity) caused one patient to withdraw from the study Pulmonary exacerbation with hospitalisation Mild and transient increases in liver function tests (ALT/AST and alkaline phosphatase)
Phase 2a Clinical Trial An open label investigation of the tolerability and pharmacokinetics of oral cysteamine in adults with Cystic Fibrosis. 10 adult CF patients with stable disease	Gastro-intestinal side effects (heartburn, halitosis, anorexia, nausea and vomiting) Erythematous rash on legs Lethargy and somnolence Dizziness/forgetfulness Impetigo Herpes Labialis Exacerbation of lung disease Haemoptysis (classed as unrelated to cysteamine) Musculoskeletal chest pain
Overdose	None reported for either clinical trial

Table 12. Key clinical and pre-clinical data pertinent to treatment of acute respiratory infections

Oral Lynovex®/NM001 (cysteamine bitartrate) clinical data in Cystic Fibrosis	
Phase 2b Clinical Trial Efficacy and Safety of oral cysteamine in adult CF patients being treated for an exacerbation of CF-associated lung disease 89 adult patients with Gram-negative pulmonary exacerbation of their CF disease 14 days of treatment	Minimum once daily dose of 450mg required for clinical benefit but 450mg BID appears to be more clinically meaningful Sputum microbial load not appropriate efficacy outcome method Clinically meaningful PROM end-point of CFRSD-CRISS was the most appropriate end-point with which to assess the impact of cysteamine (plus standard of care treatment) on the resolution of the acute infections in exacerbations of the CF-associated respiratory disease. The CRISS was supported by aspects of the CFQ-R The clinically meaningful improvements observed in the CFRSD-CRISS and the CFQ-R Respiratory Symptoms, Vitality, Health Perceptions, Weight, and Digestive Symptoms domains indicate improvement in patient outcomes. Specifically, in the CFRSD-CRISS, there was an improvement in participants in the cysteamine 450 mg BID group feeling less feverish, having less tightness in the chest, and decreased coughing compared to placebo. These patient-reported improvements were supported by laboratory measures of improvement in FEV_1 percent predicted (chest tightness domain), neutrophil elastase levels, blood leukocyte counts, and CRP in the 450 mg BID group (feverishness) compared to placebo The efficacy signals of cysteamine appear to be supported by the plasma cysteamine levels
Phase 2a Clinical Trial An open label investigation of the tolerability and pharmacokinetics of oral cysteamine in adults with Cystic Fibrosis. 10 adult CF patients with stable disease	Cysteamine was generally well tolerated at up to 450mg 3 times daily (see section 5.3.2 for full safety details). Confirmation that oral cysteamine is absorbed and it does reach the sputum- the concentration in sputum is several-fold higher than that in plasma. Data from this study demonstrated that cysteamine was able to reduce microbial sputum load and reduce sputum viscosity, both of which are highly desirable attributes.
Oral Lynovex®/NM001 pre-clinical data pertinent to the treatment of acute respiratory infections	
Mechanism of action	Cysteamine is produced naturally in the body as a breakdown product of co-enzyme A and is implicated in innate immunity. Cysteamine binds susceptible cysteine residues to form mixed - disulfides and can also react with aldehyde groups in both patients and in target pathogens. Cysteamine reversibly inhibits the glycine cleavage system in human cells which can potentiate host antiviral immunity via temporary restriction of de novo pyrimidine synthesis, increasing type I interferon responses and inhibiting Th1 inflammatory responses. Bacterial pathogens lack the enzyme cysteamine dioxygenase (ADO) which can detoxify cysteamine. The disulphide product of oxidation, cystamine depletes bacterial cellular thiol pools: nicotinamide adenine dinucleotide phosphate/ glutathione (NADPH/GSH) leaving them more susceptible to stress

	including that induced by a broad range of antibiotics. Cysteamine can also dysregulate bacterial metabolism, including inhibition of the glycine cleavage system, which potentiates antifolate antibiotics. It also has broad anti-virulence properties. Cysteamine breaks apart polymeric mucin by forming mixed disulfides, rapidly altering mucin properties, reducing viscosity and yield stress of sputum.
Ex vivo studies	Cysteamine potentiated tobramycin to reduce the microbial load of sputum from CF patients. Cysteamine treatment also reduces the Spinnbarkeit of sputum.
In vivo studies	Intravenous (iv) delivery of cysteamine potentiated/synergised ciprofloxacin in a neutropenic mouse thigh model of infection with *Pseudomonas aeruginosa*. Intratracheal delivery of dry powder for inhalation (dpi) potentiated/synergised with inhaled tobramycin in a chronic lung infection model. Neither study or route of delivery had any toxicology/safety concerns
In vitro studies	Cysteamine improves host-mediated antiviral activity and inhibits cytopathology (CPE) in Vero cells caused by infection with SARS-CoV-2 with an IC_{50} of 21.36 and also reduced the CPE in A549 lung epithelial cells induced by infection with human coronavirus 229E. Cysteamine also reduces viral load of HCoV 229E as detected by quantitative PCR in A549 cells treated with cysteamine. Dose-dependent inhibition of recombinant human neutrophil elastase activity by both cysteamine bitartrate and free base. The oxidised disulfide, cystamine, had no effect. Cystemaine also increased the A549 pulmonary epithelial cell interferon beta response to HCoV 229E infection and reduced the pro-inflammatory IL-6 response detected by ELISA and supported by molecular evidence. Cysteamine potentiates a broad range of antibiotics against pulmonary pathogens, using standardised laboratory testing methods. Cysteamine inhibits biofilm formation in a range of pathogens including *P. aeruginosa, Staphylococcus aureus* and *B. cenocepacia*, and including biofilms induced by sub-inhibitory aminoglycosides. Cysteamine can be more effective against slow-growing and stationary phase bacteria when compared to other antibiotics. Sub-inhibitory concentrations of cysteamine can inhibit virulence factor production, including the inhibition of pyocyanin, pyoverdine and exopolysaccharide production in *P. aeruginosa,* staphyloxanthin production in *S. aureus* and pyomelanin secretion in *Burkholderia cenocepacia*.

Table 13. Summary of aminothiols and the clinical indications for which they are licensed/being investigated

Aminothiol	*Indication(s) and clinical trial information*	*Route of delivery and dose range used*	*Stage of development*	*Approved in following territories*
Cysteamine bitartrate 	Infectious pulmonary exacerbations of Cystic fibrosis Initial ph2a to confirm safety and tolerability trial 2014/15 information here. Global phase 2b trial to determine efficacy and determine endpoints for registration studies information here.	Oral 150 mg TID to 450 mg QID	Ph 2b	Not yet approved - Fast-track status (US) and orphan designation (US and EU)
Cysteamine bitartrate 	Maintenance of ventilatory function in CF	Dry powder inhalation	Ph 1b/2a 2020	
Cysteamine bitartrate 	Nephropathic cystinosis in adults and pediatric patients >2yrs old. Information on trials for cystinosis and many other indications available here.	Oral capsules, (dose titration not to exceed 1.95 g/m^2), granules, and ophthalmic (eye drops)	Approved	EU, USA, CAN
N-acetylcysteine 	Acetaminophen overdose. Retrospective clinical trial information here.	Intravenous infusion	Approved	Worldwide
N-acetylcysteine 	Adjuvant therapy for patients with abnormal, viscid, or inspissated mucous secretions. Information on trials for this and many other indications available here.	Oral solution (10 or 20%) for inhalation	Approved	Worldwide
Amifostine 	Cytoprotective adjuvant therapy during radiotherapy and chemotherapy for cancer. Trial information for this and other indications available here.	Intravenous	Approved	US, EU, CAN, JAP
Carbocysteine/carbocisteine 	COPD, and as OTC mucolytic. Trial information for this and other indications available here.	Oral via powder for resuspension or liquid	Widely used, OTC in some countries	
Erdosteine 	COPD. Trial information for this and other indications available here.	Oral capsules 300 mg BID	Approved	EU

7. REFERENCE SAFETY INFORMATION FOR ASSESSMENT OF EXPECTEDNESS OF SERIOUS ADVERSE REACTIONS

As indicated in **Section 5.3.2**, there are reports in the literature of cysteamine being administered intravenously to patients (with conditions not related to CAP). A case study by Gahl *et al* reported lethargy, nausea and vomiting as adverse effects following administration of 10mg/kg cysteamine free base (equivalent to 29.46 mg/kg cysteamine bitartrate) every 6 hours to a 4-year-old boy with nephropathic cystinosis and gastrointestinal dysmotility of unknown etiology (Gahl *et al* 1995). Another case study by Bendel-Stenzel *et al* also reported vomiting and lethargy at increasing doses of IV cysteamine in a 2-year-old girl with nephropathic cystinosis and severe gastrointestinal dysmotility who was treated with up to 7mg/kg of intravenous of cysteamine hydrochloride (HCl) (equivalent to 10.31 mg/kg cysteamine bitartrate) starting at 5mg/kg (equivalent to 7.36 mg/kg cysteamine bitartrate). In addition, flushing, nausea, vomiting and drowsiness were reported as adverse events for IV cysteamine hydrochloride (5310mg, equivalent to 7820mg of cysteamine bitartrate) administered in high doses for acetaminophen poisoning in adults (Douglas *et al* 1976, Prescott *et al* 1976). There were no serious adverse events reported from these studies. These studies have used much higher doses of cysteamine compared to the 5mg/kg of cysteamine bitartrate dosing schedule in the REMAP-CAP trial.

Cysteamine bitartrate has not, to the best of our knowledge, been administered intravenously in studies other than those described above and so we are unable to assess the expectedness of serious adverse reactions listed for this IMP. We can however comment that in our two previous clinical trials conducted in respiratory disease (in patients experiencing pulmonary exacerbations of cystic fibrosis) with an oral form of cysteamine bitartrate as the IMP at 7.5 mg/kg TID for 14 days adjunct to standard of care treatment (see **Section 5.3.2** for full information, and safety summary in **Table 11**), there was only one Serious Unexpected Adverse Reaction (SUSAR) of depression reported, and this did not occur more than once. Therefore, no table has been included here for the reference safety information. All other Adverse Events (AEs) reported in the two trials were deemed not related to the study drug.

No SARs are considered expected by the sponsor for the purpose of expedited reporting of SUSARs and identification of SUSARs in the "Cumulative summary tabulation of serious adverse reactions" in the DSUR for the IMP. In the cysteamine domain of REMAP-CAP trial, all life-threatening or fatal events are to be considered unexpected and will be reported as SUSARs.

8. REFERENCES

1. Almirall J, Bolíbar I, Toran P, Pera G, Boquet X, Balanzó X, Sauca G; Community-Acquired Pneumonia Maresme Study Group. Contribution of C-reactive protein to the diagnosis and assessment of severity of community-acquired pneumonia. Chest. 2004 Apr;125(4):1335-42. doi: 10.1378/chest.125.4.1335. PMID: 15078743.
2. Bendel-Stenzel MR, Steinke J, Dohil R, Kim Y (2008) Intravenous delivery of cysteamine for the treatment of cystinosis: association with hepatotoxicity. Pediatr Nephrol (2008) 23:311–315 DOI 10.1007/s00467-007-0529-8
3. Bergamini A, Ventura L, Mancino G, Cappozzi M, Placido R, Salanitro A, Cappannoli L, Faggioli E, Stoler A, Rocchi G. (1996). *In vitro* inhibition of the replication of human immunodeficiency virus type 1 by beta-mercaptoethylamine (cysteamine). *J Infect Dis.* 174(1):214-218
4. Charrier C, Rodger C, Robertson J, Kowalczuk A, Shand N, Fraser-Pitt D, Mercer D, O'Neil D. (2014). Cysteamine (Lynovex®), a novel mucoactive antimicrobial & antibiofilm agent for the treatment of cystic fibrosis. Orphanet Journal of Rare Diseases 2014 9:189
5. Chen, W., Zheng, K.I., Liu, S. *et al.* Plasma CRP level is positively associated with the severity of COVID-19. *Ann Clin Microbiol Antimicrob* **19,** 18 (2020). https://doi.org/10.1186/s12941-020-00362-2
6. Devereux G, Fraser-Pitt, D, Robertson, J, Devlina, E, Mercer, D, O'Neil, D.(2015) Cysteamine as a Future Intervention in Cystic Fibrosis Against Current and Emerging Pathogens: A Patient-based *ex vivo* Study Confirming its Antimicrobial and Mucoactive Potential in Sputum. EBIOM 2015 Aug 10; 2 (10)
7. Devereux G, Steele S, Griffiths K, Devlin E, Fraser Pitt D, Cotton S, Norrie J, Chrystyn H, O'Neil D. (2016). An open label investigation of the pharmacokinetics and tolerability of oral cysteamine in adults with cystic fibrosisClinical Drug Investigation, 36(8), 605-612.
8. Devereux G, Wrolstad D, Bourke SJ, Daines CL, Doe S, Dougherty R, Franco R, Innes A, Kopp BT, Lascano J, Layish D, MacGregor G, Murray L, Peckham D, Lucidi V, Lovie E, Robertson J, Fraser-Pitt DJ, O'Neil DA. (2020). Oral cysteamine as an adjunct treatment in cystic fibrosis pulmonary exacerbations: An exploratory randomized clinical trial. PLOS One. 15(12): e0242945. doi: 10.1371/journal.pone.0242945.
9. Dimitrova P, Skapenko A, Herrmann ML, Schleyerbach R, Kalden JR, Schulze-Koops H. Restriction of de novo pyrimidine biosynthesis inhibits Th1 cell activation and promotes Th2 cell differentiation. (2002). *J Immunol*. 169(6):3392-9.
10. Douglas AP, Hamlyn AN, James O. (1976.) Controlled Trial of cysteamine in treatment of acute paracetamol (acetaminophem) poisoning. The Lancet vol 307, Issue 7951, 111-115.
11. Fraser-Pitt D and O'Neil D. Cystic Fibrosis – a multiorgan protein misfolding disease. 2015. Future Sci.OA (2015) FS057
12. Fraser-Pitt D, Mercer D, Lovie E, Robertson J and O'Neill D. (2016). Activity of cysteamine against the Cystic Fibrosis Burkholderia cepacian complex. Antimicrob Agents Chemo. 60(10): 6200-6206.
13. Fraser-Pitt D, Mercer D K, Smith D, Kowalczuk A, Robertson J, Lovie E, Perenyi P, Cole M, Doumith M, Hill R L R, Hopkins K L, Woodford N, O'Neill D. (2018). Cysteamine, an endogenous aminothiol, and cystamine, the disulfide product of oxidation, increase Pseudomonas aeruginosa

sensitivity to reactive oxygen and nitrogen species and potentiate therapeutic antibiotics against bacterial infection. Infec Immun 10.1128/IAI. 00947-17

14. Gahl WA, Ingelfinger J, Mohan P, Bernardini I, Hyman PE, Tangleman A. (1995). Intravenous Cysteamine Therapy for Nephropathic Cystinosis. Pediatric Research 38, 579-584.
15. Ho WZ, Zhu XH, Song L, Lee HR, Cutilli JR, Douglas SD. (1995). Cystamine inhibits HIV type 1 replication in cells of monocyte/macrophage and T cell lineages. *AIDS Res Hum Retroviruses* 11(4):451-459
16. Lucas-Hourani M, Dauzonne D, Jorda P, Cousin G, Lupan A, Helynck O, Caignard G, Janvier G, André-Leroux G, Khiar S, Escriou N, Desprès P, Jacob Y, Munier-Lehmann H, Tangy F, Vidalain PO. (2013). *PLoS Pathog*. 9(10):e1003678.
17. Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, Ye L, Xiong J, Jiang Z, Liu Y, Zhang B, Yang W. Prognostic Value of C-Reactive Protein in Patients With Coronavirus 2019. Clin Infect Dis. 2020 Nov 19;71(16):2174-2179. doi: 10.1093/cid/ciaa641. PMID: 32445579; PMCID: PMC7314209.
18. Prescott LF, Sutherland GR, Park J, Smith IJ and Proudfoot AT. (1976) Cysteamine, methionine and penicillamine in the treatment of paracetamol poisoning. The Lancet 308, 7977, 103-113 https://doi.org/10.1016/S0140-6736(76)92842-7
19. Yamashita N, Yashiro M, Ogawa H, Namba H, Nosaka N, Fujii Y, Morishima T, Tsukahara H, Yamada M. (2017). Metabolic pathway catalyzed by Vanin-1 pantetheinase plays a suppressive role in influenza virus replication in human alveolar epithelial A549 cells. *Biochem Biophys Res Commun*. 489(4):466-471
20. Zhou J, Wang D, Wong BH, Li C, Poon VK, Wen L, Zhao X, Chiu MC, Liu X, Ye Z, Yuan S, Sze KH, Chan JF, Chu H, To KK, Yuen KY. Identification and characterization of GLDC as host susceptibility gene to severe influenza. 2019. *EMBO Mol Med.* 11(1):e9528